Reappointment Materials

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Statement of Reappointment

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I. Introduction

In May of 2007 I began my first year as an Assistant Professor in the Department of Chemistry at Hamilton College. After two-and-a-half years I feel as though I have made solid efforts to move my teaching and research programs forward, while contributing to the campus community through service to Department, the College and the professional community.

In the paragraphs below, I have tried to present what I believe is a summary of my teaching experience, research endeavors and service projects in my time at the College. I would like to mention that my colleagues, especially Ian Rosenstein, Robin Kinnel, and Karen Brewer have been especially helpful. My transition from Wellesley, and subsequent Hamilton experience, would not have been nearly as successful had it not been for their guidance and support. The additional guidance and support I have received from my fellow colleagues in the chemistry and biology departments, and from the administration has also been important to my success as a junior faculty member.

II. Teaching

In my time at Hamilton College I have taught four courses, each twice: Organic Chemistry I with lab (Chemistry 190), Organic Chemistry II with lab (Chemistry 255), Research Methods (Chemistry 371) and Chemical Immunology and Immunopharmacology (Chemistry 380). I have also mentored several students through Independent Research (Chemistry 298) and the Senior Research Project (Chemistry 551/552).

In the paragraphs below, I discuss my approaches in these courses, and reflect on student and faculty feedback. In addition, you will find syllabi and supplements, lecture materials, sample assignments, quizzes, and exams for each course in the appendix. For Chemistry 190 and Chemistry 255 I have coordinated sample assignments and quizzes with the sample lectures.

Organic Chemistry I with Lab (Chemistry 190 and 190L)

I taught the first semester of organic chemistry with lab in the spring of 2008, and the lecture again in the spring of 2009. Chemistry 190 is a fast-paced course that requires students be engaged on a number of levels in both the lecture and laboratory. This course is designed to introduce students to a number of topics including the physical properties of molecules, molecular structure and chemical bonding, acid base chemistry, functional groups and functional group nomenclature, stereochemistry, the nature of chemical reactions including radical, substitution, elimination and addition reactions, as well as several spectroscopic methods used to probe carbon-based compounds. The format of this course is largely lecture-based, and because this course is the first course in a two-course series there is a set amount of information that must be covered over the fifteen week term.

In my Chemistry 190 course, students are given a number of opportunities to show they have mastered the material. Weekly problem sets and quizzes are used to gauge student learning as a function of the concepts presented in the lectures. Class participation, which counts as a significant portion of the final grade, provides students with the opportunity to engage in regular discussion about the course material. Voluntary weekly review sessions are used as a basis for reviewing concepts covered in class, but also assist students in making connections to the material at an applied level. Weekly laboratory exercises reinforce the material learned in the classroom through hands-on experiences. Finally, four regularly scheduled exams and one final exam are used to evaluate how students apply the concepts they have learned to the broader chemical discipline.

Clear and effective teaching is critical in Chemistry 190, especially in such a fast-paced environment. Providing adequate and timely feedback so students know how much they have learned is equally as important. I make every effort to engage the students on a number of levels with each and every topic covered in the lecture. I also spend a significant amount of time reinforcing the concepts the students have been exposed to on a regular basis. Weekly emails titled “This Week in Chemistry 190” provide the students with a day by day breakdown of the material we cover, as well as opportunities for review and for building problem solving skills outside the classroom.

In an effort to provide adequate and timely feedback, weekly problem sets are returned within 24 hours with few exceptions. Quizzes are distributed during Friday’s class and returned on Monday. Class participation is evaluated on a quarterly basis with exams which are always returned the day after the students sit for the exam. In addition, I meet with every student, whether he or she has performed at the A or F level, following the first and second exams in Chemistry 190. I use these meetings as an opportunity to walk through the students’ exams with them so that we can discuss their progress in context. I do not require that students meet with me after the third and fourth exams. However, 70 to 80% of the students will still choose to schedule an appointment, regardless of their grade.

One of my major goals for this course has been to make the lecture as interactive as possible. As an incentive, I count class participation the same as one full exam (10% of the course), something that is relatively uncommon in the physical sciences. Students can participate via a number of activities. During almost every lecture students are asked to work problems individually or as part of a group at their work stations or on the board in front of the rest of the class. Students are also asked to verbally respond to questions initiated by their fellow students or myself. Voluntary review sessions offer yet another opportunity to participate, especially for those students that feel uncomfortable speaking in class. Participation is assessed based on the quality and frequency of a student’s involvement in these activities.

Each time I have taught Chemistry 190, the students have shown a certain hesitation to participate in the beginning of the course. I believe much of this hesitation is due to a lack of confidence in their understanding of the material, and the

fear that they will be "judged" by their classmates for making a mistake. On occasion, I have used a "mistake" or two in my lectures to show them anyone is capable of making mistakes (they also become much less hesitant to point out my mistakes). Eventually, as the course progresses, the students become more comfortable with the material, and begin to see the value of participation. Frequent participation almost always follows, and generally, by the end of Chemistry 190 the course is almost conversational in nature.

Overall, student responses to my teaching have been very good, and improved the second time I taught the course. Most students feel I am knowledgeable and present the material in a clear and concise manner. The students also feel I am approachable and provide adequate, consistent, and appropriate feedback on their work. The majority of the students especially appreciate the opportunity to meet individually with me after each exam. I believe this personalized approach helps the students realize that I genuinely care about their intellectual development, and it is through these short fifteen minute sessions that I really get to know my students academically and personally.

I have also received positive feedback from Ian Rosenstein who has visited this course each time I have taught it. In the spring of 2008 he sat through three-quarters of my classes, and in the spring of 2009 he visited six courses over a two week period. One key suggestion Ian made early on was to make my expectations for the students clearer, especially concerning the students' preparation for lecture and my goals for assigned homework. I have worked especially hard to clarify my expectations in these areas, and I feel that I have made significant progress, although there is still room for improvement.

In the spring of 2008, I taught one section of Chemistry 190 lab. This was my first time teaching this laboratory, so I spent most of the semester teaching and evaluating the labs that have been traditionally taught, with the intention of introducing new experiments and techniques into the curriculum in the future. Overall, student responses to my teaching in the laboratory have been very positive. It is worth noting here that had taught Chemistry 255 lab in the fall of 2007, so by then I had some experience in teaching the laboratory with Hamilton College students. I was therefore able to use the feedback I received in Chemistry 255 lab to improve in Chemistry 190 lab (see below for student comments in Chemistry 255 lab).

Organic Chemistry II (Chemistry 255)

I taught the second semester of organic chemistry with lab in the fall of 2008 and the fall of 2009. Chemistry 255 is a continuation of Chemistry 190, and covers several key organic reactions that are used in the synthesis of molecules as simple as the sweetener Splenda® and as complex as anticancer agent calicheamicin. The majority of the course is lecture-based and students are given similar opportunities to illustrate their mastery of the material including weekly problem sets and quizzes, regular class participation, four scheduled exams, a final examination, and an integrative laboratory experience. I also apply the same teaching strategies I use in Chemistry 190 in Chemistry 255.

By the time most students enroll in Chemistry 255, they know what to expect in terms of my expectations and the work load. Class participation is generally not an issue as most students are used to the interactive environment from Chemistry 190. One of my biggest goals for Chemistry 255 has been to find ways to get the students to spend more time working together outside of the classroom. In an effort to reach this goal, I constructed a weekly exercise I titled "Molecule of the Week." These exercises focused on the synthesis of current pharmaceutical compounds, and by design encourage collaboration. Overall, I believe the students enjoy these exercises, and by the end of the course I believe they have both a better appreciation for the material and for each other.

The first time I taught Chemistry 255, I asked the students to write a short, five-page paper on a named organic reaction. The goal of this assignment was to teach the students how to use the chemical literature effectively, and to learn to write in a style that is used for scientific communication in the context of named organic reactions, which are ubiquitous in organic chemistry. While I still feel this project is useful, I dropped this exercise in the fall of 2009 in part because of the amount of work these students are asked to complete as a part of the lecture and laboratory experiences in Chemistry 255. There is a fine line between engaging and overburdening.

Overall, student responses to my teaching have been very good. Again most students feel I am knowledgeable, and present the material in a clear and concise manner. The students also feel I am approachable and provide adequate, consistent, and appropriate feedback on their work.

I also received largely positive feedback from my colleague, Robin Kinnel, who sat in on my class for three weeks in the fall of 2008. His biggest criticism was that I seemed to miss a few "teaching moments." I believe this is in large part due to two factors: (i) my limited experience teaching the course at Hamilton; and (ii) the amount of material I needed to cover in each lecture. I have worked especially hard to improve on this point, and while there will always be room to improve (no one can accurately predict when a "teaching moment" will occur) I feel as though I am more aware of these moments and have become more effective at addressing them.

In the fall of 2007, I taught two sections of Chemistry 255 lab. This was my first time teaching at Hamilton College, and in the organic chemistry laboratory sequence, so I spent most of the semester teaching and evaluating the labs that have been traditionally taught, with the intention of introducing new experiments and techniques into the lab curriculum in the future. My first semester in the laboratory, I developed and introduce "Critical Thinking Assignments" or CTA's. CTA's were designed as case studies that required students to apply material learned in the laboratory to a "real-world" situation.

In many cases, there was no right or wrong answers to the questions posed. Students were graded mainly on their ability to analyze the case they were given and present a feasible solution to the problem.

Overall, student responses to my teaching in the laboratory have been positive. The biggest concern amongst students in the course was that I required work above and beyond what the other instructors expected (the CTA's), and that I was a difficult grader with "extremely" high expectations. In the future I will work more closely with the other laboratory instructors to make sure that the work load is more consistent. Although I will maintain my expectations, I will be clearer about what those expectations are.

Research Methods in Chemistry (Chemistry 371)

Research Methods is a junior/senior level course that provides students with the opportunity to design an independent research project based on a central theme. The course is divided into three segments. In the first two segments of the course, students work first alone and then in groups to build practical skills including: (i) how to search the chemical literature; (ii) how to frame a question and design experiments to test a hypothesis; (iii) how to physically manipulate chemical systems in the laboratory; and (iv) how to effectively communicate their results through a short paper and oral presentation. In the third segment of the course, students individually design their own experiment, conduct research, and present their experimental results at the end of the semester. This course is team-taught, and the goal of the instructors in this course is to guide the students through their joint and independent projects.

Although I was not slated to teach this in the fall of 2008, an unexpected health issue with one of our visiting assistant professors, Bradley Wile, provided me with an opportunity to serve as an instructor for the course. While I only spent six weeks working with the students, I felt my presence had a positive impact on the students' experience and I enjoyed teaching the course.

I taught the course again as the instructor of record in both the spring and fall of 2009. Each time I have taught the course, the feedback has been very positive. In general, students feel that I am helpful, and they appreciate the insight and support they receive in the laboratory, on their written work, and during their oral presentations.

Chemical Immunology and Immunopharmacology (Chemistry 380)

Chemical Immunology and Immunopharmacology is a new course at Hamilton. I taught this course for the first time in the fall of 2008 and offered it again in the fall of 2009. I designed this course to attract students from many scientific disciplines including chemistry, biochemistry, biology and neuroscience, and as such the course combines material from these disciplines in a discussion-based lecture taught in two overlapping modules. The first module provides a basic overview of the immune system with an emphasis on the important interactions that take place between macromolecules. The second module focuses on the pathology and treatment of infectious diseases and immune disorders. Actual case studies are presented, and the primary literature is used to rationalize the molecular basis for the clinical manifestations described in the case studies.

Chemistry 380 incorporates a number of traditional exercises including a midterm, final and a final project, usually a written paper, oral presentation or both. Several nontraditional exercises, including having students write and submit weekly homework questions (with answers) that I later use as midterm and final exam questions, and an artistic presentation are also important components of the course. For the weekly homework questions, students are instructed to base their questions on the course readings, and their final submission should be representative of a ten-point essay question appropriate for a take-home exam. Students are also expected to provide a one-and-a-half to one-page answer to their question. The goal of these questions is to force the students to look deeper into the topics we are covering in class to frame a question in the context of the course content that cannot simply be "looked up." The students' ability to answer their own question is equally important. Their answers provide me with insight into how the students process what they have learned, and it is surprising how many students will initially answer their own question incorrectly, or provide an answer to a question different from the one they have asked. I provide significant feedback/corrections on these questions, and one question is selected from each student for use on the midterm and the final exams. Questions are carefully chosen to ensure the exams are representative of the material covered in class.

The artistic presentation is unusual in a physical science course. Many students are accustomed to writing papers and laboratory reports, both of which are excellent methods of disseminating practical and interesting material. However, sometimes a more visual approach to the material is desirable. This project provides students with an opportunity to explore their creative side through a work of art that reflects the nature of the material we are covering in the course. Students are asked to frame their work in such a way that they teach through art. Examples of student projects include scrapbooks, sculptures, short stories, poems, theater productions, and short videos. I have included abstracts of the students work from the artistic presentations in the appendix.

The first time I taught this course in the fall of 2008 only eighteen students were enrolled. The fast-paced nature and discussion-based environment fit well with this number of students. Student responses to the course were excellent. The students felt I was knowledgeable and had an excellent grasp of the material. The students also found the course engaging, the final oral presentation on a literature review of a pharmaceutical compound relevant to the study of immunology interesting, and the midterm and final exams challenging and appropriate for a 300-level chemistry/biochemistry course. Finally, the students appreciated and enjoyed the artistic presentation component of the

course and felt as though they learned a lot about immunology through the various forms of artwork presented by their fellow classmates. The students also made me a video at the end of the semester to thank me for organizing and teaching the course. The video can be viewed on YouTube by typing in "Thank You Prof. Snyder."

The only criticism I received from the students was that they would have liked to have seen more primary literature throughout the course. Some students also would have liked to have been assigned a more advanced textbook. My colleagues Robin Kinnel and Karen Brewer also visited the course and felt Chemistry 380 was largely a success. Robin Kinnel attended nearly all of my lectures, and while he did not write a formal review of this course, his feedback was positive. Karen Brewer also felt the lectures were engaging and the students were learning a lot through the discussion-based lecture.

I used student feedback from the fall of 2008 to improve the course this past semester. I chose a more appropriate and challenging textbook, and regularly infused the lectures with material from the primary literature. I also changed the oral presentation to provide an opportunity for students with different backgrounds (chemists, biochemists, neuroscientists, and biologists) to work in small groups to prepare an original research proposal addressing a major challenge in immunology. I felt the change to the research proposal from the literature review would help the students to think more critically about how scientists design and develop experiments to answer important questions.

The biggest difference between the fall 2008 and fall 2009 courses was the size of the course. This past fall, 33 students were enrolled in the course, almost twice the number from the fall 2008. The reason this number was so high was that a number of juniors, who might have taken the course next fall, needed to take the course this fall since the course will not be taught next fall due to my pre-tenure sabbatical. In retrospect, this course really should have been physically divided into two sections. By the third week of classes I knew this course was going to be an enormous challenge to teach. Recognizing this, I worked especially hard, almost around the clock, rewriting lectures and readjusting the syllabus to meet the needs of the students and the size of the class.

Overall, I still feel the course was still a success, and so did the students. For the most part, students felt I was knowledge and they found the material engaging. Many students especially enjoyed the artistic presentations and research proposal, two of the highlights of the course. These two components will continue to remain a part of the course in future generations. The biggest criticism I received was that the course was too large, which made it difficult for students to participate frequently during the discussion-based lectures. This concern was heighted by the fact that class participation is 15% of the final grade in the course. My experience has led me to agree, and in the future I will work to make sure that the course is kept at the optimal size (between 18-20 students) for such a discussion-based lecture course.

I also received formal feedback from Ian Rosenstein who sat through the course during the artistic presentations, and rejoined the regular discussion-based lecture for a week during our discussions on bacterial and parasitic infections. Overall, Ian enjoyed the presentations and lectures he attended. He also felt that the class was managed well, and that the students were begin engaged at an appropriate level.

III. Research

I began my independent research program at Hamilton in the summer of 2007 when I worked with two Hamilton College undergraduates in the laboratory. Since then, I have worked with students every summer through the Undergraduate Summer Research Program, and every academic year through Independent Research (Chemistry 298) and the Senior Research Project (Chemistry 551/552). I am also currently advising one Senior Fellow, Gail Corneau '10, this year.

In general, I am pleased with the progress of my research program over the past two-and-a-half years. Synthetic organic chemistry requires significant continuity in order to maintain momentum. This is especially challenging at the undergraduate level where students tend to work for shorter periods of time in the laboratory. The first year, 2007-2008, was the most difficult because the students in my laboratory were only present for one year, and by the time I had them trained, they graduated. The summer of 2008 provided me with the opportunity to work with several underclassmen that would be able to contribute to my research program over the course of their academic career. Careful selection led to a core group of students that have worked for me now for almost two years. This has provided the continuity I needed, and we are gaining momentum in several areas.

In the paragraphs below, I discuss several ongoing research projects in my laboratory and highlight the scholarship derived from these projects since I arrived at Hamilton. I also discuss the long-term future of these projects, and how they will play a role in my career at Hamilton in the years to come. At the end of this document you will find a list of students I have mentored in the laboratory. I have also included relevant examples of scholarly materials in the appendix. Finally, I have included copies of the all of the proposals I have submitted for external funding (with reviewer summaries) for my research program in the appendix of this report.

Project 1: New Routes to the Synthesis of Septanose Carbohydrates

Most natural carbohydrates possess five (furanose) or six (pyranose) atoms in the carbohydrate ring. Septanose carbohydrates contain seven atoms in the carbohydrate ring. The extra atom in the ring of these molecules provides an increased flexibility that allows them to adopt a number of different low energy conformations. For this reason, septanose carbohydrates are interesting tools for studying protein-carbohydrate interactions.

I began my work on septanose carbohydrates as a graduate student at the University of Connecticut. There, I developed a new route for the preparation of septanoses using a ring-closing metathesis approach. While I was able to prepare several septanose carbohydrates in high yields, the catalyst used to perform the ring-closing metathesis, known as Schrock's catalyst, is highly sensitive to water and air, and requires the use of specialized equipment. In an effort to circumvent the use of the Schrock's catalyst for ring-closing metathesis by using the Grubbs catalyst (a more stable catalyst that does not require specialized equipment), a new and improved synthetic route would be required.

In the summer of 2007 James Greisler '10 began working on transition metal-catalyzed approach, which entailed designing propenyl ethers from allyl ethers. We felt that this approach would allow us to use Grubbs catalyst for the key ring-closing metathesis step. Brandon Clair '08 followed up on James's work in the fall of 2008 as part of his senior thesis. Together, James and Brandon accumulated enough data to present their preliminary results at the National Meeting of the American Chemical Society in New Orleans in March 2008. Unfortunately, their combined studies led us to believe that we would not be able to prepare septanoses using this approach due to a competing side reaction that would be difficult to eliminate.

Although the transition metal-catalyzed approach to prepare septanose carbohydrates was largely unsuccessful, we are now hopeful that a relay ring-closing metathesis approach, pioneered by Thomas Hoye and coworkers at the University of Minnesota, will provide us with a new route to access these molecules. Currently Graham Hone '10 is working on this project in an effort to generate a new derivative of vancomycin incorporating a glucose residue, and is making good headway (see Project 2). He will present his results at the National Meeting of the American Chemical Society in San Francisco, California this March.

In 2008, I published a full paper in the *Journal of Organic Chemistry* in this area. This paper uses a mix of experimental and computational work to help explain the stereoselectivity in a series of epoxidation reactions performed on septanose carbohydrates. The experimental work for this manuscript was largely completed during my time as a doctoral candidate at the University of Connecticut. However, the writing of this manuscript was completed during my first year at Hamilton College.

My work in this area also led to an invitation to write a chapter titled "Ring-closing Metathesis" in *Named Reactions for Ring Formations*, edited by Jie "Jack" Li of Bristol Myers Squibb and E. J. Corey of Harvard University (Nobel Prize 1990). I was only one of three small liberal arts faculty invited to write a chapter for this book. I invited one of my undergraduate research assistants, Kevin Graepel '11, to assist me in writing and preparing the manuscript for publication. Our final, ninety page, draft was just accepted and is now in the 'proof' stage for publication in early 2010. Kevin wrote ten pages of this manuscript and reviewed the final drafts and proof. The editors were so pleased with the final product that we have been asked to contribute to the next volume in this series.

Many of our projects rely on the production of septanose carbohydrates. While we are able to prepare these molecules through several traditional routes, these methods are cumbersome and inadequate for the large scale preparations of septanoses our research requires. Therefore, we will continue to focus on the design and development of new methods for the synthesis of these compounds in the years to come.

Project 2: The Synthesis of New Derivatives of Vancomycin for the Treatment of Resistant Bacteria

Vancomycin is a broad spectrum glycopeptide antibiotic that is generally used as a "last resort" for the treatment of gram positive bacterial infections, such as those caused by *Staphylococcus* ("staph" infections). Vancomycin is composed of two bioactive components, a cyclic peptide component called an aglycon, and a functionalized disaccharide composed of a vancosamine residue and a glucose residue called a glycan. The glycan and the aglycon work together to blocking the approach of several key enzymes (transpeptidases and glycosyltransferases) involved in bacteria cell wall biosynthesis.

Over the past twenty years, several vancomycin-resistant strains of bacteria have been detected. This has led researchers to search for new and more potent derivatives of vancomycin. Several students in my research group have assisted in the preparation of two new derivatives of the vancomycin glycan to study the role of the glycan in inhibiting bacterial cell wall biosynthesis by binding to glycosyltransferases. The overall goal of this project is to design new and more potent derivatives of vancomycin for the treatment of methicillin-resistant and vancomycin-resistant bacteria.

Gail Corneau '10 and Lydia Rono '11 laid the foundation for this project in the summer of 2008. Since then, Katherine Alser '08, Jared Pienkos '08, Ryan Seewald '09, Graham Hone '10, Kevin Graepel, '11 and Rem Myers '11 have assisted in this enormous effort. Gail, Lydia, Katherine, Ryan and Rem's work has focused on the synthesis of a vancomycin analog incorporating an unnatural carbohydrate residue at the vancosamine position. Together, they are about five steps away from a testable derivative. Jared, Graham, and Kevin have focused on the synthesis of a vancomycin analog incorporating an unnatural carbohydrate residue at the glucose position. They are actually about one step from a testable derivative, but need to go back and scale up their material in order to have enough compound for characterization and biological evaluation. Gail, Lydia, and Katherine presented their preliminary results at the National Meeting of the American Chemical Society in Salt Lake City, Utah in March of 2009. The ongoing results of this study will be presented by several students at the National Meeting of the American Chemical Society in San Francisco, California this March.

Once these derivatives are prepared and fully characterized, we plan to test them against panel of methicillin-resistant *Staphylococci*, vancomycin-resistant *Staphylococci* and vancomycin resistant *Enterococci*. Derivatives that show significant activity will undergo extensive biophysical characterization with penicillin binding protein 2 (PBP2), a membrane

bound glycosyltransferase isolated from *S. aureus*. The data collected from these studies will be used to develop a more specific understanding of the factors that influence the glycan-glycosyltransferase binding interaction. In the future, this information will be used to design more potent derivatives of vancomycin that can be used to combat resistant strains of gram-positive bacteria.

Project 3: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1

Galectin-1 is expressed on cell surfaces and in extra cellular matrices, and is involved in a number of critical processes including inflammation, development, mRNA splicing, differentiation, and cell adhesion. In normal cells the expression of galectin-1 is regulated. Diseased or stressed cells have been shown to over express galectin-1. For example, galectin-1 has been found in unusually high concentrations in and around tumor cells and has been implicated in several aspects of cancer biology. Research has also suggested that galectin-1 may play an important role in the promotion of HIV infectivity.

The goal of our research group is to synthesize several inhibitors of galectin-1 to evaluate the specific factors that govern the galectin-1-ligand binding interaction. An understanding of this interaction could lead to the design of new therapeutics for the treatment of cancer and HIV. Over the past two years several students, including Jodi Raymond ’08, Ben Van Arnam ’09, Sara Miller ’10, Kevin Graepel ’11, Chris Boisvert ’12, Rachel Rothbarth ‘12, Max Yelbi ‘12, and Jack Trieu ‘11 have contributed to the design and preparation of a small library of anomeric 1,2,3-lactosyl triazoles, some of these incorporating porphyrins and phthalocyanines (Project 4). Julianne Tylko ’10 is currently evaluating these molecules against a HeLa cell line to determine the ability of these molecules to inhibit the growth of cancer cells. Derivatives that show significant activity will undergo extensive biophysical characterization with human galectin-1.

We are currently in the process of preparing a manuscript based on the preliminary results of this work, and will decide where to submit our results once we have finished our biological investigations. Jodi and Ben presented the preliminary results of synthetic work; Jodi at the Sigma Xi Regional Meeting at Cornell University in Ithaca, New York in April 2008 and Ben at National Meeting of the American Chemical Society in Salt Lake City, Utah in March of 2009. Sara, Chris, and Julianne will present our most recent results, including the inhibition studies, at the National Meeting of the American Chemical Society in San Francisco, California this March. Rachel and Max will present preliminary results on a new direction in this area at the same meeting in March.

Future studies are planned for the preparation and biological evaluation of the analogous 3’ lactosyl-1,2,3-trizaoles. The goal will be to compare the data obtained from the biological and biophysical studies of the anomeric triazoles with the 3’ triazoles to determine whether any trends exist. These trends will be used to further an understanding of the galectin-1-ligand binding interaction, and the knowledge gained through these studies will serve as a foundation for the design and preparation of therapeutics that can be used to treat cancer and HIV. Further down the road we would like to repeat these studies using inhibitors that incorporate septanose carbohydrates. Derivatives incorporating unnatural carbohydrates are especially interesting since studies suggest that the increased flexibility may allow these molecules to bind and inhibit galectin-1 preferentially over molecules incorporating pyranose sugars.

Project 4: Synthesis of Carbohydrate-Porphyrin Conjugates

In the fall of 2008, Peter Zhang, Associate Professor of Chemistry at the University of South Florida, was invited to Hamilton to present his work on the synthesis of chiral porphyrins as catalysts for asymmetric epoxidation, cyclopropanation and aziridination reactions. During his visit, I asked Peter if he had ever thought about attaching carbohydrates (naturally occurring chiral molecules) to porphyrins to generate new catalysts. My thought was that by changing the type of carbohydrate (i.e. glucose versus galactose), we could tune the catalysts to generate products with different enantio- or diastereoselectivity’s.

In the winter of 2008, we wrote a proposal to the Petroleum Research Fund to support our collaboration. Our proposal was subsequently funded, and after working with Hamilton College students for eight weeks in the summer of 2008, I spent seven weeks at the University of South Florida on a Petroleum Research Fund-Summer Research Fellowship developing a palladium-catalyzed cross-coupling approach for coupling carbohydrates to porphyrins. As part of this work, I began a catalytic study using an existing chiral catalyst, a project which led to one publication in *Organic Letters* early this year.

The success of our research in the summer of 2008, led me to offer several research assistantships in the summer of 2009 to fully explore the palladium-catalyzed cross-coupling approach in the synthesis of carbohydrate-porphyrin conjugates. Hamilton College students, including Taylor Adams ’11, Peter Garrett ’11, and Kevin Graepel ’11, collaborated to prepare a host of carbohydrate derivatives which have now been coupled to several porphyrin analogs to generate the corresponding carbohydrate-porphyrin conjugates in very high yields. We are currently in the process of writing a manuscript based on this work, have a draft in place, and plan to submit our results to *Organic Letters* in late February. In addition, Taylor and Peter will present this work at the National Meeting of the American Chemical Society in San Francisco, California this March.

Recently, our research in this area led to the biological evaluation of two of our carbohydrate-porphyrin conjugates against malaria and a cancer cell line. Preliminary results from our collaborators at the Moffitt Center in Tampa, Florida are promising and suggest that these compounds may serve useful as treatments for malaria and for certain types of cancer. This has led our group to move in the direction of preparing additional carbohydrate-porphyrin and, in the last

several weeks, carbohydrate-phthalocycanine (phthalocycanines are related to porphyrins) conjugates as potential therapeutics for the treatment of these diseases. This will be a new long-term project for our research group.

This summer, Peter and I were invited to coauthor two book chapters in the *Porphyrin Science Handbook* edited by Karl Kadish, Kevin Smith, and Richard Guillard. The *Handbook* is a very important publication in the field of porphyrin chemistry. Two additional individuals, Kimberly Fields, a graduate student at the University of South Florida, and Joshua Ruppel, a former graduate student and postdoctoral fellow in Peter's lab and current Visiting Assistant Professor at Hamilton College were invited to serve as co-authors. Kimberly and Joshua completed much of the legwork including reference gathering and organization, as well as the preparation of all of the ChemDraw figures in the text. Peter and I completed most of the writing in both chapters. These chapters are both in the 'proof' stage and will be published early this year.

Project 5: Studies of Sol-Gel Encapsulated Cellulases for the Production of Cellulosic Ethanol

The development of efficient production methods for the generation of biofuels such as cellulosic ethanol is of increasing importance in today's oil-driven economy. However, the complexity of generating biocatalysts that can efficiently convert biomass into fuel is a limiting factor in this process. Our work in this area has focused on studying cellulase, a key group of enzymes used in the production of cellulosic ethanol from cellulotic biomass. Cellulase converts cellulose into glucose, which is subsequently fermented by yeasts to make ethanol.

Unfortunately cellulase is thermally labile and denatures over a short period of time when employed as a free enzyme in solution. Research has shown that the thermal stability and activity of many enzymes increases when encapsulated in an appropriate sol-gel matrix. Andrew Boddorff '10 elected to study sol-gel encapsulated cellulase from *Trichoderma reesei* as part of this senior thesis, with the goal of increasing the thermal stability and activity of this group of enzymes. This is a new project and the success of our initial experiments using cellobiose as a substrate will be measured by the ability of the sol-gel matrix to maintain the structural integrity of the encapsulated enzyme, provide adequate access to substrate, exhibit comparable reaction kinetics to the free enzyme in solution, and be stable and reusable. Long term objectives will involve optimizing these conditions for use with cellulose.

Proposals Submitted

In an effort to maximize a 2:1 match on equipment and supplies promised in my first two years at the College, I submitted eight grant proposals. Unfortunately, only one of these proposals was funded, due in part to my lack of publications beyond my graduate work (something all new faculty or postdoctoral fellows experience), and a funding environment that has changed considerably since my arrival in 2007. The proposals I submitted and the outcomes of these proposals are highlighted below. Copies of the proposals can be found in the appendix.

Prior to my arrival at Hamilton College, I submitted two proposals: a Camille and Henry Dreyfus Faculty Startup proposal based on Project 3, and a Research Corporation-CCSA proposal related to Project 3. Unfortunately, neither of these projects were funded. While the Camille and Henry Dreyfus Foundation does not provide feedback, I was able to use the feedback provided by the Research Corporation to strengthen future submissions to this organization.

In the fall of 2007, I submitted four proposals: a National Science Foundation-RUI proposal combining Projects 1 and 3, a National Institutes of Health-AREA combining Projects 1 and 2, a Petroleum Research Fund-G based on Project 5, and a Petroleum Research Fund-SRF based on Project 4. Only the fourth proposal (Petroleum Research Fund-SRF) was funded. However, I received positive and useful feedback on the National Science Foundation-RUI proposal and the National Institutes of Health-AREA proposal. The reviewers were not so generous with my Petroleum Research Fund proposal, citing the research as "[un]exciting."

In 2008, I was advised by the Research Corporation, and subsequently the Department, to focus less on proposal submission and more on publishing the results being generated in my laboratory, as these publications would eventually help me to secure funding. Therefore, I submitted only two proposals, one to the Research Corporation, a CCSA based on Project 2, and one to the Petroleum Research Fund, a UNI based on Project 5. The Research Corporation proposal received excellent reviews, but was not funded. It became clear to me, based on the comments I received from the Petroleum Research Fund, the reviewers were not excited about this project, and I would have to submit a different project for funding if I hoped to achieve funding from the Petroleum Research Fund.

This fall I submitted three proposals. I submitted one proposal to the Research Corporation, a resubmission based on Project 2, addressing specific reviewer comments. I also submitted a Petroleum Research Fund-UNI, this time based on Project 2. I am still awaiting the outcome of these submissions, and should receive results in late February/early March for both programs.

The third proposal I submitted was to the Alexander von Humboldt Programme. This proposal will provide funding for my sabbatical year (2010-2011), which I plan to spend collaborating with Professor Peter Seeberger in his laboratory at the Max Planck Institute in Berlin (assuming I am successfully reappointed). I will know the results of this submission in July 2010.

IV. Service

In my limited time at the College, I have contributed to numerous service-based opportunities. I have highlighted these below, beginning with service to the Department, followed by service to the College and professional service.

Department Service

In the 2007-2008 academic year, I assisted the department in the search for suitable candidate for a biochemistry faculty line put in place as part of the Research Corporation Department Development Award. That search resulted in the hiring of Myriam Cotten. I also participated in a search for two visiting assistant professors, Bradley Wile and Patrick Caruana.

In the 2008-2009 academic year, I began advising eight first-year students, as well as seven students in the chemistry program and seven students in the biochemistry program. I look forward to continuing to advise first year students and students in the chemistry and biochemistry programs.

In the same academic year, I assisted the department with a search for a physical chemist to replace George Sheilds, who left the College at the end of the 2007-2008 academic year to pursue an administrative position at Armstrong Atlantic State University. The search resulted in the successful hiring of Adam Van Wynsberghe, who is now in his first year and in my opinion is off to a fantastic start at the College. I also personally assisted the department in finding a last minute replacement for Patrick Caruana, who left rather unexpectedly at the end of the summer in 2009 to pursue a career as a civilian researcher in the Navy. In August we hired Joshua Ruppel, a former graduate student and post-doctoral fellow in my collaborator, Peter Zhangs laboratory at the University of South Florida. I also helped to mentor Joshua, who arrived on campus only one day before the start of classes, through his first semester of teaching. He has shared his teaching evaluations with me, and I am happy to report that his first semester, given the circumstances, was fairly successful. I will continue to work with him this semester to ensure his first year at Hamilton is a successful one.

This past year fall, I organized a Graduate Records Examination review session for our seniors looking to take the exam in chemistry for application to graduate school. I have attached a copy of the syllabus for this review session. Six review sessions were held over the course of two months, culminating in a mock GRE exam that I graded and returned to the students in preparation for the actual exam. Bradley Wile and Adam Van Wynesberghe assisted in this effort, and the students were very appreciated of our assistance.

College Service

In the 2007-2008 academic year, I served on the Strategic Planning Subcommittee for Academic Programs. I was the second faculty member on the Strategic Planning Subcommittee for Academic Programs, and my major role on the subcommittee was to evaluate advising, pedagogy, and standards and assessment at Hamilton College. I feel I made several important contributions as a member of this committee, and helped to set the overall theme—a student centered approach.

In 2008-2009 I asked to serve on the Biochemistry and Molecular Biology Committee. I am one of six faculty members on this Committee, and feel I have made significant contributions to this program over the past two years through the continued development of my Chemical Immunology and Immunopharmacology course and research program. I have also played a significant role in advising and mentoring biochemistry majors.

In the fall of 2008, I participated in two Hamilton College events: *Admissions Saturdays* and *Science Saturdays*. During one *Admissions Saturdays* event, I met with prospective students and parents at the Suida House and then took a group of eight students on a personalized tour of the Science Center. I received correspondence from two of the students and their families letting me know how much they appreciated the personalized tour I gave them. Approximately ten students were on the tour, and it is my understanding that three of those students decided to attend Hamilton.

I also hosted *Science Saturdays*, a program that brings high school students from Utica to Hamilton to take part in science demonstrations and laboratory experiments on select Saturday mornings throughout the semester. Twenty local high school students attended this event, and I directed them through a research project in which they determined the amount of cocaine on U.S. currency. A copy of this lab can be found in the appendix. I followed the laboratory experiment with liquid nitrogen ice cream! As you can imagine, the experience was a total success with the students, and I look forward to participating in *Science Saturdays* in the future.

This year, I have taken on a larger role in the *Admissions Saturdays* program. In the fall, I volunteered to lead hour-long behind the scenes tours every *Admissions Saturday* in October and November. I also assembled a short handout that I gave to students wishing to pursue a concentration in the sciences. I have included a copy of this handout in the appendix. Long term, I have plans to put together a short pamphlet highlighting faculty, coursework and research opportunities in each of the departments. I have continued to receive personal correspondence from students and their families letting me know how much they appreciate my personalized tours. I wish to continue this work in the spring, and ultimately hope my work in this area will be beneficial for all of the sciences.

Professional Service

Since the fall of 2007, I have served as a peer reviewer for several journals including the *Journal of Carbohydrate Chemistry*, the *Journal of Organic Chemistry* and the *Journal of Medicinal Chemistry*. I am also a member of the review panel for *Scientific Journals International.*

In the spring of 2008 I organized the General Papers session for the Chemical Education Division at the National Meeting of the American Chemical Society, held in Philadelphia, Pennsylvania in August 2008. As an organizer, I was responsible for selecting papers for talks to be given at the meeting, and subsequently hosted the General Papers session. I am happy to report that the meeting was a success, and I was selected to Chair the Chemical Education Division's National program for the National Meeting of the American Chemical Society in 2010.

In the spring of 2008, I was also selected to be a District Delegate for the Syracuse Section of the American Chemical Society. I have held this position for two years. My main responsibilities are to help coordinate Section activities along with the Section Chair, Rob Stankavage and the Section Chair Elect, Michelle Boucher of Utica College.

V. Conclusions

I feel as though I have made good progress toward tenure in my two-and-a-half years at Hamilton College. My teaching in the organic chemistry sequence, Research Methods, and Chemical Immunology and Immunopharmacology has been largely successful, though I realize there will always be room for improvement. In addition, I feel my research program is off to a good start with one original research publication published to date and two additional publications in the writing stage. I am also pleased with the three book chapters currently in 'proof,' one coauthored with a Hamilton College undergraduate, and another with a Hamilton College faculty colleague. Finally, I feel as though I have contributed to the Department, College, and to the professional community through numerous opportunities in service, many of these ongoing.

I recognize that the next few years will be important in terms of maintaining good teaching and publication records. I hope that this summary makes clear my ongoing commitment to both of these areas. I will continue to address the needs of my students in the classroom and laboratory. I will also continue to develop a sustainable research program. At the current pace, I expect to publish at least one or two more manuscripts before tenure. With this momentum, I feel I will be able to continue to publish at least one manuscript every other year throughout my career.

Finally, I would like to take this opportunity to say that I have thoroughly enjoyed my first two-and-a-half years at Hamilton. My daily interactions with the students, faculty, and administration have enriched my life in so many ways, and there is really no other place I would rather be. I am looking forward to many more years as a productive member of the Hamilton College faculty.

List of Students Supervised with Titles

Academic Year 2009-2010

- **Gail Corneau—**"*Seven Membered and Sweet: Routes to Rational Antibiotic Design Using Unnatural Carbohydrates to Explore Antimicrobial Resistance in Staphylococcus Infections."* (Senior Fellowship)
- **Julianne Tylko—**"*Investigating the Nature of the Binding Interactions of a Series of Lactosyl 1,2,3-Triazoles and a Monoclonal Antibody to Human Galectin-1 using a Human Carcinoma Model."* (Senior Thesis)
- **Graham Hone—"***Synthesis of an Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Glucose Position."* (Senior Thesis)
- **Ryan Seewald—**"*Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar."* (Senior Thesis-Fall 2009)
- **Andrew Boddorff—***"Studies on the Sol-Gel Encapsulation of Cellulases for the Production of Cellulosic Ethanol."* (Senior Thesis)
- **Sara Miller—***"Synthesis and Biological Evaluation of Anomeric and 3' Lactosyl 1,2,3-Triazoles as Inhibitors of Galectin-1."* (Senior Thesis)
- **Chris Boisvert—***"Synthesis of Carbohydrate-Porphyrin Conjugates as Potential Compounds for Photodynamic Therapy." (0.5cr*, *Fall 2009-Spring 2010)*
- **Jack Trieu—***"Synthesis of Carbohydrate-Porphyrin Conjugates as Potential Compounds for Photodynamic Therapy." (0.5cr*, *Fall 2009)*
- **Kevin Graepel—***"Synthesis of Carbohydrate-Phthalocyanines Conjugates as Potential Compounds for Photodynamic Therapy."* (*0.5cr*, Spring 2010)

Summer 2009 (6 weeks)

- **Graham Hone—***"Synthesis of an Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Glucose Position."*
- **Sara Miller—***"Synthesis of Anomeric 1,2,3-Triazoles and 1,2,3,5-Tetrazoles as Inhibitors of Galectin-1."*
- **Lydia Rono—**"*Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar."*
- **Peter Garrett—***"Synthesis of Carbohydrate-Porphyrin Conjugates as Potential Compounds for Photodynamic Therapy."*
- **Kevin Graepel—***"Synthesis of an Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Glucose Position."* (7 weeks)
- **Rem Myers—**"*Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar."*
- **Taylor Adams—***"Synthesis of Carbohydrate-Porphyrin Conjugates as Potential Compounds for Photodynamic Therapy."*
- **Chris Boisvert—***"Synthesis of Anomeric 1,2,3-Triazoles and 1,2,3,5-Tetrazoles as Inhibitors of Galectin-1."*
- **Max Yelbi—**"*Synthesis of Carbohydrate-Porphyrin Conjugates for the Treatment of Malaria."*
- **Rachel Rothbarth—**"*Synthesis of Carbohydrate-Porphyrin Conjugates as Agents for Photodynamic Therapy."*

Academic Year 2008-2009

- **Ben van Arnam—***"The Synthesis of Anomeric 1,2,3-Fused Lactosyl Triazoles and 1,2,3,5-Tetrazoles as Inhibitors of Galectin-1."* (Senior Thesis)
- **Katherine Alser—**"*Synthesis and Conformational Analysis of an Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar "* (Senior Thesis)
- **Jared Pienkos—"***Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Glucose Position."* (Senior Thesis)
- **Lydia Rono—**"*Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar."* (*0.5cr*, Fall 2008-Spring 2009)
- **Gail Corneau—**"*Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar."* (*1cr*, Spring 2009)
- **Ryan Seewald—**"*Synthesis of a Vancomycin Glycan Incorporating an Unnatural Carbohydrate Residue at the Vancosamine Sugar."* (*1cr*, Spring 2009)
- **Peter Garrett—**"*Synthesis and Evaluation of Galectin-1 Inhibitors Derived from Lactose."* (*1cr*, Spring 2009)

Summer 2008 (8 weeks)

- **Ben van Arnam—***"Synthesis of Lactosyl 1,2,3,5-Tetrazoles as Tools to Study Galectin-1-Ligand Interactions"* and *"The Synthesis of Nucleoside Analogs via Click Chemistry."*

- **James Greisler—**"*Evaluation of a Transition Metal-Catalyzed Isomerization/Ring Closing Metathesis Route for the Synthesis of Carbohydrate Based Oxepines."* and *"Preparation of Glucose Acceptors for the Synthesis of Carbohydrate Bearing Porphyrins."*
- **Gail Corneau—**"*Synthesis of an Unnatural Derivative of the Vancosamine Sugar of Vancomycin."*
- **Lydia Rono—**"*Synthesis of an Unnatural Derivative of the Vancosamine Sugar of Vancomycin."*
- **Sven Oman**—*"Determining the Factors that Contribute to Red Blood Cell Fragility in a Diabetic Mouse Model."*

Academic Year 2007-2008

- **Brandon Clair—**"*Synthesis of Septanose Carbohydrates from Pyranose Precursors."* (Senior Thesis)
- **Jodi Raymond—***"The Synthesis and Characterization of 1,2,3-Lactosyl Triazoles as Potential Inhibitors of Galectin-1."* (Senior Thesis)
- **James Greisler—"***Evaluation of a Transition Metal-Catalyzed Isomerization/Ring Closing Metathesis Route for the Synthesis of Carbohydrate Based Oxepines."* and *"Preparation of Glucose Acceptors for the Synthesis of Carbohydrate Bearing Porphyrins."* (*0.5cr*, Fall 2007)

Summer 2007 (8 weeks)

- **James Greisler—**"*Evaluation of a Transition Metal-Catalyzed Isomerization/Ring Closing Metathesis Route for the Synthesis of Carbohydrate Based Oxepines."*
- **Elijah LaChance—**"P*rogress on the Synthesis of an Enediyne Conjugate Derived from 1,4-Natphthoquinone."*

Nicole Leigh Snyder

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Education:

University of Connecticut Storrs, CT
Ph.D. in Organic and Biological Chemistry December 2005

Thesis: "New Perspectives on the Synthesis and Function of Septanose Carbohydrates"
Thesis Advisor: M.W. Peczuh

Westminster College New Wilmington, PA
B.S. in Chemistry and Biology, Cum Laude May 2000

Thesis: "A Method for Determining Levels of Ergosterol in the Environment Using Gas Chromatography-Mass Spectroscopy"
Thesis Advisor: T.A. Sherwood

Professional Experience:

Hamilton College Clinton, NY
Assistant Professor of Chemistry 2007-Present

-Principal investigator of a research team focusing on the design and synthesis of natural and unnatural carbohydrate systems that can be used to probe a number of key macromolecule-carbohydrate interactions.
-Primarily responsible for lecturing the College's two semester organic chemistry sequence with laboratory and research methods.
-Designed and developed a new course on immunology and immunopharmacology.

Wellesley College Wellesley, MA
Visiting Assistant Professor of Chemistry 2005-2007

-Principal investigator of a research group that primarily focused on the production of a vancomycin derivative incorporating an unnatural carbohydrate at the vancosamine position.
-Lectured the College's two semester organic chemistry sequence with laboratory in addition to a one semester general chemistry course with laboratory.
-Designed and developed a new course on carbohydrate chemistry.

University of Connecticut Storrs, CT
Research and Teaching Assistant 2000-2005

-Developed synthetic routes towards the production of carbohydrate-based oxepines (ring expanded glycals) for use as precursors in the synthesis of septanose monosaccharides.
-Taught a number of introductory and advanced level courses at the undergraduate level including general chemistry I with lab, general chemistry II with lab, a one semester organic chemistry course for nursing and nutritional science majors, organic chemistry I with lab, and organic chemistry II with lab.
-Recruited and trained new undergraduate and graduate students in developing the skills and techniques necessary to further the research and teaching goals of the program.

Professional Activities:

Reviewer for the *Journal of Carbohydrate Chemistry*
Reviewer for the *Journal of Organic Chemistry*
Reviewer for the *Journal of Medicinal Chemistry*
Member of the Review Panel for *Scientific Journals International*
District Delegate for the Syracuse Section of the American Chemical Society

Professional Affiliations:

American Chemical Society *(2001-Present)*
American Association for the Advancement of Sciences *(2002-Present)*
The Society for Glycobiology *(2008-Present)*
Sigma Xi *(2006-Present)*
Council on Undergraduate Research *(2007-Present)*
Beta Beta Beta—Biological Honors Society *(2000-Present)*
Phi Lambda Upsilon—Chemistry Honors Society *(2002-Present)*

Grant Activity:

Petroleum Research Fund (SRF)
American Chemical Society, Summer 2008, $8,000.00

Start-up Award
Hamilton College, 2007-2010, $50,000.00

Brachman Hoffman Grant
Wellesley College, 2006, $4,300.00

Staley Small Grant
Wellesley College, 2006, $3,973.99

Faculty Awards Grant
Wellesley College, 2005-2006, $3,000.00

Professional Awards:

Elsevier Top-50 Most Cited Articles Award (2004-2007) for "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine."
Awarded at EuroCarb 2007 in Lubeck, Germany

The Society of Analytical Chemists of Pittsburgh College Chemistry Award
Awarded in Pittsburgh, Pennsylvania in May 2000

Publications:

Adams, T. P.*, Garrett, P. F.*; Fields, K. B.; Xu, X.; Graepel, K. W.*; Hone, G. B.*; Zhang, X. P.; Snyder, N. L. "The Synthesis of Carbohydrate-Porphyrin Conjugates via a Palladium-Catalyzed Cross-Coupling Approach." *Manuscript in Preparation for Submission to Organic Letters (mid-February).*

Snyder, N.L., Graepel K. W.* "Ring Closing Metathesis" in *Named Reactions for Ring Forming Reactions;* Li, J. J., Corey, E. J. Eds. (Invited Chapter—*Submitted*)

Fields, K. B.; Ruppel, J. V.; Snyder, N. L.; Zhang, X. P. "Porphyrin Functionalizaton via Palladium-Catalyzed Carbon-Heteroatom Cross-Coupling Reactions." in *The Porphyrin Science Handbook*; Kadish, K.; Smith, K.; Guillard, R. Eds. (Invited Chapter—*Submitted*)

Ruppel, J. V.; Fields, K. B.; Snyder, N. L.; Zhang, X. P. "Metalloporphyrin-Catalyzed Asymmetric Atom/Group Transfer Reactions." in *The Porphyrin Science Handbook*; Kadish, K.; Smith, K. M.; Guillard, R. Eds. (Invited Chapter—*Submitted*)

Ruppel, J. V.; Gauthier, T. J.; Perman, J.A.; Snyder, N.L.; Zhang, X.P. "Asymmetric Cobalt-Catalyzed Cyclopropanation with Succinimidyl Diazoacetate: General Synthesis of Optically Active Cyclopropyl Carboxamides." *Organic Letters* **2009**, *11,* 2273-2276.

Markad, S.D; Xia, S.; Snyder, N.L.; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxidation of Carbohydrate Based Oxepines." *Journal of Organic Chemistry* **2008***, 73(16)*, 6341-6354.

Castro, S.; Cherney, E. C.*; Snyder, N. L.; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007**, *342(10)*, 1366-1372*.*

Snyder, N.L.; Peczuh, M.W. Haines, H.M.* "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006***, 62,* 9301-9320.

Castro, S.; Duff, M.; Snyder, N.L.; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic and Biomolecular Chemistry* **2005**, *3,* 3869-3872*.*

DeMatteo, M. P.; Snyder, N.L.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38*.*

Peczuh, M.W.; Snyder, N.L.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004***, 339(6),* 1163-1171.

Peczuh, M.W.; Snyder, N.L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, 44, 4057-4061.

*Indicates undergraduate coauthor.

Lectures:

Adams, T. P.*, Garrett, P. F.*; Fields, K. B.; Xu, X.; Graepel, K. W.*; Hone, G. B.*; Zhang, X. P.; Snyder, N. L. "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts." Talk presented at Connecticut College, New London, CT in October **2009**.

Adams, T. P.*, Garrett, P. F.*; Fields, K. B.; Xu, X.; Graepel, K. W.*; Hone, G. B.*; Zhang, X. P.; Snyder, N. L. "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts." Talk presented at Connecticut College, New London, CT in October **2009**.

Adams, T. P.*, Garrett, P. F.*; Fields, K. B.; Xu, X.; Graepel, K. W.*; Hone, G. B.*; Zhang, X. P.; Snyder, N. L. "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts." Talk presented at Hartwick College, Oneonta, NY in September **2009**.

Adams, T. P.*, Garrett, P. F.*; Fields, K. B.; Xu, X.; Graepel, K. W.*; Hone, G. B.*; Zhang, X. P.; Snyder, N. L. "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts." Poster presented at the Gordon Conference, Tilton, NH in June **2009**.

Snyder, N.L.; Alser, K. A.*; Corneau, G. C.*; Pienkos, J. A.*; Rono, L. J.*, Seewald, R. H.* "Understanding the Role of the Vancomycin Glycan in Binding Glycosyltransferases: The Design and Synthesis of Two Novel Glycan Derivatives of Vancomycin with the Potential for Combating Antibiotic Resistance." Poster presented at the Sigma Xi Regional Meeting, Oswego, NY in April **2009**.

Snyder, N.L.; Van Arnam, B. J.* "The Synthesis and Characterization of 1,2,3-Lactostyl Triazoles and 1,2,3,5-Lactosyl Tetrazoles as Potential Inhibitors of Galectin-1." Poster presented at the 237th ACS National Meeting, Salt Lake City, UT in March **2009**.

Snyder, N.L.; Alser, K. A.*; Corneau, G. C.*; Pienkos, J. A.*; Rono, L. J.* "Understanding the Role of the Vancomycin Glycan in Binding Glycosyltransferases: The Design and Synthesis of Two Novel Glycan Derivatives of Vancomycin with the Potential for Combating Antibiotic Resistance." Poster presented at the 237th ACS National Meeting, Salt Lake City, UT in March **2009**.

Snyder, N.L. "Adventures in Carbohydrate Chemistry: The Design, Synthesis, and Biological Evaluation of Carbohydrate-based Therapeutics." Talk presented at Colgate University, Hamilton, NY, September **2008**.

Snyder, N.L.; Raymond, J. C.* "The Synthesis and Characterization of 1,2,3-Lactosyl Triazoles as Potential Inhibitors of Galectin-1." Poster presented at the Sigma Xi Regional Meeting at Cornell University, Ithaca, NY in April **2008**.

Snyder, N.L.; Greisler, J.J.*; Clair, B. C.* La Chance, E. T.* "Evaluation of a Transition Metal-Catalyzed Isomerization/Ring Closing Metathesis Route for the Synthesis of Septanose Carbohydrates." Poster presented at the 235th ACS National Meeting, New Orleans, LA in April **2008**.

Snyder, N.L "Seven Member Ring Sugars: An Expanded View of Carbohydrates." Talk presented at Westminster College, New Wilmington, PA in March **2007**.

Snyder, N.L.; Haines, H. M.*; Hewitt, A. E.* "Carbohydrate-Based Vaccines Targeted at Galectin-1." Poster presented at the 233rd ACS National Meeting, Chicago, IL in March **2007**.

Snyder, N.L.; de Guillebon, A.M.*; Ngai, K.-Y.*; Hewitt, A. E.* "Progress Towards a Vancomycin Derivative Containing a Septanose Residue at the Vancosamine Position." Poster presented at the 233rd ACS National Meeting, Chicago, IL in March **2007**.

Snyder, N.L.; Hogan, M.F.*; de Guillebon, A.M.* "Progress Towards the Preparation of a Vancomycin Derivative Containing a Septanose Residue at the Vancosamine Position." Poster presented at the 231st ACS National Meeting, Atlanta, GA in March **2006**.

Castro, S.; Duff, M.; Snyder, N.L.; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." Poster presented at the RT Major Lecture Series, Storrs, CT in March **2005**.

DeMatteo, M. P.; Snyder, N.L.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." Talk presented at the 228th ACS National Meeting, Philadelphia, PA in August **2004**.

Snyder, N.L.; Peczuh, M.W. "Accessing Septanose Carbohydrates: The Synthesis and Reactivity of Carbohydrate Based Oxepines." Poster presented at the RT Major Lecture Series, Storrs, CT in April **2004**.

Peczuh, M.W.; Snyder, N.L. “Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates.” Poster presented at the RT Major Memorial Lecture Series, Storrs, CT in April **2003**.

Peczuh, M.W.; Snyder, N.L. “Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates.” Poster presented at the 224th ACS National Meeting, Boston, MA in August **2002**.

*Indicates undergraduate coauthor.

A Philosophy of Teaching Inspired by the Unified Field Theory

Nicole L. Snyder, Ph.D.

> "If you do the job in a principled way, with diligence, energy and patience, if you keep yourself free of distractions, and keep the spirit inside you un-damaged, as if you might have to give it back at any moment. If you can embrace this without fear or expectation—can find fulfillment in what you're doing now, as Nature intended, and in superhuman truthfulness (every word, every utterance)—then your life will be happy. No one can prevent that."
>
> -Marcus Aurelius (*Meditations*)

Almost every educator would agree that learning for the sake of acquiring knowledge is the main purpose of a formal education. Learning is the goal of every student, and therefore the job of every educator is to teach the students "in a principled way, with diligence, energy, and patience." My thoughts and feelings on what makes for a successful educational experience are best understood by examining the following lesson in science on the basis for the unified field theory.

The entire universe rests on four fundamental components: (i) gravitational forces; (ii) electromagnetic forces; (iii) strong forces; and (iv) weak forces. At lower temperatures, such as those observed on Earth, the four forces appear as separate, yet interconnected entities. At higher temperatures, the electromagnetic force and the weak force appear as one force—the electroweak force. At even higher temperatures, the electromagnetic force, weak force and strong force combine to form the strong electroweak force. At temperatures as high as those believed to have created the universe all four forces merge and act as one force.

As an educator, I feel that learning, much like the unified field theory, is a process comprised of four fundamental components: (i) introduction; (ii) reinforcement; (iii) development; and (iv) application. An initial observation of these components might lead one to believe that they are individual stages in the process of an education. However, with the right catalyst, all of these units begin to converge until the product is one single force—knowledge. An educator plays an integral role as the catalyst that serves to join the four components of knowledge. The following breakdown illustrates my own retrosynthetic analysis for assisting students in their quest.

Introduction. The most important role of the educator should be to instill an interest (or at the very least an appreciation) for the material that is being taught. Initially, the educator, who has acquired a great deal of knowledge in his or her subject area, serves as a guide. The educator introduces students to new material in a clear and concise manner, and provides direction for those students who would like to explore the material in more detail. Equally important is the ability of the educator to understand the dynamics of the audience. Every student in the classroom has a unique style of learning formed through a lifetime of experiences. Recognizing different styles of learning and capitalizing on life experiences enriches the learning environment and provides a platform for student success.

I feel that the best way to introduce new material is through a multifaceted approach involving lectures, demonstrations, and discussion sessions. Each of my lectures begins with a clear set of objectives that defines what material must be mastered in order to succeed. The content of each lecture presents the key concepts covered in the assigned readings, and best serve those who learn through a more cognitive approach. Frequent hands on demonstrations of relevant scientific principles are an integral part of my courses, and serve to benefit those students who learn more effectively through a visual approach. Regular discussion sessions and review sessions provide adequate time to focus on material that requires a considerably higher level of critical thinking.

The material presented in the lecture is supplemented with problem sets and laboratory exercises outside of the classroom. These activities are designed to provoke critical thinking in context, a skill that is developed over time and with continued exposure to the material. Students may be asked to work alone or as part of a group to complete these exercises. Independent work provides an opportunity for students to reflect on their own development. Group work provides an opportunity for students to learn the importance of collaboration in solving difficult and thought provoking problems.

Reinforcement. Research has shown time over that students generally only retain about 30% of the material disseminated in any given lecture. Therefore, in order to ensure students are learning the material, key concepts must be reinforced. At this point, the interaction between the educator and the students becomes especially important. The educator should act as an advisor and should make him or herself available to the students through regularly scheduled office hours, and discussion and review sessions outside of class. Regularly scheduled office hours provide the educator with the opportunity to meet with students one-on-one. Discussion sessions and review sessions are useful for meeting with smaller groups of students. Through these interactions, the educator and students begin to foster a meaningful relationships built on maintaining the personal and professional goals of both parties.

I reinforce concepts in the classroom through short formal discussion sessions where student work through a series of problems in groups and present their results on the board and to their classmates. In addition, I use regular, formal in-class review sessions to establish an outline of the key "take-home" concepts that the students should have already mastered in order to move on to the next topic. Students are encouraged to ask questions in these sessions if they feel there are still gaps in their knowledge base. I also use the in-class review sessions as an opportunity to engage the students in a discussion on how the material we have covered is interconnected to concepts we have learned and will learn. Overall, these sessions allow me to assess the student dynamic and the needs of the students as a whole. I use the information I glean from these sessions to provide a more personalized approach in the lecture.

Outside of the classroom, I offer several office hours per week and encourage students to meet to set goals, ask questions, make comments, exchange ideas or simply talk about school, life, careers, or just about anything relevant to their intellectual growth and development. If a student has a conflict with scheduled office hours, alternative arrangements are made to accommodate the student. I also offer an informal voluntary two-hour review session in the evenings once per week. The first hour is generally used to reinforce the concepts discussed in class. The second hour is used to take the material learned in class to the next level through a series of critical thinking exercises. Students are instructed to come to these sessions with questions for the first half of the review session. I prepare several exercises for the second half.

Development. As educators guide students through the process of an education, they must also assess their development along the way. Problem sets, quizzes, papers, presentations and exams that focus on the course material are extremely valuable tools for gauging a student's ability to learn and understand the material that is being taught.

In my courses, I use a mix of problem sets, quizzes, papers, presentations, and exams to evaluate student learning. Problem sets reinforce the key concepts discussed in lecture. Quizzes reinforce the problem sets. Papers and presentations provide a platform for students to broaden their understanding of one or more aspects of the discipline. Finally, exams are used as tools to determine which students are able to think about the concepts they have learned in context. I also believe that returning these materials in a timely manner with appropriate feedback is important for student development. Therefore, I make every effort to grade and return student assignments and exams within two days of receipt, and most often within 24 hours.

These forms of assessment are reasonable for determining whether a student has mastered the material. However, I believe the following additional criteria should also be used to asses a students' personal and professional development: (i) a zeal for learning; (ii) an ability to think and perform both independently and as part of a group; (iii) a willingness to accept criticism and grow from it; (iv) and a questioning attitude that tests what is said in lecture and takes it beyond the bounds of the classroom. In order to asses these criteria, I take every opportunity to get to know my students personally and professionally, inside and outside of the classroom.

Application. Intellectual development does not end at the conclusion of a course. Educators should continue to involve themselves in projects that capture the students' interest and allow them to successfully apply their knowledge. In the sciences this is best done through research.

As an educator, I encourage students to apply the knowledge they have learned in the classroom and laboratory in the context of an independent research project. Research assistants in my laboratory are offered a stimulating environment that fosters intellectual and social development. Students participate fully in the experimental design phase, and learn first-hand how to master the technical skills needed to conduct the experiments required of their project through a collaborative, hands-on approach. Students also learn how to properly maintain a research notebook, how to search and interpret the chemical literature, and how to organize and present the data they obtain in the laboratory. Weekly group meetings allow students to share ideas and validate them with others.

Every student in my research group is also given the opportunity to present their research publicly at one or more local or national meetings (for example, an American Chemical Society or Sigma Xi meeting). If their experimental results are publishable, students participate in manuscript preparation, submission, and copy editing. This experience allows students to follow a project through from design and development, through implementation, and finally dissemination to a broader audience.

Finally, I feel that teaching is not just a job. Teaching is a gift that should be "embraced …without fear or expectation…as if you might have to give it back at any moment." Teaching can be a highly rewarding experience. When a student thanks you for believing in them and seeing them through at time when they had trouble believing in themselves, it is a source of fulfillment unlike any other. If you "can find fulfillment in what you're doing now, as Nature intended, and in superhuman truthfulness (every word, every utterance)—then your life will be happy. No one can prevent that."

Hamilton College Glycoscience Group Research Summary

Nicole L. Snyder, Ph.D.

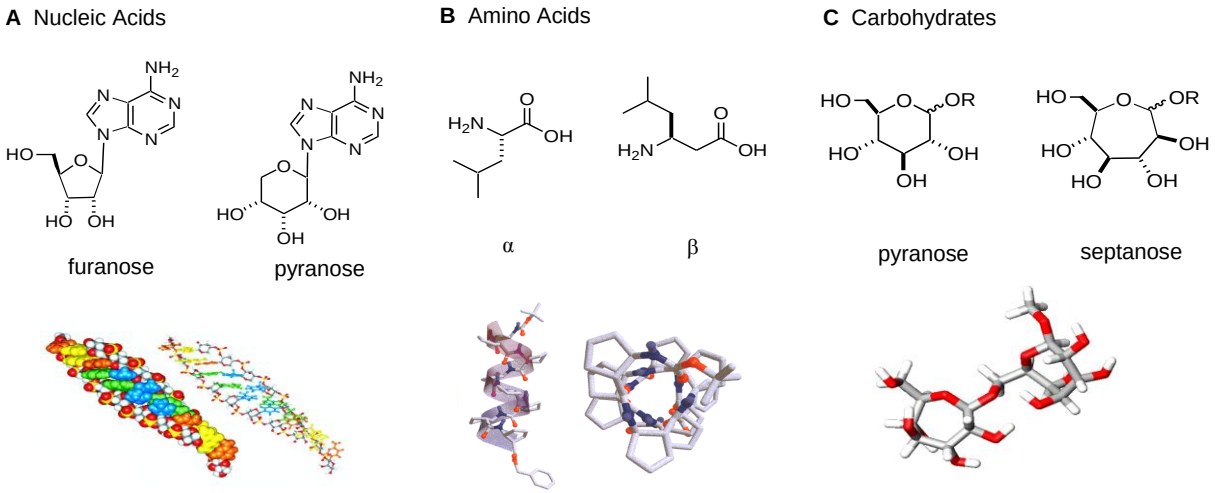
Introduction

The unique topologies afforded to carbohydrates have allowed them to play critical roles in a number of biological recognition events including cell trafficking, growth factor recognition, immunological recognition, and metastasis. By studying fundamental interactions between carbohydrates and other biomolecules in these processes, a better understanding of the natural functions of carbohydrates has been realized. This understanding has helped enlighten fundamental biochemical knowledge, and has played a significant role in the construction of designed carbohydrate-based systems. Much of this work has focused on the preparation of mono- and oligosaccharide structures that can serve as biological probes, and has led to the development of a variety of therapeutic strategies and new classes of pharmaceutical compounds that have been used to treat diabetes, viral infections, and cancer.

Recently, the construction of expanded biopolymers, specifically oligodeoxynucleotides and oligopeptides, has gained considerable attention. These molecules are the result of a formal one-carbon homologation of their respective monomers and have been shown to exhibit interesting biological activities. For example, it is well known that nucleotide sugars in DNA and RNA occur as five member furanose ring systems. Eschenmoser[1] has observed that when these five member furanose sugars are expanded by one carbon to pyranoses, an alternative base-pairing and heteroduplex shape is observed (Figure 1A). Similarly, Gellman[2] and Seebach[3] have studied the homologation of α-amino acids to β-amino acids. Oligomers constructed from β-amino acids have been shown to adopt defined conformations that complement natural structures (Figure 1B). Characterization of these oligomers has led to the design of a variety of biologically important β-peptides from which amphipathic α-helix mimics have been synthesized.[4] These mimics have been shown to selectively disrupt bacterial cell membranes over mammalian cell membranes.

The aforementioned research has provided a foundation for the design of synthetic glycoconjugates through the construction of a class of ring expanded carbohydrates known as septanose carbohydrates. As shown in Figure 1C, septanose carbohydrates are unnatural, ring expanded homologs of pyranose carbohydrates. The extra atom in the ring of these molecules provides an increased flexibility that allows them to adopt a number of different low energy conformations. For this reason, septanose carbohydrates are interesting tools for studying protein-carbohydrate interactions. In fact, binding studies involving a number of different septanose carbohydrates have already revealed interesting information about the biological activity of these molecules.[5]

Figure 1. Ring expanded biopolymers.



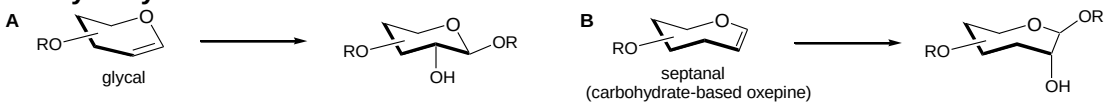
My research interests lie in the preparation and characterization of designed structures that incorporate natural and unnatural carbohydrate residues. My students and I use these systems to investigate a number of key carbohydrate interactions of biological interest. Our major research projects are described below. The uniting goals of these projects are:

1. To optimize existing synthetic routes for the preparation of functionalized natural and unnatural carbohydrates.
2. To generate rationally designed structures incorporating natural and/or unnatural carbohydrate residues that can be used to study carbohydrate-protein, carbohydrate-DNA, and carbohydrate-carbohydrate interactions of biological interest.
3. To investigate the functional consequences of the structures described above using state of the art chemical, biochemical and spectroscopic techniques.

Project 1: New Routes Towards the Synthesis of Septanose Carbohydrates

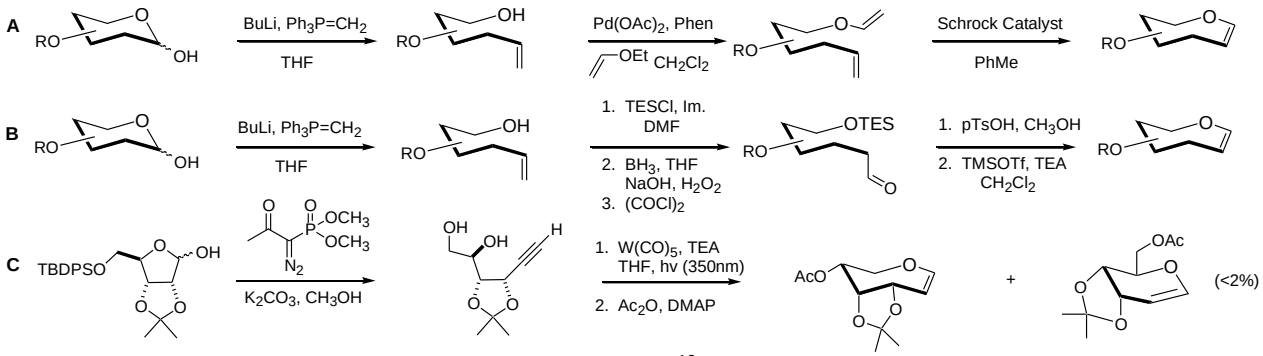
Glycals, a group of cyclic enol ethers, have been extensively employed as synthons to access a number of important carbohydrate derivatives and glycosylated natural products.[6] Septanals, 1,2 unsaturated derivatives of septanose carbohydrates, have recently gained attention as useful starting materials for the synthesis of septanose sugars. Septanals, more commonly referred to as carbohydrate-based oxepines, are functionally analogous to glycals and show similar reactivity profiles in a number of glycosylation reactions (Figure 2).[7] For this reason, researchers have increasingly focused on methods to produce carbohydrate-based oxepines for use as building blocks in the preparation of septanose containing oligosaccharides and glycoconjugates.

Figure 2. Reactivity of cyclic enol ethers.



Synthetic approaches to oxepines, including carbohydrate-based oxepines, have recently been reviewed.[8] Three syntheses involving the preparation of carbohydrate-based oxepines with functional equivalence to glycals are especially noteworthy. The first synthesis, reported by Peczuh and Snyder,[9] uses a Schrock catalyzed ring-closing metathesis of dienes derived from substituted pyranose lactols (Scheme 3A). This route has been successfully employed in the preparation of a number of carbohydrate-based oxepines in high yields. However, this method is limited by the fact that the catalyst employed requires an inert atmosphere free of oxygen to function properly, and the expired catalyst is difficult to separate from the products once the reaction is complete. The author's attempts to use the more robust Grubbs' catalyst with these substrates gave consistently poor results, presumably as a result of the reaction of the ruthenium catalyst with the more accessible enol-ether to form an unreactive ruthenium alkylidene.

Figure 3. Recent advances in the production of carbohydrate-based oxepines.

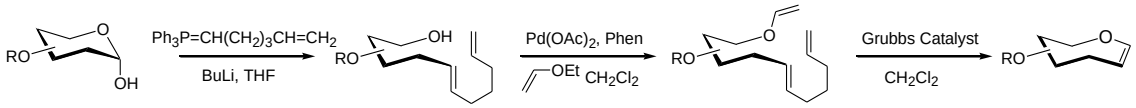


The second synthesis, reported by Peczuh and Castro,[10] uses a cyclization elimination approach to access carbohydrate-based oxepines (Scheme 3B). This method has been successfully employed to prepare gram quantities of glucose and 2-deoxy glucose based oxepines without the use of expensive organometallic reagents. However, this sequence has shown limited extension in the production of carbohydrate-based oxepines other than those reported.

More recently, McDonald and coworkers[11] reported the tungsten catalyzed cycloisomeraization of furanose based alkynols prepared from substituted furanose lactols (Scheme 3C). This route has proven effective for producing carbohydrate-based oxepines deoxygenated at the C-6 position (glucose numbering). However, the C-6 hydroxymethyl group has been shown to play an important role in a number of biological processes.

In an effort to provide a more globally accessible route to carbohydrate-based oxepines, and therefore septanose carbohydrates, undergraduate students in my research group are currently exploring a new relay ring-closing metathesis route based on work by Hoye and coworkers.[12] Relay ring-closing metathesis employs the use of a tether to make one double bond of a polyunsaturated substrate more readily susceptible to chelation by the ruthenium catalyst, thus inhibiting the formation of a ruthenium alkylidene species. A general approach for the synthesis of a substrate incorporating a tether is outlined in Figure 4. Wittig olefination of the appropriately protected lactol provides the corresponding alcohol. Functionalization of the free alcohol using ethyl vinyl ether gives the desired diene precursor which undergoes relay ring-closing metathesis in the presence of a catalytic amount of Grubbs catalyst to produce the product oxepine.

Figure 4. Relay ring-closing metathesis.



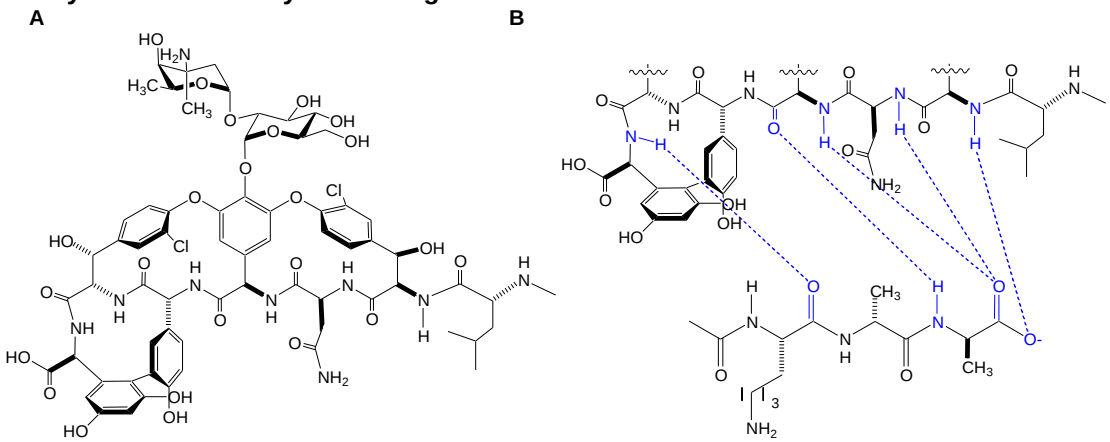
We are hopeful that this method will allow us to access carbohydrate-based oxepines for incorporation into several other systems we are currently investigating, including the synthesis of two new derivatives of a vancomycin glycan (Project 2), and several galaectin-1 inhibitors (Project 3).

Project 2: Synthesis of a Vancomycin Derivative Incorporating an Unnatural Carbohydrate at the Vancosamine Position

Vancomycin (Figure 5A) is a glycopeptide antibiotic used in the clinical setting for the treatment of methicillin-resistant *Staphylcocci* and *Enterococci.* Vancomycin is composed of two bioactive components, a cyclic peptide component (aglycon) and a functionalized peripheral carbohydrate (glycan), that work together to inhibit the biosynthesis of peptidoglycan, a major component of the cell wall of gram-positive bacteria.[13]

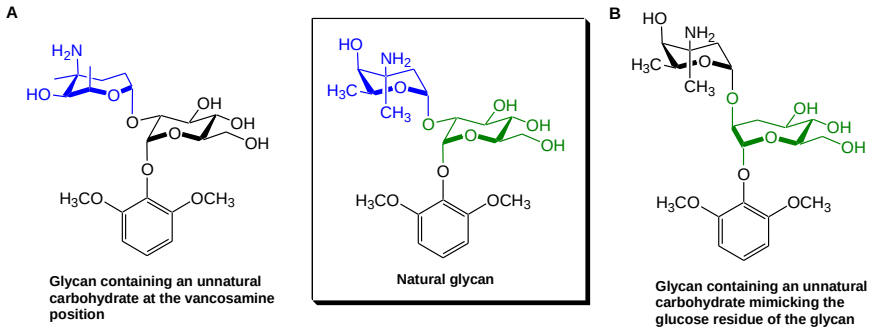
The role of the aglycon is well established. The aglycon functions by binding peptidoglycan precursors terminating in the amino acid sequence D-ala-D-ala (Figure 5B).[14] This essentially blocks the approach of several key enzymes involved in the transglycosylation and transpeptidation steps of peptidoglycan synthesis. The role of the glycan is less understood. Experimental evidence suggests that the glycan plays an important role in the conformational maintenance of the aglycon.[15] The glycan is also believed to assist the aglycon in dimerization and membrane anchoring events that act cooperatively to create a chelating effect that increases the affinity of the aglycon for D-ala-D-ala.[16] Recent research has also revealed that the glycan may be involved in a direct binding event with the glycosyltransferases involved in transglycosylation, regardless of a petidoglycan binding event.[17] However the specific role of the glycan in combating resistant strains of bacteria is not well understood.

Figure 5. Vancomycin and vancomycin binding D-ala-D-ala.



Students in my research group are currently preparing two new derivatives of the vancomycin glycan as a model to study the specific factors that govern the glycan-glycosyltransferase binding interaction (Figure 6). The first derivative incorporates an unnatural (septanose) residue at the vancosamine position of the glycan (Figure 6A). The second derivative incorporates an septanose residue at the glucose position (Figure 6B). Previous studies involving septanose carbohydrates suggest that they may require less energy than their natural (pyranose) counterparts to access the half-chair conformation required to bind the specific enzymes involved in bacterial cell wall biosynthesis.

Figure 6. Target derivatives.

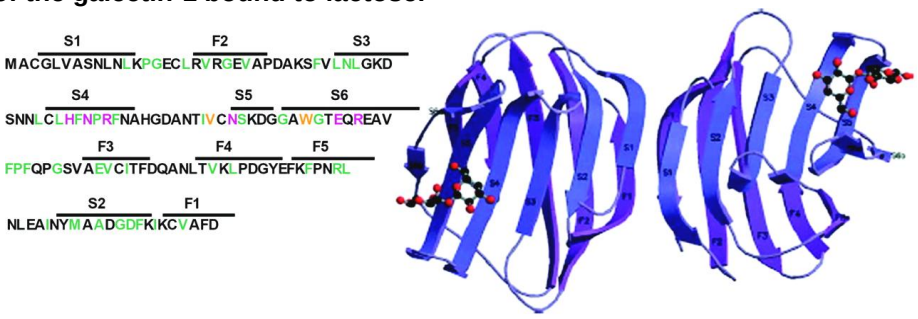


Once these derivatives are prepared and fully characterized, we plan to test them against panel of methicillin-resistant *Staphylococci*, vancomycin-resistant *Staphylococci* and vancomycin resistant *Enterococci*. Derivatives that show significant activity will undergo extensive physical characterization with penicillin binding protein 2 (PBP2), a membrane bound glycosyltransferase isolated from *S. aureus*. The data collected from these studies will be used to develop a more specific understanding of the factors that influence the glycan-glycosyltransferase binding interaction. In the future, this information will be used to design more potent derivatives of vancomycin that can be used to combat resistant strains of gram-positive bacteria.

Project 3: Exploring the Nature of Ligand Binding in the Active Site of Galectin-1 Using Natural and Unnatural Carbohydrates

Human galectin-1 (Figure 7) is expressed on cell surfaces and in extra cellular matrices, and is involved in a number of critical processes including inflammation, development, mRNA splicing, differentiation, and cell adhesion.[18] In normal cells the expression of galectin-1 is regulated. Diseased or stressed cells have been shown to over express galectin-1. For example, galectin-1 has been found in unusually high concentrations in and around tumor cells and has been implicated in several aspects of cancer biology including tumor transformation,[19] apoptosis,[20] cell growth regulation,[21] and metastasis.[22] Research has also shown that galectin-1 may play an important role in protecting tumor cells from immune attack,[23] and recent studies have suggested that galectin-1 may play an important role in the promotion of HIV infectivity.[24]

Figure 7. Structure of the galectin-1 bound to lactose.[18c]

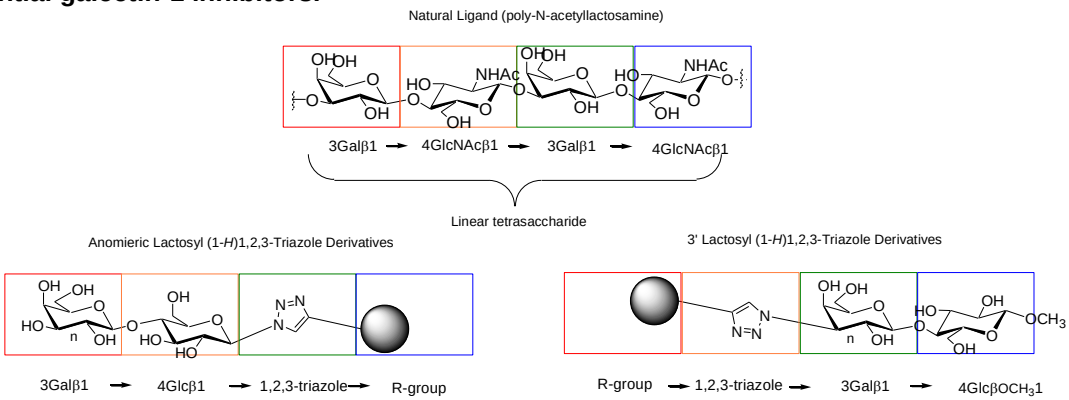


Notes: Amino acids highlighted in green illustrate highly conserved residues. Amino acid residues highlighted in pink are known to interact with bound carbohydrates via hydrogen bonding interactions. Amino acids highlighted in orange are known to interact with bound carbohydrates via vDW forces.

Because of the biological role galectin-1 plays in a number of diseases, considerable research has been devoted to the design and synthesis of specific galectin-1 inhibitors. Such ligands are desirable since they can be used to study how galectin-1 plays a role in these processes. Many recent efforts have capitalized on the fact that galectin-1 is somewhat promiscuous, having an affinity for multiple ligands including lactose, *N*-acetyllactosamine, and naturally occurring branched polylactosamine and poly-*N*-acetyllactosamine derivatives.[25] The only trend established thus far is that lactose derivatives bind galectin-1 better than galactose or glucose derivatives alone. Unfortunately, significant differences in substitution patterns (anomeric *vs* 3 or 3') and substituents between the derivatives that have been prepared, has made it difficult to evaluate the specific factors that govern the galectin-1-ligand-binding interaction

In an effort to address this issue, students in my laboratory are currently preparing several derivatives of natural and unnatural carbohydrate triazoles that mimic poly-*N*-acetyllactosamine (Figure 8). The rationale for the proposed derivatives is based on three parameters we would like to investigate: (i) how the position of the triazoles (anomeric *vs* 3') affects the binding of these molecules; (ii) how the nature of triazole substituent R (flexible *vs* nonflexible, aromatic *vs* non aromatic and carbohydrate *vs* non carbohydrate) influences binding interactions and (iii) whether the nature of the reducing galactose residue (natural *vs* unnatural) affects the binding profile for galectin-1.

Figure 8. Potential galectin-1 inhibitors.



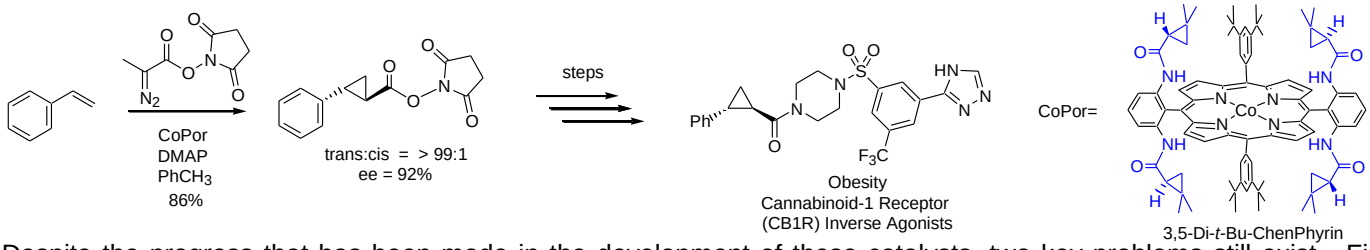
Future studies are planned for the preparation and biological evaluation of the analogous 3' lactosyl-1,2,3-trizaoles. The goal will be to compare the data obtained from the biological and biophysical studies of the anomeric triazoles with the 3' triazoles to determine whether any trends exist. These trends will be used to further an understanding of the galectin-1-ligand binding interaction, and the knowledge gained through these studies will serve as a foundation for the design and preparation of therapeutics that can be used to treat cancer and HIV. Further down the road we would like to repeat these studies using inhibitors that incorporate septanose carbohydrates. Derivatives incorporating unnatural carbohydrates are especially interesting since studies suggest that the increased flexibility may allow these molecules to bind and inhibit galectin-1 preferentially over molecules incorporating pyranose sugars.

Project 4: Synthesis of Carbohydrate-Porphyrin Conjugates.

Chiral cyclopropane rings are found in a number of biologically relevant natural products. For example, the antitumor curacin A,[26] and antifungal ambruticin[27] contain chiral cyclopropane units that are critical to the biological functions of these compounds. Because of the importance of chrial cyclopropane rings, a number of reactions have been developed for their synthesis.[28] However, the development of catalysts that can catalyze asymmetric cyclopropanation with a variety of substrates in high yield and with excellent diastereo- and enantioselectivity is still a major area of research in the field.

The recent development of palladium-catalyzed cross-coupling reactions between mono-, di-, and tetrasubstituted bromo-porphyrins and amides, amines, alcohols, and thiols has allowed for the preparation of a number of chiral amino-, amido-, oxo- and mercaptoporphyrins.[29] The cobalt derivatives of these chiral porpyrins have, in turn, been used to catalyze a number of key functional group transformations including the asymmetric cyclopropanation of aromatic and electron-deficient olefins using diazo reagents with good diastereo- and enantioselectivity.[30] For example, we recently demonstrated that 3,5-di-*t*-Bu-ChenPhyrin (Figure 9) can serve as an effective catalyst for the asymmetric cyclopropanation of aromatic olefins such as styrene, using succinimidyl diazoacetate.[31] The corresponding cyclopropyl carboxamides can serve as important building blocks for pharmaceutics such as the obesity cannabinoid-1 receptor agonist CB1R.[32]

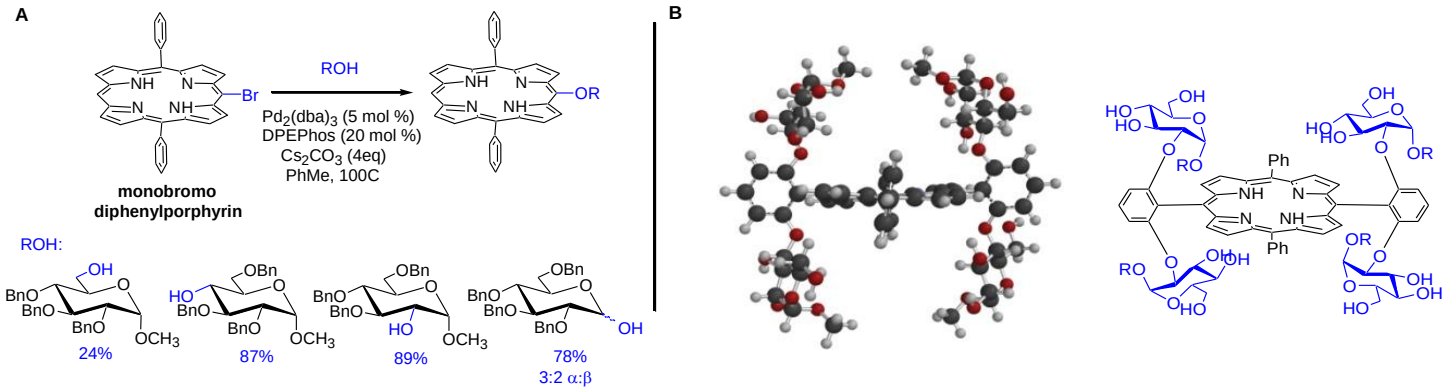
Figure 9. Asymmetric cyclopropanation of styrene.



Despite the progress that has been made in the development of these catalysts, two key problems still exist. First, many of the porphyrin catalysts currently in use exhibit low solubility in polar solvents such as water, limiting substrate scope and making them unsuitable for use in green chemistry applications. In addition, the aromatic nature of these catalysts often facilitates pi stacking resulting in metalloporphyrin aggregation in solution, leading to decreased catalytic efficiency. In an effort to address these problems, we have developed a program for synthesizing novel porphyrins bearing carbohydrate residues. The polarity and predicted conformation of the carbohydrate-porphyrin conjugates render them excellent candidates to obviate the challenges described above.

The carbohydrate-porphyrin conjugates we have synthesized thus far have been prepared by cross-coupling bromoporphyrin synthons with selectively functionalized carbohydrates using tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) as a source of palladium and bis(2-dipheneylphosphinophenyl) ether (DPEphos) as a ligand source in the presence of cesium carbonate (Cs_2CO_3). Through these preliminary studies, we were able to assess the ability of performing palladium-catalyzed cross-couplings with glucose via different carbohydrate linkages (Figure 10A).

Figure 6. Sample carbohydrate-porphyrin conjugates.



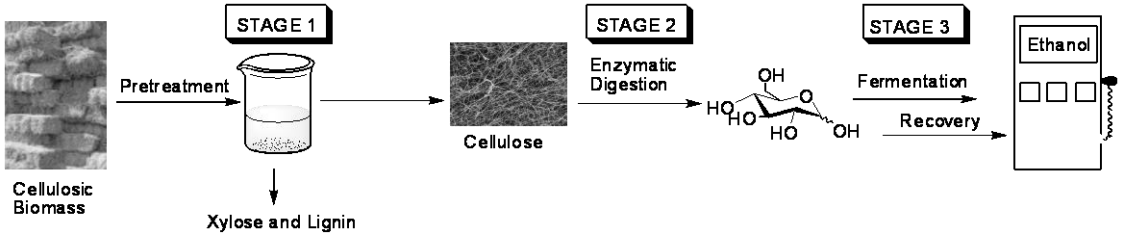
The results obtained from these experiments have provided us with a foundation for the preparation of additional carbohydrate-porphyrin conjugates containing multiple carbohydrate ligands that can serve as effective and efficient catalyst for the asymmetric synthesis of cyclopropane rings. Examples of these carbohydrate-porphyrin conjugates can be seen in Figure 10B below. The complexes prepared in this study will eventually be evaluated for their ability to serve as catalysts in asymmetric cyclopropanation reactions.

Recently, several of the carbohydrate-porphyrin conjugates we have prepared have been evaluated as potential therapeutics for the treatment of malaria and cancer, and the initial results look promising. This has opened a new vein of research for our group, and we have already begun preparing additional carbohydrate-porphyrin analogs for the treatment of these diseases. This project will continue to be important component of our research program in the years to come.

Project 5: Studies of Sol-Gel Encapsulated Cellulases for the Production of Cellulosic Ethanol

Recently, cellulose has gained attention as a potential source of ethanol for use as an alternative fuel.[33] Agricultural residues, municipal solid wastes, herbaceous energy crops, and hardwood all provide cheap and easily accessible sources of cellulose. cellulosic ethanol is currently produced in three major stages from cellulosic biomass (Figure 11). In the first stage, cellulosic biomass is pretreated to release the cellulose from lignin. In stage two, pretreated cellulosic biomass is subjected to enzymatic degradation by a number of different enzymes collectively known as cellulase to produce free sugars. In the third stage, yeasts are employed to ferment the free sugars into ethanol, which is then recovered by distillation.

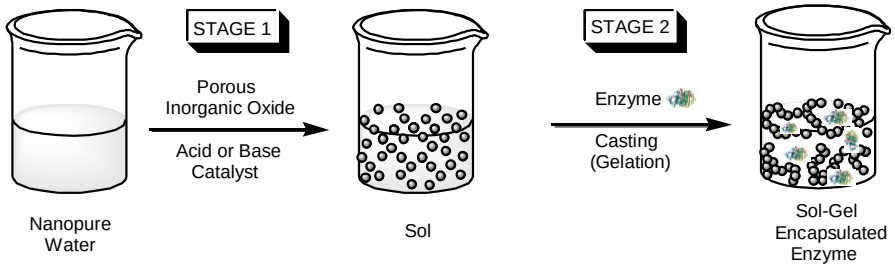
Figure 11. Production of cellulosic ethanol.



One of the major obstacles associated with the production of cellulosic ethanol is the efficient enzymatic hydrolysis of cellulose into glucose (Stage 2). Methods that commonly employ the cellulotic enzymes required for this process often leave the enzymes susceptible to exposure which results in proteolytic degradation over time, reducing turnover. An additional problem is the efficient recovery of the desired products, in this case glucose, from the reaction mixture.

Non-covalent processes for the immobilization of enzymes in silica gels have gained considerable attention over the past twenty years, and a number of enzymes have been successfully immobilized using the sol-gel method.[34] As shown in Figure 12, the sol-gel method of encapsulating enzymes involves two major stages. In the first stage, a porous metal oxide material (usually tetraethoxy orthosilicate (TEOS) or tetramethoxy orthosilicate (TMOS)) is hydrolyzed in water using either an acidic or basic catalyst to form a sol solution. In the second stage, an enzyme-buffer solution is introduced to the sol and cast to from an optically transparent sol-gel monolith, thin film, powder, and/or fiber.

Figure 12. The sol-gel process for encapsulating enzymes.



The resulting sol-gel creates a microporous environment that preserves the structure and function of the encapsulated enzyme. Carefully designed sol-gels have been shown to protect the active site, prevent unfolding, and assist in the stabilization of many key electrostatic interactions important for protein structure and function. These advantages allow most enzymes to retain their activity over prolonged periods of time in comparison to the free enzyme. In addition, the reusability and physical nature of the sol-gel (which provides for facile recovery of the reaction products) makes these materials especially attractive as catalysts for bioconversion.

One of the major challenges still remaining in this area is the encapsulation of enzymes that target larger substrates, such as cellulase. The microporous nature of most conventional sol-gel systems hinders the ability of larger substrates to access the enzymes inside of the sol-gel matrix.[35] Optimum pore sizes should be large enough to allow for unrestricted transport of substrate molecules and reaction products, but should also be small enough to prevent leakage of the encapsulated enzyme. Theoretically, the ability to control the pore size distribution, geometry, morphology and polarity would aid in the preparation of sol-gel materials that can process larger substrates.

Students in my research group are currently focusing on the design of an appropriate sol-gel matrix for the encapsulation of cellulase from *Trichoderma reesei*. A number of different sol-gel media are currently being screened in order to determine the appropriate conditions for the immobilization of cellulase. This is a new project, and the success of our initial experiments using cellobiose as a substrate will be measured by the ability of the sol-gel matrix to maintain the structural integrity of the encapsulated enzyme, provide adequate access to substrate, exhibit comparable reaction kinetics to the free enzyme in solution, and be stable and reusable. Long term objectives will involve optimizing these conditions for use with cellulose. Cellulose is a considerably more challenging substrate, as it is a much larger in size and adopts well defined secondary structures which may make it difficult traverse the sol-gel network.

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Appendix

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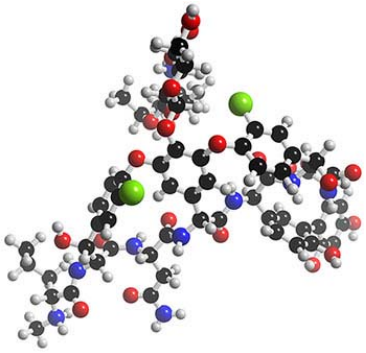
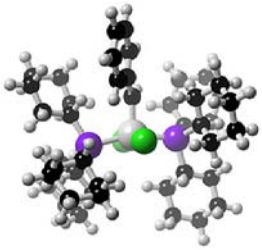
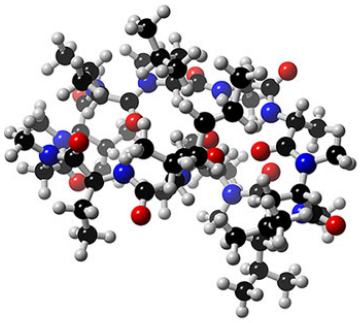
Department of Chemistry
Hamilton College
January 10, 2010

Teaching Materials—Chemistry 190







Organic Chemistry I
Chem 190
Course Syllabus—Spring 2008
January 21, 2008-May 09, 2008

Instructor Information:

Nicole L. Snyder, Ph.D. (Call me Nicole)

Office: SCCT 1072
Lab: SCCT 1068
Office Hours: Tuesday: 9:00am until 10:00am
Wednesday: 11:00am until 12:00pm
Thursday: 3:00pm until 5:00pm
Friday: 11:00am until 12:00pm
….and also by appointment
Office Phone: (315) 859-4742
Email: nsnyder@hamilton.edu

Course Objectives:

This course is designed to introduce you to the exciting field of organic chemistry and its everyday application. We will begin by reviewing a number of topics covered in your introductory chemistry course including the physical properties of molecules, molecular structure, and chemical bonding. Acid base chemistry will also be reviewed and extended to include the discussion of organic compounds. It is my hope that our initial discussions will help lay the foundation for developing a strong approach to the study of organic chemistry.

Once we have reviewed, we will begin to establish a system of communication for discussing organic compounds and chemical reactions. This may seem like learning a new language for some of you, and I will try to demystify the process as much as possible. Functional groups and functional group nomenclature will be discussed in detail followed by a number of current spectroscopic techniques—IR, UV-Vis and MS—used to determine the structural components (and ultimately the complete structure) of organic compounds.

As we progress through the course, we will explore many new and interesting topics. We will converse about stereochemistry and the nature of chemical reactions involving stereogenic (and non stereogenic) centers. We will learn about the chemistry of radicals, substitution reactions, elimination reactions and a number of addition reactions. Finally, we will dive head first into organic synthesis using the information you have acquired to build complex and important organic molecules. After a thorough investigation of each topic, we will apply the new information we have acquired to explain how organic chemistry (and chemistry in general) plays a role in almost everything we are exposed to on a daily basis.

As you may already know, organic chemistry is not always an easy subject to learn. You cannot simply learn organic chemistry by reading a book much as you would learn many of the other topics you will tackle in some of your other classes. Instead, you will need to solve problems inside and outside of class, and you need to acquire hands on experience through laboratory experimentation. This course is designed to help you do just that!

Remember that even though the study of organic chemistry can at times be difficult, it can also be a highly rewarding experience. You are here to learn and I am here to teach you. Therefore, I will always try to make sure that you understand the material and you are always welcome to drop by, email, or call me with any questions or concerns you may have. I hope that in the end, each of you will take away something new from this class be it an improved study habit or a new outlook on the world in which we live.

*Images taken from chem3D.com database

Course Requirements:

Students taking Chemistry 190 are required to gain access to the course and lab textbooks, a model kit, and a laboratory notebook. More information on each of these items is provided below.

I. ***Textbook***—I will assign readings and problems from the textbook and other resources that I feel are necessary to convey a particular topic. The required textbook for this class is **"Organic Chemistry" (6th edition) by Leroy G Wade.** Please make sure that you are using the sixth edition for this course.

II. ***Model set***—Access to a molecular model set for organic chemistry is essential to this course and is therefore required. Prentice Hall molecular model sets are available at the bookstore and online. There are also a number of other good (and cheap) molecular model sets out there. Please see me if you would like some suggestions.

III. ***Laboratory textbook and notebook***—Laboratory handouts will be handed out in class and posted on Blackboard. A required supplementary text for the laboratory, **"The Organic Chem Lab Survivial Manual" (7th edition) by Zubrick et. al.**, can be purchased in the college bookstore. You should be able to produce a copy of the lab (printed from the web) and the text during your assigned laboratory period. In addition, you will be required to purchase and maintain a Freeman Laboratory Notebook specifically for use in the laboratory portion of the course. A handout for keeping the laboratory notebook will be provided during your designated laboratory period and requirements may vary by instructor.

Class Format:

Each week I will provide a general overview of the material that we will be covering in class. I have proposed the following schedule that is subject to change based on Holidays and other important school related functions:

Monday:	-Homework problems from previous week collected and select problems discussed
	-General lecture
Tuesday:	-Evening review session 7:30pm until 9:30pm (SCCT 3040)
***Wednesday*:**	-General lecture
	-In-class problem set
***Friday*:**	-Quiz
	-General lecture

Class Policies:

It is expected that all students in this section of Chemistry 190 will take note of the following policies:

I. ***Attendance***—Attendance is strongly encouraged. If you need to miss a class for any reason, please contact me in advance so that I can arrange to have missed work delivered to you in a timely fashion. Also note that class participation is considered for 10% of your final grade (see below). Frequently missing class can (and will) negatively impact your grade.

II. ***Class and lab participation***—Every member of the class is expected to participate in lecture and in lab. This means that you must verbally interact with me during class by answering questions. You must also interact with your fellow classmates during group discussions, lab, and through the use of Blackboard when appropriate. Class participation is worth 10% of the final grade. Failing to participate will result in the reduction of your final grade in the course.

III. ***Late work or missed assignments***—Work that is not completed on time will be marked late unless arrangements are made **in advance** to turn the work in at an alternate time other than the due date. In addition, a percentage of points will be deducted as outlined below:

One day late = 10% deduction
Two days late = 25% deduction
Three days late = 50% deduction
Four days late = 75% deduction
Five or more days late = no credit

IV. ***Academic honesty***— Each individual is expected to follow the academic conduct code (Honor Code) set forth by Hamilton College.

V. ***Learning disabilities***— In accordance with the Americans with Disabilities Act, any student who has a documented learning disability will be provided with reasonable accommodations designed to meet his/her needs. Before any such assistance can occur, it is the responsibility of the student to see that documentation is on file with the appropriate individual. Please see me as soon as possible to discuss any need for accommodations.

Student Evaluation:

You will be evaluated in this course on a regular basis. The basis for course evaluation is provided below with explanations:

Quizzes:	10%
Problem Sets::	10%
Lab Reports:	25%
Hourly Exams:	30%
Final Exam:	15%
Class Participation:	10%

I *Quizzes*—There will be a short 10 point quiz given the Friday of each week unless an exam is scheduled for that week. Each quiz is designed to test your knowledge of the material covered in the previous week's classes. Overall, there will be ten quizzes given. Ten quizzes will be scored for a total of 100 points or 10% of the final grade.

II. *Problem Sets*—Problem sets will be assigned at the beginning of each week and will correspond to the material that we will be covering throughout that week. Problem sets will be collected and graded on a weekly basis, generally the following Monday of the week the assignment was made. Each assignment must be turned in on time and will be worth ten points. Ten problem sets will be counted towards your final grade. This comprises 100 points or 10% of the final grade.

III. *Lab reports*—Completing lab is essential to understanding organic chemistry. Throughout the term we will we will complete a total of eleven experiments that relate to material covered in lecture. These assignments are designed to help you maximize your lecture experience and should be thought of as a supplement to your in class lecture. Lab assignments will be graded separately by your individual lab instructor and will count towards 25% of the final grade. Please see your laboratory instructor for details about the grading of laboratory assignments.

Note: Failure to turn in two or more lab reports will constitute and automatic failure of the course!

IV. *Hourly exams*—There will be four scheduled 100 point hourly exams given throughout the semester. The dates for these exams are given below:

Exam I—Thursday, February 21, 2008 (7:00pm-9:00pm)
Exam II—Thursday, March 13, 2008 (7:00pm-9:00pm)
Exam III—Thursday, April 10, 2008 (7:00pm-9:00pm)
Exam IV—Thursday, May 01, 2008 (7:00pm-9:00pm)

In most cases, each exam will reflect material that we have covered in class up to the week prior to the exam. At the end of the semester, I will drop the lowest exam score. The remaining three exams will count for 300 points or 30% of the final grade. Exams will not be curved and **you must take all four exams** in order for me to drop your lowest exam score..

V. *Final Exam*—There will be one final exam worth 150 points or 15% of the final grade. The exam will be cumulative, but will focus heavily on the material covered in the last quarter of the course. **The final exam is schedule for Thursday, May 15, 2008 from 9:00am until 12:00am.** More details will be provided as we approach the final exam period.

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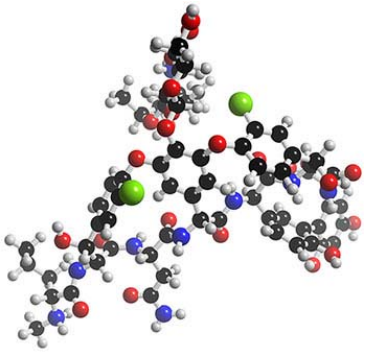
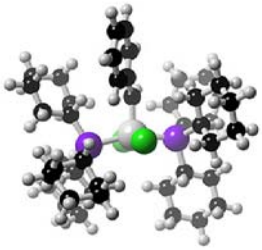
Schedule of Events:*

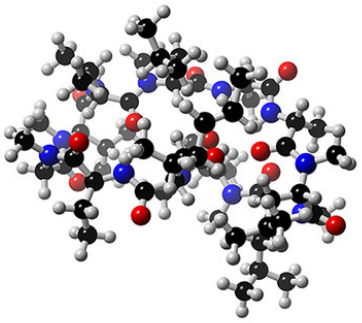
*This schedule is subject to change based on inclement weather.

Class	Monday	Wednesday	Friday
Week One **January 21** **Review**	-Introductions	-Chapter 1 (1.1-1.9)	**-QUIZ I (Gen Chem)** -Chapter 1 (1.10-1.14)
Week Two **January 28** **Organic Structures; IR and MS**	**-PROBLEM SET I DUE (Chapter 1)** -Chapter 2 (2.1-2.8)	-Chapter 2 (2.9-2.13)	**-QUIZ II (Chapter 1)** -Chapter 12 (12.1-12.11)
Week Three **February 4** **IR and MS; Conformational Analysis of Alkanes**	**- PROBLEM SET II DUE (Chapter 2)** -Chapter 12 (12.12)	-Chapter 12 (12.13-12.15) -In class worksheet	**-QUIZ III (Chapter 2)** - Chapter 3 (3.1-3.5)
Week Four **February 11** **Conformational Analysis of Alkanes**	**- PROBLEM SET III DUE (Chapter 12)** -Chapter 3 (3.6-3.9)	-Chapter 3 (3.10-3.13) -In class worksheet	**-QUIZ IV (Chapter 12)** -Chapter 3 (3.14-3.16)
Week Five **February 18** **Chemical Reactions**	**- PROBLEM SET IV DUE (Chapter 3)** -Chapter 4 (4.1-4.7)	-Chapter 4 (4.8-4.11) **-Review for Exam I (Chapters 1, 2, 12, 3)**	-Chapter 4 (4.12-4.16)
Week Six **February 25** **NMR**	**- PROBLEM SET V DUE (Chapter 4)** -Chapter 13 (13.1-13.4)	- Chapter 13 (13.5-13.7) -In class worksheet	**-QUIZ V (Chapter 4)** -Chapter 13 (13.8-13.11)
Week Seven **March 3** **NMR; Stereochemistry**	- Chapter 13 (13.12-13.14)	- Chapter 13 (Problem Session) -In class worksheet	**-QUIZ VI (Chapter 13)** - Chapter 5 (5.1-5.5)
Week Eight **March 10** **Stereochemistry**	**- PROBLEM SET VI DUE (Chapter 13)** - Chapter 5 (5.6-5.9)	- Chapter 5 (5.10-5.12) **-Review for Exam II (Chapters 4, 13)**	- Chapter 5 (5.13-5.16)
Week Nine **March 17**	No Class- **Spring Break**	No Class- **Spring Break**	No Class- **Spring Break**
Week 10 **March 24**	No Class- **Spring Break**	No Class- **Spring Break**	No Class- **Spring Break**
Week 11 **March 31** **Substitution and Elimination Reactions**	-Chapter 6 (6.1-6.7)	-Chapter 6 (6.8-6.15) -In class worksheet	**-QUIZ VII (Chapter 5)** -Chapter 6 (6.17-6.20)
Week 12 **April 7** **Substitution and Elimination Reactions; Alkenes**	**- PROBLEM SET VII DUE (Chapters 5 and 6)** -Chapter 6 (6.16,6.21) -In class worksheet	-Chapter 7 (7.1-7.5) **- Review for Exam III (Chapters 5, 6)**	-Chapter 7 (7.6-7.10)
Week 13 **April 14** **Reactions of Alkenes**	**- PROBLEM SET VIII DUE (Chapter 7)** -Chapter 8 (8.1-8.4)	-Chapter 8 (8.5-8.7) -In class worksheet	**-QUIZ VIII (Chapter 7)** -Chapter 8 (8.8-8.11)
Week 14 **April 21** **Reactions of Alkenes/Alkynes**	**- PROBLEM SET IX DUE (Chapter 8)** -Chapter 8 (8.12-8.16)	-Chapter 9 (9.1-9.6) -In class worksheet	**-QUIZ IX (Chapter 8)** -Chapter 9 (9.7-9.10)
Week 15 **April 28** **Structure and Synthesis of Alcohols**	**- PROBLEM SET X DUE (Chapter 9)** -Chapter 10 (10.1-10.6)	-Chapter 10 (10.7-10.10) **-Review for Exam IV (Chapters 7, 8, 9)**	-Chapter 10 (10.11-10.12)
Week 16 **May 5** **Reactions of Alcohols**	**- PROBLEM SET XI DUE (Chapter 10)** - Chapter 11 (11.1-11.4)	- Chapter 11 (11.5-11.9) -In class worksheet	**-QUIZ X (Chapter 10)** -Chapter 11 (11.10-11.14)

Note: The final exam is schedule for Thursday, May 15, 2008 from 9:00am until 12:00am.







Organic Chemistry I
Chem 190
Course Syllabus—Spring 2009
January 19, 2009-May 08, 2009

Instructor Information:

Nicole L. Snyder, Ph.D. (Call me Nicole)

Office: SCCT 1072
Lab: SCCT 1068
Office Hours: Tuesday: 8:00am until 10:00am
Wednesday: 10:00am until 11:00am
Thursday: 5:00pm until 7:00pm
Sunday: 6:00pm until 8:00pm
….and also by appointment
Office Phone: (315) 859-4742
Email: nsnyder@hamilton.edu

Course Objectives:

This course is designed to introduce you to the exciting field of organic chemistry and its everyday application. We will begin by reviewing a number of topics covered in your introductory chemistry course including the physical properties of molecules, molecular structure, and chemical bonding. Acid base chemistry will also be reviewed and extended to include the discussion of organic compounds. It is my hope that our initial discussions will help lay the foundation for developing a strong approach to the study of organic chemistry.

Once we have reviewed, we will begin to establish a system of communication for discussing organic compounds and chemical reactions. This may seem like learning a new language for some of you, and I will try to demystify the process as much as possible. Functional groups and functional group nomenclature will be discussed in detail followed by a number of current spectroscopic techniques—IR, UV-Vis and MS—used to determine the structural components (and ultimately the complete structure) of organic compounds.

As we progress through the course, we will explore many new and interesting topics. We will converse about stereochemistry and the nature of chemical reactions involving stereogenic (and non stereogenic) centers. We will learn about the chemistry of radicals, substitution reactions, elimination reactions and a number of addition reactions. Finally, we will dive head first into organic synthesis using the information you have acquired to build complex and important organic molecules. After a thorough investigation of each topic, we will apply the new information we have acquired to explain how organic chemistry (and chemistry in general) plays a role in almost everything we are exposed to on a daily basis.

As you may already know, organic chemistry is not always an easy subject to learn. You cannot simply learn organic chemistry by reading a book much as you would learn many of the other topics you will tackle in some of your other classes. Instead, you will need to solve problems inside and outside of class, and you need to acquire hands on experience through laboratory experimentation. This course is designed to help you do just that!

Remember that even though the study of organic chemistry can at times be difficult, it can also be a highly rewarding experience. You are here to learn and I am here to teach you. Therefore, I will always try to make sure that you understand the material and you are always welcome to drop by, email, or call me with any questions or concerns you may have. I hope that in the end, each of you will take away something new from this class be it an improved study habit or a new outlook on the world in which we live.

*Images taken from chem3D.com database

Course Requirements:

Students taking Chemistry 190 are required to gain access to the course textbook, a model set, and a laboratory notebook. More information on each of these items is provided below.

I. *Textbook*—I will assign readings and problems from the textbook and other resources that I feel are necessary to convey a particular topic. The required textbook for this class is **"Organic Chemistry" (5th edition) by Marc Loudon.** Please make sure that you are using the fifth edition for this course.

II. *Model set*—Access to a molecular model set for organic chemistry is essential to this course and is therefore required. Prentice Hall molecular model sets are available at the bookstore and online. There are also a number of other good (and cheap) molecular model sets out there. Please see me if you would like some suggestions.

III. *Laboratory notebook*—Laboratory handouts will be handed out in class and posted on Blackboard. In addition, you are required to purchase and maintain a Freeman Laboratory Notebook specifically for use in the laboratory portion of the course. A handout for keeping the laboratory notebook will be provided during your designated laboratory period and requirements may vary by instructor.

Class Format:

Each week I will provide a general overview of the material that we will be covering in class. I have proposed the following schedule that is subject to change based on Holidays and other important school related functions:

Monday:	-General lecture
Tuesday:	-Evening review session 7:00pm until 9:00pm in SCCT 2048
***Wednesday*:**	-General lecture
	-Problem set collected
Thursday:	-Problem set returned
***Friday*:**	-Quiz
	-General lecture

Class Policies:

It is expected that all students in this section of Chemistry 190 will take note of the following policies:

I. *Attendance*—Attendance is strongly encouraged. If you need to miss a class for any reason, please contact me in advance so that I can arrange to have missed work delivered to you in a timely fashion. Also note that class participation is considered for 10% of your final grade (see below). Frequently missing class can (and will) negatively impact your grade.

II. *Class and lab participation*—Every member of the class is expected to participate in lecture and in lab. This means that you must verbally interact with me during class by answering questions. You must also interact with your fellow classmates during group discussions, lab, and through the use of Blackboard when appropriate. Class participation is worth 10% of the final grade. Failing to participate will result in the reduction of your final grade in the course.

III. *Late work or missed assignments*—Work that is not completed on time will be marked late unless arrangements are made **in advance** to turn the work in at an alternate time other than the due date. In addition, a percentage of points will be deducted as outlined below:

One day late = 10% deduction
Two days late = 25% deduction
Three days late = 50% deduction
Four days late = 75% deduction
Five or more days late = no credit

IV. *Academic honesty*— Each individual is expected to follow the academic conduct code (Honor Code) set forth by Hamilton College.

V. *Learning disabilities*— In accordance with the Americans with Disabilities Act, any student who has a documented learning disability will be provided with reasonable accommodations designed to meet his/her needs. Before any such assistance can occur, it is the responsibility of the student to see that documentation is on file with the appropriate individual. Please see me as soon as possible to discuss any need for accommodations.

Student Evaluation:

You will be evaluated in this course on a regular basis. The basis for course evaluation is provided below with explanations:

Quizzes:	10%
Problem Sets:	10%
Lab Reports:	25%
Hourly Exams:	30%
Final Exam:	15%
Class Participation:	10%

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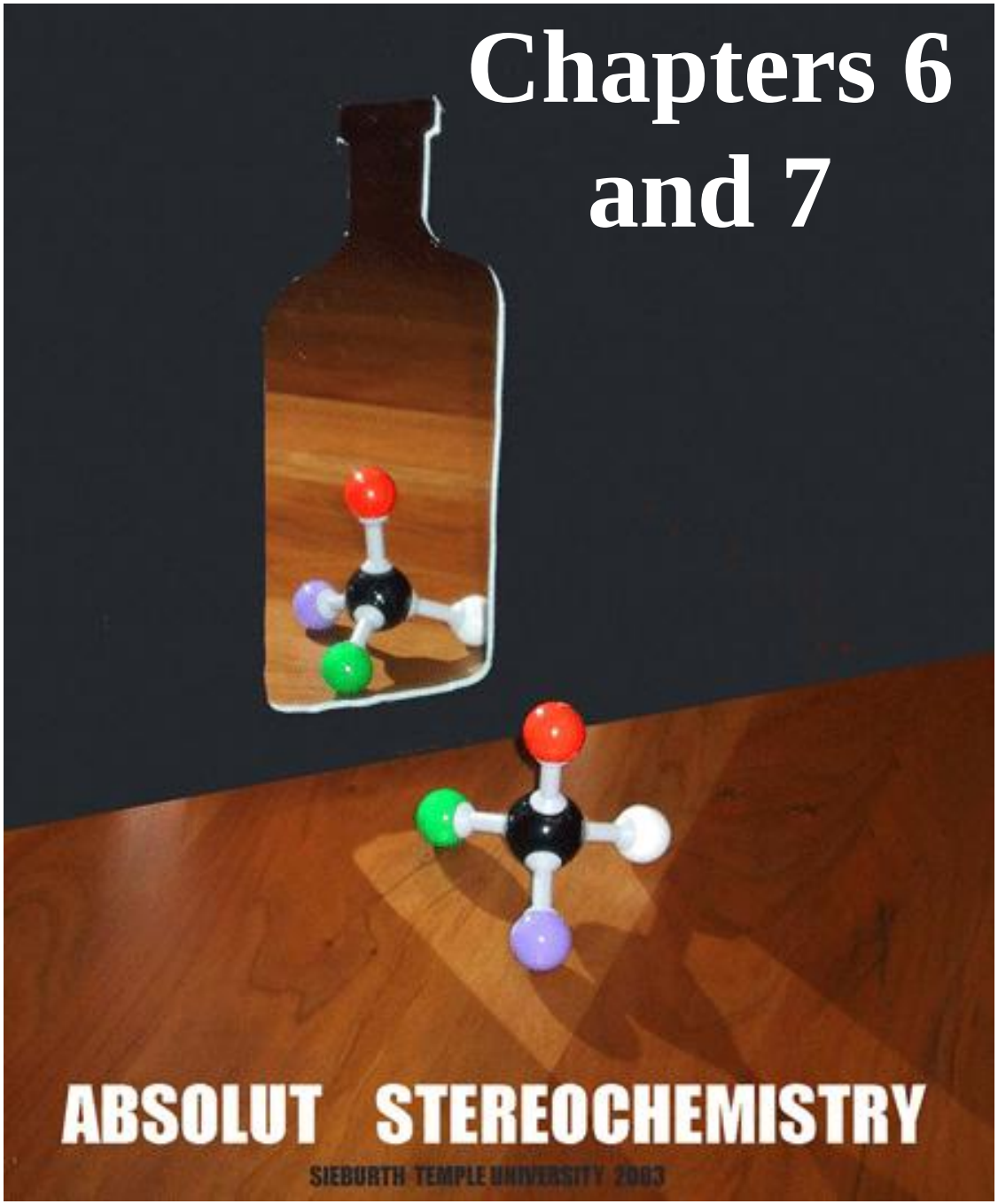
Schedule of Events:

Class	Monday	Wednesday	Friday
Week One **January 19** **Review**	-Introductions	-Chapter 1 (1.1-1.4)	**-QUIZ I (Gen Chem)** -Chapter 1 (1.5-1.9)
Week Two **January 26** **Organic Structures; Acid-Base Chemistry**	-Chapter 2 (2.1-2.5)	**-PROBLEM SET I DUE (Chapter 1)** -Chapter 2 (2.6-2.9)	**-QUIZ II (Chapter 1)** -Chapter 3 (3.1-3.3)
Week Three **February 2** **Acid-Base Chemistry; Infrared Spectroscopy**	-Chapter 3 (3.4-3.6)	**-PROBLEM SET II DUE (Chapter 2)** -Chapter 12 (12.1-12.2)	**-QUIZ III (Chapter 2)** - Chapter 12 (12.3-12.4)
Week Four **February 9** **MS; NMR**	-Chapter 12 (12.6)	**-PROBLEM SET III DUE (Chapter 3)** -Chapter 13 (13.1-13.3, 13.10)	**-QUIZ IV (Chapter 3)** -Chapter 13 (13.4-13.6)
Week Five **February 16** **NMR; Stereochemistry**	-Chapter 13 (13.7-13.8)	**-PROBLEM SET IV DUE (Chapter 12)** -Chapter 13 (13.9-13.10) **-Review for Exam I (Chapters 1, 2, 3, 12)**	-Chapter 6 (6.1-6.2)
Week Six **February 23** **Stereochemistry**	-Chapter 6 (6.3-6.5)	**-PROBLEM SET V DUE (Chapter 13)** -Chapter 6 (6.6-6.8)	**-QUIZ V (Chapter 13)** -Chapter 6 (6.9-6.12)
Week Seven **March 2** **Conformational Analysis**	-Chapter 7 (7.1-7.3)	**-PROBLEM SET VI DUE (Chapter 6)** - Chapter 7 (7.4-7.5) -In class worksheet	**-QUIZ VI (Chapter 6)** -Chapter 7 (7.6-7.8)
Week Eight **March 9** **Substitution and Elimination**	-Chapter 8	**-PROBLEM SET VII DUE (Chapter 7)** -Chapter 9 (9.1-9.3) **-Review for Exam II (Chapters 13, 6, 7)**	-Chapter 9 (9.4)
Week Nine (March 16)	No Class- **Spring Break**	No Class- **Spring Break**	No Class- **Spring Break**
Week 10 (March 23)	No Class- **Spring Break**	No Class- **Spring Break**	No Class- **Spring Break**
Week 11 **March 30** **Substitution and Elimination**	-Chapter 9 (9.5)	-Chapter 9 (9.6)	**-QUIZ VII (Chapters 8-9)** -Chapter 9 (9.7-9.8)
Week 12 **April 6** **Alkenes**	-Chapter 4 (4.1-4.5)	**-PROBLEM SET VIII DUE (Chapters 8 and 9)** -Chapter 4 (4.6-4.9) **- Review for Exam III (Chapters 8, 9)**	-Chapter 5 (5.1-5.3)
Week 13 **April 13** **Alkenes**	-Chapter 5 (5.4, 7.9)	-Chapter 5 (5.5 and handout)	**-QUIZ VIII (Chapter 4)** -Chapter 5 (5.6-5.7)
Week 14 **April 20** **Alkynes**	-Chapter 14 (14.1-14.4)	**-PROBLEM SET IX DUE (Chapters 4 and 5)**- Chapter 14 (14.5-14.6)	**-QUIZ IX (Chapter 5)** -Chapter 14 (14.7-14.8)
Week 15 **April 27** **Alcohols**	-Chapter 10 (10.1-10.4)	**- PROBLEM SET X DUE (Chapter 14)** -Chapter 10 (10.5-10.7) **-Review for Exam IV (Chapters 4, 5, 14)**	-Reduction Reactions (handout)
Week 16 **May 4** **Alcohols**	- Chapter 11 (11.1-11.3)	**- PROBLEM SET XI DUE (Chapter 10)** -Chapter 11 (11.4-11.6)	**-QUIZ X (Chapters 10-11)** -Chapter 11 (11.7-11.10)

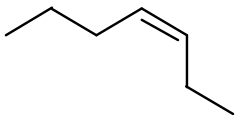
Chapters 6 and 7

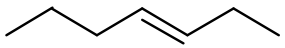


Stereoisomers

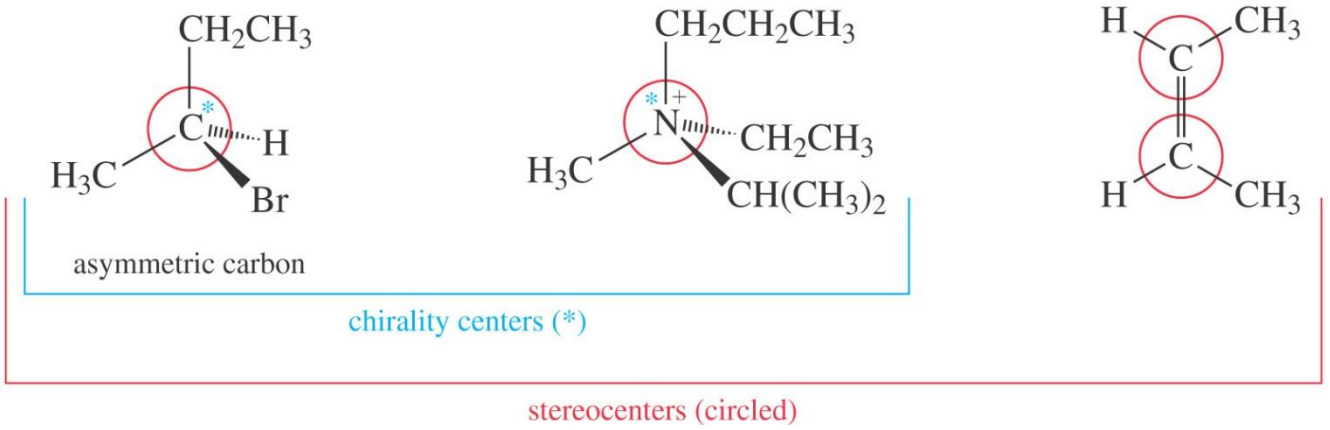
Compounds with the same connectivity, but different arrangements of the atoms in three dimensional space.

-Example: *cis* versus *trans* isomerism





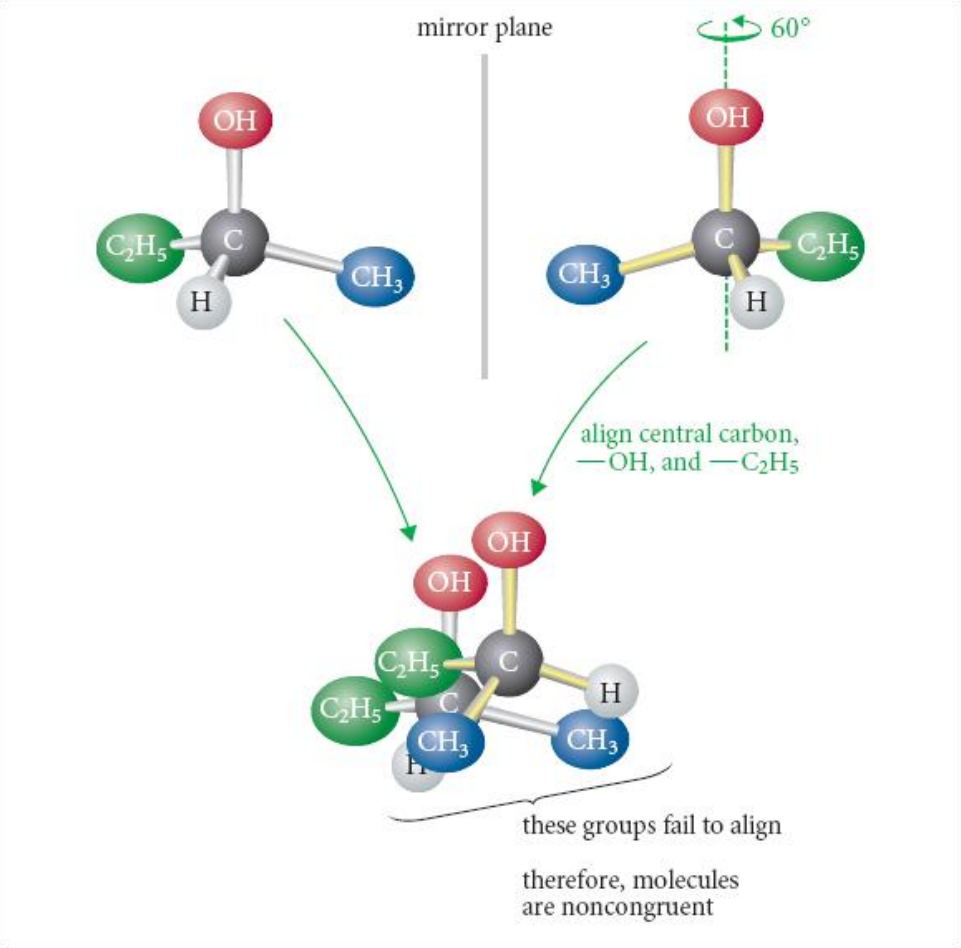
-The study of stereoisomers is called stereochemistry.



Chiral Molecule

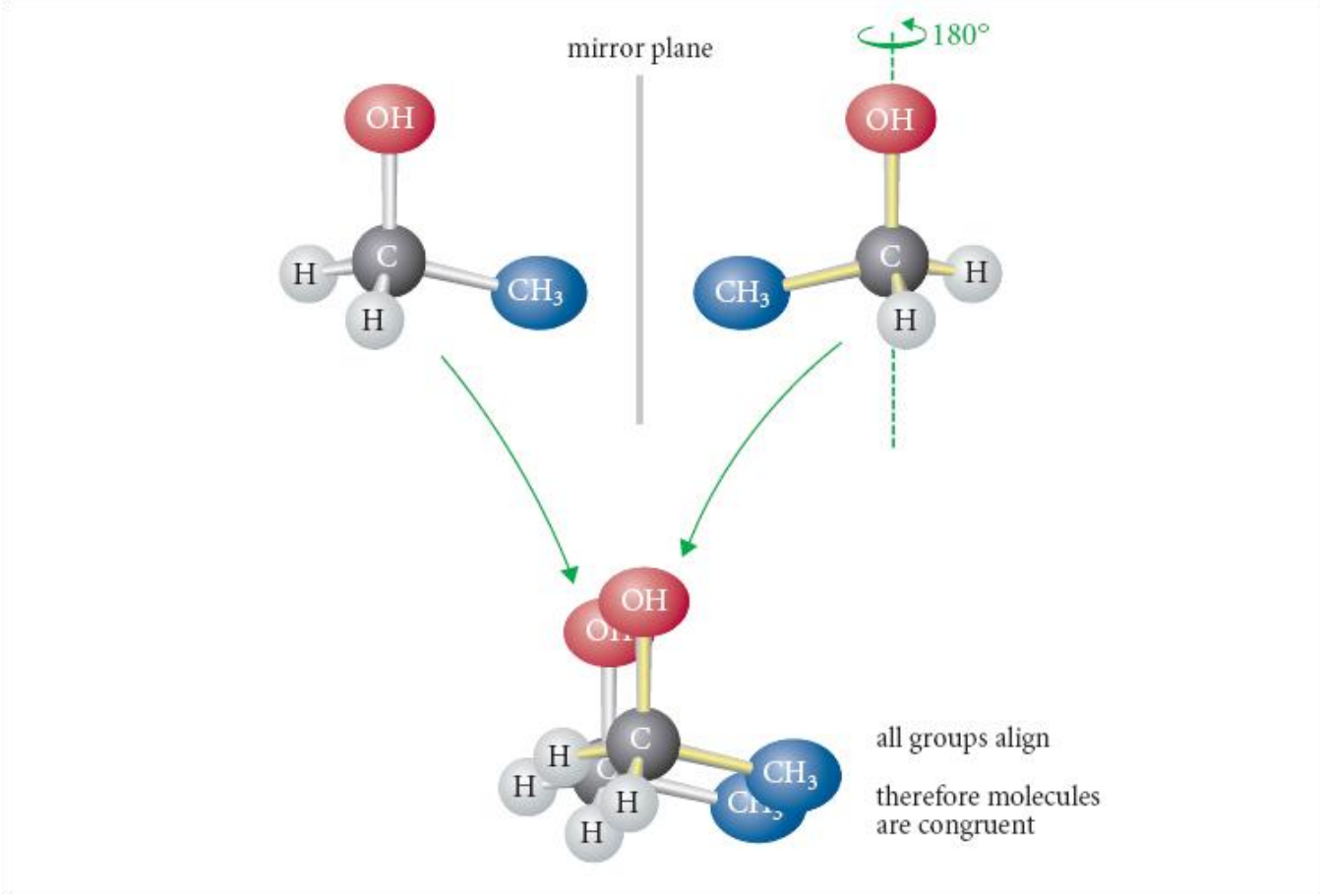
Molecules that contains at least one stereocenter.

-Chiral molecules are said to have a “handedness.”



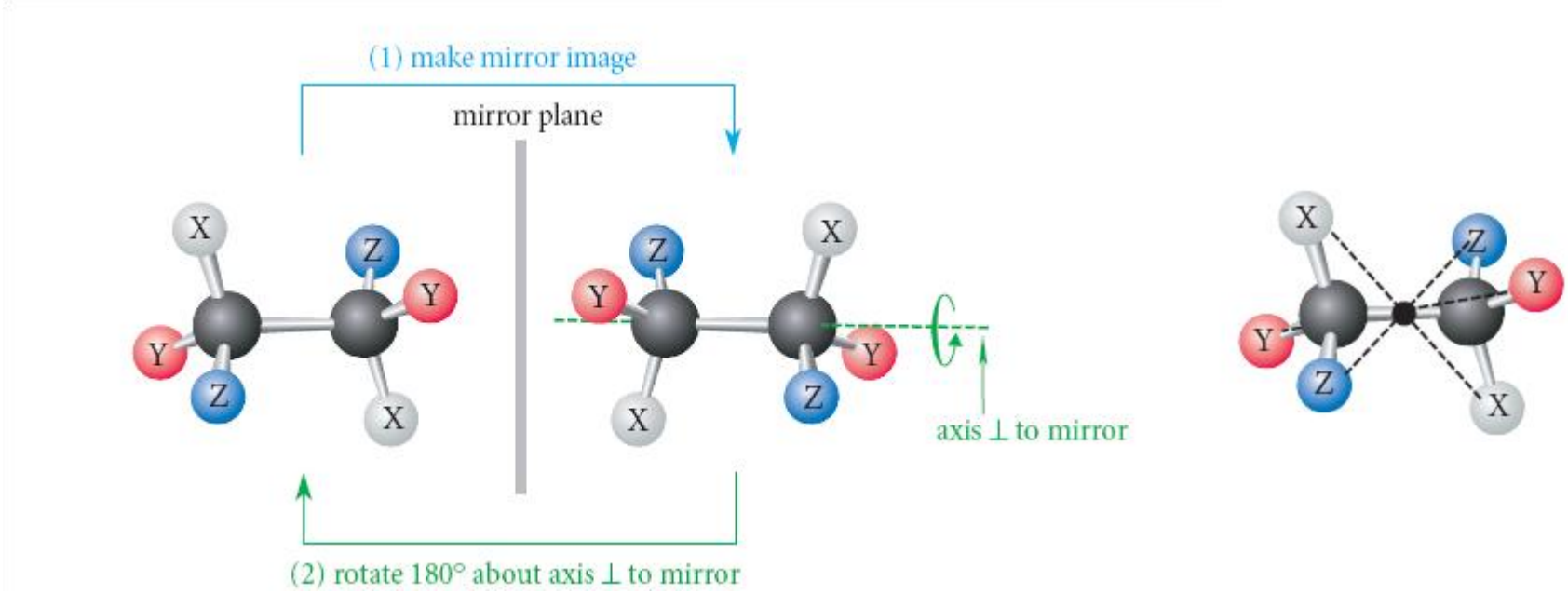
Chiral Molecule

Molecules without an stereocenter are generally achiral.



Chiral Molecule

Molecules without a plane or point of symmetry are said to be achiral.

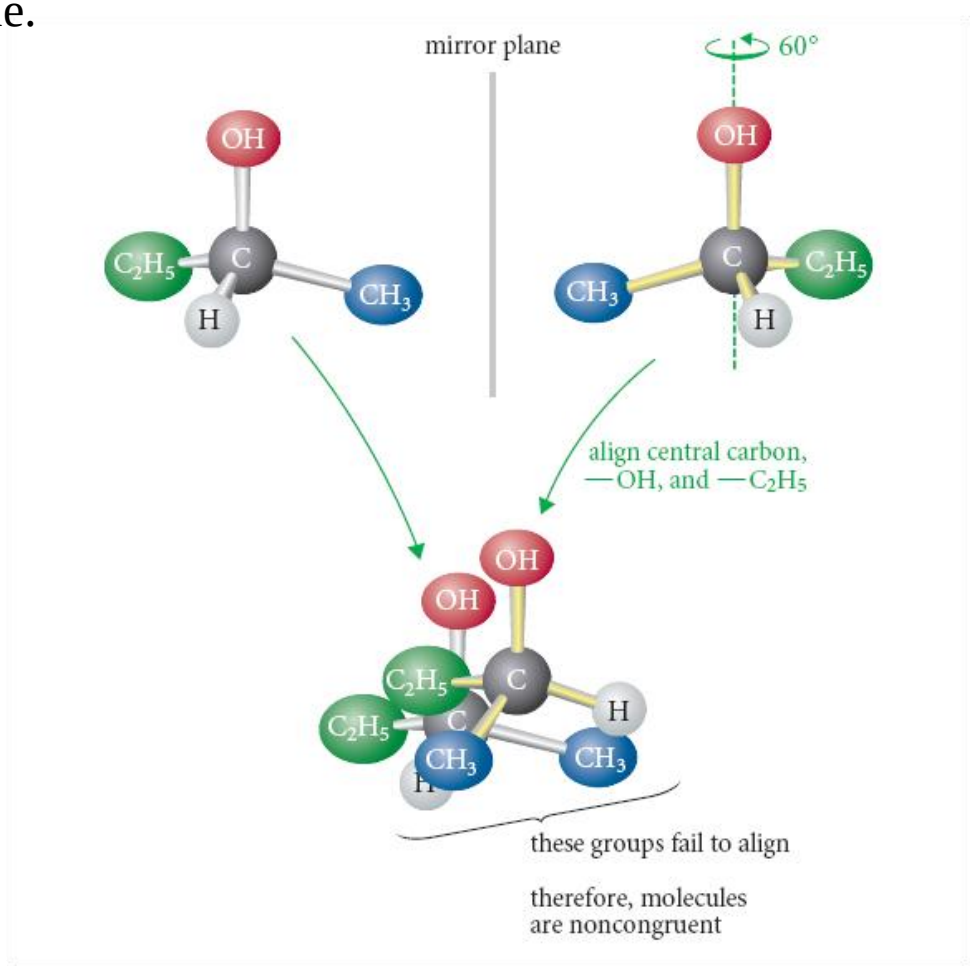


Enantiomers

Enantiomers- chiral molecules that are nonsuperimposable mirror images.

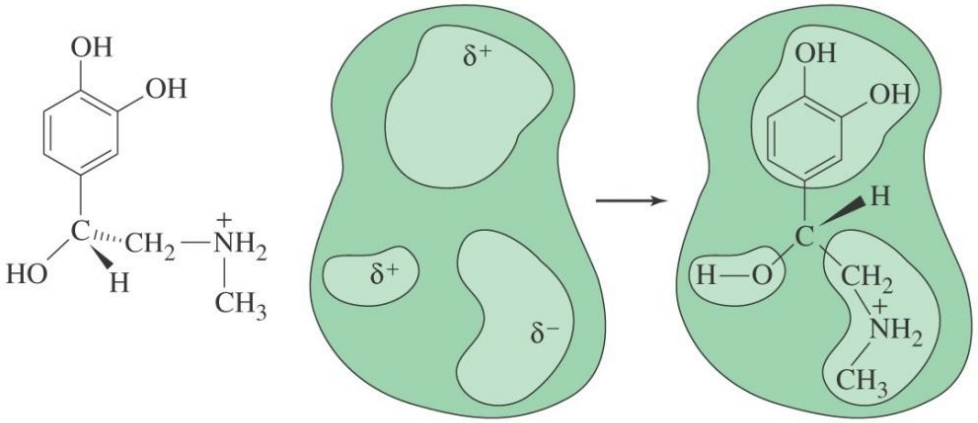
-Enantiomers contain an at least one steroecenter, usually carbon.

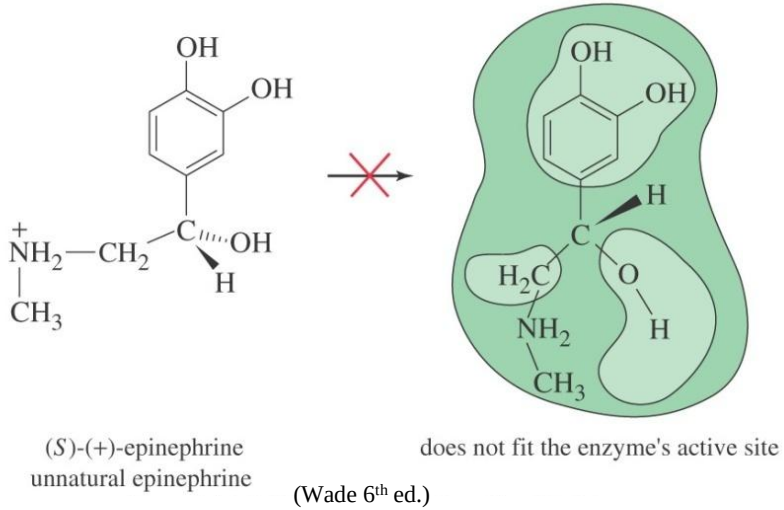
-Stereocenter is a center at which the interchange of two groups give a different molecule.



The Importance of Enantiomers

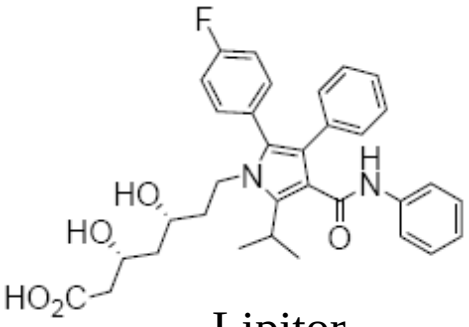
Biological recognition is key!



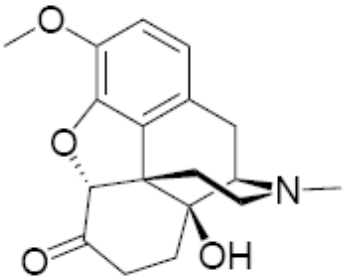


(Wade 6th ed.)

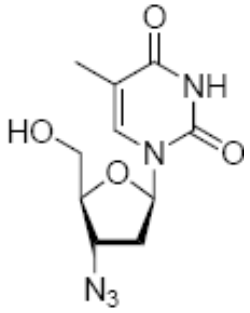
Examples



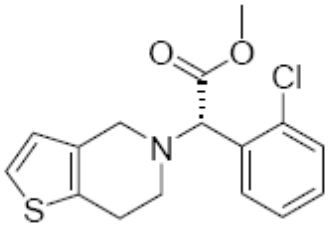
Lipitor



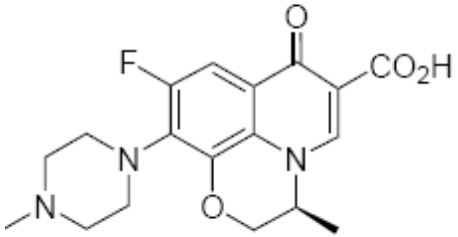
OxyContin



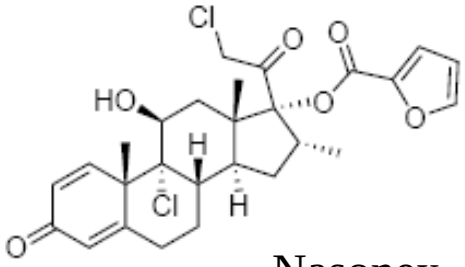
AZT



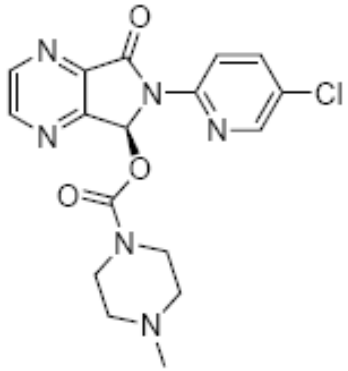
Plavix



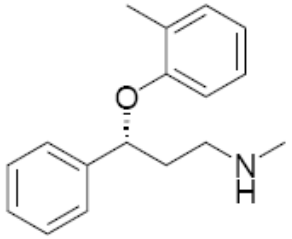
Levaquin



Nasonex



Lunesta



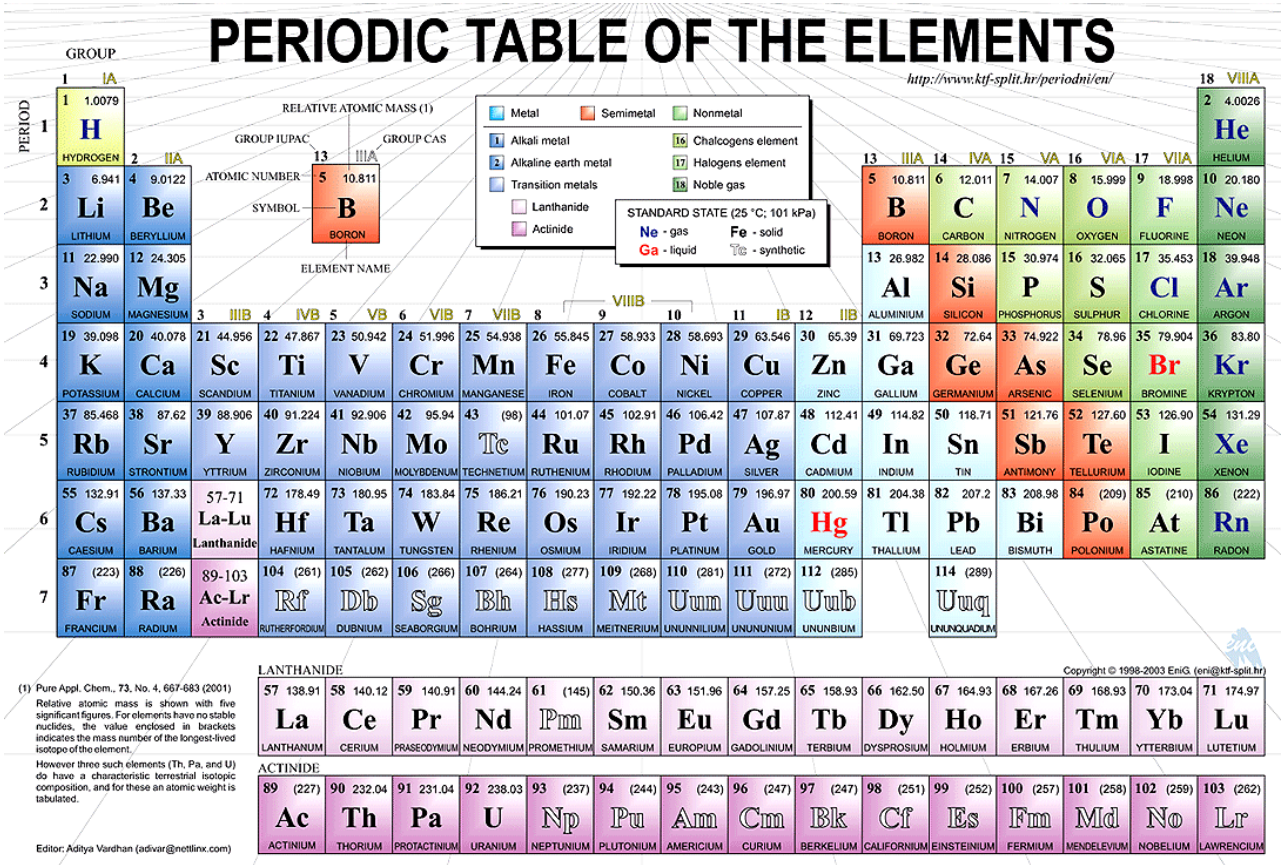
Strattera

Cahn-Ingold-Prelog Notation

Used to describe the configuration or arrangement of the atoms around a stereocenter.

-Assigns priorities to the groups around the stereocenter and assigns a notation (R or S) based on the configuration of the atoms.

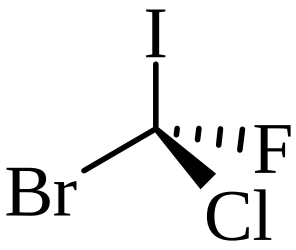
-Assignments are largely based on atomic number.



Cahn Ingold Prelog Notation

The higher the atomic number, the higher the priority.

-Example: I > Br > Cl > F

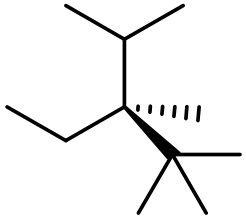


Cahn Ingold Prelog Notation

For saturated carbon groups the first point of difference must used to determine priority.

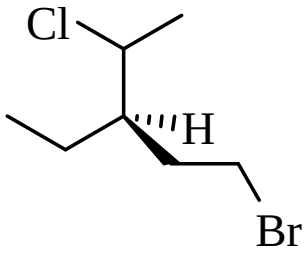
-For saturated carbons substituted only with carbon and hydrogen, at the first point of difference, a tertiary carbon will take priority over a secondary, which will take priority over a primary carbon.

-Example: $-(CH_3)_3 > -CH(CH_3)_2 > CH_2CH_3 > CH_3$



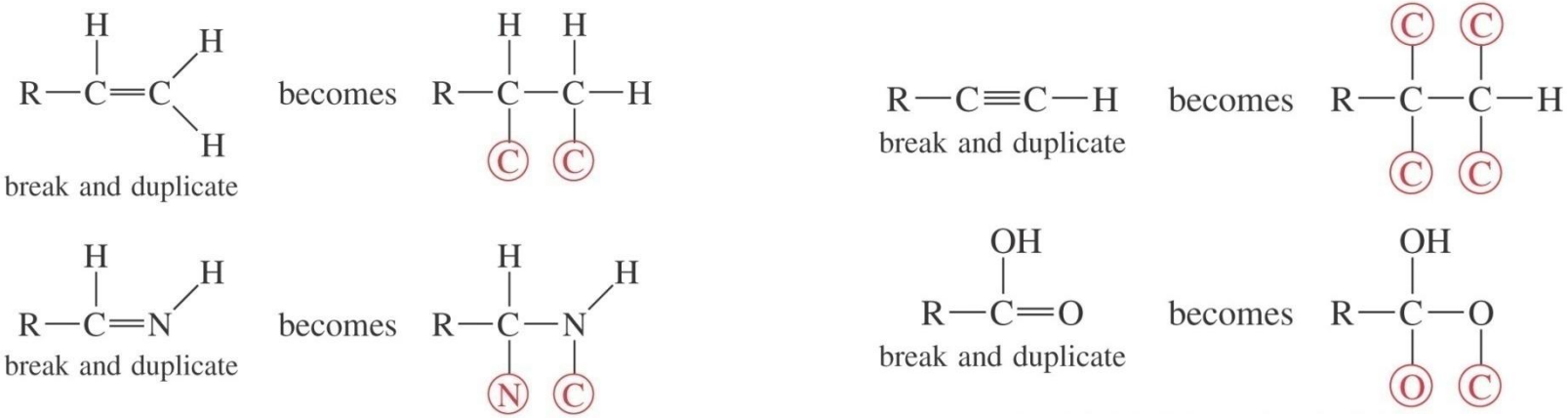
-For saturated carbons groups substituted with atoms other than carbon or hydrogen, the carbon atom containing the atom with the highest atomic number gets the higher priority.

-Example: $-CH_3CH_2I > -CH_3CH_2Br > -CH_3CH_2Cl > -CH_3CH_2F$

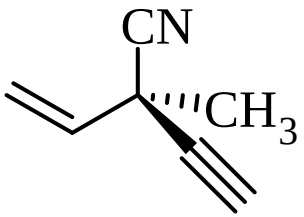


Cahn Ingold Prelog Notation

For unsaturated carbon groups, each unsaturated atom should be treated as if it were singly bonded to the other atom.



-Example:

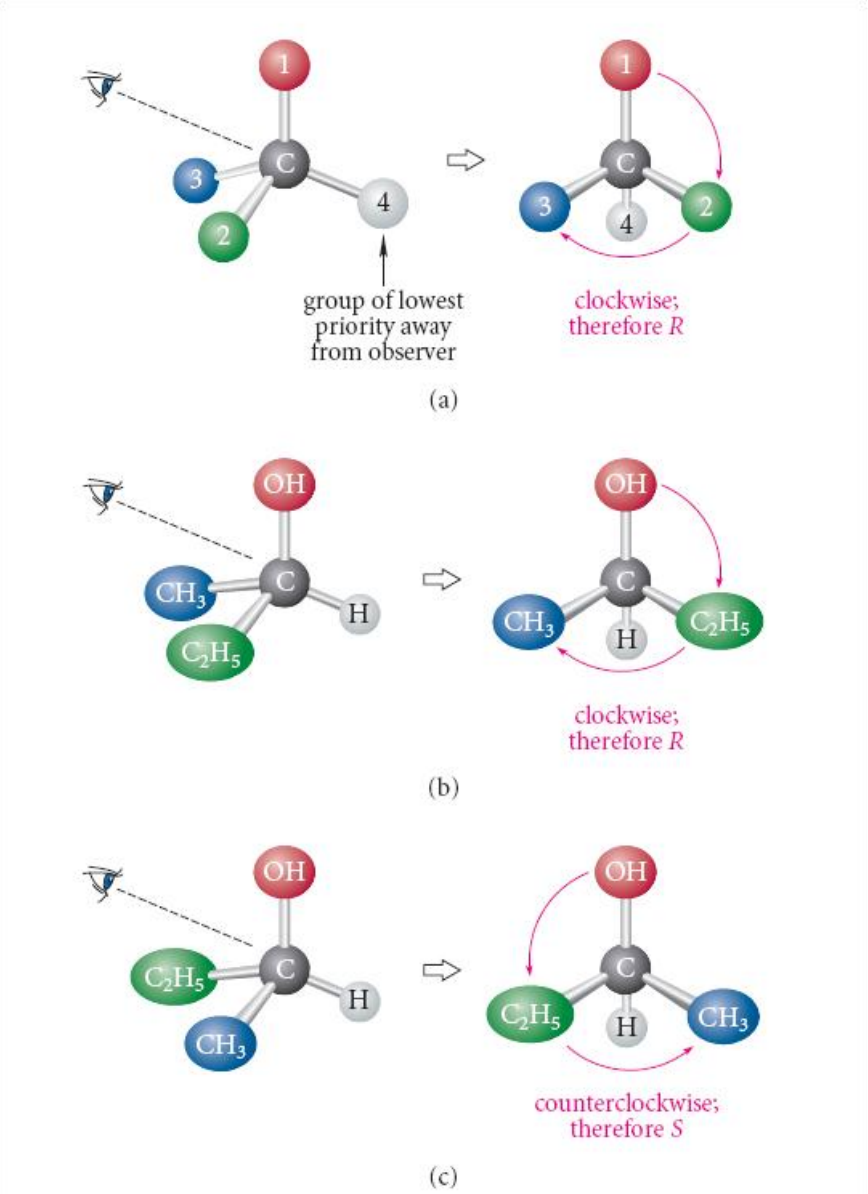


Cahn Ingold Prelog Notation

Once prioritization is accomplished, the lowest (fourth) priority atom is placed in the back, and an arrow is drawn from the first priority group to the second priority group and then to the third group.

-If the arrow is clockwise, the configuration is R.

-If the arrow is counterclockwise, the configuration is S.



Cahn Ingold Prelog Notation

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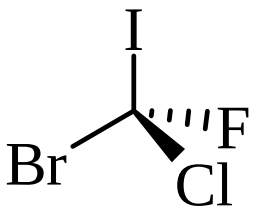
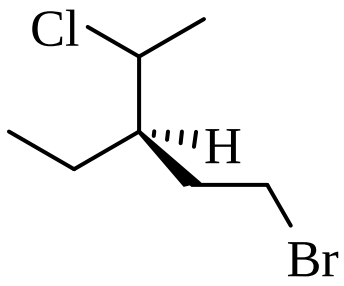
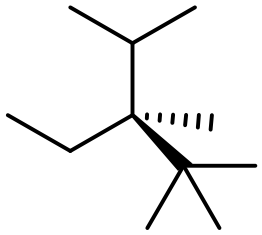
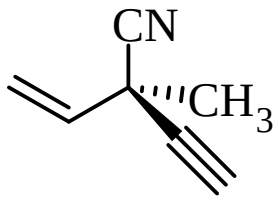
-Examples:

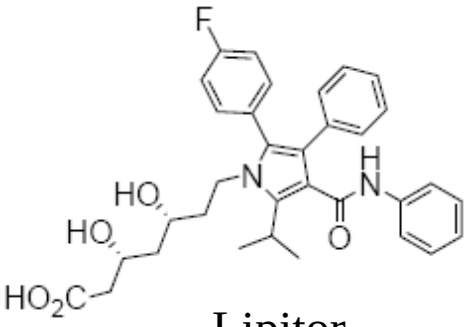




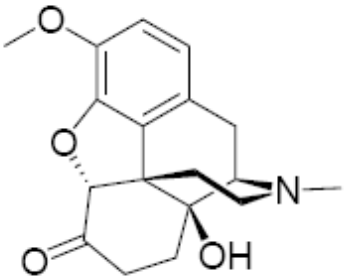




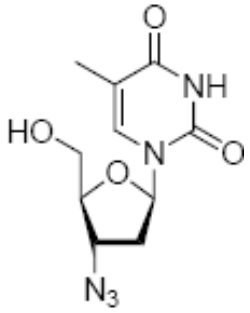
Examples



Lipitor



OxyContin



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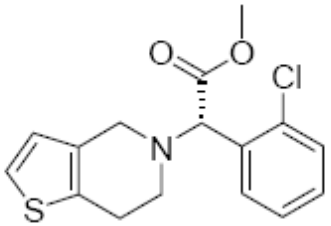
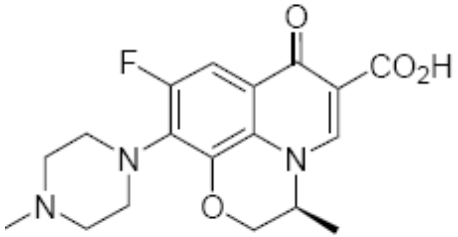
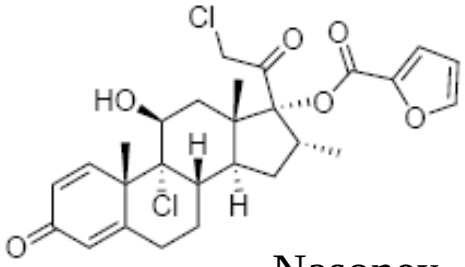


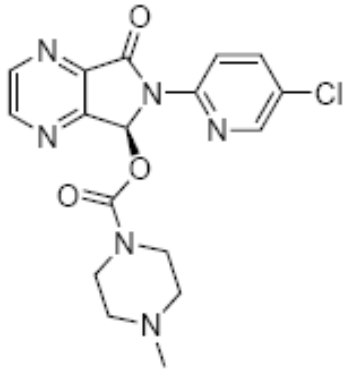
Plavix



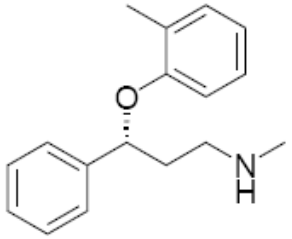
Levaquin



Nasonex



Lunesta



Strattera

Diastereomers

Stereoisomers that contain multiple chiral centers.

-Diastereomers are not mirror images (usually they are geometric isomers).

-Diasteromers have at least on stereocenter between them that is the same.

-Two major types:

1. Cis/Trans isomers.
2. Molecules with more than one asymmetric center.

-Diasteromers, unlike enantiomers, have different physical properties.

Diastereomers

Cis/Trans isomers.

-Example: cis-2-butene vs trans 2-butene

-Example: cis-1,2-dimethycyclopentane vs trans-1,2-dimethylcyclopentane

Diastereomers

Molecules with more than one asymmetric center.

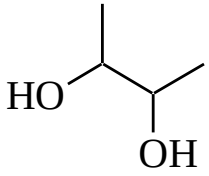
-Example: 2-bromo-2-chlorobutane

-Example: glucose

Meso Compounds

Achiral compounds containing more than one stereocenter and a plane or point of symmetry.

-Example: butan-2,3-diol



-Best studied using Fischer Projections.

Fischer Projections

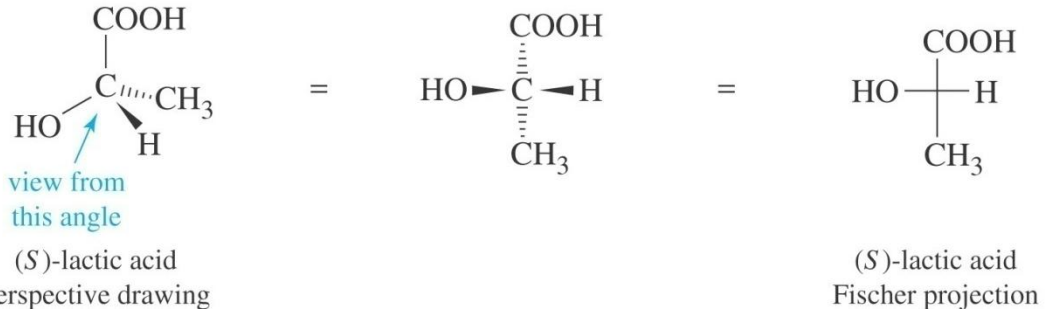
Method for representing acyclic molecules without using wedge and dash notation.

-Uses lines to represent bonds and bond orientation.

-Horizontal lines are “wedges.”

-Vertical lines are “dashes.”

-Example:



(*S*)-lactic acid
perspective drawing

(*S*)-lactic acid
Fischer projection

Fischer Projections

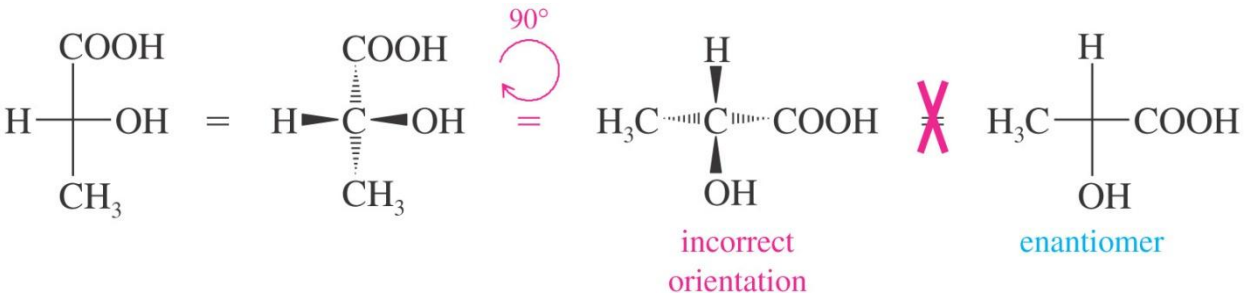
Rules for working with Fischer projections:

1. Fisher projections are usually drawn with the most oxidized carbon at the top of the chain.

   -Based on IUPAC nomenclature.

2. Fisher projections may not be flipped and must be rotated in the plane of the paper.
3. Fisher projections may only be rotated by 180 degrees.

   -Rotation by 90 degrees changes the configuration.

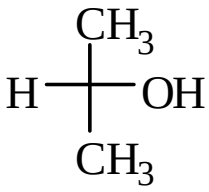


Fischer Projections

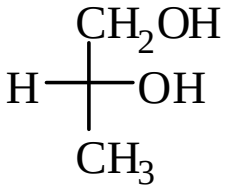
One can determine the chirality of a molecule in a Fisher projection by drawing the mirror image and rotating it 180 degrees to see if it is super imposable.

-Examples:

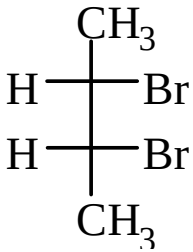
2-propanol



1,2-propanediol



2,3-dibromobutane (meso)



The Cahn-Inglold-Prelog convention for assigning absolute configuration may also be applied to Fischer projections.

Polarimetry

Measures the rotation of polarized light upon exposure to a sample containing a chiral molecule.

-Chrial molecules have the ability to rotate a plane of polarized light (are optically active).

-Enantiomers will rotate the plane of polarized light in opposite directions (equal in magnitude, but opposite in sign).

-For diastereomers the rotation is not as well defined but is still useful.

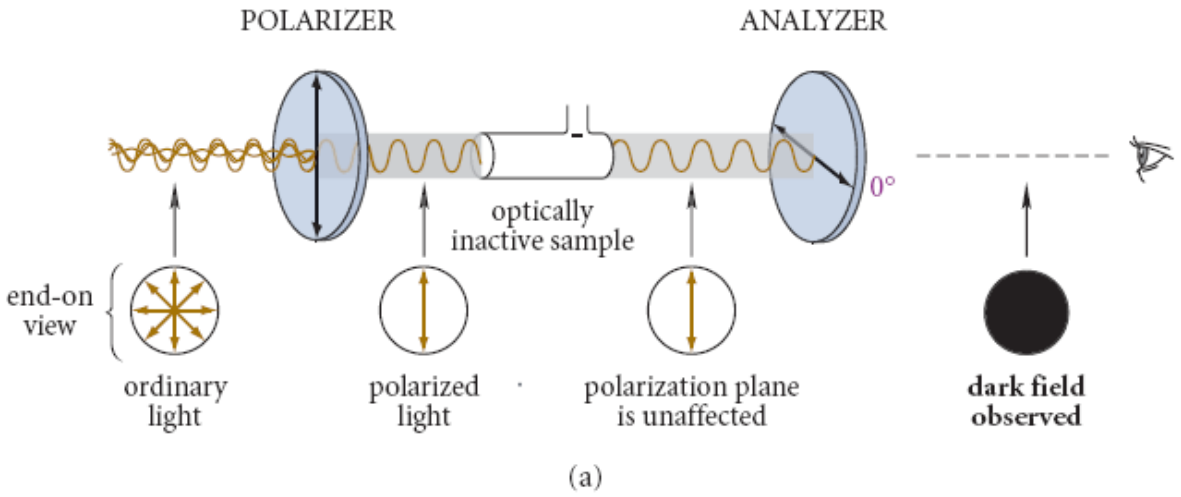
Requires the use of a polarimeter.

Compounds that rotate the plane of polarized light to the right (clockwise) are called dextrorotatory and are noted as *d* or +.

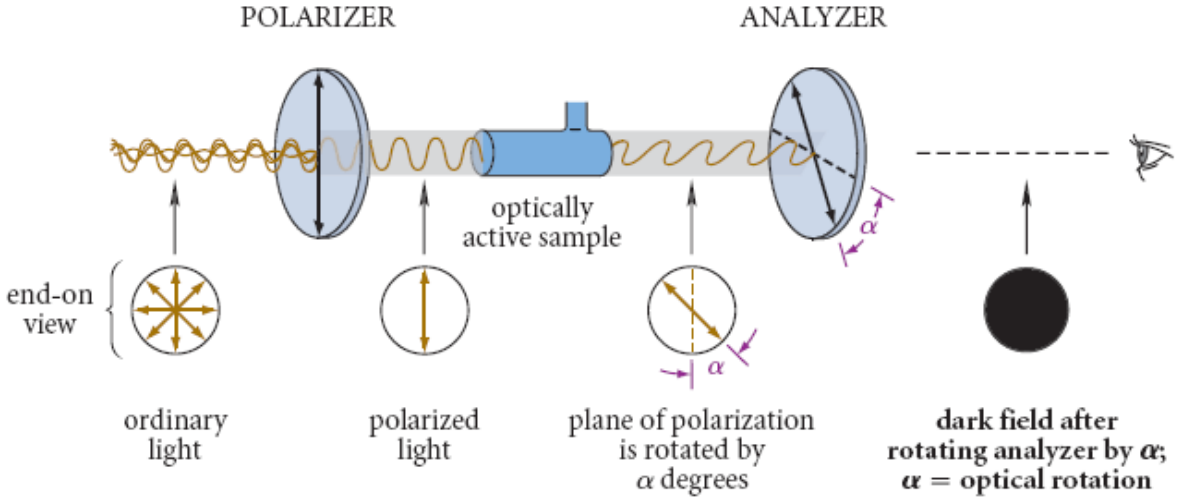
Compounds that rotate the plane of polarized light to the left (counter clockwise) are called levorotatory and are noted as *l* or -.

The degree that a compound will rotate a plane of polarized light can not be predicted and must be measured.

Polarimetry



(a)



(b)

Polarimetry

The observed rotation, symbolized by α, is depended on four factors:

1. Temperature (usually 25°C)
2. Wavelength of light (usually 589 nm)
3. Path length of the cell used for the measurement (usually10 dm)
4. Concentration (varies)

The specific rotation, symbolized $[\alpha]_D^{25}$, is a standardization of the observed rotation for reporting purposes.

Given by: $[\alpha]_D^{25} = \alpha_{(obs)} / c \times l$

where c = concentration

l = path length

Polarimetry

For mixtures of enantiomers, the enantiomeric excess (e.e.) of one enantiomers with respect to the other can be calculated.

Given by: $e.e. = (I\,d - l\,I)/(d + l) \times 100\%$

where d and l are the proportions of the two enantiomers

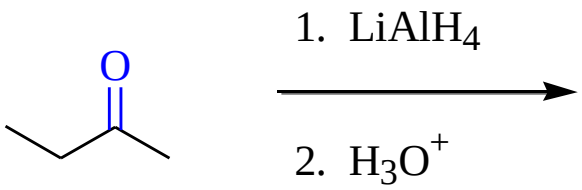
Sample Problem: (see handout)

Polarimetry

Racemic mixtures (equimolar mixtures of two enantiomers) do not rotate the plane of polarized light.

-Formed when achiral molecules undergo a reaction to produce chiral molecules.

-Example: reduction of 2-butanone



-Achiral molecules do not rotate the plane of polarized light.

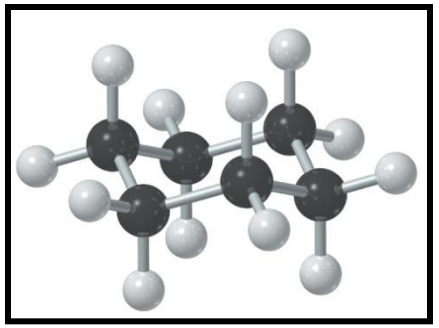
Conformationally Mobile Systems and Chirality

Conformationally mobile systems- systems such as rings, which interconvert freely, and acyclic molecules with chiral centers that are able to rotate may or may not be chiral.

-The energy of the system must be considered.

Example: Cyclohexane- six membered ring alkane that exhibits no ring strain.

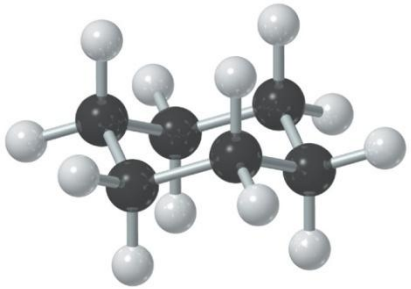
-Cyclohexane is commonly found in carbohydrates, steroids, plant products and pesticides.

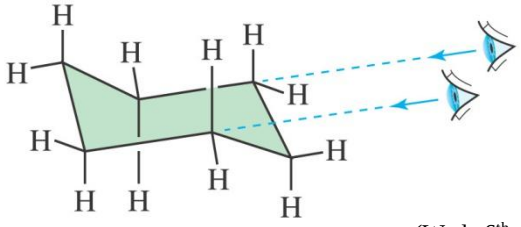


Structure and Conformation-Cyclohexane

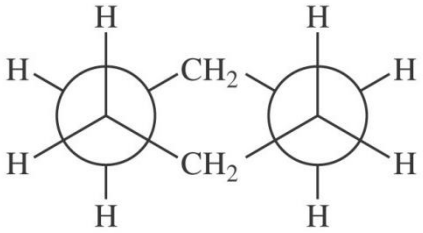
Cyclohexane is found in three major conformations:

I. Chair conformation:

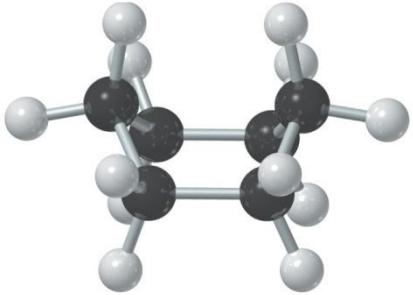


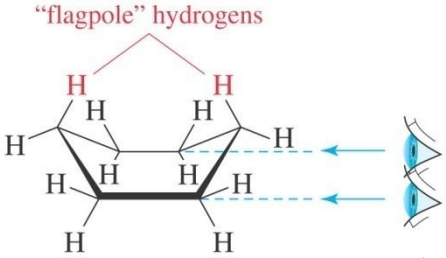


(Wade 6th ed.)

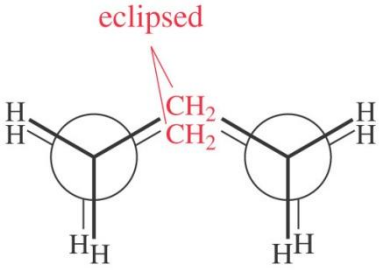


II. Boat conformation:

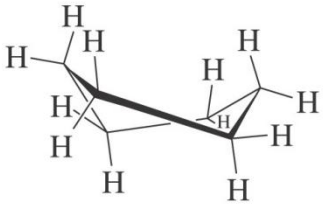




(Wade 6th ed.)



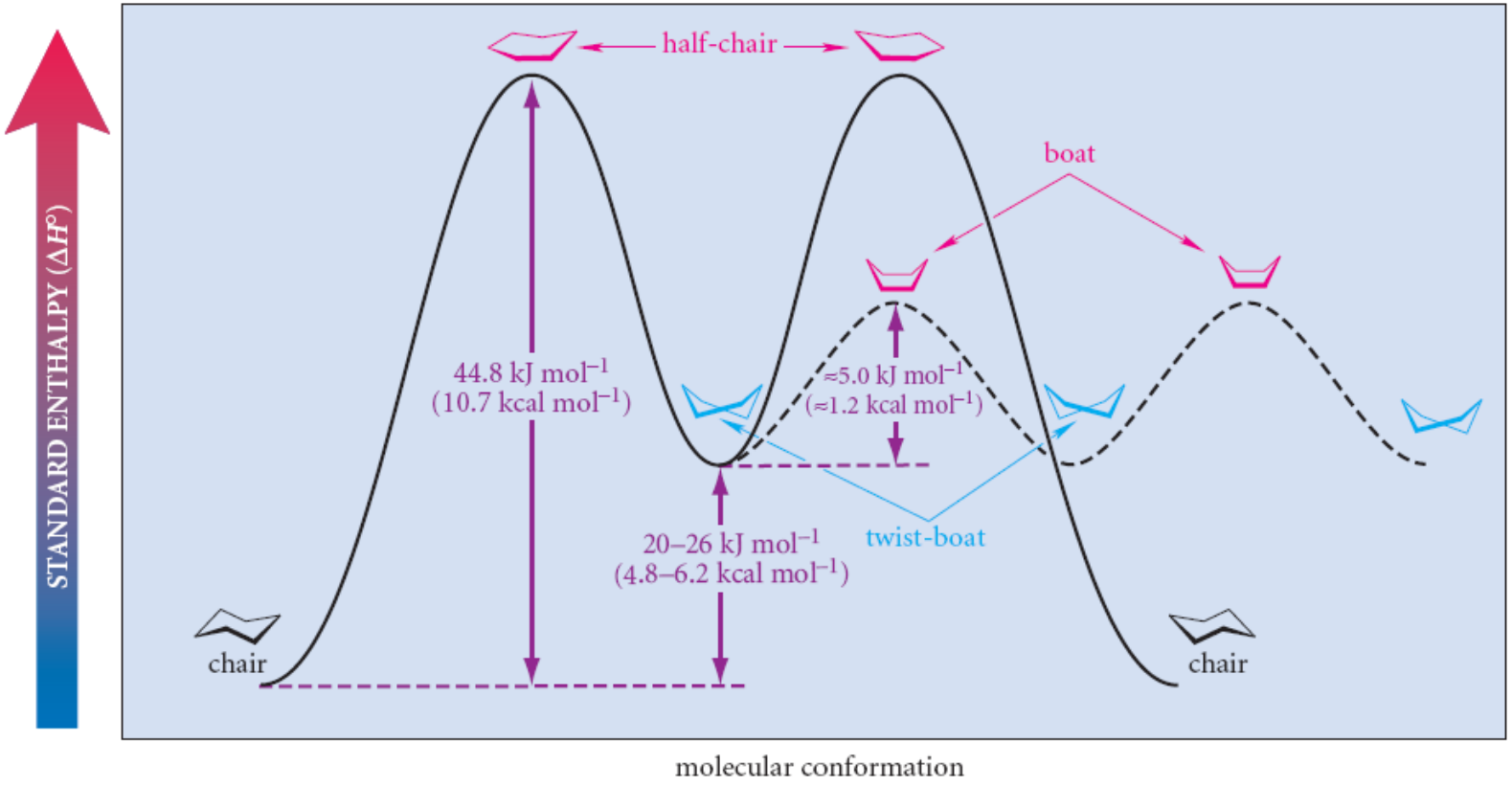
III. Twist boat conformation:



(Wade 6th ed.)

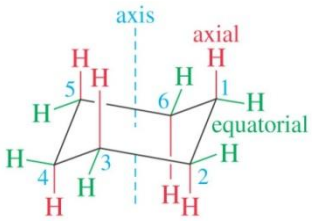
Structure and Conformation-Cyclohexane

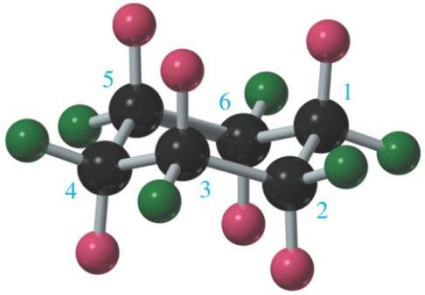
Conformational analysis of cyclohexane can be used to determine the most stable (most populated) conformer is chair conformer.

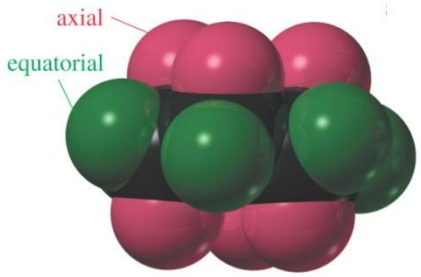


Structure and Conformation-Cyclohexane

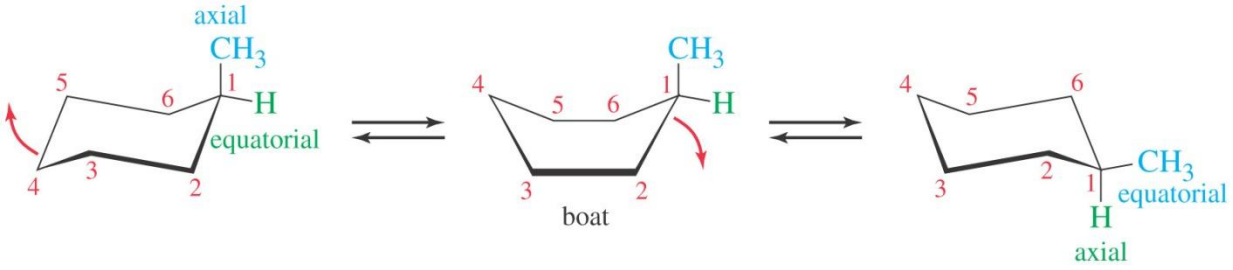
Substituents can occupy both axial and equatorial positions.

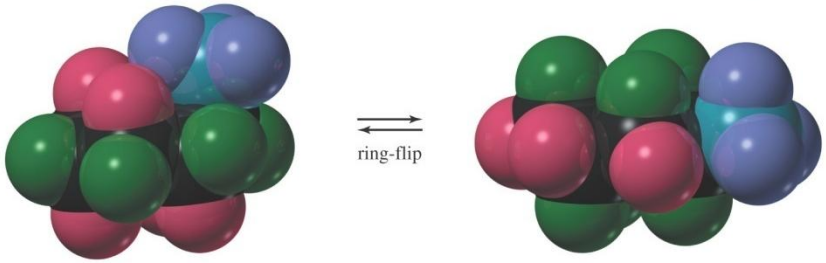






-The ring interconvert's at room temperature and both conformations can exist.



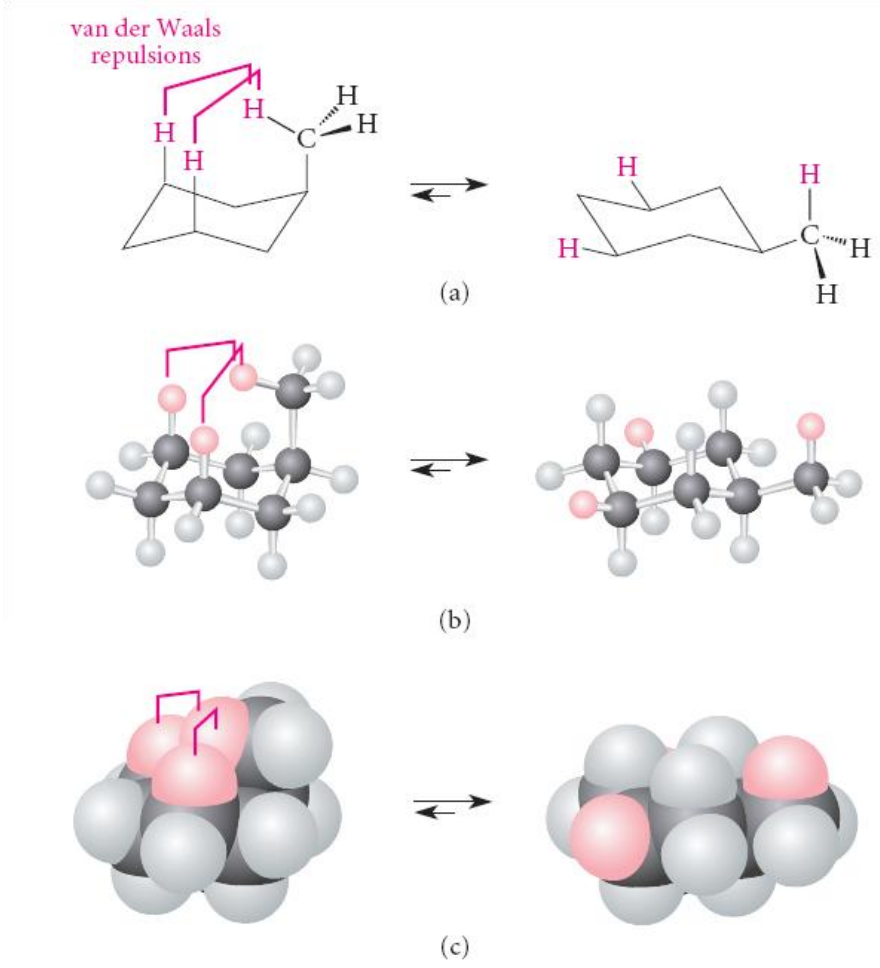


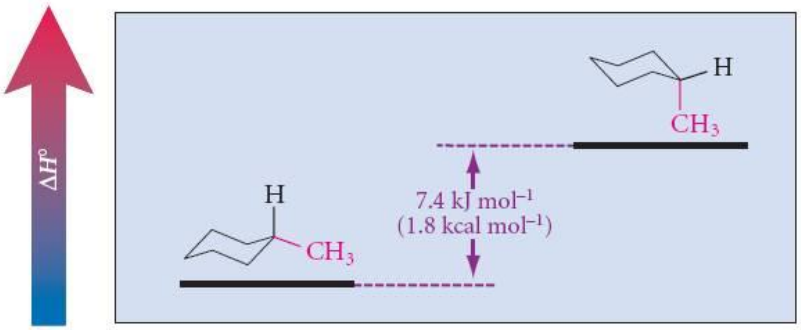
(Wade 6th ed.)

Conformationally Mobile Systems and Chirality

Monosubsituted cyclohexanes prefer to occupy a conformation where the bulkier substituent is an equatorial position.

-Placing the bulkier substituent in an equatorial position reduces the amount of torsional strain by limiting 1,3-diaxial interactions with the hydrogen atoms in axial positions.





Structure and Conformation-Cyclohexane

While both conformers exist at room temperature, the equilibrium of the system lies toward the more stable conformer with the substituent in the equatorial position.

-The larger the substituent, the greater the 1,3-diaxial interaction.

X	ΔG (kJ/mol)
-F	0.8
-CN	0.8
-Cl	2.1
-Br	2.5
-OH	4.1
-COOH	5.9
$-CH_3$	7.6
$-CH_2CH_3$	7.9
$-CH(CH_3)_2$	8.8
$-C(CH_3)_3$	23

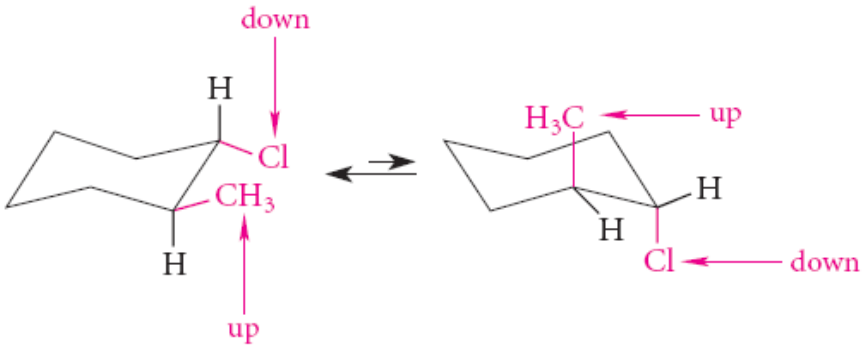
-Larger substituents are almost always found in the equatorial position.

-Smaller substitutents equilibrate between conformations where the substitutents are in the axial and equatorial positions.

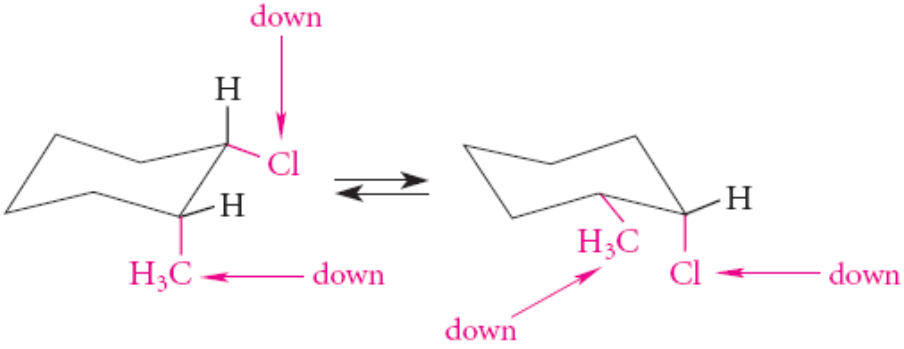
-Relative distribution depends on the size of the substituent.

Structure and Conformation-Cyclohexane

Disubstituted cyclohexanes prefer to occupy a conformation where both groups are in the equatorial position (when possible).



-If it is not possible to place both substitutents into equatorial positions, then the bulkier of the two substituents generally occupies the equatorial position.



Conformationally Mobile Systems and Chirality

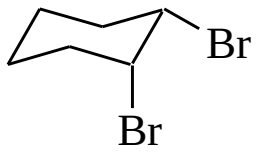
Systems such as rings, which interconvert freely, and acyclic molecules with chiral centers that are able to rotate may or may not be chiral.

-The energy of the system must be considered.

For four, five and six membered rings, this process can be simplified by treating the ring as flat and looking for planes or points of symmetry.

-If a plane or point of symmetry is present then the system is achiral.

-Example: *cis*-1,2-dibromocyclohexane *vs trans*-1,2-dibromocyclohexane



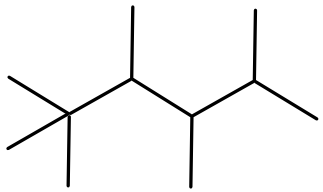
Conformationally Mobile Systems and Chirality

Acyclic molecules must be taken on a case by case basis.

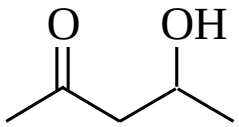
-Most linear molecules with asymmetric centers prefer one conformation over the other.

-Influence may be steric or electronic.

-Example: 2-t-butyl-3-isopropyl butane



-Example: 4-hydroxy pentan-2-one

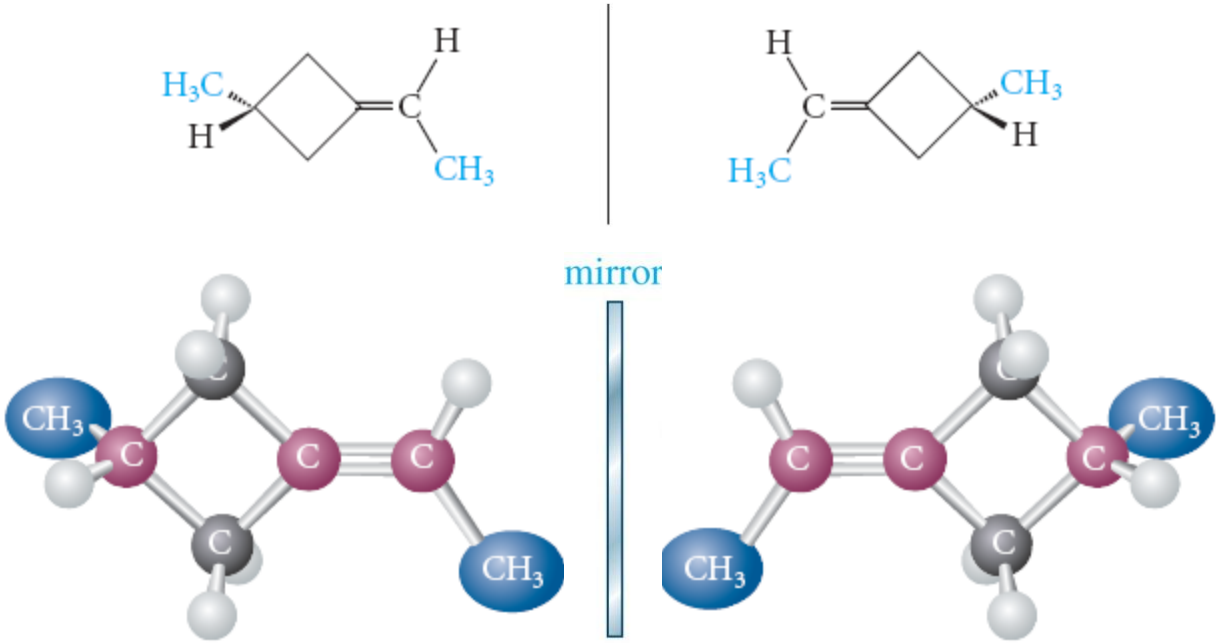


Conformationally Mobile Systems and Chirality

Some acyclic molecules without stereogenic centers are chiral.

-Conformational enantiomers- molecules that do not contain an asymmetric center, but are chiral based on the fact that they are locked into a specific conformation.

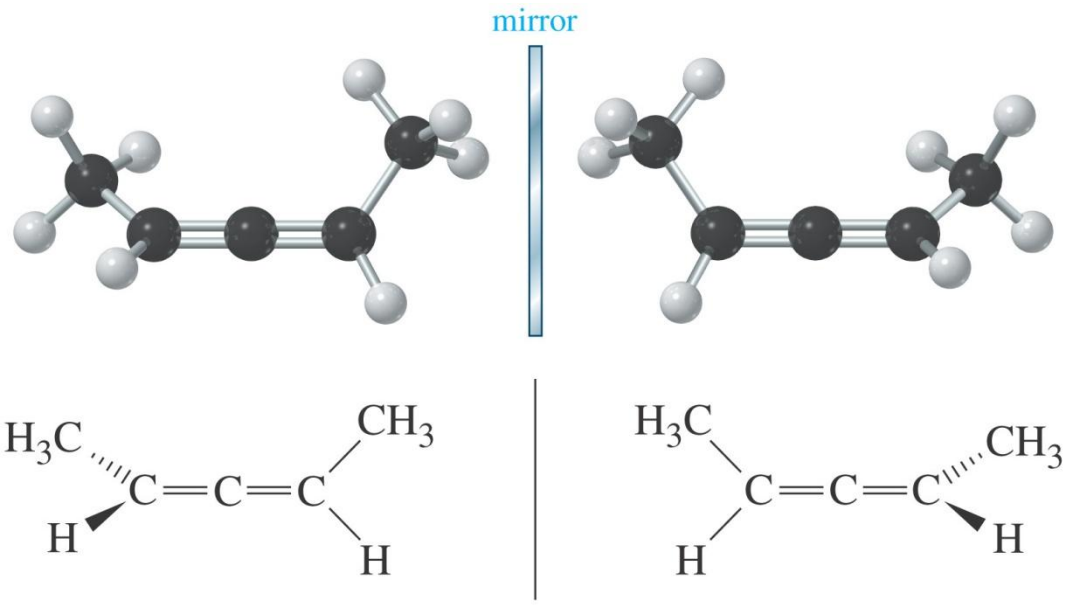
-Example: conformationally restricted alkenes (Figure 6.15)



Conformationally Mobile Systems and Chirality

Allene derivatives- molecules that do not contain an asymmetric center, but are chiral based on the fact that they are locked into a conformation that puts the substituents at either end into a perpendicular conformation (extended tetrahedron).

-Example: 2.-3-pentatdiene



enantiomers of 2,3-pentadiene

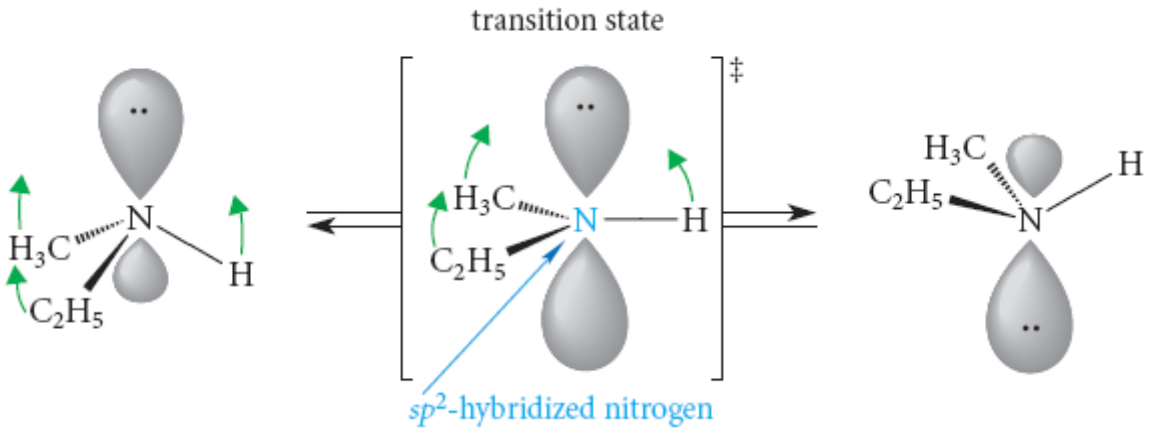
(Wade 6th ed.)

Conformationally Mobile Systems and Chirality

Atoms other than carbon may also be chiral.

-Nitrogen can be chiral or achiral depending on the groups surround the atom.

-Example: Figure 6.17

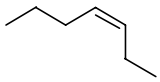


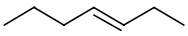
-If the groups on the nitrogen are small enough, rapid interconversion can occur making the molecule achiral.

-If the groups on the nitrogen are large enough, interconversion cannot take place and the molecule is considered chiral.

Chapter Six and Seven—Stereochemistry

A. Stereoisomers- compounds with the same connectivity, but different arrangements of the atoms in three dimensional space.
   -Example: *cis* versus *trans* isomerism





   -The study of stereoisomers is called stereochemistry.

B. Chiral molecule- molecule that contains at least one stereocenter.
   -Chiral molecules are said to have a "handedness."
      -Example: Figure 6.2

   -Molecules without a stereocenter are generally achiral.
      -Example: Figure 6.1

   -Molecules with a plane or point of symmetry are achiral.
      -Example: Figure 6.4

   -Molecules with more than one stereocenter may be chiral or achiral

C. Enantiomers- chiral molecules that are nonsuperimposable mirror images.
   -Enantiomers contain at least one stereocenter, usually carbon.
      -Stereocenter is a atom where the interchange of two groups give a different molecule.
         -Includes asymmetric carbons (carbons with four different groups attached) and carbons involved in *cis*/*trans* isomerism.
      -Example: Figure 6.2

-Examples:

-Enantiomers have indistinguishable physical properties.

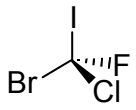
D. Cahn-Ingold-Prelog (CIP) notation for stereocenters- used to describe the configuration or arrangement of the atoms around a stereocenter.

-Assigns priorities to the groups around the stereocenter as R or S (absolute conformation) based on the configuration of the atoms.

-Assignments are largely based on atomic number.

-The higher the atomic number, the higher the priority.

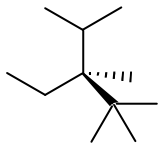
Example: I > Br > Cl > F



-For saturated carbon groups the first point of difference must be used to determine priority.

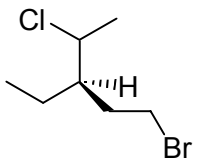
-For saturated carbons substituted only with carbon and hydrogen, at the first point of difference, a tertiary carbon will take priority over a secondary, which will take priority over a primary carbon.

-Example:



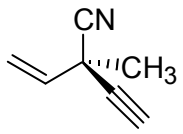
-For saturated carbons groups substituted with atoms other than carbon or hydrogen, the carbon atom containing the atom with the highest atomic number gets the higher priority.

-Example: $-CH_3CH_2I$ > $-CH_3CH_2Br$ > $-CH_3CH_2Cl$ > $-CH_3CH_2F$



-For unsaturated carbon groups, each unsaturated atom should be treated as if it were singly bonded to the other atom.

-Example:



-Once prioritization is accomplished, the lowest (fourth) priority atom is placed in the back, and an arrow is drawn from the first priority group to the second priority group and then to the third group.

-If the arrow is clockwise, the configuration is R.

-If the arrow is counterclockwise, the configuration is S.

-Example: Figure 6.5

Examples:

E. Diastereomers- stereoisomers that contain multiple chiral centers.

-Diastereomers are not mirror images (usually they are geometric isomers).

-Diastereomers have at least one stereocenter that is the same.

-Two major types:

1. Cis/Trans isomers.

-Example: *cis*-2-butene *vs trans* 2-butene

-Example: *cis*-1,2-dimethycyclopentane *vs trans*-1,2-dimethylcyclopentane

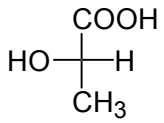
2. Molecules with more than one asymmetric center.
    -Example: 2, 3-pentanediol

    -Example: glucose

-Diastereomers, unlike enantiomers, have different physical properties.

F. Meso Compounds- achiral compounds containing more than one stereocenter and a plane or point of symmetry.
    -Example: butan-2,3-diol

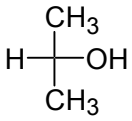
-Best studied using Fischer Projections.
    -Method for representing acyclic molecules without using wedge and dash notation.
    -Uses lines to represent bonds and bond orientation.
        -Horizontal lines are “wedges.”
        -Vertical lines are “dashes.”
        -Example:



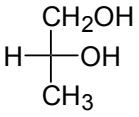
    -Rules for working with Fischer projections:
        1. Fisher projections are usually drawn with the most oxidized carbon at the top of the chain.
            -Based on IUPAC nomenclature.
        2. Fisher projections may not be flipped and must be rotated in the plane of the paper.
        3. Fisher projections may only be rotated by 180 degrees.
            -Rotation by 90 degrees changes the configuration.

-One can determine the chirality of a molecule in a Fisher projection by drawing the mirror image and rotating it 180 degrees to see if it is super imposable.

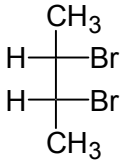
-Example: 2-propanol



-Example: 1,2-propanediol



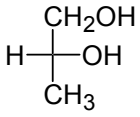
-Example: 2,3-dibromobutane



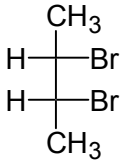
-This compound is meso (molecule that contains asymmetric centers, but is achiral due to an internal plane or point of symmetry.

-The Cahn-Inglold-Prelog convention for assigning absolute configuration may also be applied to Fischer projections.

-Example: 1,2-propanediol



-Example: 2,3-dibromobutane



G. Polarimetry- method used to characterize chiral molecules.

-Chiral molecules have the ability to rotate a plane of polarized light (are optically active).

-Enantiomers will rotate the plane of polarized light in opposite directions (equal in magnitude, but opposite in sign).

-For diastereomers the rotation is not as well defined but is still useful.

-Requires the use of a polarimeter: Figure 6.8

-Measures the rotation of polarized light upon exposure to a sample containing a chiral molecule.

-Compounds that rotate the plane of polarized light to the right (clockwise) are called dextrorotatory and are noted as *d* or +.

-Compounds that rotate the plane of polarized light to the left (counter clockwise) are called levorotatory and are noted as *l* or -.

-The degree that a compound will rotate a plane of polarized light can not be predicted and must be measured.

-The observed rotation, symbolized by α, is depended on four factors:

1. Temperature (usually 25C)
2. Wavelength of light (usually 589nm)
3. Path length of the cell used for the measurement (usually10dm)
4. Concentration (varies)

-The specific rotation, symbolized $[\alpha]_D^{25}$, is a standardization of the observed rotation for reporting purposes.

-Given by: $\alpha_{(obs)}$/ c x l

Where c = concentration

l = path length

-For mixtures of enantiomers, the enantiomeric excess (e.e.) of one enantiomers with respect to the other can be calculated.

-Given by: e.e. = (l d – l l)/ (d + l) x 100%

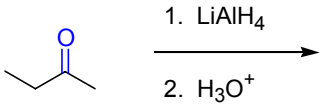
where d and l are the proportions of the two enantiomers

Sample Problem: (see handout)

-Racemic mixtures (equimolar mixtures of two enantiomers) do not rotate the plane of polarized light.

-Formed when achiral molecules undergo a reaction to produce chiral molecules.

-Example: reduction of 2-butanone



H. Conformationally mobile systems- systems such as rings, which interconvert freely, and acyclic molecules with chiral centers that are able to rotate may or may not be chiral.

-The energy of the system must be considered.

-Cyclohexane- six membered ring alkane that exhibits no ring strain.

-Cyclohexane is commonly found in carbohydrates, steroids, plant products and pesticides.

-Cyclohexane is found in three major conformations:

I. Chair conformation- conformation that is free of any ring strain.
   -Example: Figure 7.4

II. Boat conformation- conformation that exhibits no angle strain, but some torsional strain due to steric hindrance.
   -Example: Figure 7.4

III. Twist boat conformation- conformation that exhibits no angle strain, but less torsional strain than the boat conformation.
   -Example: Figure 7.4

-Conformational analysis of cyclohexane can be used to determine the most stable (most populated) conformer is chair conformer.
   -Example: Figure 7.5

-Substituents can occupy both axial and equatorial positions.

-Example: Figures 7.6 and 7.7

-The ring interconvert's at room temperature and both conformations can exist.

-Monosubsituted cyclohexanes prefer to occupy a conformation where the bulkier substituent is an equatorial position.

-Example: Figure 7.7

-Placing the bulkier substituent in an equatorial position reduces the amount of torsional strain by limiting 1,3-diaxial interactions with the hydrogen atoms in axial positions.

-Example: Figure 7.9

-While both conformers exist at room temperature, the equilibrium of the system lies toward the more stable conformer with the substituent in the equatorial position.

-The larger the substituent, the greater the 1,3-diaxial interaction.

X	**ΔG (kJ/mol)**
-F	0.8
-CN	0.8
-Cl	2.1
-Br	2.5
-OH	4.1
-COOH	5.9
$-CH_3$	7.6
$-CH_2CH_3$	7.9
$-CH(CH_3)_2$	8.8
$-C(CH_3)_3$	23

-Larger substituents are almost always found in the equatorial position.

-Smaller substitutents equilibrate between conformations where the substitutents are in the axial and equatorial positions.

-Relative distribution depends on the size of the substituent.

-Disubstituted cyclohexanes prefer to occupy a conformation where both groups are in the equatorial position (when possible).

-Example: Loudon p. 281

-If it is not possible to place both substitutents into equatorial positions, then the bulkier of the two substituents generally occupies the equatorial position.

-Example: Loudon p. 282

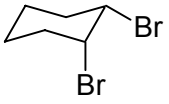
cis-1-chloro-2-methylcyclohexane *trans*-1-chloro-2-methylcyclohexane

-In order to determine chirality for ring systems such as six membered rings, one must consider the ability of the molecule to interconvert to produce its, mirror image.

-For four, five and six membered rings, this process can be simplified by treating the ring as flat and looking for planes or points of symmetry.

-If a plane or point of symmetry is present then the system is achiral.

-Example: *cis*-1,2-dibromocyclohexane *vs trans*-1,2-dibromocyclohexane

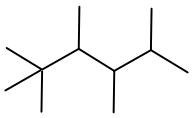


-Acyclic molecules must be taken on a case by case basis.

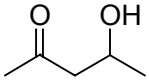
-Most linear molecules with asymmetric centers prefer one conformation over the other.

-Influence may be steric or electronic.

-Example: 2-t-butyl-3-isopropyl butane



-Example: 4-hydroxy pentan-2-one



-Some acyclic molecules without stereogenic centers are chiral.

-Conformational enantiomers- molecules that do not contain an asymmetric center, but are chiral based on the fact that they are locked into a specific conformation.

-Example: conformationally restricted alkenes (Figure 6.15)

-Allene derivatives- molecules that do not contain an asymmetric center, but are chiral based on the fact that they are locked into a conformation that puts the substituents at either end into a perpendicular conformation (extended tetrahedron).

-Example: 2,3-pentatdiene

-Atoms other than carbon may also be chiral.

-Nitrogen can be chiral or achiral depending on the groups surround the atom.

-Example: Figure 6.17

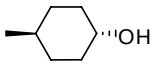
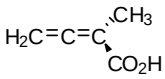
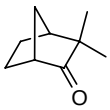
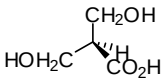
-If the groups on the nitrogen are small enough, rapid interconversion can occur making the molecule achiral.

-If the groups on the nitrogen are large enough, interconversion cannot take place and the molecule is considered chiral.

Chemistry 190
Spring 2009
Problem Set 5
Due Date: Wednesday, March 11, 2009

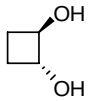
Name:_____

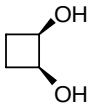
1. Determine whether the following molecules are chiral or achiral.

Molecule	Classification
	
	
	
	
	

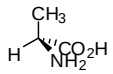
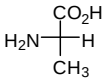
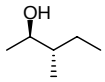
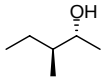
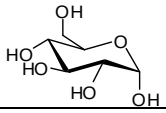
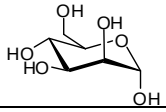
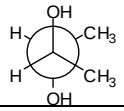
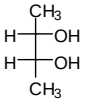
2. Circle the molecules that are chiral. For the achiral molecules, briefly explain what makes them achiral.

a. 

b. 

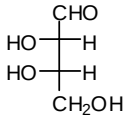
c. 

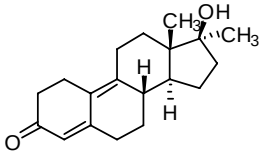
3. Determine the relationship (enantiomers, diasteromers, the same or different) between the following sets of molecules.

	Molecules		Relationship
a.		and 	
b.		and 	
c.		and 	
d.		and 	
e.		and 	

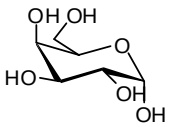
4. Label each asymmetric carbon in the following molecules as *R* or *S*.

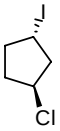
a. b. c.

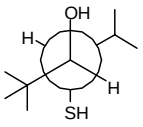




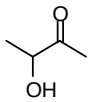
d. e. f.







5. Answer the following questions with respect to lactic acid (the general structure is given below).



a. Draw the structure of D-(-)-lactic acid. (Note: The stereocenter in D-(-)-lactic acid is *R*)

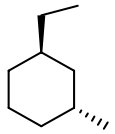
b. Draw the enantiomer of D-(-)-lactic (L-(+)-lactic acid).

c. If the optical rotation of pure D-(-)-lactic acid is -3.8°, what is the optical rotation of pure L-(+)-lactic acid?

d. Calculate the e.e. of a mixture containing 7.5g of pure L-(+)-lactic acid and 2.5g or pure D-(-)-lactic acid. Please show your work.

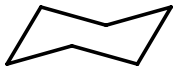
e. Bonus: How could you separate a racemic mixture of D-(-)-lactic acid and L-(+)-lactic acid? Briefly outline your experiment below.

6. Answer the following questions with respect to the structure of 1-ethyl-3-methyl cyclohexane shown below.

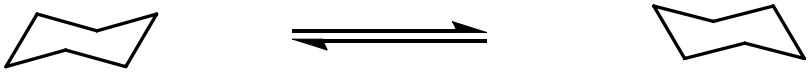


a. Are the substituents in the molecule above in the cis or trans conformation?

b. Using the skeleton below, draw the most stable chair conformation for 1-methyl-3-ethyl cyclohexane.



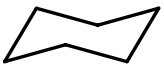
c. Using the skeletons below, show the resulting chair conformation when cyclohexane derivative you drew in "b" above interconverts (undergoes a ring flip).



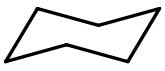
molecule drawn in "b"

7. Draw the most stable chair conformations for *trans*-1,3-difluoro cyclohexane and *trans*-1,3-dimethyl cyclohexane using the skeleton structures below. Which molecule would you predict to be more stable and why? Assign R and S configuration to any stereocenters below.

a. *trans*-1,3-difluoro cyclohexane



b. *trans*-1,3-dimethyl cyclohexane

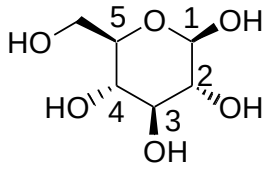


**Additional problems to consider for the exam: 6.30-6.33, 6.36, 6.42, 6.43-6.46, 6.51, 7.36, 7.37, 7.41-7.43, 7.48, 7.50, 7.54, 7.59, 7.62

Quiz Seven
Stereochemistry

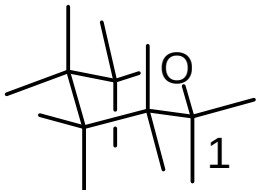
Name:_____

The following questions correspond to β-D-glucose, the structure of which is drawn below:

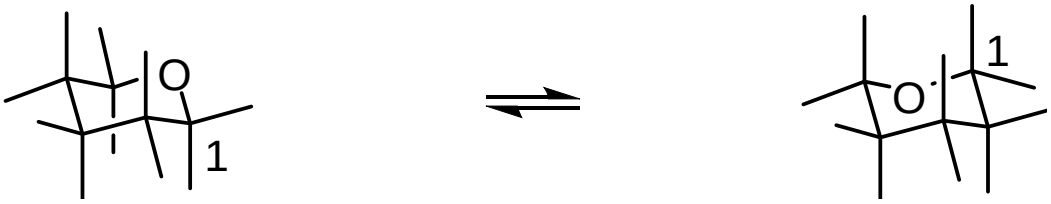


β-D-glucose

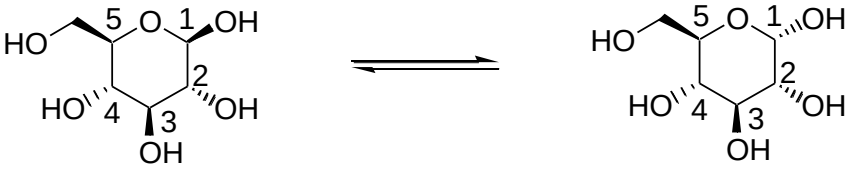
a. Convert the line-wedge structure of β-D-glucose into a chair conformation using the structure below. (2 points)



b. Show what happens to the structure of β-D-glucose when the ring flip occurs. (5 points)



c. In an aqueous solution, pure β-D-glucose is converted to an equilibrium mixture of β-D-glucose and α-D-glucose. What is the relationship between these two molecules (constitutional isomers, enantiomers, or diasteromers)? (3 points)



β-D-glucose α-D-glucose

Chemistry 190
Exam One
Spring 2008

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		5
3		5
4		10
5		10
6		10
7		5
8		5
9		20
10		10
11		10
BONUS		

There are ten pages, a periodic table, and a page of infrared spectral frequencies in this exam. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems (resonance/acid base questions) you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

Good Luck ☺

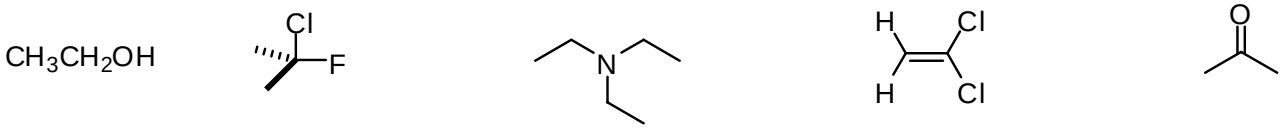
Oh yeah…..and DU = (2C-H-X+N+2)/2

"Not everything that counts can be counted, and not everything that can be counted counts."

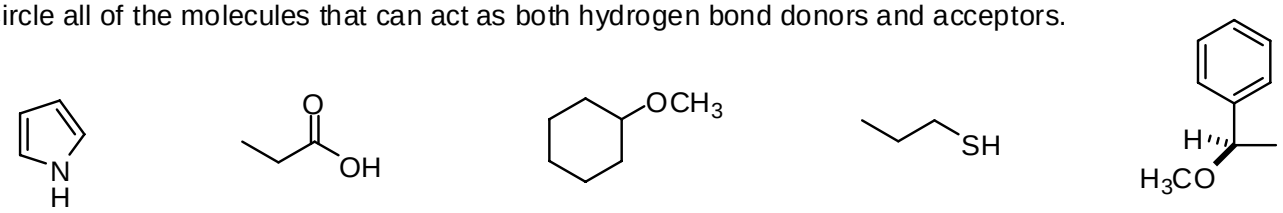
(Sign hanging in Einstein's office at Princeton)

1. In each case circle the correct answer(s). A half of a point will be deducted for each extra answer. (10 points)

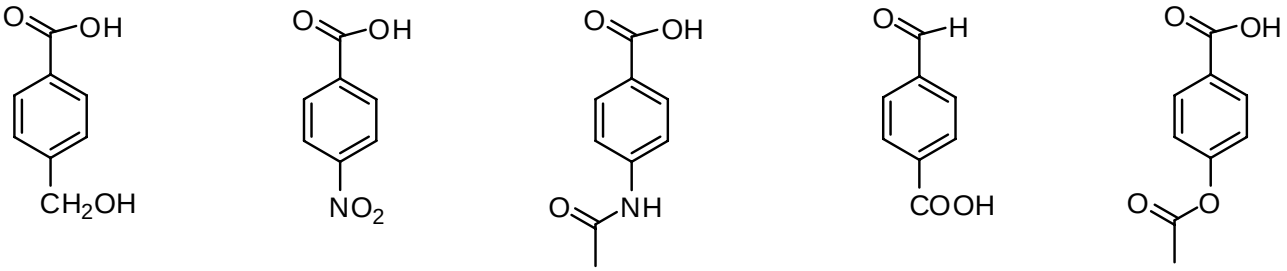
a. Circle all nonpolar molecules.



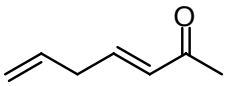
b. Circle all of the molecules that can act as both hydrogen bond donors and acceptors.

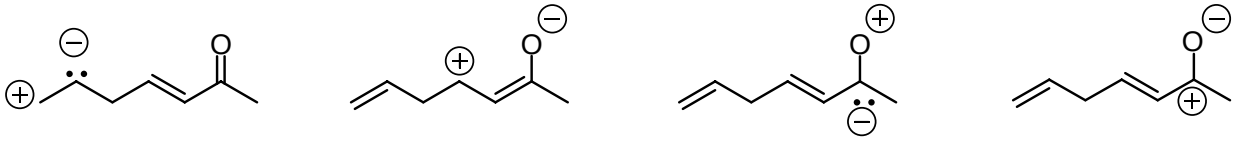


c. Circle the strongest carboxylic acid.



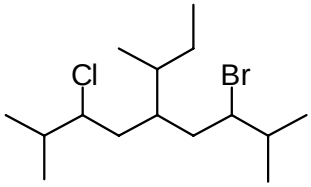
d. Circle the most unstable resonance structure for the following molecule:

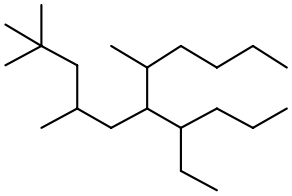




e. Which structure results from the deprotonation of 2-bromo-5-chloro-pentanol.

2. Provide complete IUPAC names for the following compounds. (5 points)

a. 

b. 

_____ _____

3. Provide structures for the following compounds based on the IUPAC name. (5 points)

a. 2-bromo-4-*sec*butyl-6-iodo heptane

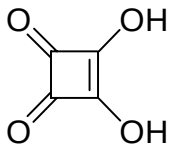
b. 1-fluoro-2-ethyl-3,5,7-trimethyl nonane

4. Write contributing resonance structures, showing formal charges when necessary, for isocyanic acid (HNCO). Indicate the most and least stable structures and give reasons for your choices. (10 points)

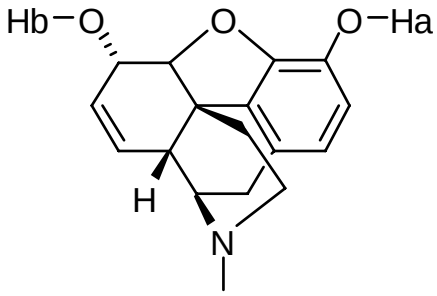
Hint: There are three resonance structures to be drawn.

5. Squaric acid (shown below) is a diprotic acid with both protons being more acidic than acetic acid. In the dianion obtained after the loss of both protons, all of the carbon-carbon bonds are the same length as well as all of the carbon-oxygen bonds. Using resonance structures with curved arrows to show electron flow and one or two brief sentences provide a reasonable response for this observation. (10 points)

Hint: You will first need to draw the dianion obtained after the loss of both protons. Overall, you will need to show a total of four resonance structures (including the dianion) in order to receive full credit for this problem.



6. The following molecule Kadian, is an analagesic used to treat moderate to severe pain. Please answer the following questions with respect to Kadian.



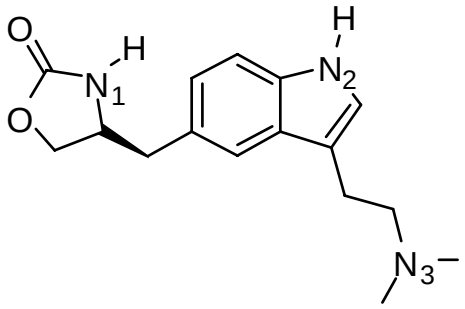
a. Which proton, a (H_a) or b (H_b) is more acidic? Use resonance structures to support your explanation. (5 points)

Note: You do not have to draw out the entire molecule to answer this question. You may draw out only the relevant portions of the molecule.

b. What major stretching frequencies would you observe were you to take an infrared spectrum of Kadian? Please use the table below to organize your work. Note that there may be more spaces in the table then necessary. (5 points)

Functional Group	Approximate Stretching Frequency

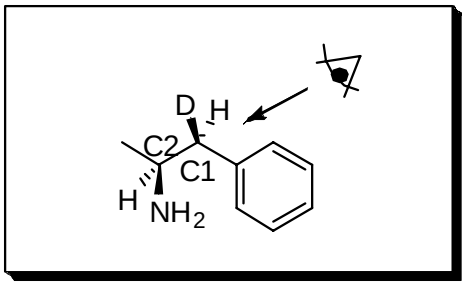
7. The following molecule, Zomig, is a serotonin receptor antagonist used to treat severe migraines. Please answer the following question with respect to Zomig.

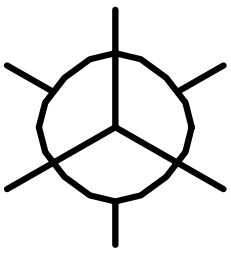


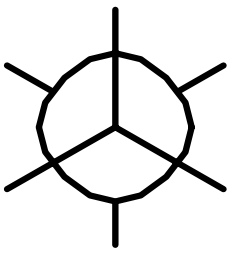
Rank the three labeled nitrogen atoms, N_1, N_2 or N_3 in order of basicity and provide a brief explanation for your reasoning. (5 points)

Rank	Nitrogen	Explanation
1 (most basic)		
2		
3 (least basic)		

8. Using the skeletal structure below, draw the most and least stable staggered Newman projections for a deuterated form Adderall, an amphetamine used to treat ADHD (shown in the box below) looking down the C1-C2 bond axis. Briefly discuss your answer. (5 points)



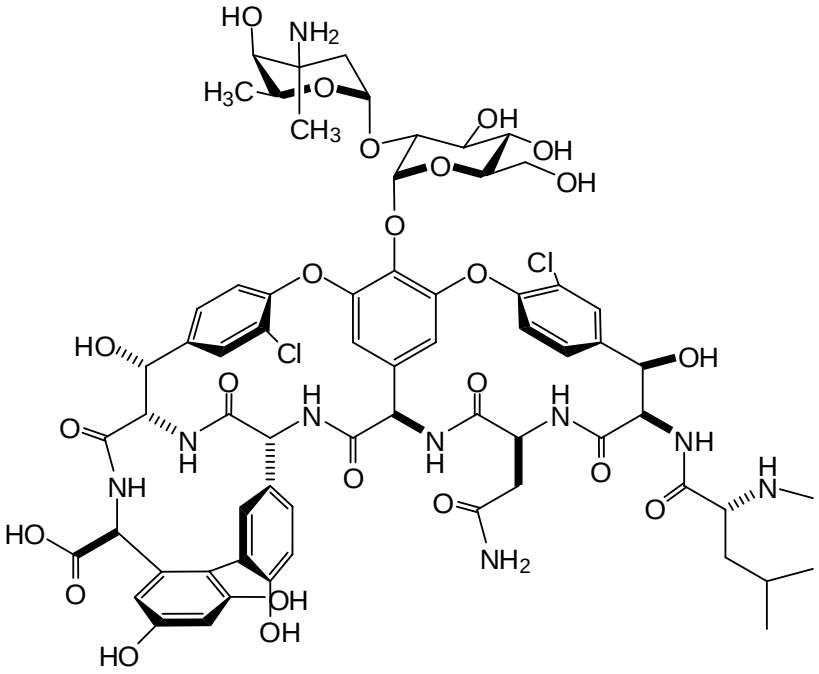




Most Stable

Least Stable

9. The following structure is vancomycin, a glycopeptide antibiotic. Answer the following questions about this molecule in the space provided. (20 points)



a. How many alcohol groups does this molecule contain?_____

b. How many carboxylic acid groups does this molecule contain?_____

c. How many ether groups does this molecule contain?_____

d. How many amine groups does this molecule contain?_____

e. How many amide groups does this molecule contain?_____

f. How many carboxylic acid groups does this molecule contain?_____

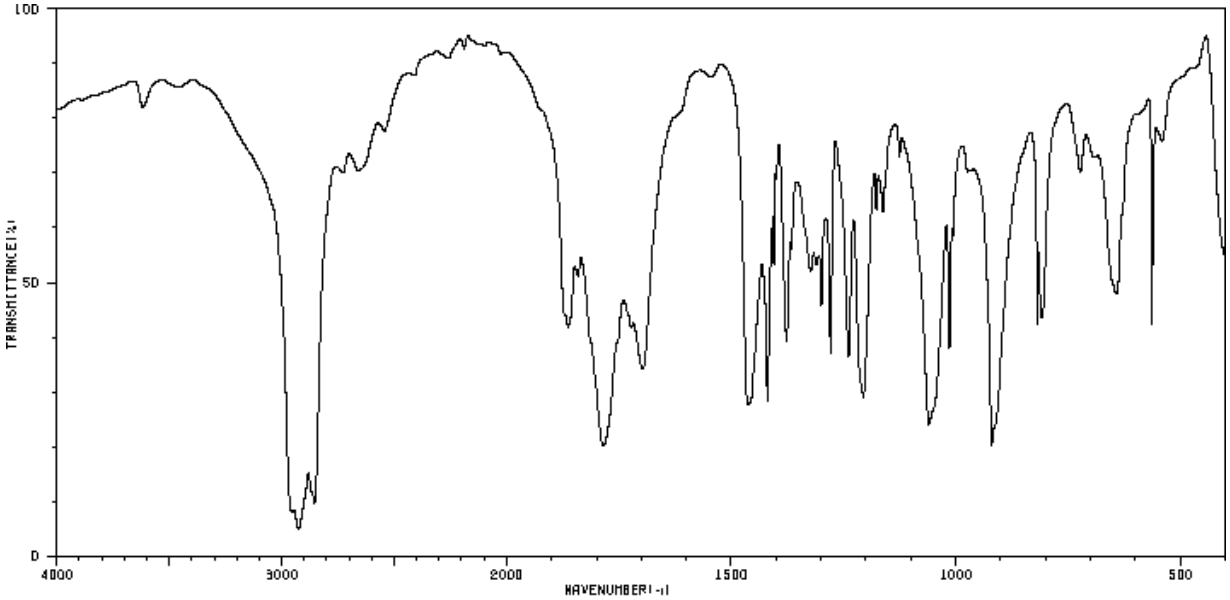
g. How many nitrile groups does this molecule contain?_____

h. How many aromatic rings does this molecule contain?_____

i. How many pi bonds does this molecule contain?_____

j. How many sp^2 hybridized atoms does this molecule contain?_____

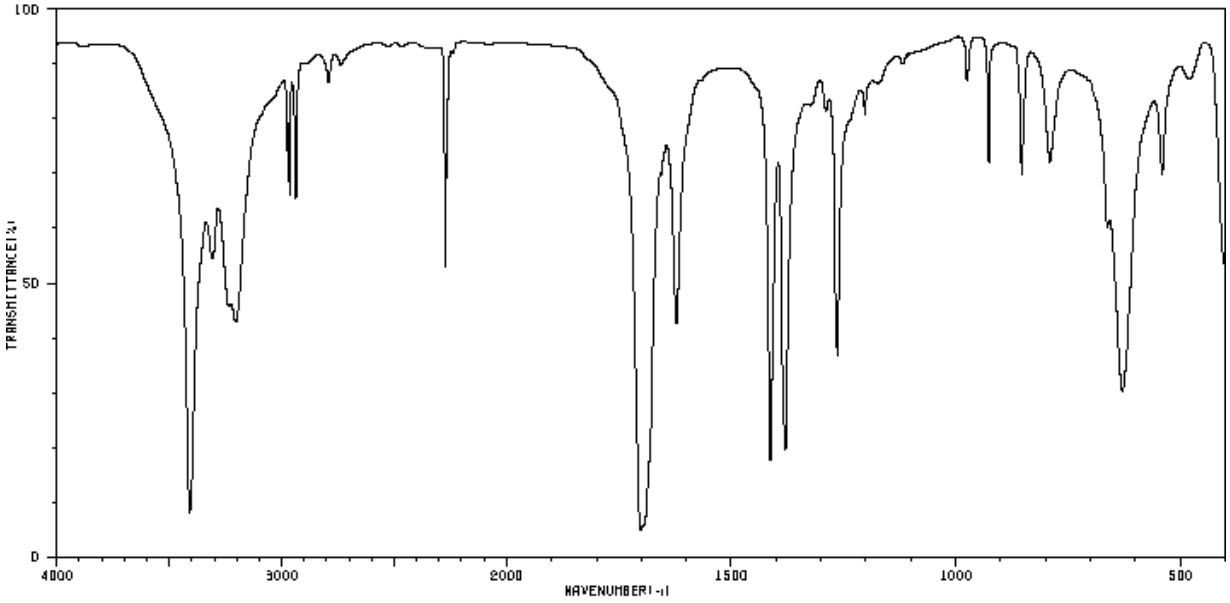
10. Provide a plausible structure for a molecule with a molecular formula of $C_4H_4O_3$ and the following infrared spectrum. Please place your final answer in the box provided. In order to receive full credit you must show all of you work and justify each major functional group on the molecule you have drawn. (10 points)



2954	7	1786	19	1366	53	1178	60	819	41		
2925	4	1697	33	1323	50	1163	60	809	42		
2854	9	1461	26	1311	50	1125	70	723	56		
2728	68	1420	26	1300	44	1061	29	643	46		
2660	68	1406	50	1279	35	1014	36	584	41		
1862	39	1399	66	1239	35	1007	55	552	74		
1841	49	1377	37	1206	27	920	19	541	72		

Answer:

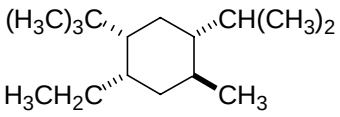
11. Provide a plausible structure for a molecule with a molecular formula of $C_3H_4N_2O$ and the following infrared spectrum. Please place your final answer in the box provided. In order to receive full credit you must show all of you work and justify each major functional group on the molecule you have drawn. (10 points)

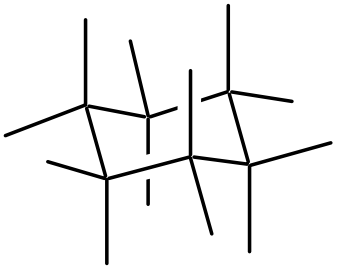


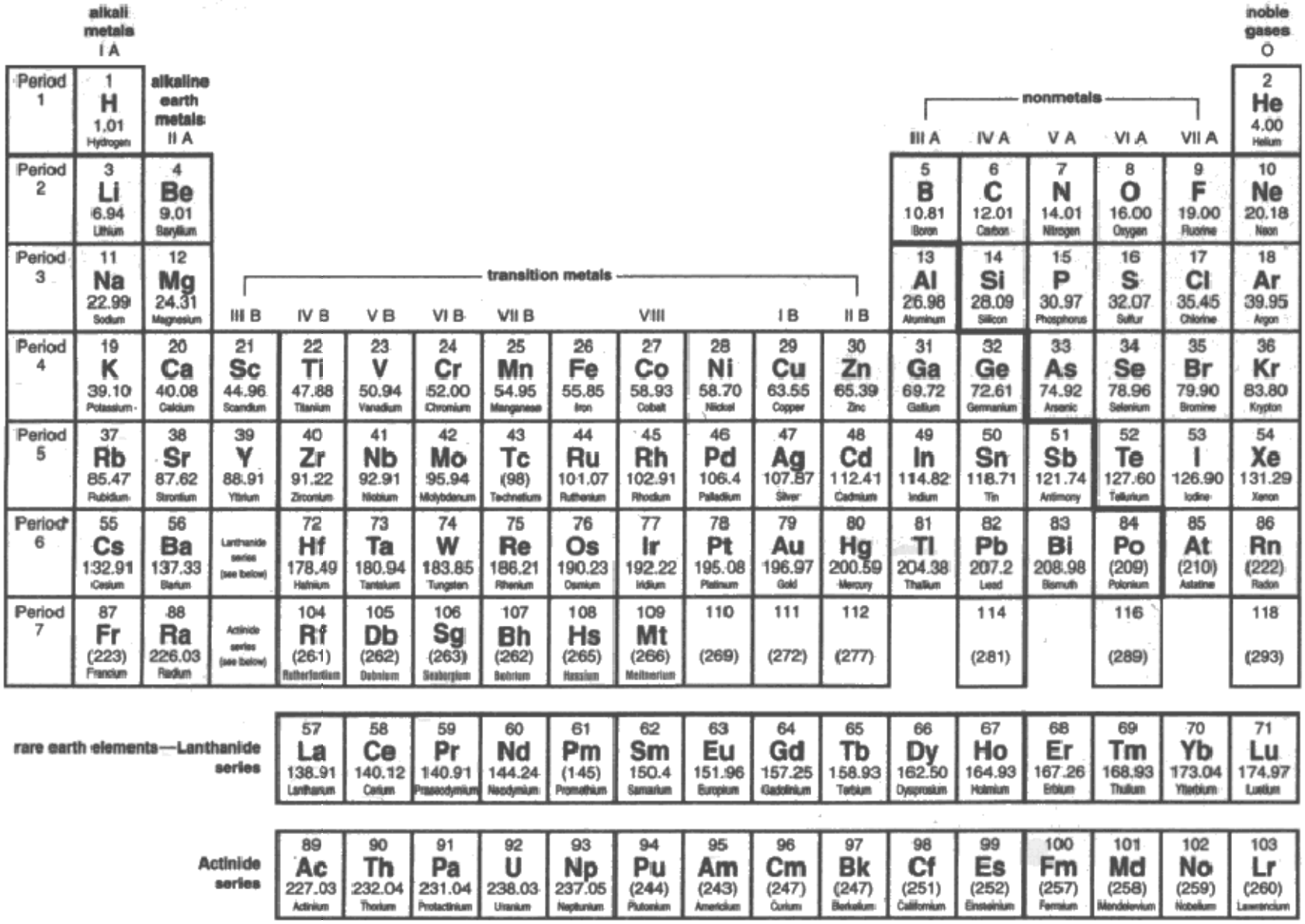
3408	7	2736	86	1264	36	629	29
3308	52	2273	52	1203	77	541	68
3234	44	1701	4	976	84	480	84
3209	41	1622	41	927	70		
2966	64	1413	17	853	68		
2937	62	1380	18	791	70		
2791	84	1289	79	663	68		

Answer:

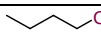
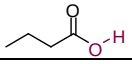
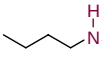
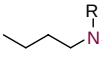
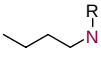
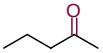
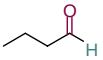
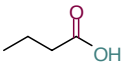
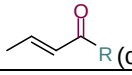
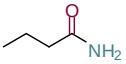
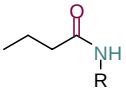
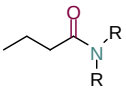
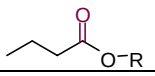
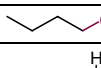
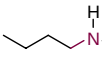
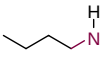
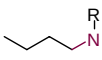
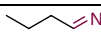
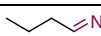
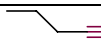
BONUS (3 points): Using the skeletal structure below, draw the most stable chair conformation of the following molecule. Be sure to draw in all of the hydrogen's about the ring.






alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
III A
IV A
V A
VI A
VII A
noble gases 0
III B
IV B
V B
VI B
VII B
VIII
I B
II B
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

Infrared Stretching Frequencies

Category	Type of Bond	$\tilde{\nu}$ (cm^{-1})	Notes
carbon-carbon		1200	
	 (isolated)	1640-1680	
	 (conjugated)	1620-1640	
	 (aromatic)	~1600	
	 (terminal)	2100-2200	
	 (internal)	2100-2200	may be weak or absent
carbon-hydrogen	 (sp^3)	2800-3000	
	 (sp^2)	3000-3100	
	 (sp)	3300	
oxygen-hydrogen	 (alcohol)	3300	broad
		3000	broad
nitrogen-hydrogen	 (1°)	3300	two sharp spikes
	 (2°)	3300	one sharp spike
	 (3°)	3300	may be weak or absent
carbon-oxygen		1710	may appear ~1785 if strained
		1710	will also see peaks at 2700 and 2800 for the aldehyde C-H stretching
		1710	will also see peaks between 2500-3500 for the O-H stretching
	 (conjugated)	1685-1690	will also see peaks for the R group.
	 (1°)	1640-1680	will also see two spikes around 3300 for the NH stretching
	 (2°)	1640-1680	will also see one spike around 3300 for the NH stretching
	 (3°)	1640-1680	
		~1735	
		~1200	
carbon-nitrogen	 (1°)	1200	will also see two spikes around 3300 for the NH stretching
	 (2°)	1200	will also see one spike around 3300 for the NH stretching
	 (3°)	1200	
		1660	will also see one spike around 3300 for the NH stretching
		1660	
		>2200	

Chemistry 190
Exam Four
Spring 2008

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		10
3		5
4		10
5		40
6		10
7		5
8		10
BONUS		

There are fourteen pages, including a periodic table, two pages of NMR shifts and a blank piece of scratch paper in this exam. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems (resonance/acid base questions) you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

Good Luck ☺

Oh yeah…..and DU = (2C-H-X+N+2)/2

On a cosmic scale, our life is insignificant, yet this brief period when we appear in the world is the time in which all meaningful questions arise.

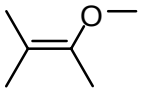
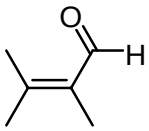
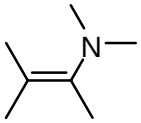
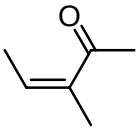
-**Paul Ricoeur**

1. In each case circle the correct answer(s). A half of a point will be deducted for each extra answer. (10 points)

a. Circle the reagent(s) that could be used to perform an oxidative cleavage on an alkene.

$KMnO_4/NaOH\ \Delta$ MCPBA in CH_2Cl_2 $O_3/(CH_2)S$ OsO_4/H_2O_2

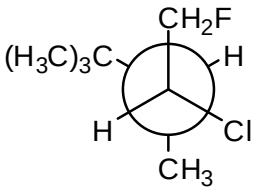
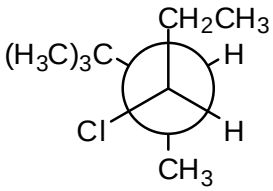
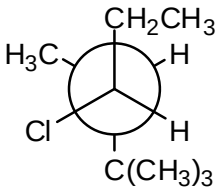
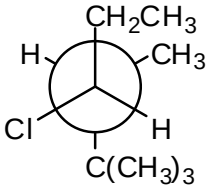
b. Circle the most reactive alkene in an electrophilc addition reaction.

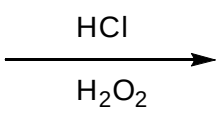
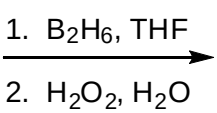
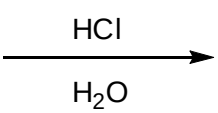
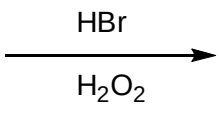
c. Which of the following conditions would give rise to a cation intermediate in the course of a reaction.

OsO_4/H_2O_2 HBr/H_2O_2 MCPBA/NaOH $Hg(OAc)_2/H_2O$

d. Cirlce the conformation(s) below that will give rise to an E product in an E_2 elimination reaction.

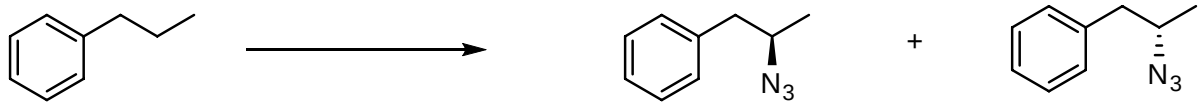





f. Circle the condition(s) that will give anti-Markovnikov addition to an alkene.

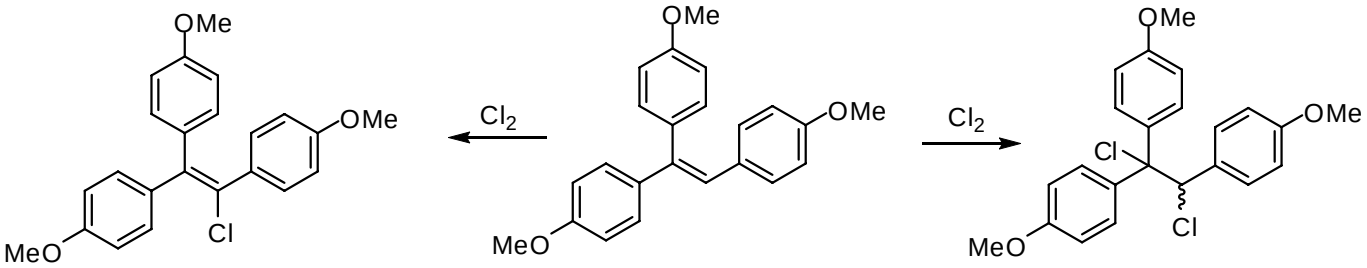





2. Chose one of the following problems and provide a complete synthesis. (10 points)

a. 

b. 

3. Chlorination of the triarylethylene derivative below leads to a chloroalkene A rather than the expected dichloroalkane B. Suggest a mechanism and an explanation for this phenomenon. (5 points)



product observed

A

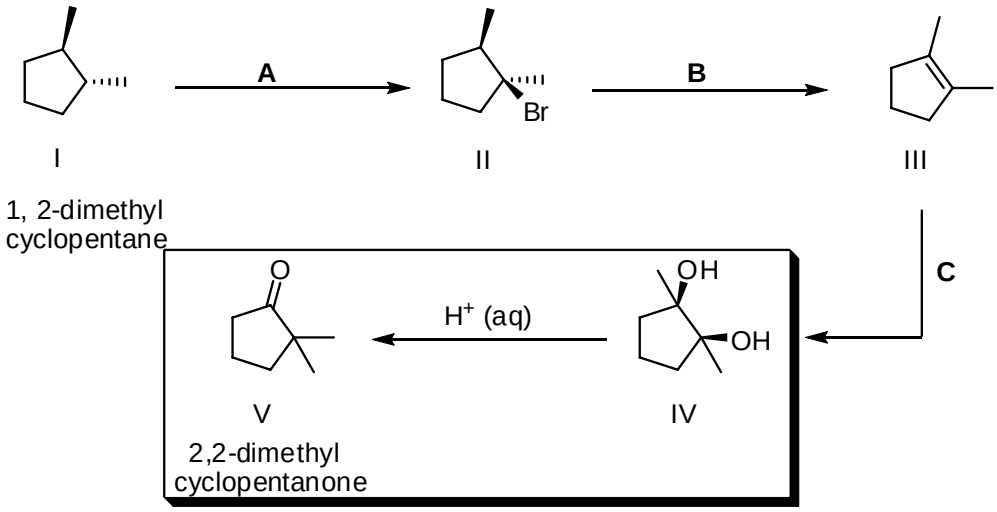
triarylethylene

product not observed

B

4. The following questions pertain to the reaction sequence below. (10 points

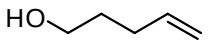
a. Complete the reagents needed in steps A through C of the sequence.



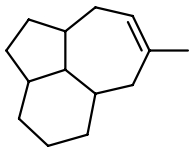
Step	Reagents
A	
B	
C	

b. Provide a complete mechanism for the conversion of IV to V (boxed in above).

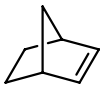
5. In each case below, give the major product(s) of the reaction. In most cases there will be only one product, but in each case there should be no more than two products. If there is no reaction write N.R. Be sure to show stereochemistry where appropriate. (40 points)

a. 

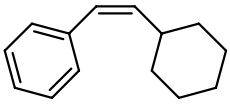
$\xrightarrow[2.\ NaBH_4]{1.\ Hg(OAc)_2}$

b. 

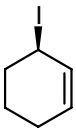
$\xrightarrow[heat]{KMnO_4}$

c. 

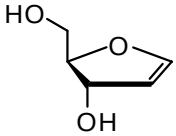
$\xrightarrow[NaOH]{CHBr_3}$

d. 

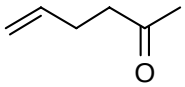
$\xrightarrow[CCl_4]{ICl}$

e. 

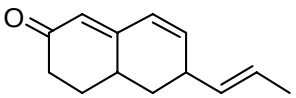
$\xrightarrow[2.\ NaOH,\ H_2O]{1.\ OsO_4,\ H_2O_2}$

f. 

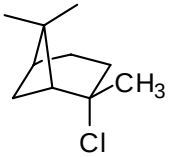
$\xrightarrow[2.\ (CH_3)_2S]{1.\ O_3}$

h. 

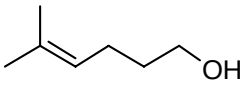
1. NBS, CCl_4
2. LDA

i. 

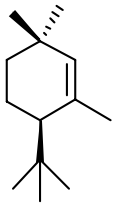
$KMnO_4$
dilute, 0 C

j. 

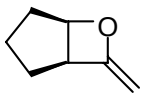
1. KOtBu
   HOtBU
2. HBr
   H_2O_2

k. 

Br_2
CCl_4

l. 

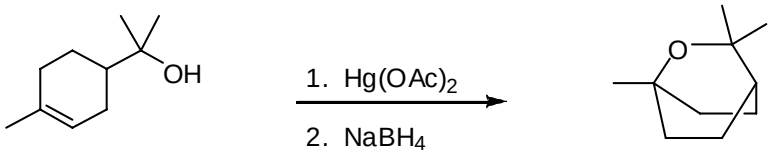
H_2
Pt

m. 

1. MCPBA
2. NaH (source of H-)
3. H_2O workup

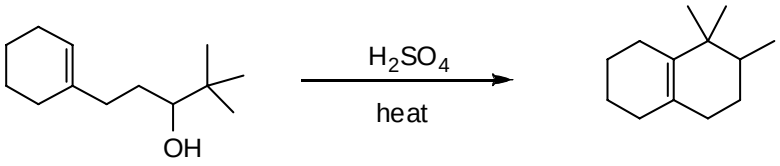
6. Choose one of the following reactions, and write a complete and detailed mechanism. Note: You do not need to show the reduction step. (10 points)

a.

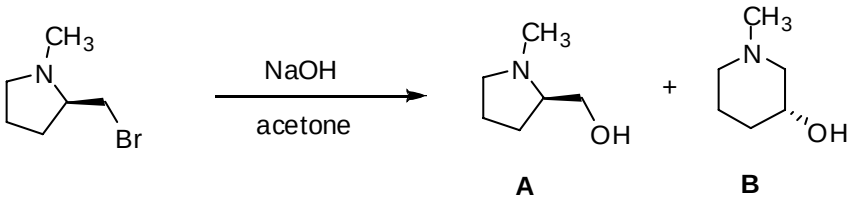


Note: You do not need to show the reduction step of this reaction.

b.



7. When 2-bromomethyl-1-methylpyrrolidine is dissolved in acetone and treated with sodium hydroxide, 2-hydroxymethyl-1-methylpyrrolidine (A) and molecules B and C are produced as a result. (5 points)



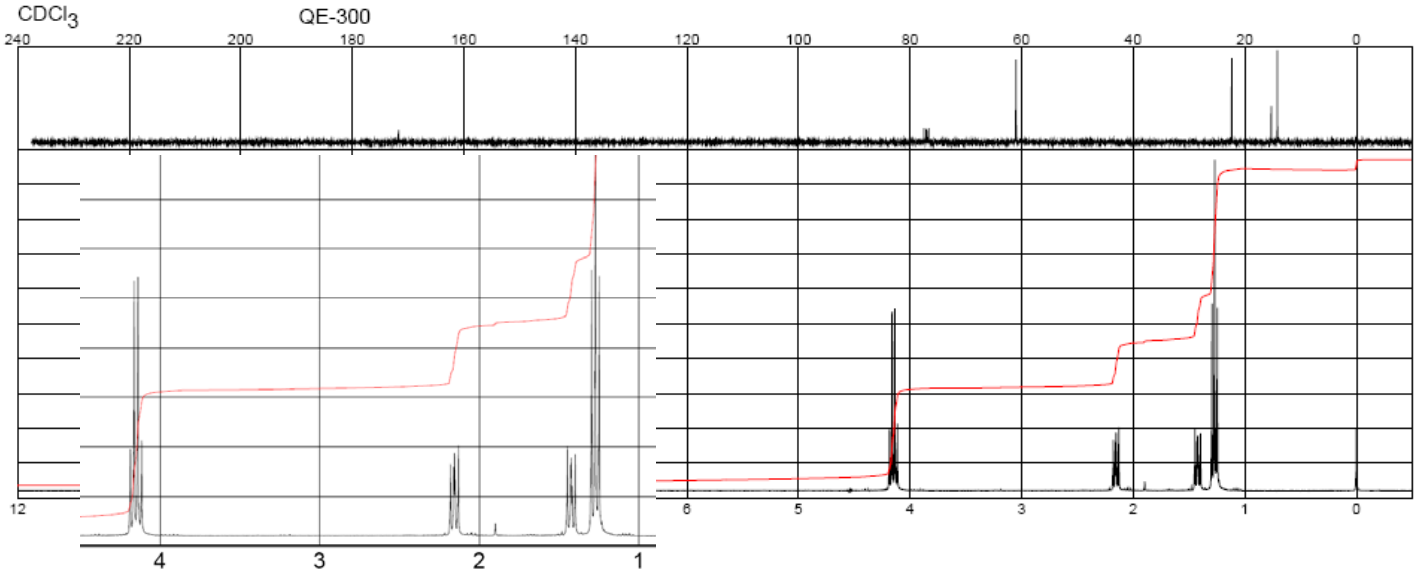
A **B**

a. Write a complete mechanism to show the formation of products B and C.

b. What is the relationship between 2-hydroxymethyl-1-methylpyrrolidine (A) and product B?

8. Professor Snyder conducted a Simmons Smith reaction on unknown molecule (starting material). Upon workup and isolation, she obtained a mass spectrum and learned that the molecular formula of her product ($C_9H_{14}O_4$). She then obtained both ^1H and ^{13}C NMR spectra. The spectra she obtained are shown below:

^1H signals: 1.3ppm (t, 6H), 1.4ppm 9 (dd, 2H), 2.2ppm (dd, 2H), 4.2ppm (q, 4H)
^{13}C signals: 173ppm, 62ppm, 23ppm, 17ppm, 15ppm



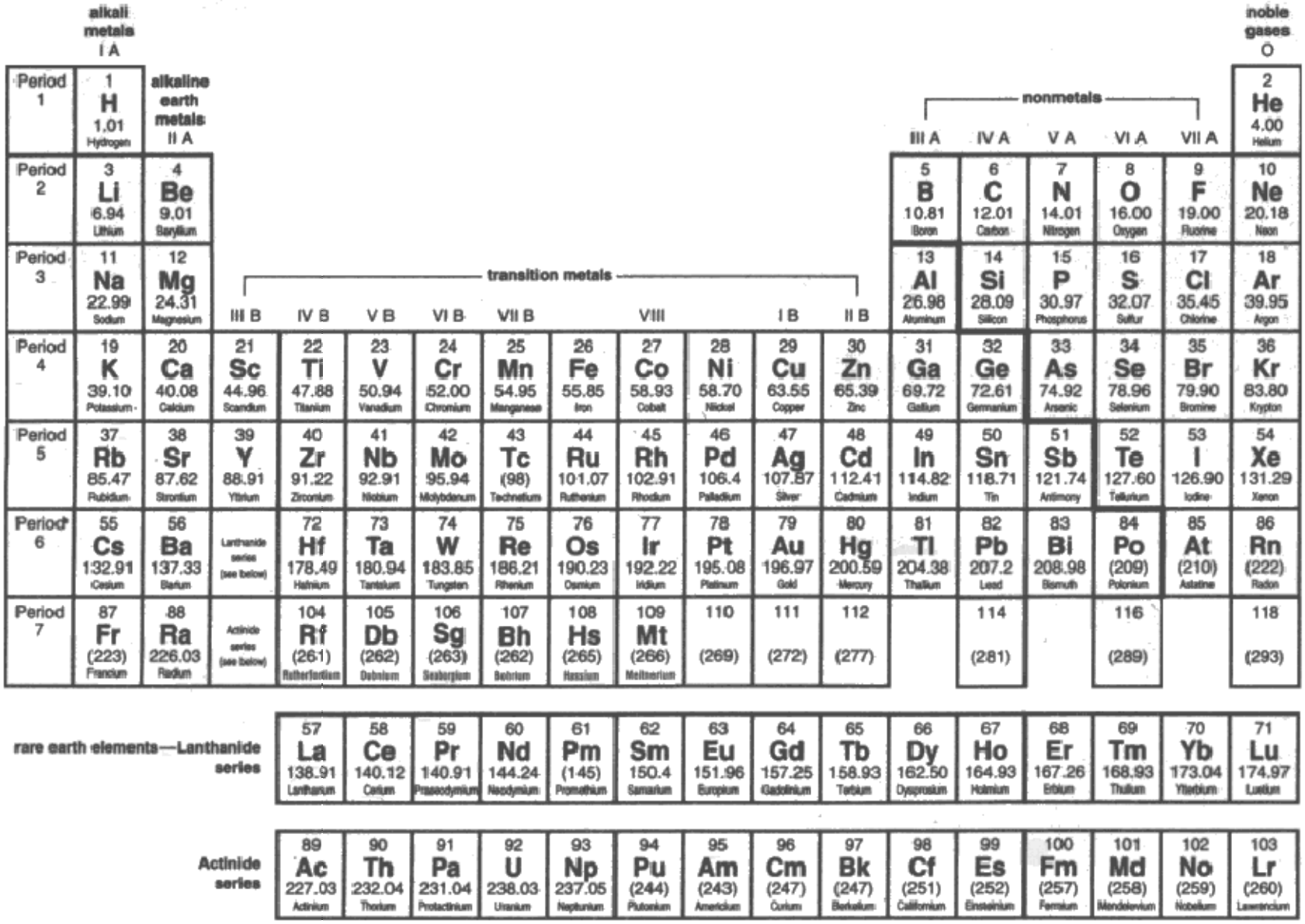
What is the structure of the product Professor Snyder obtained from the Simmons-Smith reaction? To answer this problem you should give each spectral component a complete treatment by showing the following work:

a. For the IR, labeling the major functional groups on the spectra.
b. For the ^1H NMR, label each of the protons on your final structure as H_a, H_b, H_c etc and identify those protons on the spectrum.
c. For the ^{13}C NMR, label each of the carbons on your final structure as C_1, C_2, C_3 etc and identify those carbons on the spectrum.

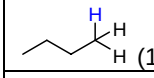
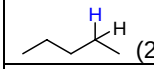
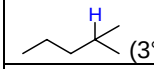
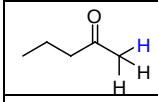
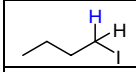
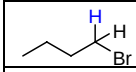
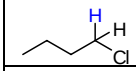
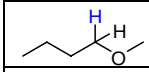
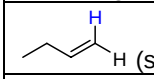
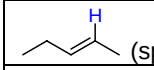
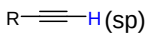
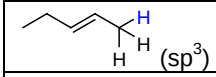
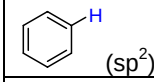
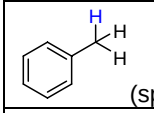
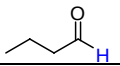
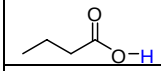
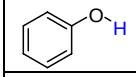
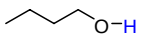
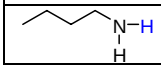
If no work is shown, no credit will be given. Place your final answer in the box provided. (10 points)

Answer:

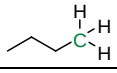
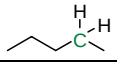
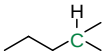
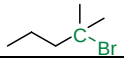
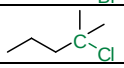
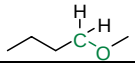
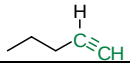
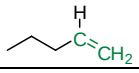
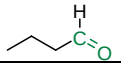
Bonus (5 points): What is the identity of the unknown starting material?


alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
III A
IV A
V A
VI A
VII A
noble gases 0
III B
IV B
V B
VI B
VII B
VIII
I B
II B
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

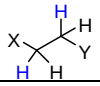
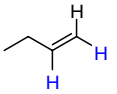
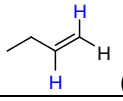
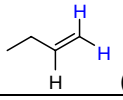
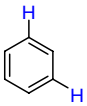
Characteristic ^{1}H NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0.8-1.0	
 (2°)	1.2-1.4	
 (3°)	1.4-1.7	
	2.1	
	3.1-3.3	
	3.4-3.6	
	3.6-3.8	
	3.3-4.0	
 (sp^2)	4.6-5.0	
 (sp^2)	5.2-5.7	
 (sp)	2.5	
 (sp^3)	1.7	
 (sp^2)	6.0-9.5	
 (sp^3)	2.2-2.5	
	9-10	
	10-13	Can be broad and may exchange
	4.5-7.7	Can be broad and may exchange
	0.5-6.0	Can be broad and may exchange
	1.0-5.0	Can be broad and may exchange

Characteristic ^{13}C NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0-35	
 (2°)	0-35	
 (3°)	0-35	
	30-70	
	30-70	
	85-90	
	75-100	
	100-150	
 (sp^2 aromatic)	115-150	
	165-210	May take a while to relax (signals may be weak)

Coupling Constant Values

Type of coupling	J-value (Hz)	Notes
	2-12 (7)	The actual J value depends on the dihedral angle and the nature of the R groups
 (diasterotopic)	12-15	
 (cis)	7-12	
 (trans)	12-15	
 (geminal)	0.5-3	
 (allylic)	3-11	The actual J value depends on the dihedral angle
 (ortho)	6-9	
 (meta)	1-3	

Chemistry 190
Exam One
Spring 2009

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		3
3		2
4		4
5		15
6		16
7		10
8		10
9		10
10		10
11		10
BONUS		

There are eleven pages, a periodic table, a page of infrared spectral frequencies, and a blank piece of scrap paper in this exam (fourteen pages total). Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems (resonance/acid base questions) you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

Good Luck ☺

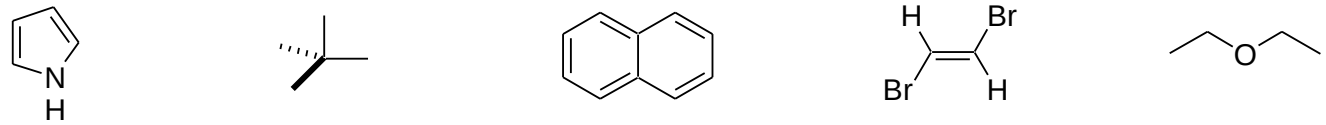
Oh yeah…..and DU = (2C-H-X+N+2)/2

Twenty years from now you will be more disappointed by the things you didn't do than by the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails. Explore. Dream. Discover.

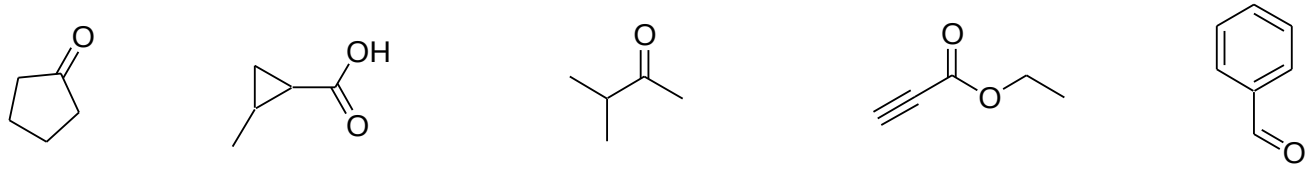
Mark Twain

1. In each case circle the correct answer(s). A half of a point will be deducted for each extra answer. (10 points)

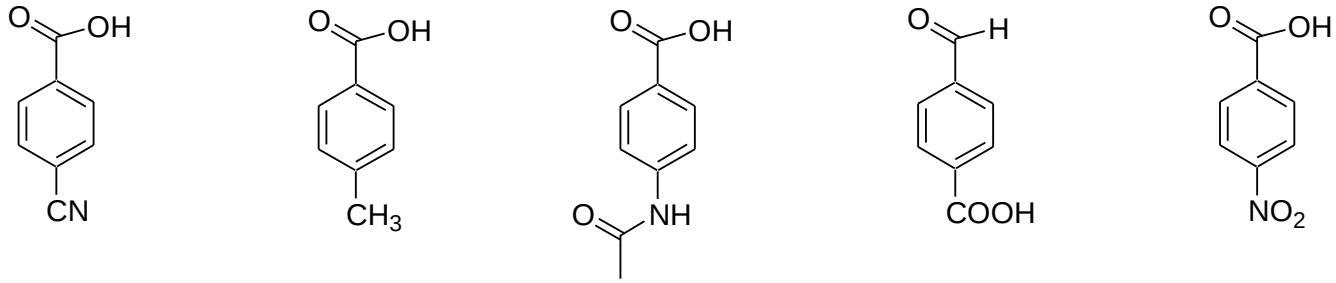
a. Circle all polar molecules.



b. Circle all of the molecules that contain a ketone.

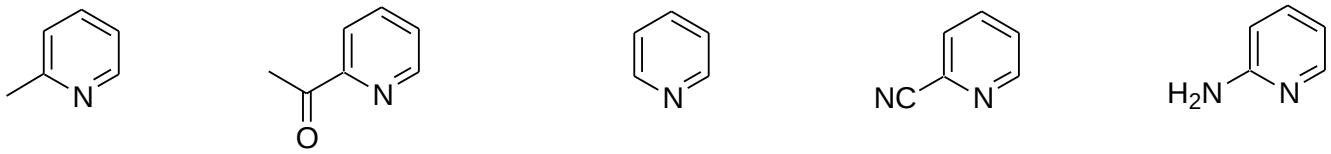


c. Circle the weakest carboxylic acid.

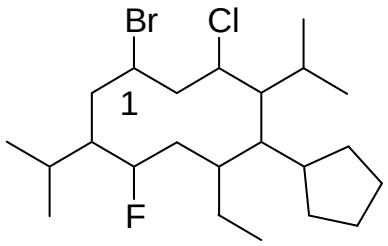


d. Circle the most stable resonance structure for an aliphatic azide (CH_3-N_3):

e. Circle the most basic pyridine derivative.



2. Provide complete IUPAC names for the following compound. Notice that the first carbon of the ring has been numbered for you. (3 points)



_____

3. Provide a structure for the following compound based on the IUPAC name provided. (2 points)

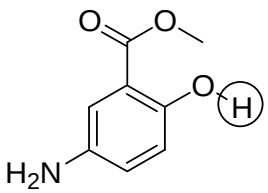
1,1-dibromo-2,3-diethylcylopropane

4. Write contributing resonance structures, showing formal charges when necessary, for methyl isothiocyanate (CH_3NCS). Indicate the most and least stable structures and give reasons for your choices. (4 points)

Hint: There are three resonance structures to be drawn.

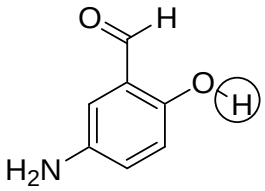
5. For the following sets of molecules below, circle the most acidic molecule based on the indicated proton, and provide a brief explanation for your answer in the space provided. (15 points)

a.

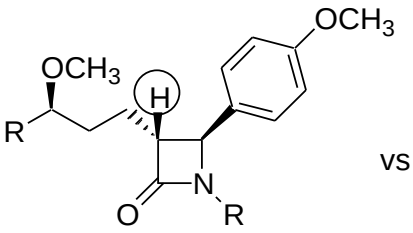


Asacol

vs

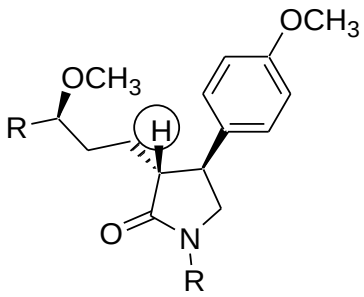


b.

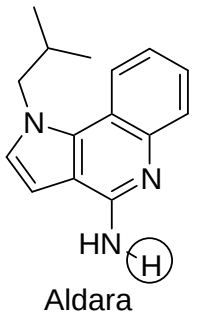


Zetia (OCH_3)

vs

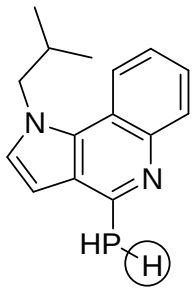


c.

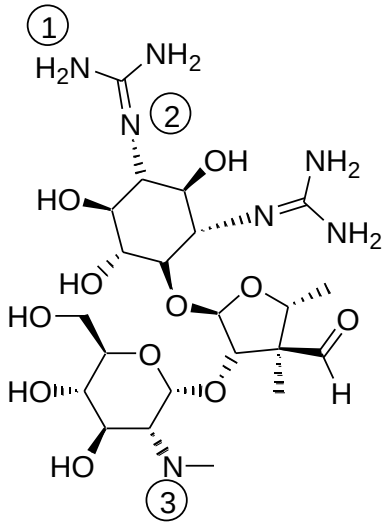


Aldara

vs



6. The following molecule, streptomycin, is an antibiotic used to treat tuberculosis. Please answer the following question with respect to streptomycin. (16 points)



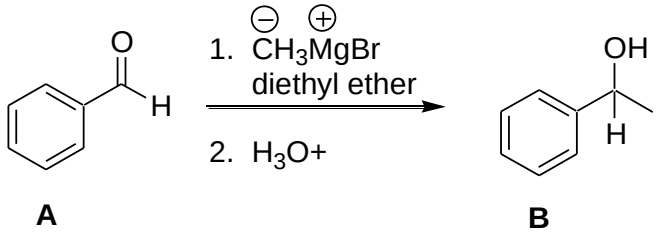
Rank the three labeled nitrogen atoms, N_1, N_2 or N_3 in order of basicity and provide a brief explanation for your reasoning. (6 points)

Rank	Nitrogen	Explanation
1 (most basic)		
2		
3 (least basic)		

b. What major stretching frequencies other than C-C-H sp^3 stretching would you expect to see were you able to take an infrared spectrum of streptomycin? Please use the table below to organize your work. Note that there may be more spaces in the table then necessary. (10 points)

Functional Group	Approximate Stretching Frequency

7. A Grignard reaction, named after Victor Grignard, a Nobel Prize laureate, can be used to perform substitution reactions at the face of a carbonyl as shown below:



A **B**

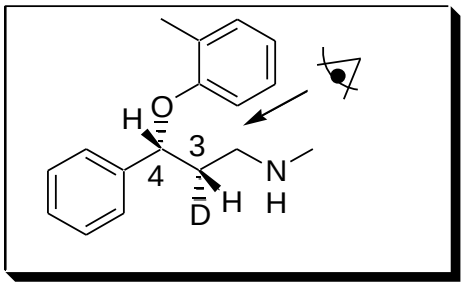
In the reaction above, the aldehyde (A) is serving as an electrophile and methyl magnesium bromide is serving as a nucleophile. The intermediate produced after step one of the reaction above is an anion.

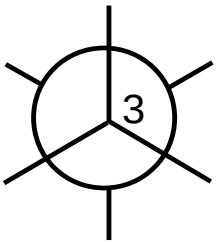
a. Use curved arrow notation to show how methyl magnesium bromide (CH_3MgBr) adds to the carbonyl to produce the intermediate anion (not shown). (3 points)

b. What is the purpose of adding acid in the second step? (2 points)

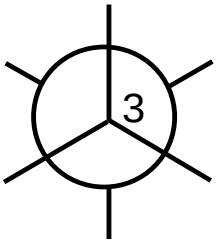
c. How would you use infrared spectroscopy to determine if your reaction had occurred? What stretching frequencies that occur in the starting material would be missing, and what new stretching frequencies would be present? (5 points)

8. Using the skeletal structure below, draw the most and least stable staggered Newman projections for a deuterated form Strattera, a selective norepinephrine uptake inhibitor used to treat ADHD (shown in the box below) looking down the C3-C4 bond axis. Briefly discuss your answer. (10 points)



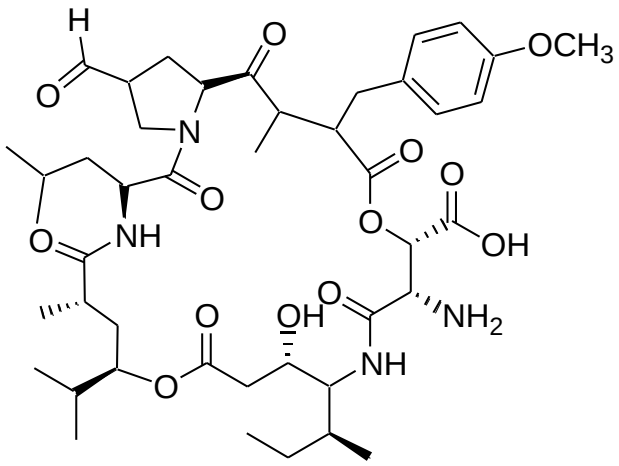


Most Stable



Least Stable

9. The following structure is a derivative of didemins, a macrolide antibiotic. Answer the following questions about this molecule in the space provided. (10 points)



a. How many alcohol groups does this molecule contain?_____

b. How many carboxylic acid groups does this molecule contain?_____

c. How many ether groups does this molecule contain?_____

d. How many amine groups does this molecule contain?_____

e. How many amide groups does this molecule contain?_____

f. How many carboxylic acid groups does this molecule contain?_____

g. How many nitrile groups does this molecule contain?_____

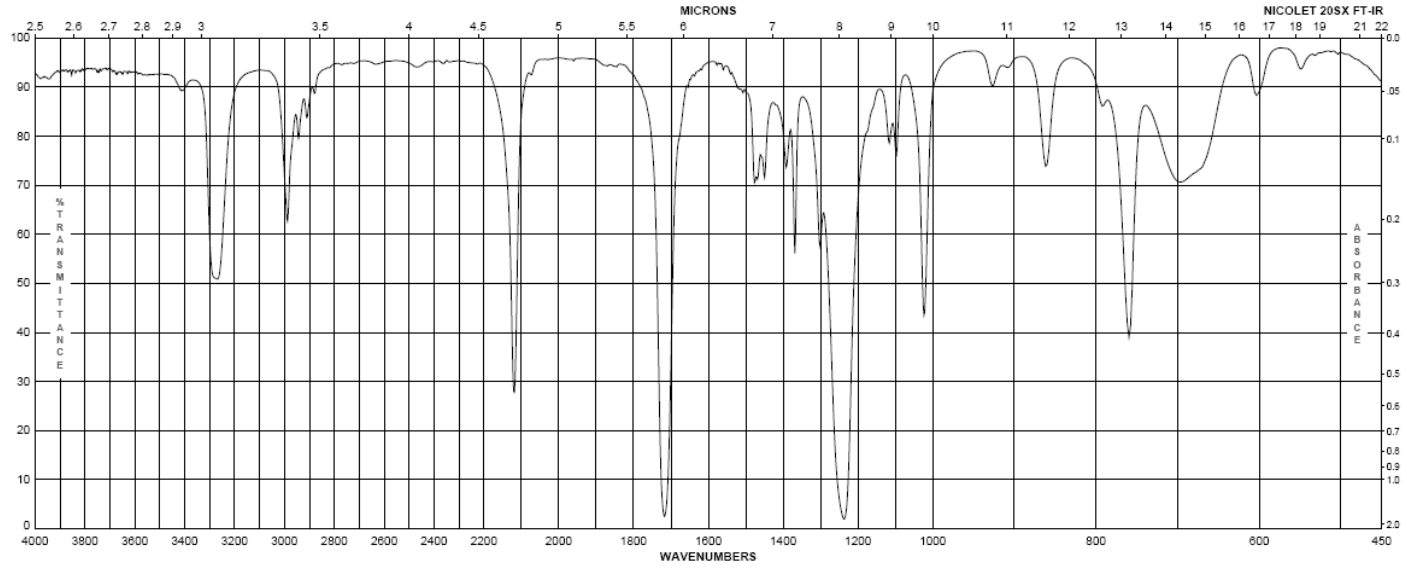
h. How many aromatic rings does this molecule contain?_____

i. How many pi bonds does this molecule contain?_____

j. How many sp^2 hybridized atoms does this molecule contain?_____

10. Provide a plausible structure for a molecule with a molecular formula of $C_5H_6O_2$ and the following infrared spectrum. Please place your final answer in the box provided. In order to receive full credit you must show all of you work and justify each major functional group on the molecule you have drawn. (10 points)

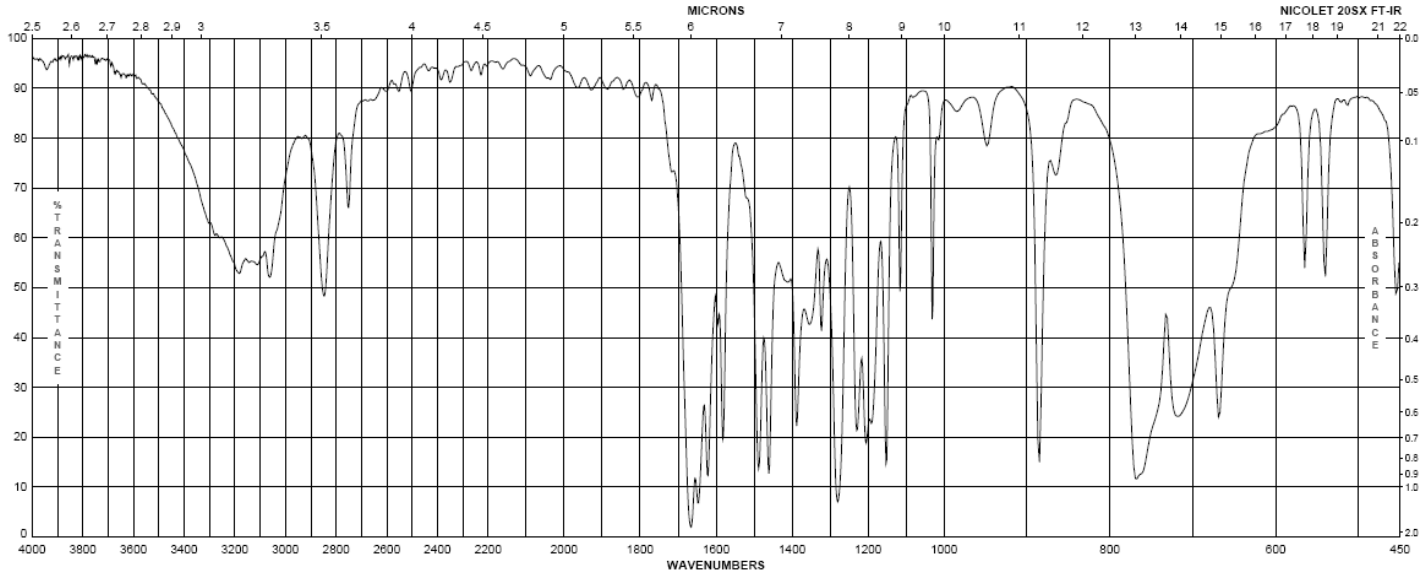
Major Stretches: 3280, 2990, 2120, 1730, 1220



Answer:

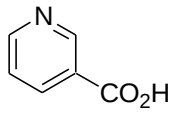
11. Provide a plausible structure for a molecule with a molecular formula of $C_7H_6O_2$ and the following infrared spectrum. Please place your final answer in the box provided. In order to receive full credit you must show all of you work and justify each major functional group on the molecule you have drawn. (10 points)

Major Stretches: 3200, 2850, 2750, 1650

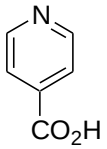


Answer:

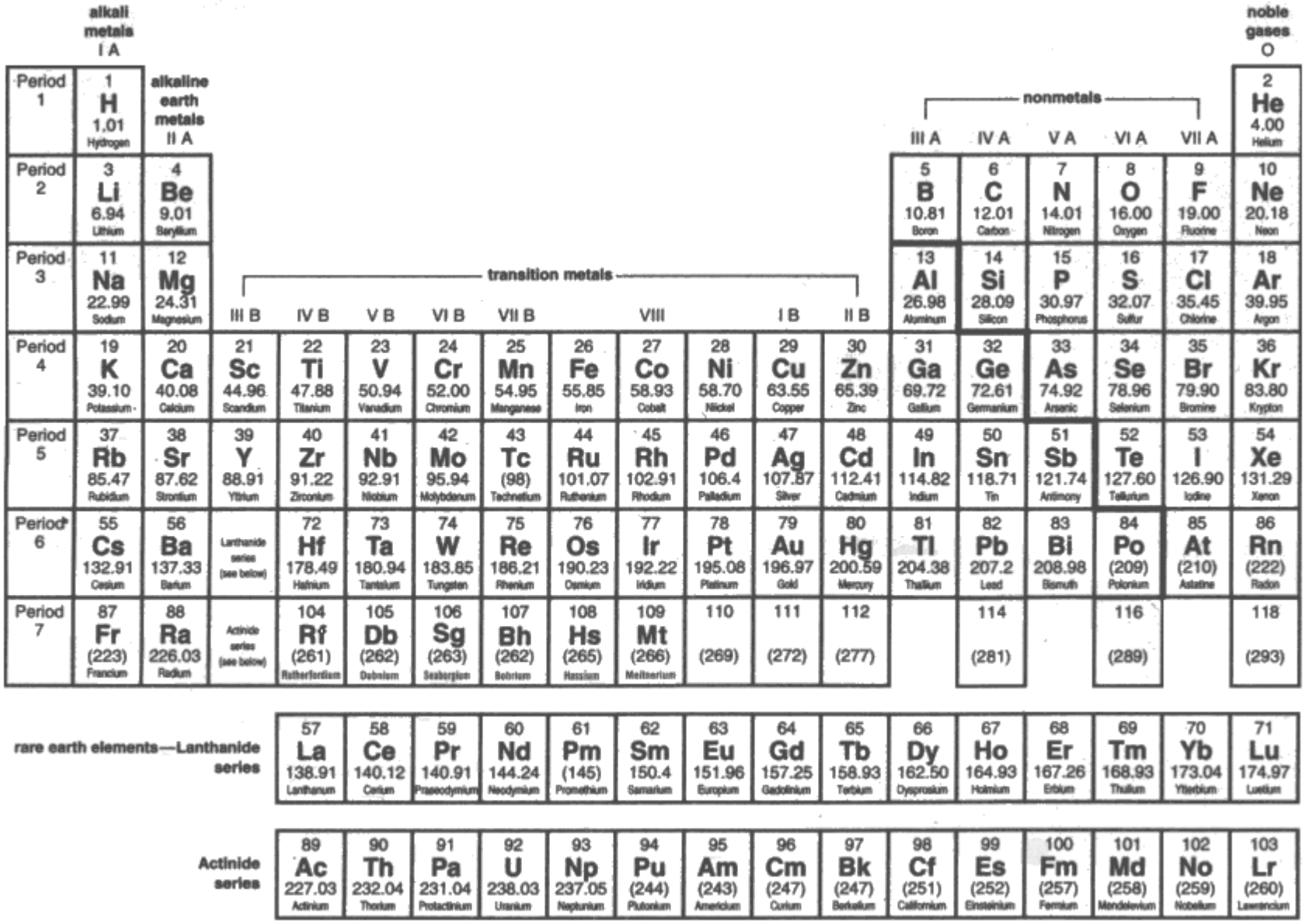
BONUS (5 points): Which molecule would you predict would be more basic, Niaspan (A) a cholesterol lowering compound, or its derivative B. You must use resonance to support your answer.



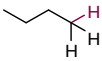
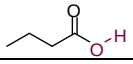
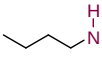
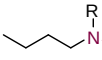
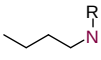
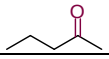
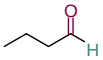
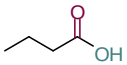
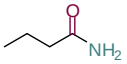
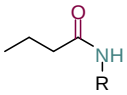
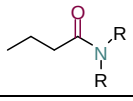
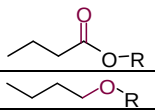
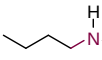
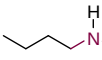
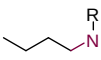
Niaspan (A)



B


alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
noble gases O
III A
IV A
V A
VI A
VII A
III B
IV B
V B
VI B
VII B
VIII
I B
II B
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

Infrared Stretching Frequencies

Category	Type of Bond	$\tilde{\nu}$ (cm^{-1})	Notes
carbon-carbon		1200	
	 (isolated)	1640-1680	
	 (conjugated)	1620-1640	
	 (aromatic)	~1600	
	 (terminal)	2100-2200	
	 (internal)	2100-2200	may be weak or absent
carbon-hydrogen	 (sp^3)	2800-3000	
	 (sp^2)	3000-3100	
	 (sp)	3300	
oxygen-hydrogen	 (alcohol)	3300	broad
		3000	broad
nitrogen-hydrogen	 (1°)	3300	two sharp spikes
	 (2°)	3300	one sharp spike
	 (3°)	3300	may be weak or absent
carbon-oxygen		1710	may appear ~1785 if strained
		1710	will also see peaks at 2700 and 2800 for the aldehyde C-H stretching
		1710	will also see peaks between 2500-3500 for the O-H stretching
	 (conjugated)	1685-1690	will also see peaks for the R group.
	 (1°)	1640-1680	will also see two spikes around 3300 for the NH stretching
	 (2°)	1640-1680	will also see one spike around 3300 for the NH stretching
	 (3°)	1640-1680	
		~1735	
		~1200	
carbon-nitrogen	 (1°)	1200	will also see two spikes around 3300 for the NH stretching
	 (2°)	1200	will also see one spike around 3300 for the NH stretching
	 (3°)	1200	
		1660	will also see one spike around 3300 for the NH stretching
		1660	
		>2200	

Chemistry 190
Exam Four
Spring 2009

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		10
3		10
4		40
5		10
6		10
7		10
BONUS		

There are twelve pages, including a periodic table and two pages of NMR shifts in this exam. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems (resonance/acid base questions) you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

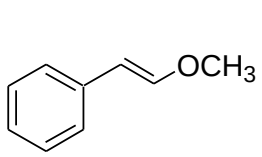
Good Luck ☺

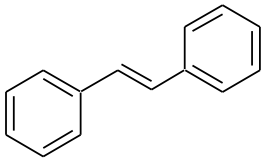
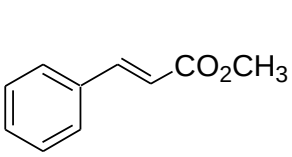
Oh yeah…..and DU = (2C-H-X+N+2)/2

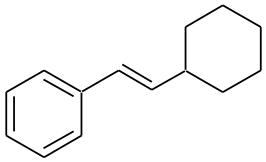
If your actions inspire others to dream more,
learn more, do more and become more,
you are a leader.
-John Quincy Adams

1. In each case circle the correct answer(s). A half of a point will be deducted for each extra answer. (10 points)

a. Circle the reagent that would react the fastest in an electrophilic addition reaction.



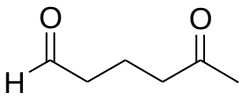



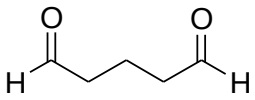


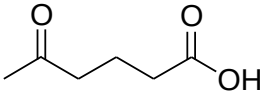
b. Circle the conditions that give rise to syn products [exclusively].

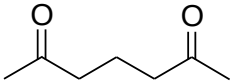
OsO_4/H_2O_2 BH_3, THF/H_2O_2, OH- mCPBA/NaOH Br_2/H_2O

c. Cirlce the product(s) formed when 1-methyl cylclopentene reacts with hot, concentrated potassium permanganate.





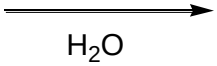


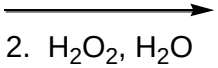


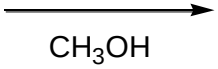
d. Circle the conditions that can be used to form a cyclopropane ring.

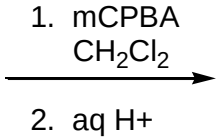
$CH_2I_2/Zn(Cu)$ mCPBA/CH_2Cl_2 $CHBr_3$/NaOH CH_2N_2/CH_2Cl_2

f. Circle the condition(s) that will give rise to Markovnikov products when reacted with an alkene.

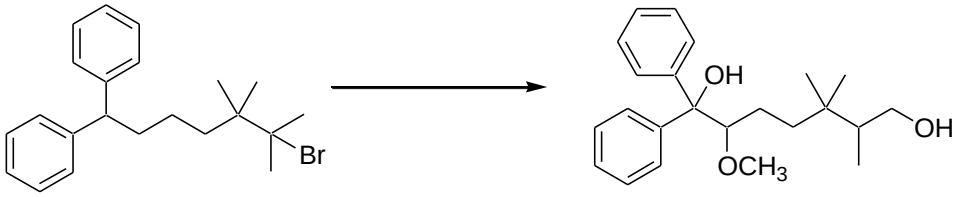






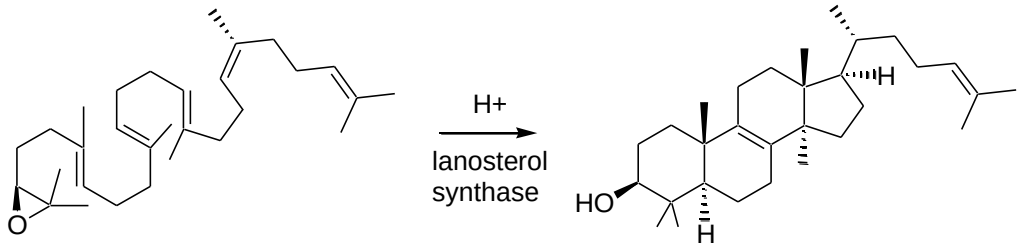


2. Provide a synthesis for the following molecule. This synthesis can be accomplished in six steps, but you can use as many steps as you like. (10 points)



3. The enzyme lanosterol synthase plays a key role in the formation of lanosterol (**B**), an important intermediate in the production of cholesterol from squalene oxide (**A**) as shown below. The enzyme uses a number of acidic amino acid residues in the active site in order to affect the reaction. Provide a plausible mechanism for the transformation of squalene oxide to lanosterol. (10 points)

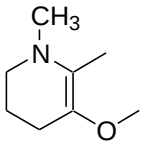
NOTE: There will be a point in the mechanism where a number of hydride and methyl shifts occur simultaneously.



squalene oxide
A

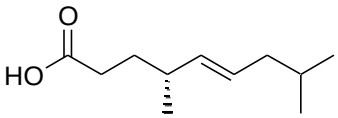
lanosterol
B

4. In each case below, give the major product(s) of the reaction. In most cases there will be only one product, but in each case there should be no more than two products. If there is no reaction write N.R. Be sure to show stereochemistry where appropriate. (40 points)

a. 

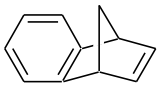
1. O_3

2. $(CH_3)_2S$

b. 

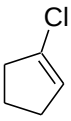
1. $Hg(OAc)_2$

2. $NaBH_4$

c. 

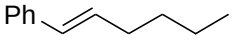
CH_2I_2

$Zn(Cu)$

d. 

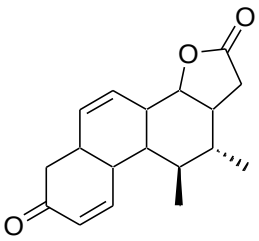
1. mCPBA, CH_2Cl_2

2. aq NaOH

e. 

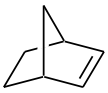
Br_2

H_2O

f. 

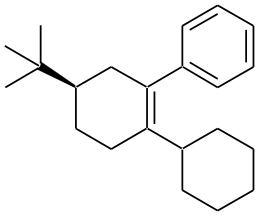
1. OsO_4

2. H_2O_2

h. 

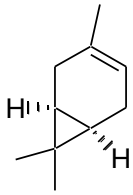
$KMnO_4$ (conc.)

heat

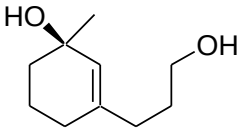
i. 

1. BH_3 THF

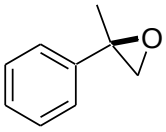
2. H_2O_2, OH-

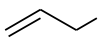
j. 

H_2, Pt

k. 

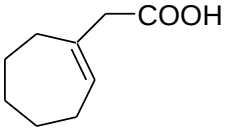
aq H+

l. 

1.  MgBr

Et_2O

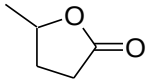
2. aq H+

m. 

1. I_2, CH_2Cl_2

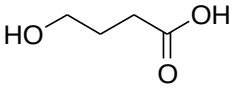
2. $NaHCO_3$

5. Gamma-valerolactone (**A**) is a cyclic ester used in the flavor and fragrance industry and provides the flavor and fragrance of coffee, chocolate and honey. Gamma-valerolactone has also been subject to abuse due to the fact that it is an analog of GHB (**B**), the date rape drug.



gamma valero lactone

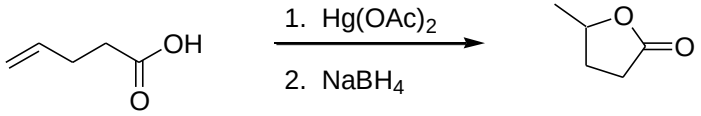
A



GHB

B

a. Gamma-valerolactone can be produced from readily available pent-4-enoic acid (**C**) by oxymercuration-demercuration as shown below. Provide a complete mechanism for this reaction. (5 points)



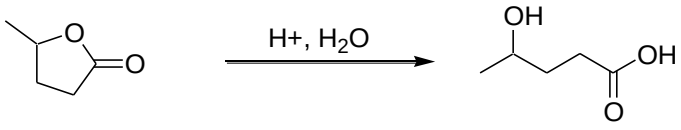
pent-4-enoic acid

C

gamma valero lactone

A

b. Under acidic conditions (such those found in the stomach) gamma-valerolactone can be converted to 4-hydroxy-pentanoic acid (**D**), an analog of GHB. Provide a complete mechanism for this reaction. (5 points)



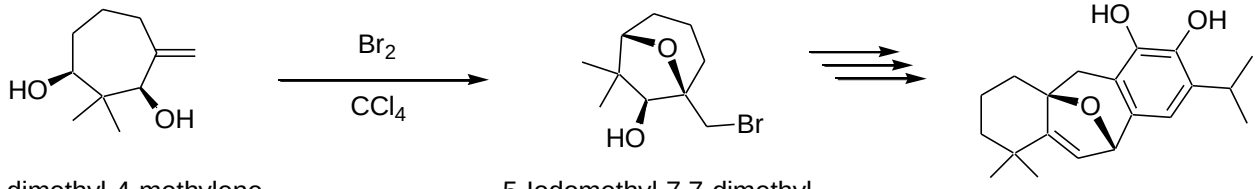
gamma valero lactone

A

4-hydroxy-pentanoic acid

D

6. Bridged bicyclic furan systems are important intermediates in the synthesis of analogs of natural products such as salviasperanol. One way to access these systems is to treat a diol, such as diol **A**, with bromine in the presence of an inert solvent to produce the corresponding bicyclic system. Using curved arrow notation, provide a complete mechanism for this reaction. (10 points)



2,2-dimethyl-4-methylene-cycloheptane-1,3-diol

A

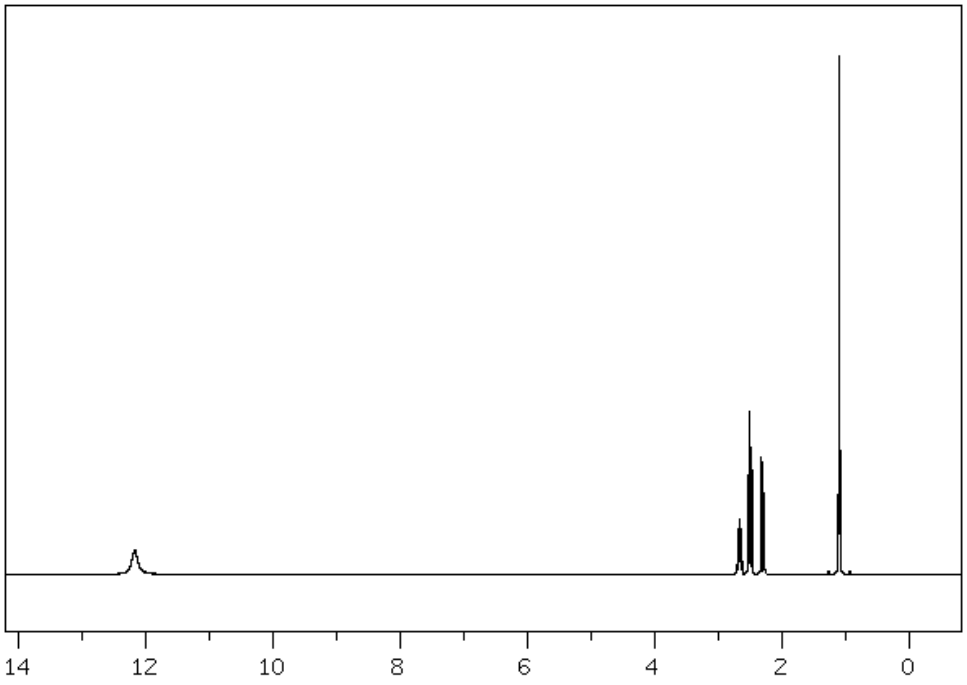
5-Iodomethyl-7,7-dimethyl-8-oxa-bicyclo[3.2.1]octan-6-ol

B

Salviasperanol

7. Professor Snyder conducts an ozonolysis on an unknown molecule (starting material). Upon workup and isolation, she obtained a mass spectrum and learned that molecular formula of her product ($C_5H_8O_4$). She then obtained both ^1H and ^{13}C NMR spectra. The spectra she obtained are shown below:

^1H signals: 1.1ppm (d, 3H), 2.3ppm, (dd, 1H), 2.5ppm (dd, 1H), 2.7ppm (m, 1H), 12.2 (bs, 2H)
^{13}C signals: 176.3ppm, 172.9ppm, 37.3ppm, 35.2ppm, 16.8ppm



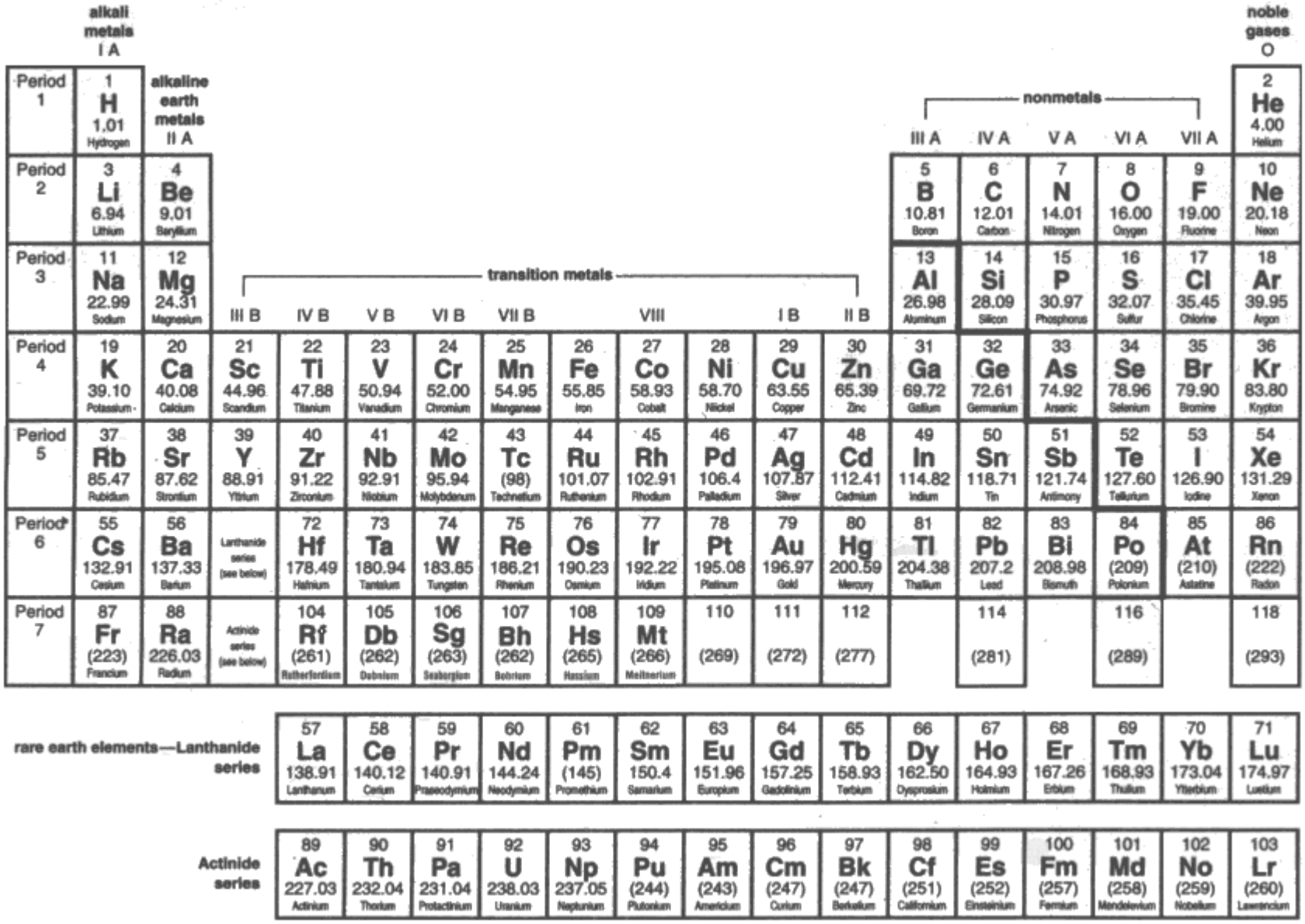
What is the structure of the product Professor Snyder obtained from the ozonolysis reaction? To answer this problem you should give each spectral component a complete treatment by showing the following work:

a. For the ^1H NMR, label each of the protons on your final structure as H_a, H_b, H_c etc and identify those protons on the spectrum.
b. For the ^{13}C NMR, label each of the carbons on your final structure as C_1, C_2, C_3 etc and identify those carbons on the spectrum.

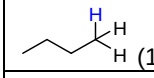
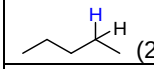
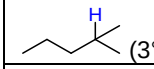
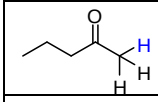
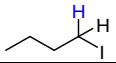
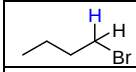
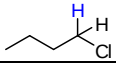
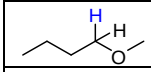
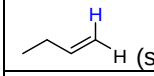
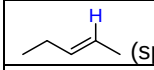
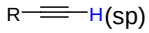
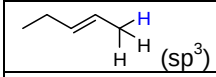
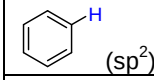
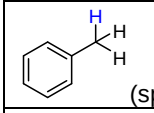
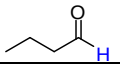
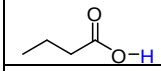
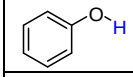
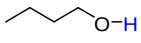
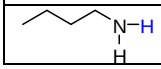
If no work is shown, no credit will be given. Place your final answer in the box provided. (10 points)

Answer:

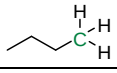
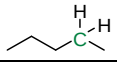
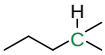
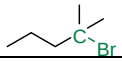
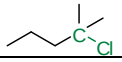
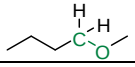
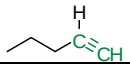
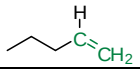
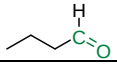
Bonus (5 points): What is the identity of the unknown starting material and what are the conditions for the ozonolysis?


alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
noble gases O
III A
IV A
V A
VI A
VII A
III B
IV B
V B
VI B
VII B
VIII
I B
II B
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

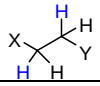
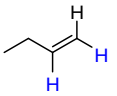
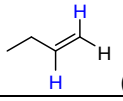
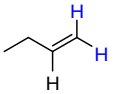
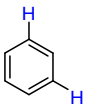
Characteristic ^{1}H NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0.8-1.0	
 (2°)	1.2-1.4	
 (3°)	1.4-1.7	
	2.1	
	3.1-3.3	
	3.4-3.6	
	3.6-3.8	
	3.3-4.0	
 (sp^2)	4.6-5.0	
 (sp^2)	5.2-5.7	
 (sp)	2.5	
 (sp^3)	1.7	
 (sp^2)	6.0-9.5	
 (sp^3)	2.2-2.5	
	9-10	
	10-13	Can be broad and may exchange
	4.5-7.7	Can be broad and may exchange
	0.5-6.0	Can be broad and may exchange
	1.0-5.0	Can be broad and may exchange

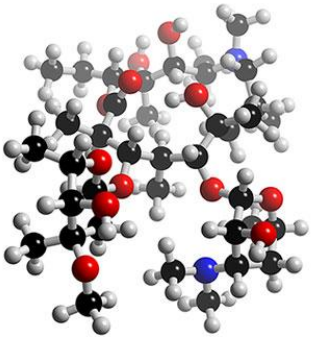
Characteristic ^{13}C NMR Chemical Shifts

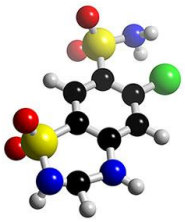
Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0-35	
 (2°)	0-35	
 (3°)	0-35	
	30-70	
	30-70	
	85-90	
	75-100	
	100-150	
 (sp^2 aromatic)	115-150	
	165-210	May take a while to relax (signals may be weak)

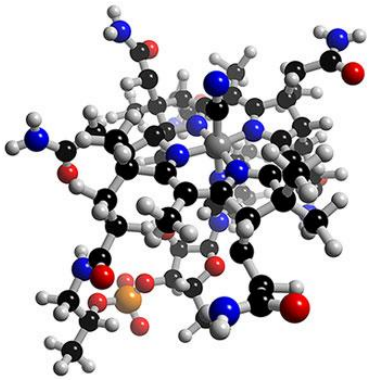
Coupling Constant Values

Type of coupling	J-value (Hz)	Notes
	2-12 (7)	The actual J value depends on the dihedral angle and the nature of the R groups
 (diasterotopic)	12-15	
 (cis)	7-12	
 (trans)	12-15	
 (geminal)	0.5-3	
 (allylic)	3-11	The actual J value depends on the dihedral angle
 (ortho)	6-9	
 (meta)	1-3	

Teaching Materials—Chemistry 255







Organic Chemistry II
Chem 255
Course Syllabus—Fall 2008
August 28, 2008-December 12, 2008

Instructor Information:

Nicole L. Snyder, Ph.D. (Call me Nicole)

Office: SCCT 1072
Lab: SCCT 1068
Office Hours: Tuesday: 8:00am until 10:00am
Wednesday: 3:00pm until 5:00pm
Thursday: 10:00am until 12:00pm
Sunday: 7:00pm until 9:00pm
….and also by appointment
Office Phone: (315) 859-4742
Email: nsnyder@hamilton.edu

Course Objectives:

The second semester of organic chemistry is designed to introduce you to a number of key organic reactions that are used in the synthesis of molecules as simple as Splenda and as complex as calicheamicin. We will begin this course by quickly reviewing a number of topics covered in the first semester, including acid-base chemistry, molecular spectroscopy, and many of the key substitution and elimination reactions you learned before you left for the summer. It is my hope that our initial discussions will help lay the foundation for developing a strong approach to the study of chemical reactions and organic synthesis.

As the semester progresses, we will build upon the concepts you learned in the first semester of organic chemistry, and apply them to many new reactions including pericyclic reactions, carbonyl chemistry, and amine and amide reactivity. Some of the reactions we learn will be named after the organic chemist(s) who discovered them and will have interesting back stories. Other reactions will have significant implications in the synthesis and preparation of pharmaceutical compounds that make our everyday lives easier. When possible, I will make every attempt to connect this material to the current literature, and will highlight the scientists invested in the project and illustrate key reactions that have been used to prepare synthons of chemical or biochemical interest.

In the last few weeks of the course we will discuss how the chemistry you have learned applies to biological systems. We will begin with a systematic approach to the organic and biosynthetic pathways used to prepare complex carbohydrates and proteins that play an important role in our everyday lives. We will learn how these molecules come together at the molecular level to form complex interactions that have vast implications on several processes going on in your body as you read this introduction. We will also explore how several of the compounds we have discussed throughout the course interact with biomolecules to either inhibit or aid in their activity.

Your success in this course will depend heavily on your ability to think mechanistically about the reactions we learn in this course, and apply them to the synthesis of simple and complex molecules and molecular systems. In many cases, the work you do will seem like a large puzzle. The reactions you have learned will be the pieces of the puzzle, and you will need to carefully fit the pieces together to form the final product. Many of you will ultimately find this to be a very rewarding experience, but will probably be frustrated with the process initially. Please do not be discouraged. Your hard work and efforts in this course will eventually pay off, and you will learn skills in this course that will last you a lifetime.

Throughout our journey through the second semester of organic chemistry, I will serve as your guide. I promise to work hard each day to help make sure you understand the material, and you are always welcome to drop by, email, or call me with any questions or concerns you may have. I hope that in the end, you will look back on this experience with fond memories of your time spent learning how the world around you works from a molecular perspective.

*Images taken from chem3D.com database

Course Requirements:

Students taking Chemistry 255 are required to gain access to the course and lab textbooks, a model kit, and a laboratory notebook. More information on each of these items is provided below.

I. *Textbook*—I will assign readings and problems from the textbook and other resources that I feel are necessary to convey a particular topic. The required textbook for this class is **"Organic Chemistry" (6th edition) by Leroy G Wade.** Please make sure that you are using the sixth edition for this course.

II. *Model set*—Access to a molecular model set for organic chemistry is essential to this course and is therefore required. Prentice Hall molecular model sets are available at the bookstore and online. There are also a number of other good (and cheap) molecular model sets out there. Please see me if you would like some suggestions.

III. *Laboratory textbook and notebook*—Laboratory handouts will be handed out in class and posted on Blackboard. A required supplementary text for the laboratory, **"The Organic Chem Lab Survivial Manual" (7th edition) by Zubrick et. al.**, can be purchased in the college bookstore. You should be able to produce a copy of the lab (printed from the web) and the text during your assigned laboratory period. In addition, you will be required to purchase and maintain a Freeman Laboratory Notebook specifically for use in the laboratory portion of the course. A handout for keeping the laboratory notebook will be provided during your designated laboratory period and requirements may vary by instructor.

Class Format:

Each week I will provide a general overview of the material that we will be covering in class. I have proposed the following schedule that is subject to change based on Holidays and other important school related functions:

Monday:	-Homework and molecule of the week distributed
	-General lecture
Tuesday:	-Evening review session 7:30pm until 9:30pm
***Wednesday*:**	-Homework problems from previous week collected and select problems discussed
	-General lecture
	-In class problem session
Thursday:	-Homework graded and returned outside my office door by 9:00pm
***Friday*:**	-Quiz
	-Molecule of the week collected.
	-General lecture

Class Policies:

It is expected that all students in this section of Chemistry 255 will take note of the following policies:

I. *Attendance*—Attendance is strongly encouraged. If you need to miss a class for any reason, please contact me in advance so that I can arrange to have missed work delivered to you in a timely fashion. Also note that class participation is considered for 10% of your final grade (see below). Frequently missing class can and will negatively impact your grade.

II. *Class and lab participation*—Every member of the class is expected to participate in lecture and in lab. This means that you must verbally interact with me during class by answering questions. You must also interact with your fellow classmates during group discussions, lab, and through the use of Blackboard when appropriate. Class participation is worth 10% of the final grade. Failing to participate will result in the reduction of your final grade in the course.

III. *Late work or missed assignments*—Work that is not completed on time will be marked late unless arrangements are made **in advance** to turn the work in at an alternate time other than the due date. In addition, ten percentage of the point total for the assignment will be deducted for each day the assignment is late. If the assignment is more than five days past due, no credit will be given.

IV. *Academic honesty*— Each individual is expected to follow the academic conduct code (Honor Code) set forth by Hamilton College. The honor code will be strictly enforced in this course and there will be no warnings.

V. *Learning disabilities*— In accordance with the Americans with Disabilities Act, any student who has a documented learning disability will be provided with reasonable accommodations designed to meet his/her needs. Before any such assistance can occur, it is the responsibility of the student to see that documentation is on file with the appropriate individual. Please see me as soon as possible to discuss any need for accommodations.

Student Evaluation:

You will be evaluated in this course on a regular basis. The basis for course evaluation is provided below with explanations:

Quizzes:	5%
Problem Sets:	5%
Molecule of the Week:	10%
Lab Reports:	25%
Hourly Exams:	30%
Final Exam:	10%
Named Reaction Summary	5%
Class Participation:	10%

I *Quizzes*—There will be a short five point quiz given the Friday of each week unless an exam is scheduled for the Thursday of that week. Each quiz is designed to test your knowledge of the material covered in the previous week's classes. Overall, there will be ten quizzes scored for a total of 50 points or 5% of the final grade.

II. *Problem Sets*—Problem sets will be assigned at the beginning of each week and will correspond to the material that we will be covering throughout that week. Problem sets will be collected and graded on a weekly basis, generally the following Wednesday of the week the assignment was made. Each assignment must be turned in on time and will be worth ten points. Ten problem sets will be counted towards your final grade. This comprises 50 points or 5% of the final grade.

III. *Molecule of the Week*—Every week you and several of your classmates will be assigned a small molecule and will be asked to provide a short synthesis (usually less than ten steps) of the compound you have been assigned. Molecules will be distributed on Monday, and will be collected and graded at the end of the week. You are strongly encouraged to work with your classmates on this assignment and there will be at least fifteen to twenty minutes of class time per week to work in groups on your assigned molecule. Each assignment must be turned in on time and will be worth ten points. Ten assignments will be counted towards your final grade. This comprises 100 points or 10% of the final grade.

IV. *Lab reports*—Completing lab is essential to understanding organic chemistry. Throughout the term we will complete a total of eleven experiments that relate to material covered in lecture. These assignments are designed to help you maximize your lecture experience and should be thought of as a supplement to your in class lecture. Lab assignments will be graded separately by your individual lab instructor and will count towards 25% of the final grade. Please see your laboratory instructor for details about the grading of laboratory assignments.

Note: Failure to turn in two or more lab reports will constitute and automatic failure of the course!

V. *Hourly exams*—There will be four scheduled 100 point hourly exams given throughout the semester. The dates for these exams are given below:

Exam I—Thursday, September 25, 2008 (7:00pm-9:00pm)
Exam II—Thursday, October 23, 2008 (7:00pm-9:00pm)
Exam III—Thursday, November 13, 2008 (7:00pm-9:00pm)
Exam IV—Thursday, December 04, 2008 (7:00pm-9:00pm)

In most cases, each exam will reflect material that we have covered in class up to the week prior to the exam. At the end of the semester, I will drop the lowest exam score. The remaining three exams will count for 300 points or 30% of the final grade. Exams will not be curved and **you must take all four exams** in order for me to drop your lowest exam score.

VI. *Final Exam*—There will be one final exam worth 100 points or 10% of the final grade. The exam will be cumulative. **The final exam is schedule for Tuesday, December 16, 2008 from 2:00pm until 5:00pm.** More details will be provided as we approach the final exam period.

VII. *Named Reactions Summary*—You will be required to complete a short 3-5 page summary (including figures) on a named reaction that you will select during the first few weeks of the course. Details for the content of this summary will be discussed during the first day of class. The final paper will be due **no later than midnight on December 07, 2008**. This assignment is worth 50 points or 5% of the final grade.

VII. *Class participation*—Frequent participation in class is required and counts for 10% of the final grade. There are two major ways to participate in class: (i.) during lectures; and (ii.) during group problem sessions. Throughout the lecture, students will be asked to provide answers to problems that are presented. Initially, I will ask for volunteers. If there are no volunteers, I will call on a student at random. The student that is chosen will have the opportunity to consult with another classmate or two before answering the question. During group problem sessions, students will be asked to work on a particular problem or set of problems. After a given period of time, each group will be asked to choose a group member to represent the group and present the groups solution to their assigned problem(s). Class participation will count for 100 points or 10% of the final grade.

Schedule of Events:

Class	Monday	Wednesday	Friday
Week One **August 26th** **Introduction and Review**	**-No Class**	**-No Class**	- Introduction - Quick review of key reactions - Introduction to synthesis
Week Two **September 01** **Conjugated Systems**	- Epoxidation (Handout) - Chapter 15 (15.1-15.4)	- Chapter 15 (15.5-15.9)	- **QUIZ I (Review)** - **Molecule of the Week Due** - Chapter 15 (15.10-15.11)
Week Three **September 08** **Conjugated Systems and Aromatic Compounds**	- Chapter 15 (15.10-15.11) and supplement	- **PROBLEM SET I DUE (Chapter 15)** - Chapter 16	- **QUIZ II (Chapter 15)** - **Molecule of the Week Due** - Chapter 17 (17.1-17.4)
Week Four **September 15** **Aromatic Compounds**	- Chapter 17 (17.5-17.9)	- **PROBLEM SET II DUE (Chapter 16)** - Chapter 17 (17.10-17.11)	- **QUIZ III (Chapter 16)** - **Molecule of the Week Due** - Chapter 17 (17.12-17.14)
Week Five **September 22** **Ketones and Aldehydes**	- Chapter 19 (19.11, 19.18)	- **PROBLEM SET III DUE (Chapter 17. 19.18)** - Chapter 18 (18.1-18.8) - **Review for Exam I (Chapters 15-17, 19.18, 19.11)**	- Chapter 18 (18.9-18.12)
Week Six **September 29** **Ketones and Aldehydes**	-Chapter 18 (18.13-18.14)	- **Named Reaction Selection Due** - Chapter 18 (18.15-18.17)	- **QUIZ IV (Chapter 18 though 18.14)** - **Molecule of the Week Due** - Chapter 18 (18.18-18.21)
Week Seven **October 06** **Amines**	- Chapter 19 (19.1-19.10, 19.12-19.13)	- **PROBLEM SET IV DUE (Chapters 18-19)** Chapter 19 (19.15-19.17)	- **QUIZ V (Chapter 18 and 19 through 19.13)** - **Molecule of the Week Due** - Chapter 19 (19.19-19.21)
Week Eight **October 13** **Carboxylic Acids and Carboxylic Acid Derivatives**	- Chapter 20 (20.1-20.10)	- Chapter 20 (20.11-20.15) - **QUIZ VI (Chapter 20—Take Home)**	**No Class-Fall Break**
Week Nine **October 20** **Carboxylic Acid Derivatives**	- Chapter 21 (21.1-21.5) - **QUIZ VI Due** - **Molecule of the Week Due (Take Home)**	- **PROBLEM SET V DUE (Chapter 20)** - Chapter 21 (21.6-21.9) - **Review for Exam II (Chapters 18-20)**	- Chapter 21 (21.10-21.16)
Week 10 **October 27** **Enolates**	- Chapter 22 (22.1-22.4)	- **PROBLEM SET VI DUE (Chapter 21)** - Chapter 22 (22.5-22.6)	- **QUIZ VII (Chapter 21)** - **Molecule of the Week Due** - Chapter 22 (22.7-22.8)
Week 11 **November 03** **Enolates**	- Chapter 22 (22.9-22.11)	- **PROBLEM SET VII DUE (Chapter 22 through 22.11)** - Chapter 22 (22.12-22.14)	- **QUIZ VIII (Chapters 21 through 22.11)** - **Molecule of the Week Due** - Chapter 22 (22.15-22.17)
Week 12 **November 10** **Enolates**	- Chapter 22 (22.18-22.19)	- **PROBLEM SET VIII DUE (Chapter 22 through 22.19)** - **Review for Exam III (Chapter 21-22)**	- Chapter 23 (23.1-23.8)
Week 13 **November 17** **Carbohydrates**	- Chapter 23 (23.9-23.12 and supplement)	- **Draft of Named Reaction Due** - Chapter 23 (23.18-23.19)	- **QUIZ IX (Chapter 23 through 23.12)** - **Molecule of the Week Due** - Chapter 23 (23.20-23.24 and supplement)
Week 14 (November 24)	**No Class-Holiday**	**No Class-Holiday**	**No Class-Holiday**
Week 15 **December 01** **Carbohydrates and Amino Acids**	- **Draft of Named Reaction Returned** - Chapter 24 (24.1-24.4)	- **PROBLEM SET IX DUE (Chapter 23 and 24 through 24.4)** - Chapter 24 (24.5, 24.7, 24.11) - **Review for Exam IV (Chapters 22-23)**	- Chapter 24 (24.8-24.9, 24.13)
Week 16 **December 08** **Amino Acids and Review**	- **Named Reaction Summary Due 12/07/08 by 12:00am** - Additional reactions (Handout)	- **PROBLEM SET X DUE (Chapter 24)** - **Review for Final**	- **QUIZ X (Chapter 24)** - **Molecule of the Week Due** - **Review for Final**

Chemistry 255
Named Reactions Summary
Fall 2008

Named reactions are an important element of organic chemistry and are essential to understanding spoken and written communications in the field. Throughout the semester, each of you will work on a short research paper that is themed towards named reactions in organic synthesis. In general, this paper will teach you about the importance of named reactions in synthetic organic chemistry (they are everywhere). This paper will also teach you how to utilize the chemical literature effectively and learn to write in a style that is used for scientific communication.

The due dates for this paper are listed in the syllabus, but are reiterated below:

Wednesday, September 31, 2008—Latest date for topic selection
Thursday, November 17, 2008—First draft due
Monday, December 07, 2008—Final draft due

I have also set forth some guidelines for the paper in the following text. Please remember that these guidelines should be strictly adhered to but you are also free to contribute additional ideas and/or resources that you think are appropriate.

Choosing a Named Reaction:

You can choose any named reaction that interests you. An important note to make is that no two persons in the class can choose to write on the same named reaction. Therefore, you want to get your choices in to me as soon as possible.

Here are a few representative internet resources that you can use to get you started:

Named Organic Reactions: An Interactive Guide (Oxford University)
http://www.chem.ox.ac.uk/vrchemistry/nor/

Named Reactions (Organic Chemistry Porthole)
http://www.organic-chemistry.org/namedreactions/

Classic Organic Reactions (ChemPen Software)
http://www.chempensoftware.com/organicreactions.htm

Named Reactions in Organic Chemistry (Michael B. Smith—University of Connecticut)
http://orgchem.chem.uconn.edu/namereact/named.html

Named Reactions List (Marcus Brackeen—MonomerChem Inc.)
http://www.monomerchem.com/display4.html

Here are a few printed resources that you can use to access named reactions in organic chemistry:

Li, Jie Jack. "Named Reactions: A Collection of Detailed Reaction Mechanisms (Third Edition)." 2006 (Springer, ISBN-13: 9783540300304)

Kurti, Laszlo; Czako, Barbara "Strategic Applications of Named Reactions in Organic Synthesis: Background and Detailed Mechanisms." 2005 (Elsevier, ISBN-13; 9780124297852)

Laue, Thomas; Plagens, Andreas; Vogel, Claus. "Named Organic Reactions." 2005 (Wiley, ISBN-13: 9780470010419)

Mundy, Bradford P.; Ellerd, Michael G.; Favaloro, Frank G. "Named Reactions and Reagents in Organic Synthesis." 2005 (Wiley, ISBN-13: 9780471228547)

Corey, E. J.; Li, Jie Jack. "Named Reactions of Functional Group Transformations." 2006 (Wiley, ISBN-13: 9780471748687)

As you will see from the resources cited above, there are hundreds of named reactions. The key here is that you want to narrow your topic to one that is manageable so that you don't have too much information to digest, but you also want to be careful that you choose a named reaction that has been used enough to accumulate the amount of information necessary to write your paper.

Outlining the Paper:

Once you have chosen a topic you should begin to outline your paper keeping in mind that the following items should appear somewhere in your writing:

1. A brief discussion of the individual(s) responsible for the authoring the reaction.
   - -You should include a brief biography of the individual(s) credited with the named reaction (i.e. What institution where they employed during the time the named reaction was developed? Where did this development take them?) and so on and so forth.
   - -You should discuss any other important contributions of the individual(s) involved with your named reaction to the field of organic chemistry (though detailed accounts here are not necessary).
2. The circumstances under which the reaction was created.
   - -Was the named reaction created on purpose or discovered serendipitously?
   - -What were the authors doing at the time of the creation/discovery of the named reaction (total synthesis, methodology, etc.)?
3. The accepted mechanism for the reaction.
   - -There may be one or more proposed mechanisms, and while you may feel free to include these mechanisms, I am most interested in the mechanism that is accepted by the scientific community at the time of this writing.
4. The scope and limitations of your named reaction.
   - -What conditions are used to carry out the named reaction?
   - -Are there any special precautions associated with the reaction?
5. A complete and thorough discussion of at least three instances where your named reaction was used in the preparation of an important natural product or pharmaceutically relevant reagent.
   - -The initial creation/discovery does not count here. You must use three additional circumstances in which your named reaction was used.
   - -One of the three instances should be historical in nature (in other words the Julia Olefination was used as a key step in the synthesis of vitamin D but more recently vitamin D-2).
   - -The other two instances should be relatively new, reported in the literature within the past eight years (2000 or greater).

While there is no formal bonus assigned for this paper, special consideration will be given to those students choosing to highlight recent advances in synthetic organic chemistry where their reaction is presented as a key step in the synthesis of a particular molecule or set molecules with some biological relevance. Recent is defined as published between June 2008 and the commencement of the class in December of 2008.

Writing the Paper:

The following guidelines should be followed when putting together the rough and final drafts of your paper for submission:

***Title Page*:** You should include a title page with your name and the course information typed and centered about half way down the page. You may be creative with the title page if you would like.

***Body*:** Your paper should be 3 to 5 pages in length double spaced in Arial or Times Roman font size 11. Margins should be half an inch on each side and the pages should be numbered accordingly. Relevant figures, schemes, and tables should be included in your writing and should be referenced appropriately.

***References*:** References should include the following:

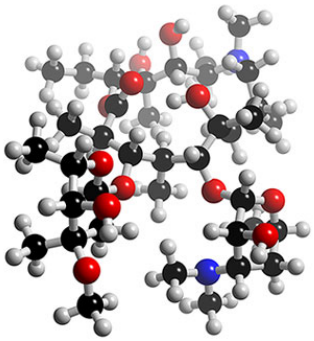
1. At least three current (2000 or later) journal articles.
2. At least five references total with at least four from primary sources including scientific journals from chemistry, biology, physics, or other disciplines.
3. Proper citation using either end notes or footnotes using superscript Arabic numerals. References should be made in proper format according to the *ACS Style Guide*. Recent examples may be found in the *Journal of the American Chemical Society* (JACS) as well as the *Journal of Organic Chemistry* (JOC). To review the *ACS Style Guide* please drop by my office. Copies of JACS and JOC can be found in the library.

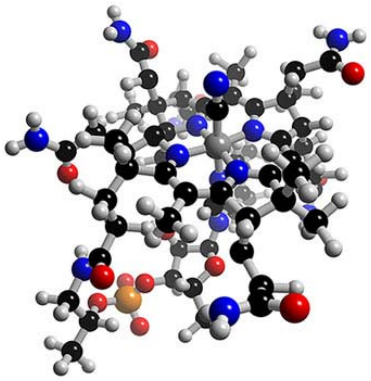
Due Date:

The paper is due no later than midnight on December 07, 2008. Papers should be submitted both electronically (MS Word or PDF) and via hard copy. Late papers will be accepted and will receive a reduction in the overall points as outlined in the class syllabus.

Grading:

The paper is worth 50 points. You will receive a score out of 50 points, a percentage, and a letter grade for your paper. Papers will be returned during the final examination period.





Organic Chemistry II
Chem 255
Course Syllabus—Fall 2009
August 27, 2009-December 11, 2009

Instructor Information:

Nicole L. Snyder, Ph.D. (Call me Nicole)

Office: SCCT 1072
Lab: SCCT 1068
Office Hours: Monday: 10:00am until 11:00am
Wednesday: 3:00pm until 5:00pm
Thursday: 8:00am until 9:00am
Sunday: 5:00pm until 6:00pm
….and also by appointment
Office Phone: (315) 859-4742
Email: nsnyder@hamilton.edu

Course Objectives:

The second semester of organic chemistry is designed to introduce you to a number of key organic reactions that are used in the synthesis of molecules as simple as Splenda and as complex as calicheamicin. We will begin this course by quickly reviewing a number of topics covered in the first semester, including acid-base chemistry, molecular spectroscopy, and many of the key substitution and elimination reactions you learned before you left for the summer. It is my hope that our initial discussions will help lay the foundation for developing a strong approach to the study of chemical reactions and organic synthesis.

As the semester progresses, we will build upon the concepts you learned in the first semester of organic chemistry, and apply them to many new reactions including pericyclic reactions, carbonyl chemistry, and amine and amide reactivity. Some of the reactions we learn will be named after the organic chemist(s) who discovered them and will have interesting back stories. Other reactions will have significant implications in the synthesis and preparation of pharmaceutical compounds that make our everyday lives easier. When possible, I will make every attempt to connect this material to the current literature, and will highlight the scientists invested in the project and illustrate key reactions that have been used to prepare synthons of chemical or biochemical interest.

In the last few weeks of the course we will discuss how the chemistry you have learned applies to biological systems. We will begin with a systematic approach to the organic and biosynthetic pathways used to prepare complex carbohydrates and proteins that play an important role in our everyday lives. We will learn how these molecules come together at the molecular level to form complex interactions that have vast implications on several processes going on in your body as you read this introduction. We will also explore how several of the compounds we have discussed throughout the course interact with biomolecules to either inhibit or aid in their activity.

Your success in this course will depend heavily on your ability to think mechanistically about the reactions we learn in this course, and apply them to the synthesis of simple and complex molecules and molecular systems. In many cases, the work you do will seem like a large puzzle. The reactions you have learned will be the pieces of the puzzle, and you will need to carefully fit the pieces together to form the final product. Many of you will ultimately find this to be a very rewarding experience, but will probably be frustrated with the process initially. Please do not be discouraged. Your hard work and efforts in this course will eventually pay off, and you will learn skills in this course that will last you a lifetime.

Throughout our journey through the second semester of organic chemistry, I will serve as your guide. I promise to work hard each day to help make sure you understand the material, and you are always welcome to drop by, email, or call me with any questions or concerns you may have. I hope that in the end, you will look back on this experience with fond memories of your time spent learning how the world around you works from a molecular perspective.

*Images taken from chem3D.com database

Course Requirements:

Students taking Chemistry 255 are required to gain access to the course and lab textbooks, a model kit, and a laboratory notebook. More information on each of these items is provided below.

I. *Textbook*—I will assign readings and problems from the textbook and other resources that I feel are necessary to convey a particular topic. The required textbook for this class is **"Organic Chemistry" (5th edition) by Marc Loudon.** Please make sure that you are using the fifth edition for this course.

II. *Model set*—Access to a molecular model set for organic chemistry is essential to this course and is therefore required. Prentice Hall molecular model sets are available at the bookstore and online. There are also a number of other good (and cheap) molecular model sets out there. Please see me if you would like some suggestions.

III. *Laboratory textbook and notebook*—Laboratory handouts will be handed out in class and posted on Blackboard. A required supplementary text for the laboratory, **"Making the Connections" by Padias**, can be purchased in the college bookstore. You should be able to produce a copy of the lab (printed from the web) and the text during your assigned laboratory period. In addition, you will be required to purchase and maintain a Freeman Laboratory Notebook specifically for use in the laboratory portion of the course. A handout for keeping the laboratory notebook will be provided during your designated laboratory period and requirements may vary by instructor.

Class Format:

Each week I will provide a general overview of the material that we will be covering in class. I have proposed the following schedule that is subject to change based on Holidays and other important school related functions:

Monday:	-Homework and molecule of the week distributed -General lecture
Tuesday:	-Evening review session 7:00pm until 9:00pm
***Wednesday*:**	-Homework problems from previous week collected and select problems discussed -General lecture
Thursday:	-Homework graded and returned outside my office door by 9:00pm
***Friday*:**	-Quiz -Molecule of the week collected. -General lecture

Class Policies:

It is expected that all students in this section of Chemistry 255 will take note of the following policies:

I. *Attendance*—Attendance is strongly encouraged. If you need to miss a class for any reason, please contact me in advance so that I can arrange to have missed work delivered to you in a timely fashion. Also note that class participation is considered for 10% of your final grade (see below). Frequently missing class can and will negatively impact your grade.

II. *Class and lab participation*—Every member of the class is expected to participate in lecture and in lab. This means that you must verbally interact with me during class by answering questions. You must also interact with your fellow classmates during group discussions, lab, and through the use of Blackboard when appropriate. Class participation is worth 10% of the final grade. Failing to participate will result in the reduction of your final grade in the course.

III. *Late work or missed assignments*—Work that is not completed on time will be marked late unless arrangements are made **in advance** to turn the work in at an alternate time other than the due date. In addition, ten percentage of the point total for the assignment will be deducted for each day the assignment is late. If the assignment is more than five days past due, no credit will be given.

IV. *Academic honesty*— Each individual is expected to follow the academic conduct code (Honor Code) set forth by Hamilton College. The honor code will be strictly enforced in this course and there will be no warnings.

V. *Learning disabilities*— In accordance with the Americans with Disabilities Act, any student who has a documented learning disability will be provided with reasonable accommodations designed to meet his/her needs. Before any such assistance can occur, it is the responsibility of the student to see that documentation is on file with the appropriate individual. Please see me as soon as possible to discuss any need for accommodations.

Student Evaluation:

You will be evaluated in this course on a regular basis. The basis for course evaluation is provided below with explanations:

Quizzes:	10%
Problem Sets:	5%
Molecule of the Week:	10%
Lab Reports:	25%
Hourly Exams:	30%
Final Exam:	10%
Class Participation:	10%

I *Quizzes*—There will be a short ten point quiz given the Friday of each week unless an exam is scheduled for the Thursday of that week. Each quiz is designed to test your knowledge of the material covered in the previous week's classes. Overall, there will be ten quizzes scored for a total of 100 points or 10% of the final grade.

II. *Problem Sets*—Problem sets will be assigned at the beginning of each week and will correspond to the material that we will be covering throughout that week. Problem sets will be collected and graded on a weekly basis, generally the following Wednesday of the week the assignment was made. Each assignment must be turned in on time and will be worth five points. Ten problem sets will be counted towards your final grade. This comprises 50 points or 5% of the final grade.

III. *Molecule of the Week*—Every week you will be assigned a small molecule and will be asked to provide a short synthesis (usually less than ten steps) of the compound you have been assigned. Molecules will be distributed on Monday, and will be collected and graded at the end of the week. You are strongly encouraged to work with your classmates on this assignment and there will be at least fifteen to twenty minutes of class time per week to work in groups on your assigned molecule. Each assignment must be turned in on time and will be worth ten points. Ten assignments will be counted towards your final grade. This comprises 100 points or 10% of the final grade.

IV. *Lab reports*—Completing lab is essential to understanding organic chemistry. Throughout the term we will complete a total of eleven experiments that relate to material covered in lecture. These assignments are designed to help you maximize your lecture experience and should be thought of as a supplement to your in class lecture. Lab assignments will be graded separately by your individual lab instructor and will count towards 25% of the final grade. Please see your laboratory instructor for details about the grading of laboratory assignments.

Note: Failure to turn in two or more lab reports will constitute and automatic failure of the course!

V. *Hourly exams*—There will be four scheduled 100 point hourly exams given throughout the semester. The dates for these exams are given below:

Exam I—Thursday, September 24, 2009 (7:00pm-9:00pm)
Exam II—Thursday, October 22, 2009 (7:00pm-9:00pm)
Exam III—Thursday, November 05, 2009 (7:00pm-9:00pm)
Exam IV—Thursday, December 03, 2009 (7:00pm-9:00pm)

In most cases, each exam will reflect material that we have covered in class up to the week prior to the exam. At the end of the semester, I will drop the lowest exam score. The remaining three exams will count for 300 points or 30% of the final grade. Exams will not be curved and **you must take all four exams** in order for me to drop your lowest exam score.

VI. *Final Exam*—There will be one final exam worth 100 points or 10% of the final grade. The exam will be cumulative. **The final exam is scheduled for Wednesday, December 16, 2009 from 2:00pm until 5:00pm.** More details will be provided as we approach the final exam period.

VII. *Class participation*—Frequent participation in class is required and counts for 10% of the final grade. There are two major ways to participate in class: (i.) during lectures; and (ii.) during group problem sessions. Throughout the lecture, students will be asked to provide answers to problems that are presented. Initially, I will ask for volunteers. If there are no volunteers, I will call on a student at random. The student that is chosen will have the opportunity to consult with another classmate or two before answering the question. During group problem sessions, students will be asked to work on a particular problem or set of problems. After a given period of time, each group will be asked to choose a group member to represent the group and present the groups solution to their assigned problem(s). Class participation will count for 100 points or 10% of the final grade.

Schedule of Events:

Class	Monday	Wednesday	Friday
Week One **August 24th** **Introduction and Review**	**-No Class**	**-No Class**	- Introduction - Quick review of key reactions - Introduction to synthesis
Week Two **August 31** **Conjugated Systems and Aromatic Compounds**	- Epoxidation (Handout) - Chapter 15 (15.1-15.3)	- Chapter 15 (15.4-15.5)	- **QUIZ I (Review)** - **Molecule of the Week Due** - Chapter 15 (15.7)
Week Three **September 07** **Aromatic Compounds**	- Chapter 16 (16.1-16.3)	- **PROBLEM SET I DUE (Chapter 15)** - Chapter 16 (16.4-16.5)	- **QUIZ II (Chapter 15)** - **Molecule of the Week Due** - Chapter 17 (select topics)
Week Four **September 14** **Transition Metal Catalysis**	- Chapter 18 (18.1-18.4, 18.9)	- **PROBLEM SET II DUE (Chapter 16-17)** - Chapter 18 (18.5)	- **QUIZ III (Chapter 16-17)** - **Molecule of the Week Due** - Chapter 18 (18.6)
Week Five **September 21** **Ketones and Aldehydes**	- Chapter 19 (19.1-19.6)	- **PROBLEM SET III DUE (Chapter 18)** - Chapter 19 (19.7-19.9, 19.12, 19.14) - **Review for Exam I (Chapters 15-18)**	- Chapter 19 (19.10-19.11)
Week Six **September 28** **Ketones and Aldehydes** **Carboxylic Acids**	-Chapter 19 (19.13)	- **PROBLEM SET IV DUE (Chapter 19)** - Chapter 20 (20.1-20.7)	- **QUIZ IV (Chapter 19)** - **Molecule of the Week Due** - Chapter 20 (20.8-20.11)
Week Seven **October 05** **Carboxylic Acids and Carboxylic Acid Derivatives**	- Chapter 21 (21.1-21.6)	- **PROBLEM SET V DUE (Chapter 20)** Chapter 21 (21.7-21.8)	- **QUIZ V (Chapter 20)** - **Molecule of the Week Due** - Chapter 21 (21.9-21.10)
Week Eight **October 12** **Enolates**	- Chapter 22 (22.1-22.2)	- **PROBLEM SET VI DUE (Chapter 21)** - Chapter 22 (22.3) - **QUIZ VI (Chapter 21—Take Home)**	**No Class-Fall Break**
Week Nine **October 19** **Enolates**	- Chapter 22 (22.4-22.5) - **QUIZ VI Due** - **Molecule of the Week Due (Take Home)**	- **Review for Exam II (Chapters 19-21)**	- Chapter 22 (22.7)
Week 10 **October 26** **Enolates**	- Chapter 22 (22.8)	- Chapter 22 (22.9-22.10)	- **QUIZ VII (Chapter 22 (through 22.8)** - **Molecule of the Week Due** - Chapter 22 (22.11)
Week 11 **November 02** **Amines**	- Chapter 23 (23.1-23.5)	- **PROBLEM SET VII DUE (Chapter 22)** - **Review for Exam III (Chapter 22)**	- Chapter 23 (23.6-23.7, 23.11)
Week 12 **November 09** **Carbohydrates**	- Chapter 23 (23.8-23.10)	- **PROBLEM SET VIII DUE (Chapter 23)** - Chapter 24 (24.1-24.4)	- **QUIZ VIII (Chapter 23)** - **Molecule of the Week Due** - Chapter 24 (24.6 + supplement)
Week 13 **November 16** **Carbohydrates**	- Chapter 24 (24.6 + supplement)	- **PROBLEM SET IX DUE (Chapter 23)** - Chapter 24 (24.11 + supplement)	- **QUIZ IX (Chapter 24 through 24.6)** - **Molecule of the Week Due** - Chapter 24 (24.11 + supplement)
Week 14 (November 23)	**No Class-Holiday**	**No Class-Holiday**	**No Class-Holiday**
Week 15 **November 30** **Amino Acids**	- Chapter 26 (26.1-26.2)	- **PROBLEM SET X DUE (Chapter 24)** - Chapter 26 (26.3) - **Review for Exam IV (Chapters 23-24)**	- Chapter 26 (26.4-26.7)
Week 16 **December 07** **Amino Acids and Review**	- Chapter 26 (26.8-26.9)	- **PROBLEM SET X DUE (Chapter 26 through 26.9)** - Chapter 26 (26.10 + supplement)	- **QUIZ X (Chapters 24 and 26 through 26.9)** - **Molecule of the Week Due** - Chapter 26 (26.10 + supplement)

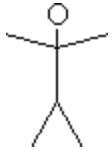
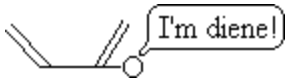
Chapter 15

Conjugated Systems and Aromatic Compounds

Carbon Nanotubes
Image courtesy of Mail Online (August 28, 2009)

Conjugated Systems

What happens to an organic chemistry student during an exam?

Organic Chemistry Student Before Exam	Organic Chemistry Student After Exam
	

Conjugated, Isolated, and Cumulated Systems

A. Conjugated systems- systems where double bonds, separated by a single bond, can interact with each other.

-Example: 1,3 pentadiene

B. Isolated systems- systems where double bonds are separated by more than one single bond and can no longer interact with one another.

-Example: 1,6 heptadiene

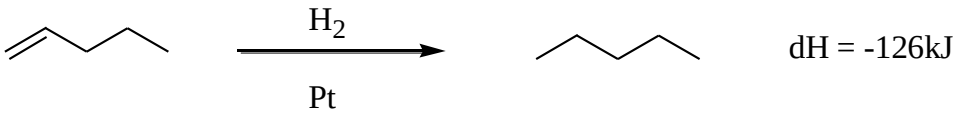
C. Cumulated systems- systems where carbon-carbon double bonds are directly next to one another.

-Example: allene

Diene Stability

Stability- heats of hydrogenation are used to compare the relative stabilities of alkenes.

-Examples: monoalkenes



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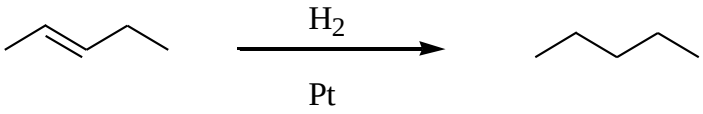
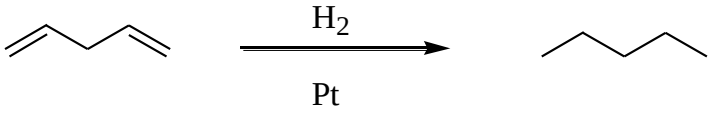


dH = -116kJ

-Examples: dienes



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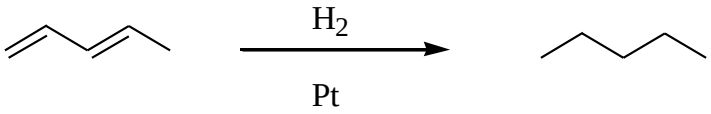
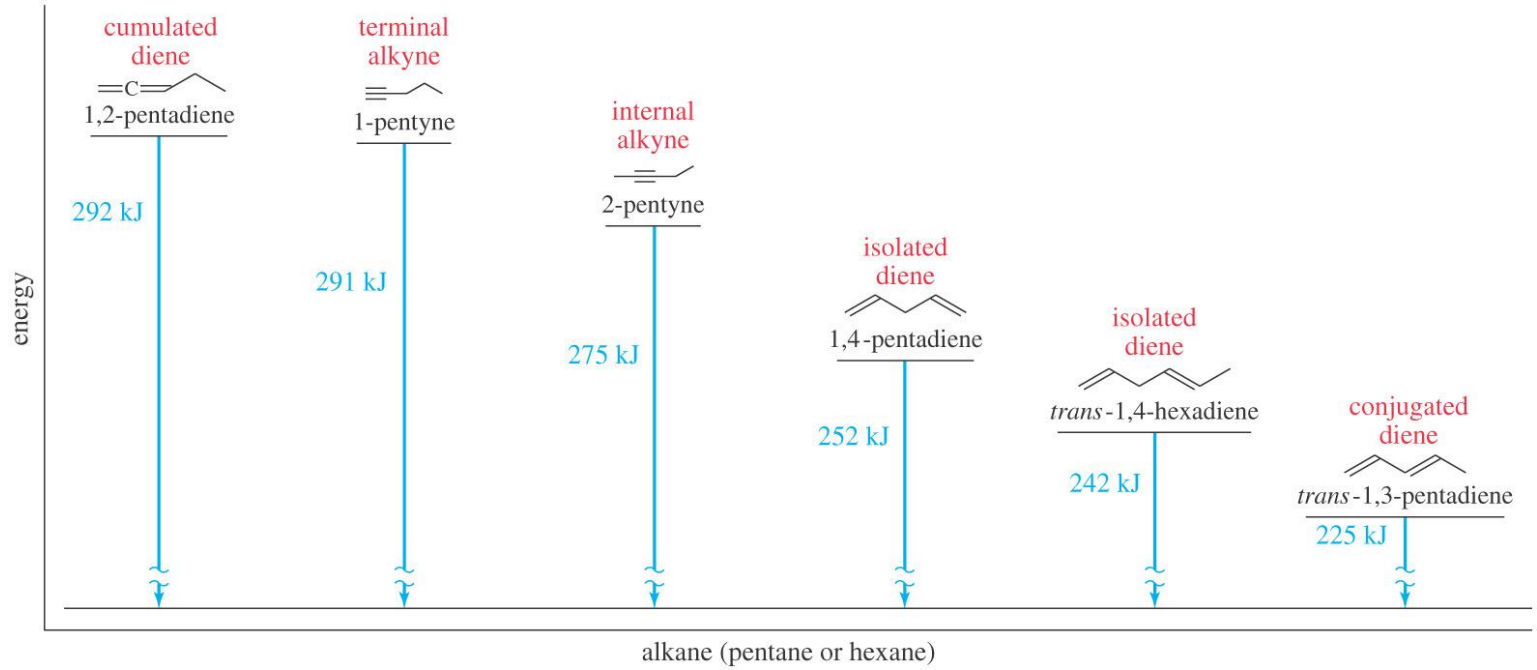


dH = -225kJ

Diene Stability

Conjugated systems are more stable due to resonance energy.

-Summary

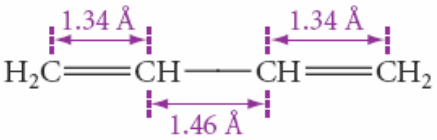


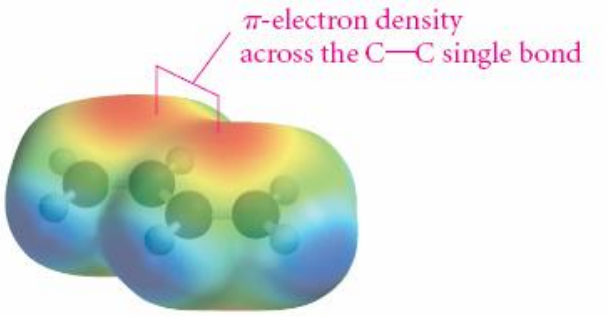
(Wade 6th ed.)

Structure and Bonding of 1, 3 Butadiene

The electrons in the pi system of 1,3 butadiene are delocalized over there bonds instead of two.

-Example:





EPM of 1,3-butadiene

A Molecular Orbital Picture of 1,3 Butadiene

General points to keep in mind:

1. Each p orbital consists of two lobes with opposite phases of the wave function.

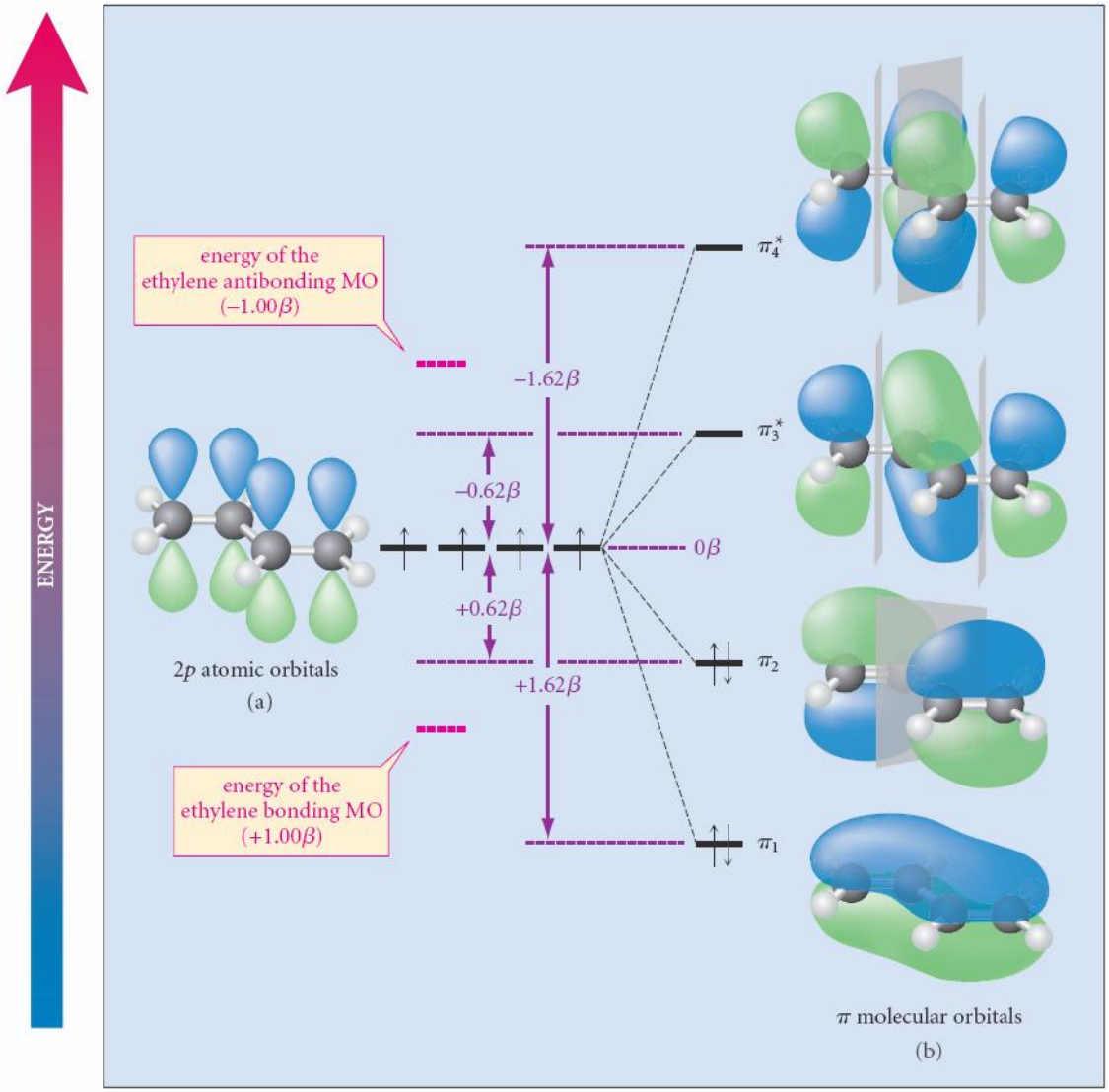
2. The orbital's can be arranged in a number of different ways, but the number of pi molecular orbital's must always equal the number of p orbital's in the system.

3. Each molecular orbital can hold two electrons.

4. Most stable molecules have filled bonding molecular orbital's and empty antibonding molecular orbital's.

A Molecular Orbital Picture of 1,3 Butadiene

1,3 butadiene

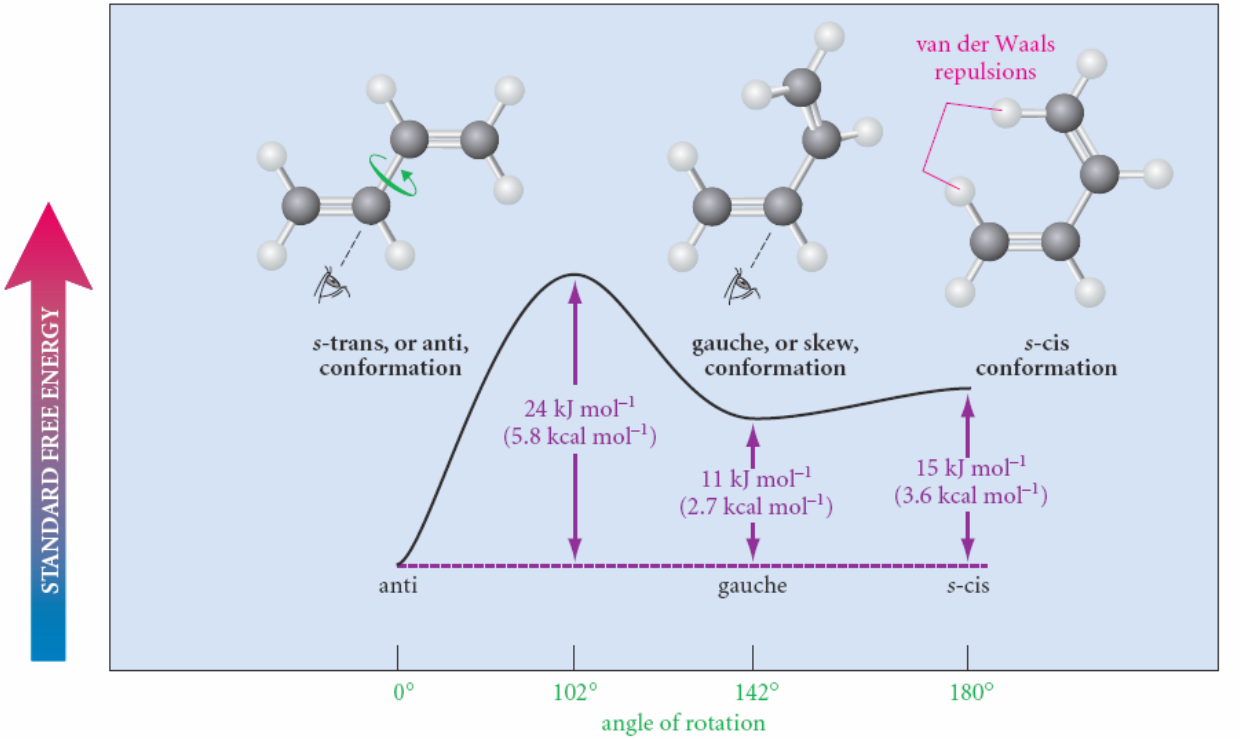


A Molecular Orbital Picture of 1,3 Butadiene

1,3 butadiene

-In the planar conformation, 1,3-butadiene can exist as two unique conformers, s-cis or s-trans.

-Example: s-cis *vs* s-trans



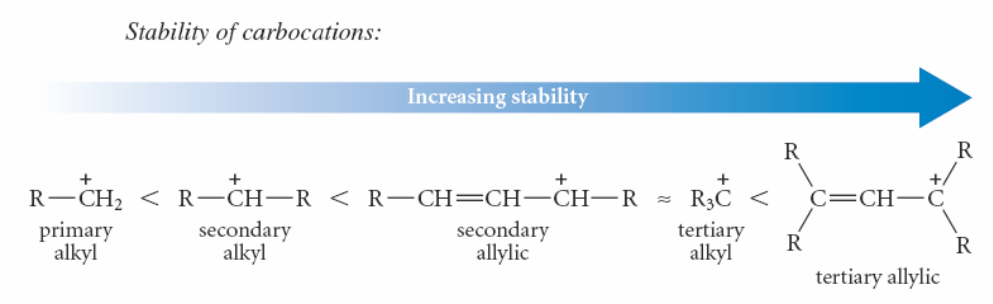
-s-trans is more stable due to steric interactions.

-s-trans and s-cis are able to interconvert at room temperature.

Allylic Groups

Cation's, anion's, and radical's generated from allyl substrates are stabilized through resonance.

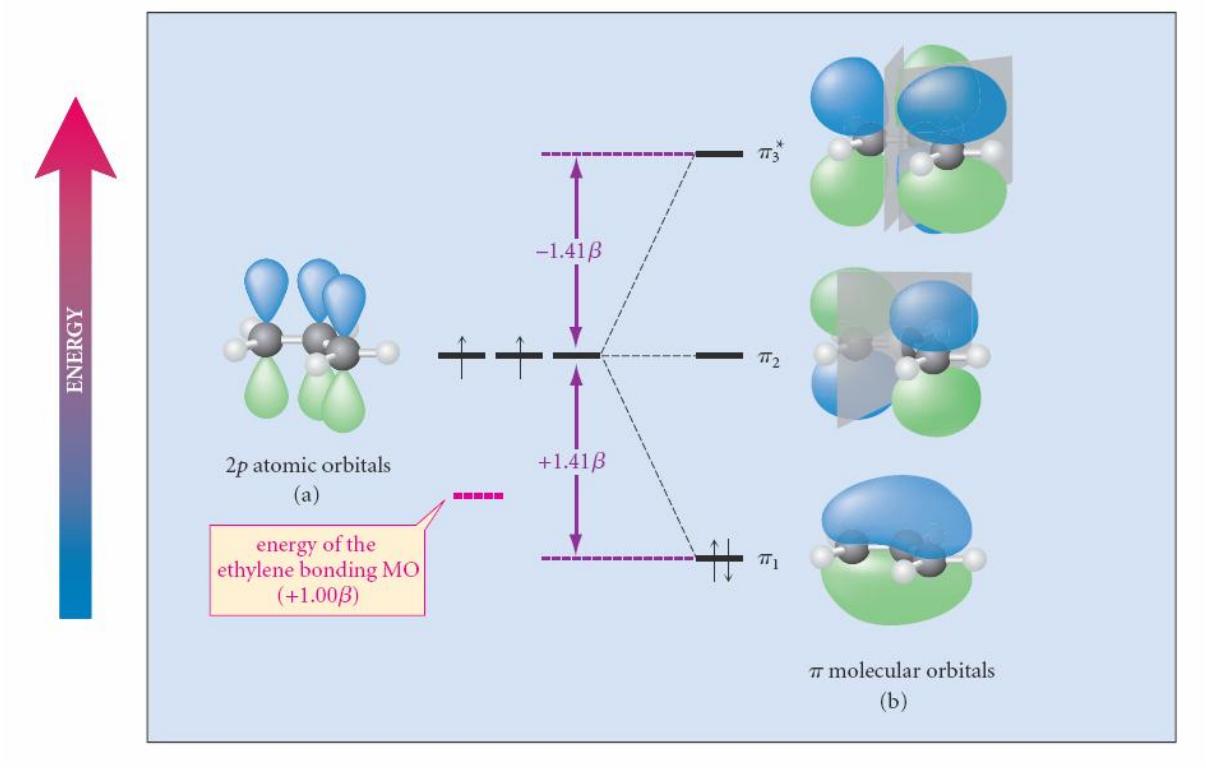
-General comparison:

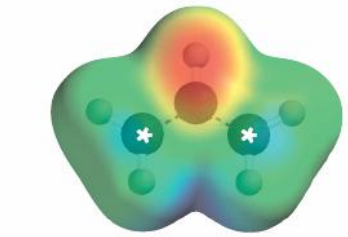


-Allylic carbocations are actually about as stable as secondary carbocations.

Allylic Groups

Example: the allyl cation





EPM of the allyl cation

Addition Reactions to Conjugated Dienes

Types of Addition Reactions- reactions with conjugated dienes may be 1,2 or 1,4 in nature.

-1,2 addition- occurs when an electrophile adds across a carbon-carbon double bond.

-Example: reaction of 1,3 butadiene with HBr

-1,4 addition- addition across a conjugated system.

-Example: reaction of 1,3 butadiene with HBr

Addition Reactions to Conjugated Dienes

Control of 1,2 and 1,4 addition reactions- whether or not a 1,2 or 1,4 addition reaction occurs depends on the temperature of the system.

-1,2 addition reactions predominate at lower temperatures (the products are said to be the kinetic products).

-The 1,2 addition reaction is faster due to a lower activation energy.

-Lower temperatures also limit the number of collisions that take place.

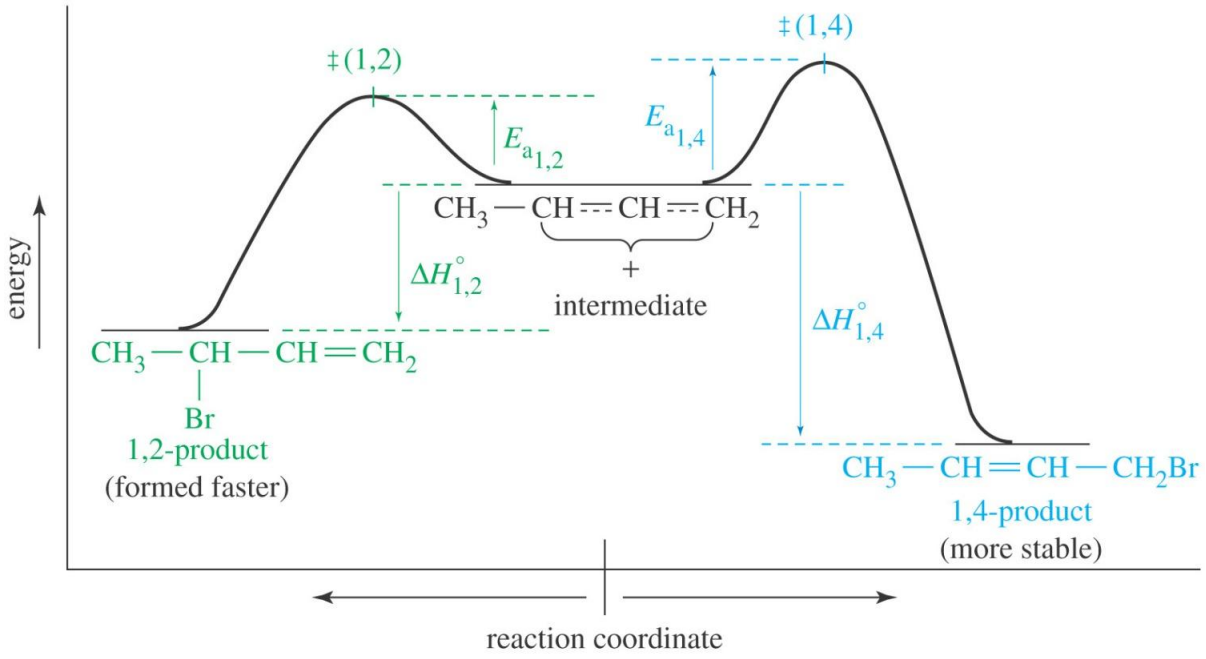
-1,4 addition reactions predominate at higher temperatures (the products are said to be the thermodynamic products).

-The 1,4 addition reaction is slower due to a higher activation energy.

-The reaction takes place because molecular collisions are more likely and the reverse reaction is likely to occur.

Addition Reactions to Conjugated Dienes

1,2 *vs* 1,4 Additions

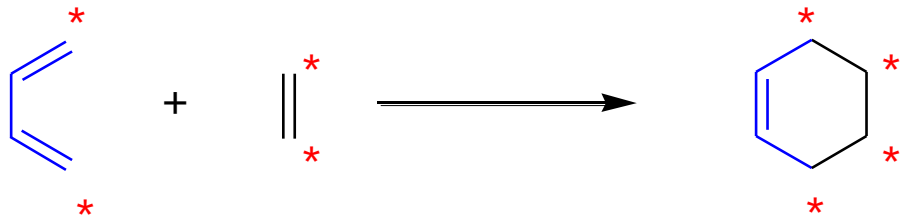


(Wade 6th ed.)

The Diels Alder Reaction

4 + 2 cycloaddition reaction between an electron rich diene and an electron poor alkene or alkyne known as a dienophile.

Reaction:



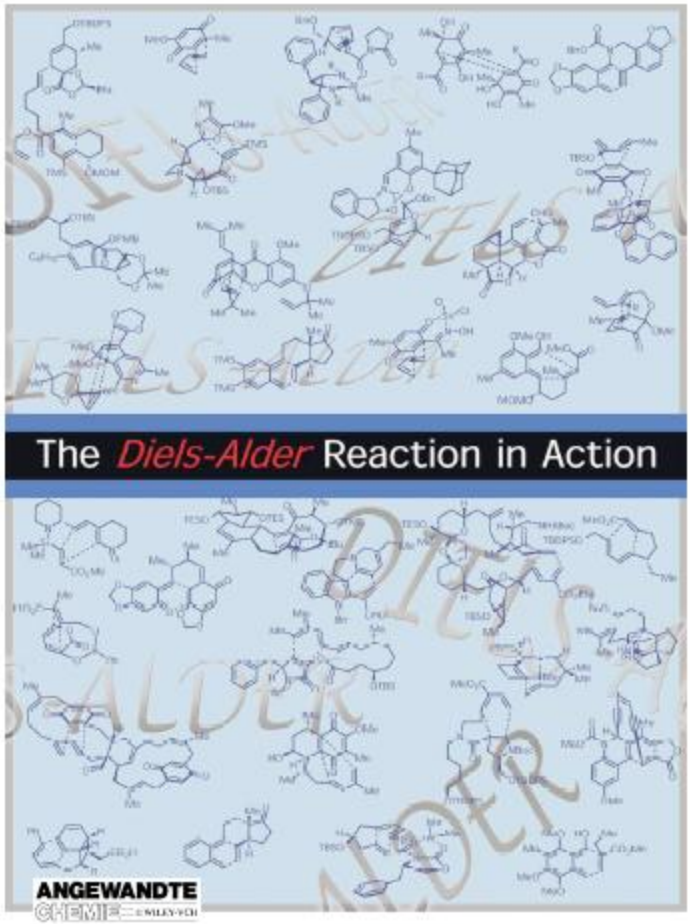
Mechanism:

The Diels Alder Reaction

One of the most important reactions in synthetic organic chemistry

Syntheses include:

- Cortisone (Woodward)
- Cholesterol (Woodward)
- Myrocin C (Danishefsky)
- Giberellic acid (Corey)
- Prostaglandin F_{2a} (Corey)
- Estrone (Volhardt)
- Taxol (Nicolaou)



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The Diels Alder Reaction

Father was a Professor of philology at the University of Berlin, where he earned his doctorate in chemistry under Emil Fischer.

Taught until 1916 University of Berlin and from 1916-1945 at the University of Kiel.

Awarded the Nobel Prize in Chemistry "for their discovery and development of the diene synthesis."

The Diels Alder Reaction

Born in the industrial area of Konigshutte.

Studied chemistry at the University of Berlin from 1922, and later at the University of Kiel where his PhD was awarded in 1926 for work supervised by Diels.

Alder was appointed reader for chemistry at Kiel (1930), and promoted to lecturer in 1934.

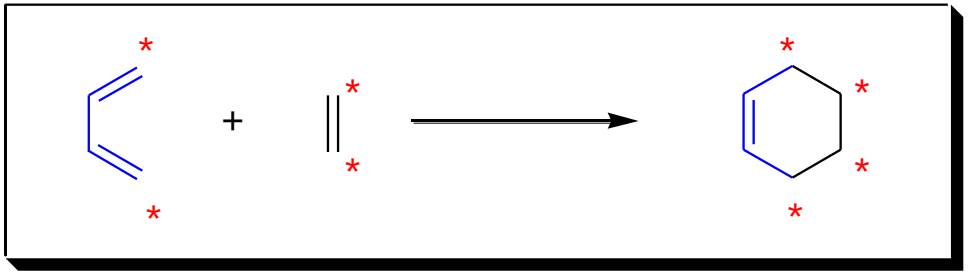
Joined IG Farben Industrie in 1936 where he worked on synthetic rubber.

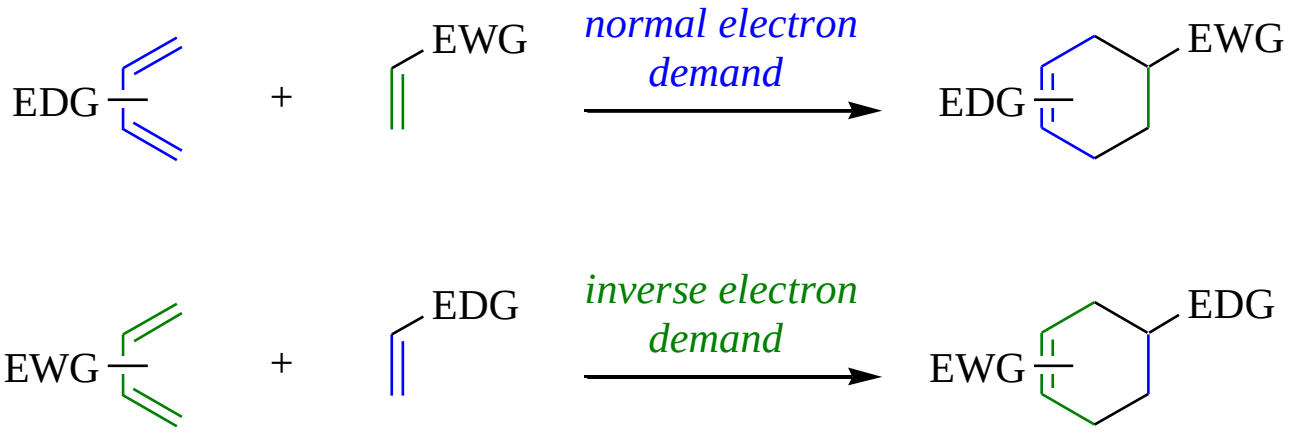
Appointed Professor of Experimental Chemistry and Chemical Technology at the University of Cologne in 1940.

Alder received several honorary degrees and other awards, most famously the 1950 Nobel Prize in Chemistry.

The lunar crater Alder is named in his honor.

The Diels Alder Reaction





EDG (Electron Donating Groups): Alkyl, O-alkyl, N-alkyl

EWG (Electron Withdrawing Groups): CN, NO_2, CHO, COR, COAr, CO_2H, CO_2R

The Diels Alder Reaction

Considerations

-The reaction is concerted (occurs in one step).

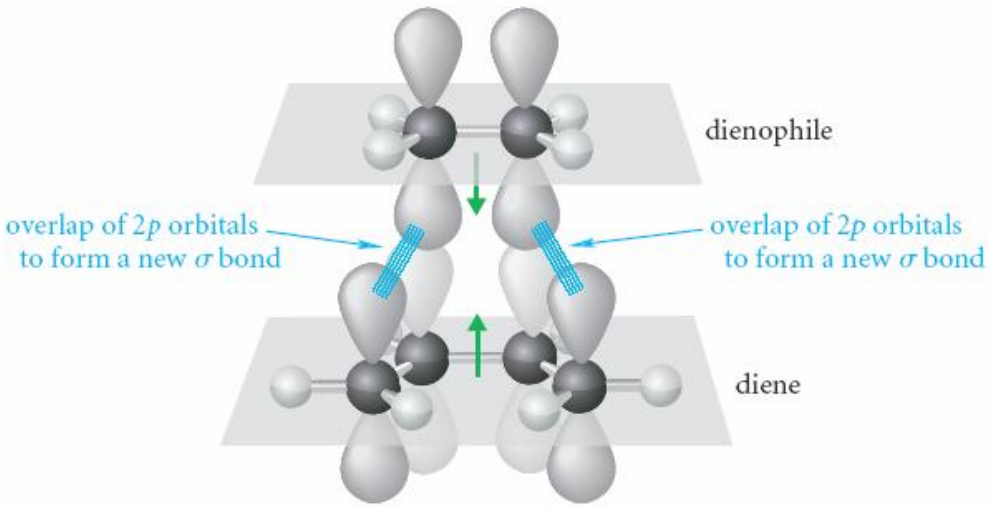
-The transition state requires and appropriate geometry.

-The p orbital's of the diene must be able to overlap with the p orbital's of the dienophile.

-In order for this to occur, the highest occupied molecular orbital (HOMO) of the diene reacts with the lowest unoccupied orbital (LUMO) of the dienophile so that the electrons may flow smoothly from one molecule to the other (Woodward-Hoffman rules).

-The diene must be in an s-cis conformation from proper overlap.

-Example:



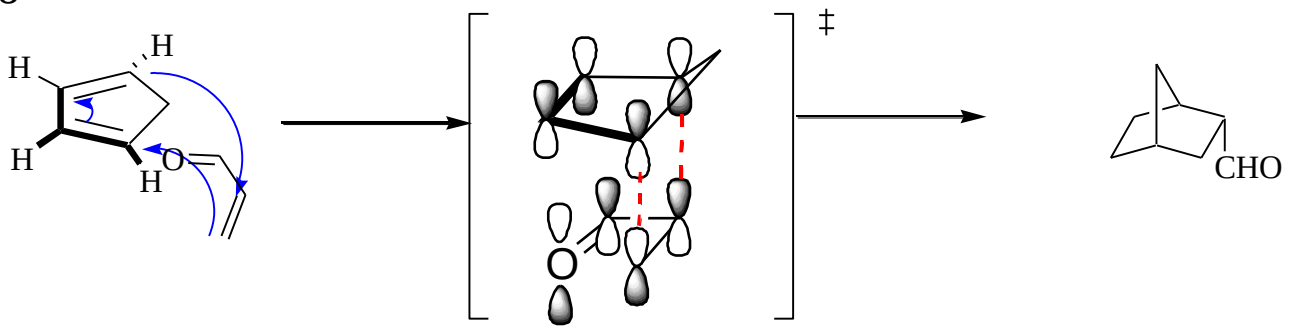
The Diels Alder Reaction

Considerations

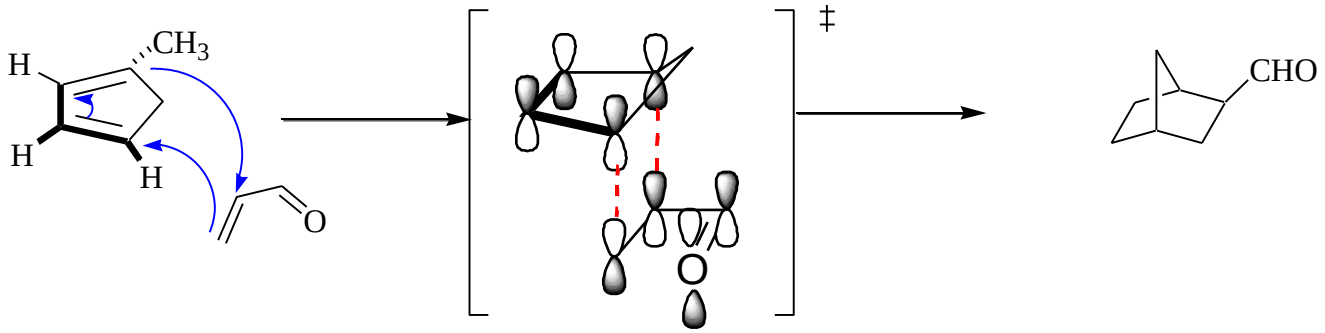
-Dienophile's containing pi bonds show considerable secondary overlap with the electron rich diene in the transition state resulting primarily in endo products.

-Example:

Endo



Exo



The Diels Alder Reaction

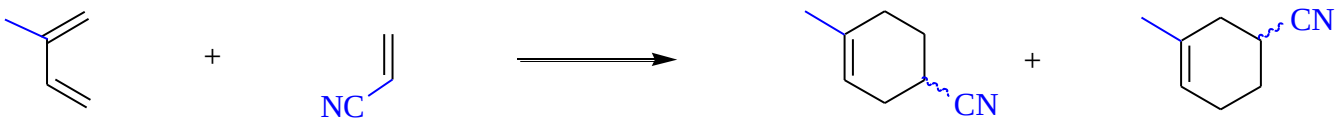
Considerations

-When unsymmetrical reagents are used, mixtures of products are formed, but one tends to predominate.

-The major product is based on the electronics of the dienophile.

-For internally substituted dienes, the 1,4 product predominates.

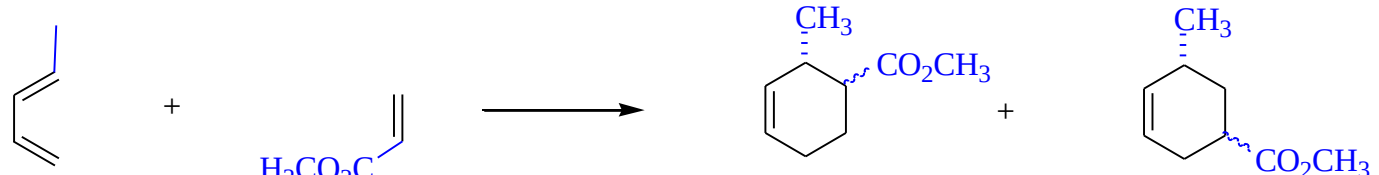
-Example:



70 30

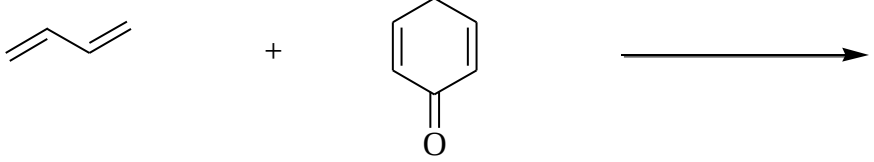
-For terminally substituted dienes, the 1,3 product predominates.

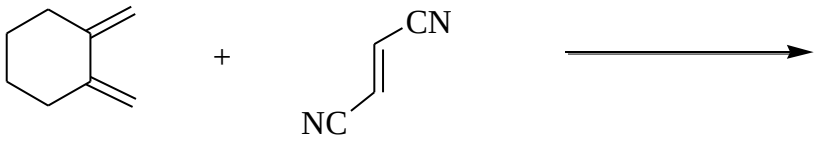
-Example:

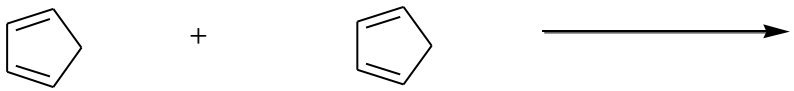


90 10

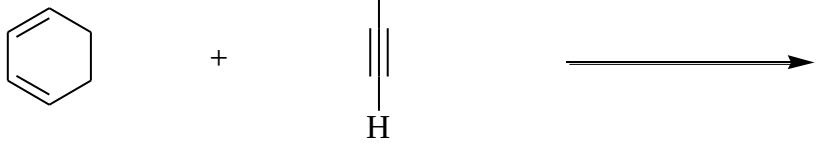
Practice Problems

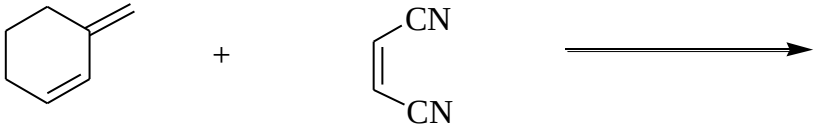
1. 

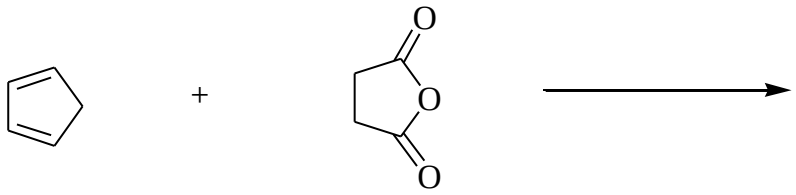
2. 

3. 

Practice Problems

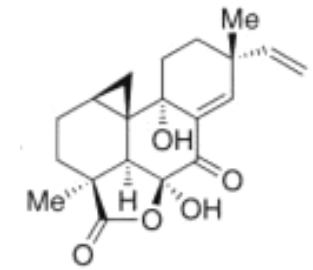
4. 

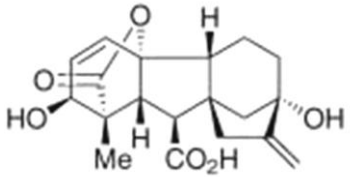
5. 

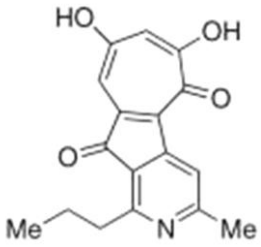
6. 

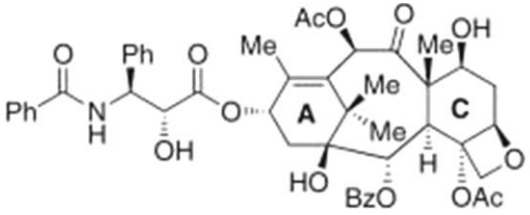
The Diels Alder Reaction in Action

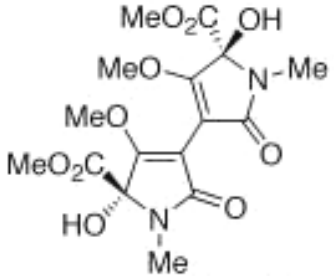
Natural Products Synthesis:

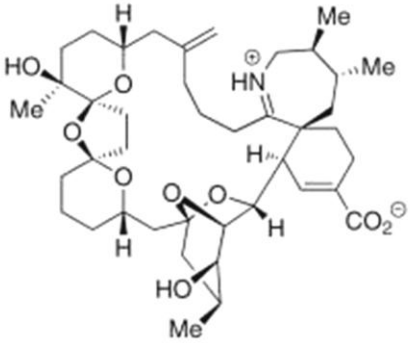


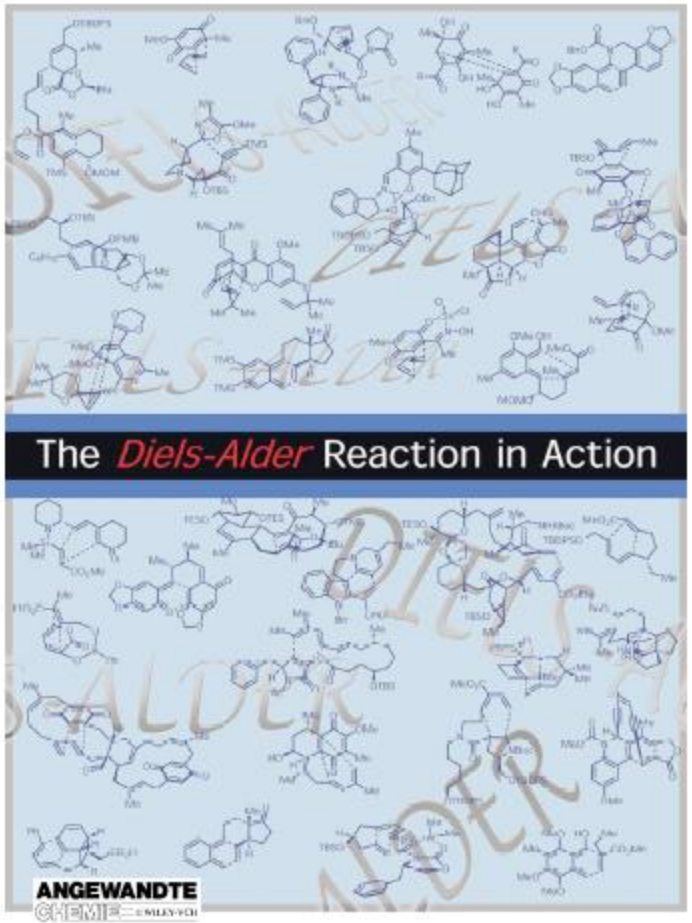












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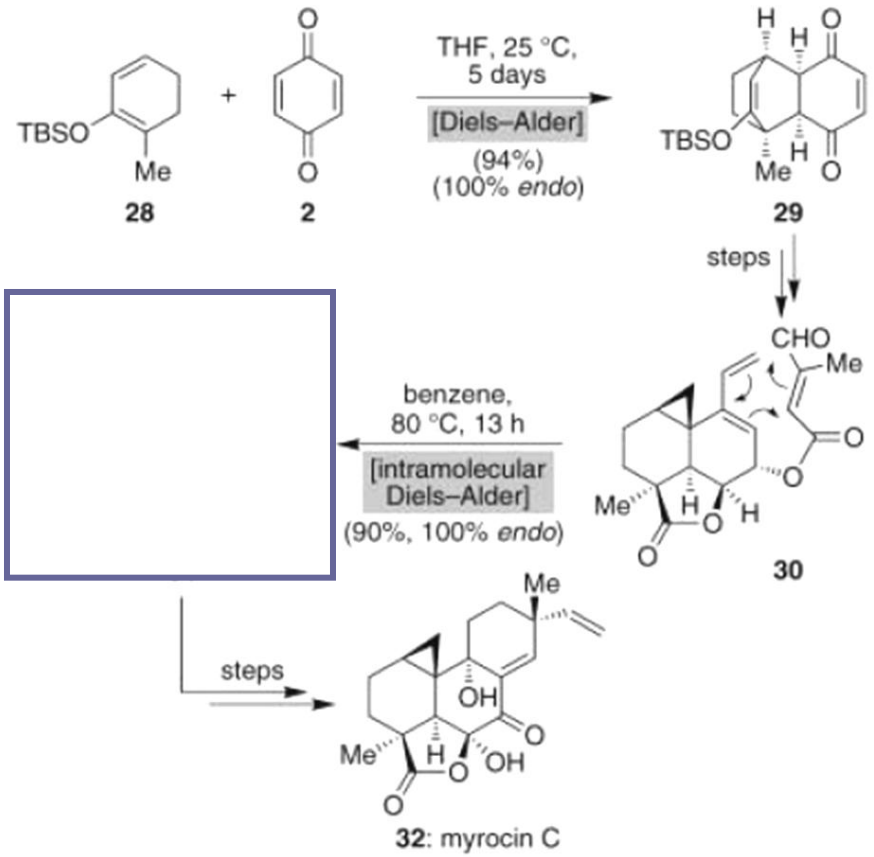
The Diels Alder Reaction in Action

Myrocin C (Danishefsky)

-Antitumor anitbiotic.



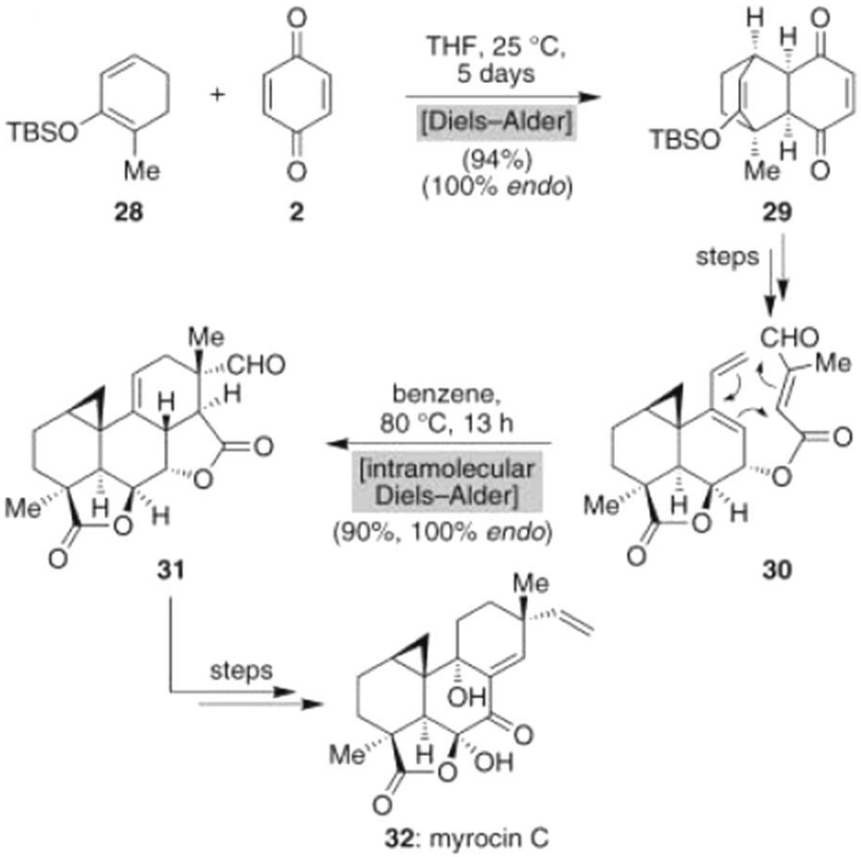
B.S. Yeshiva University

Ph.D. Harvard

Currently at Columbia

The Diels Alder Reaction in Action

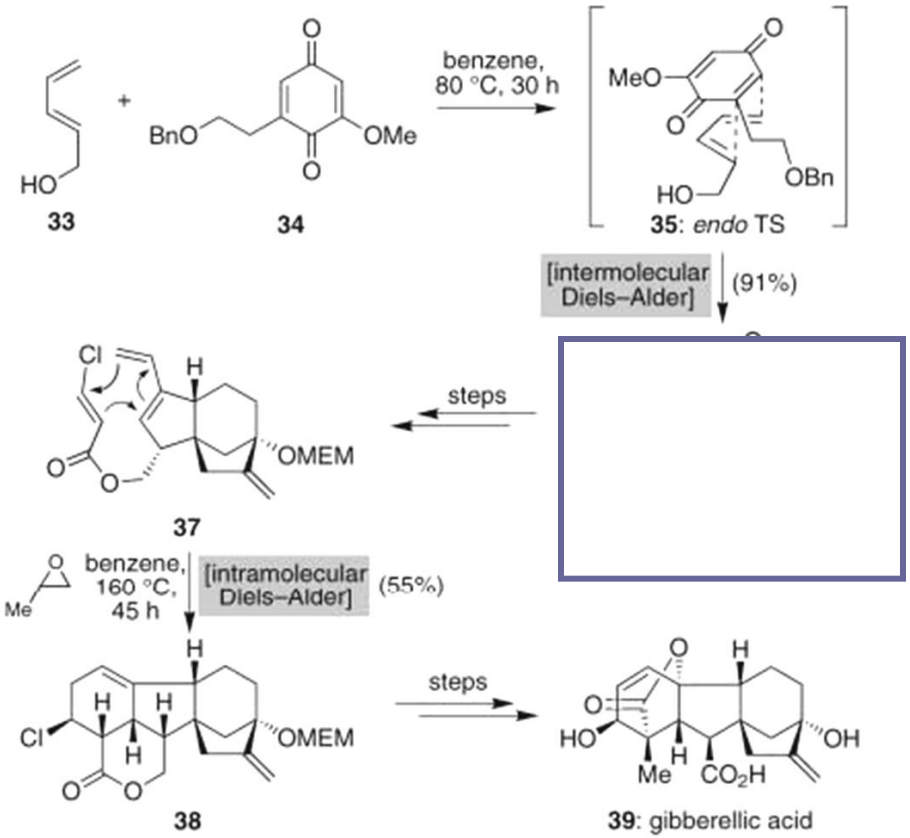
Myrosin C (Danishefsky)



The Diels Alder Reaction in Action

Gibberelic acid: (E. J. Corey)

-Plant hormone that promotes growth and elongation.



B.S. Massachusetts Institute of Technology

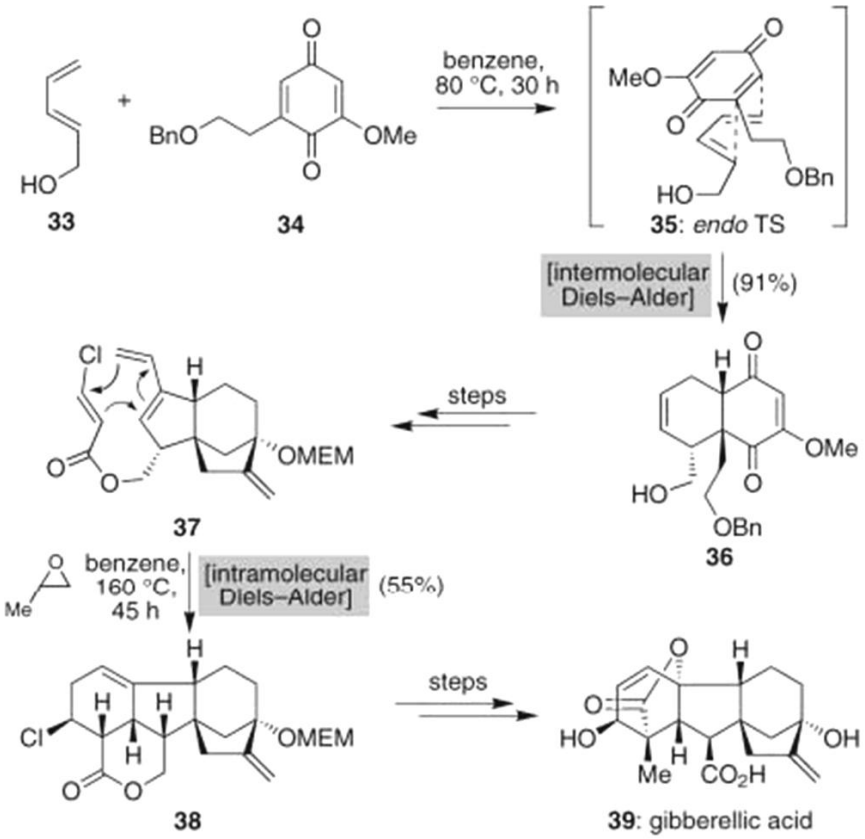
Ph.D. Massachusetts Institute of Technology

Nobel Prize 1990

Currently at Harvard

The Diels Alder Reaction in Action

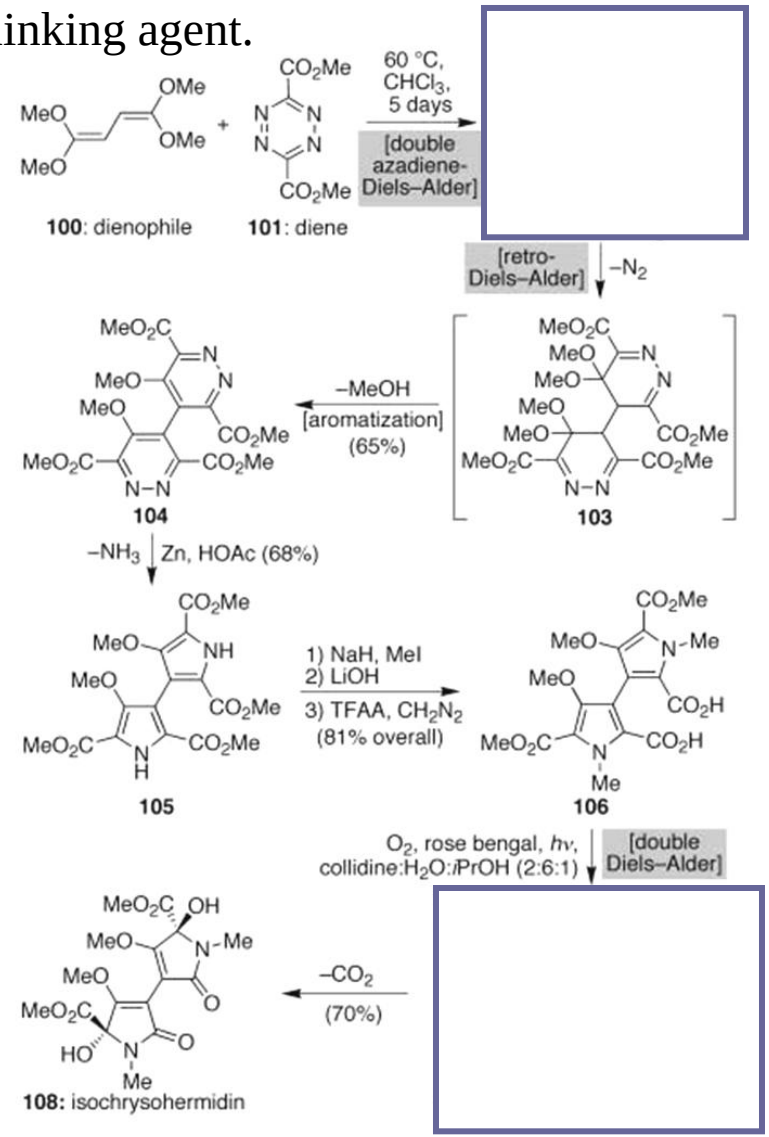
Gibberelic acid: (E. J. Corey)



The Diels Alder Reaction in Action

Isochrysohermidin: (Dale Boger)

-DNA cross linking agent.



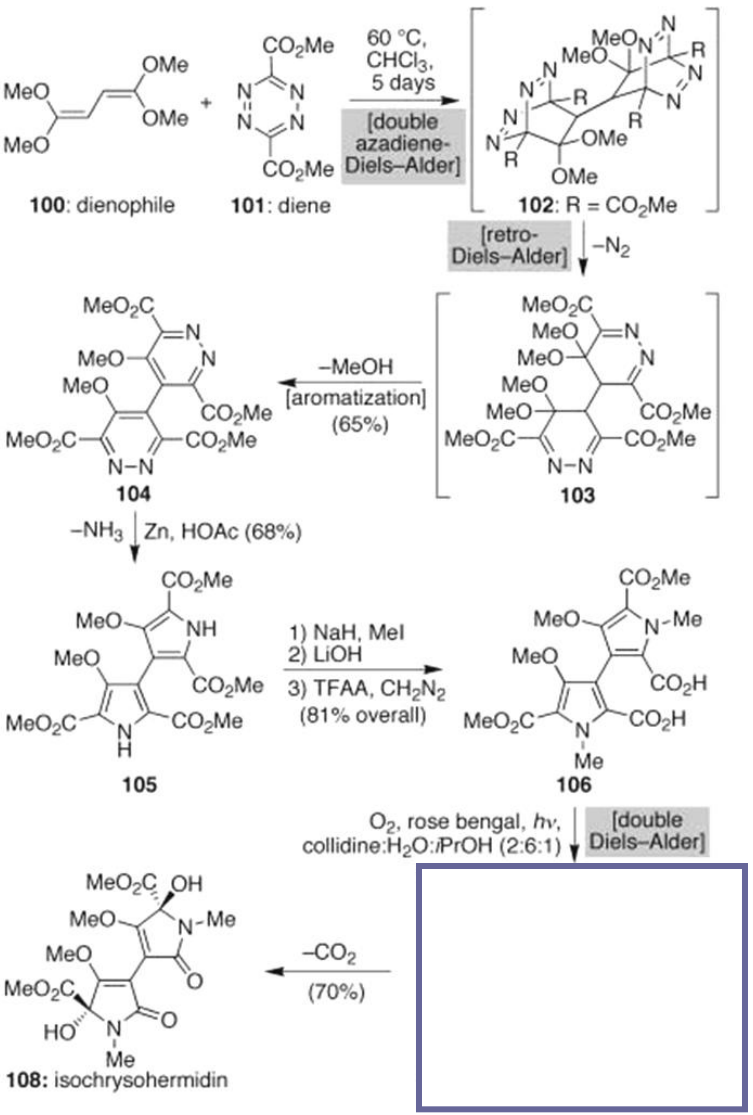
B.S. University of Kansas

Ph.D. Harvard

Currently at Scripps

The Diels Alder Reaction in Action

Isochrysohermidin: (Dale Boger)



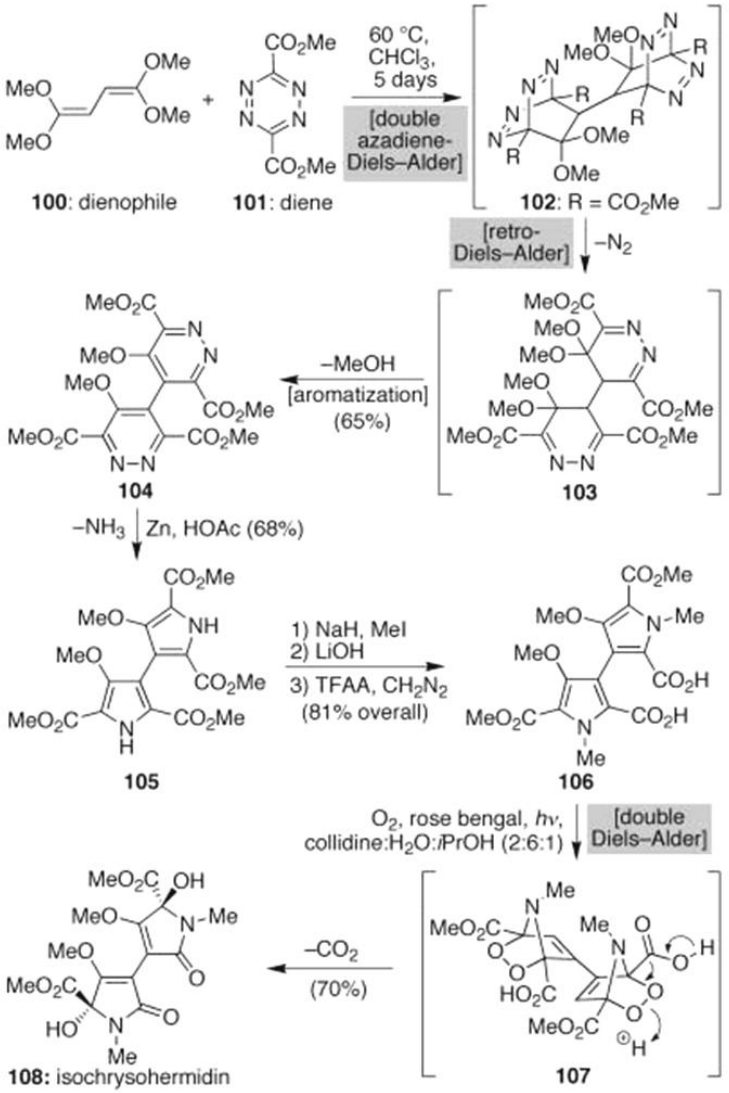
B.S. University of Kansas

Ph.D. Harvard

Currently at Scripps

The Diels Alder Reaction in Action

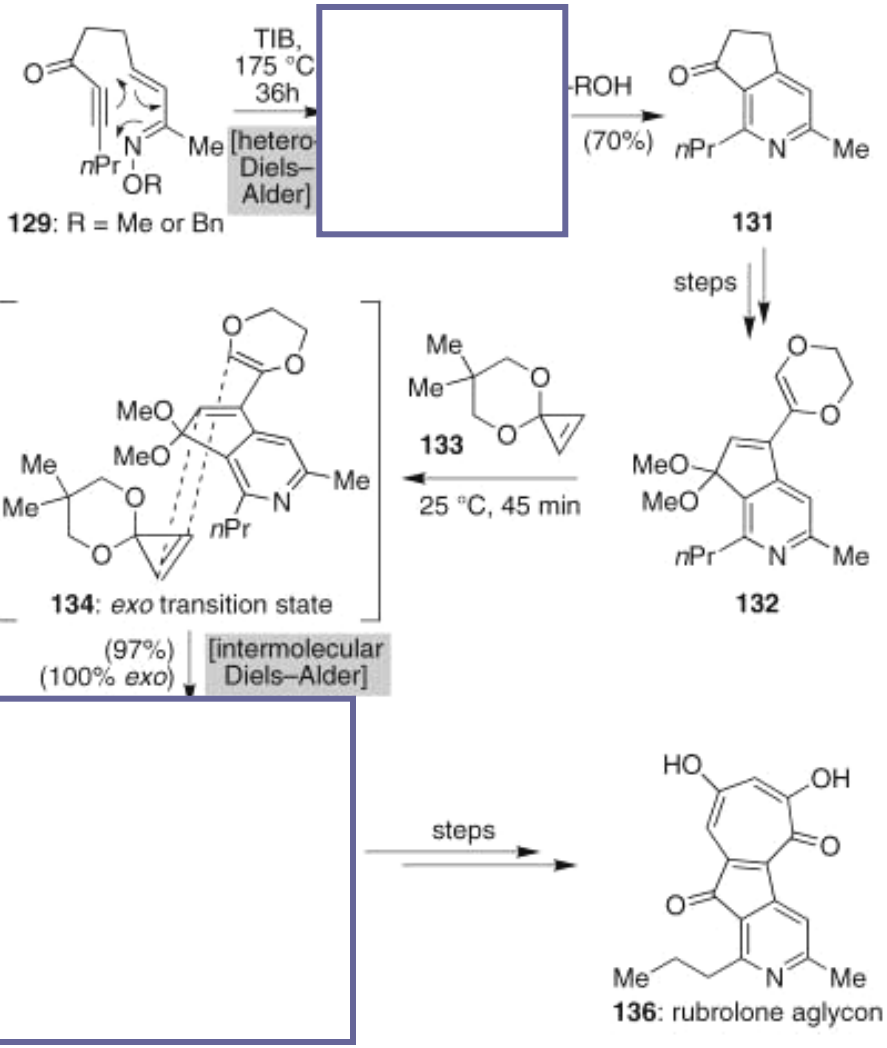
Isochrysohermidin: (Dale Boger)



The Diels Alder Reaction in Action

Rubrolone: (Dale Boger)

-Red pigment produced by Streptomycese.



B.S. University of Kansas

Ph.D. Harvard

Currently at Scripps

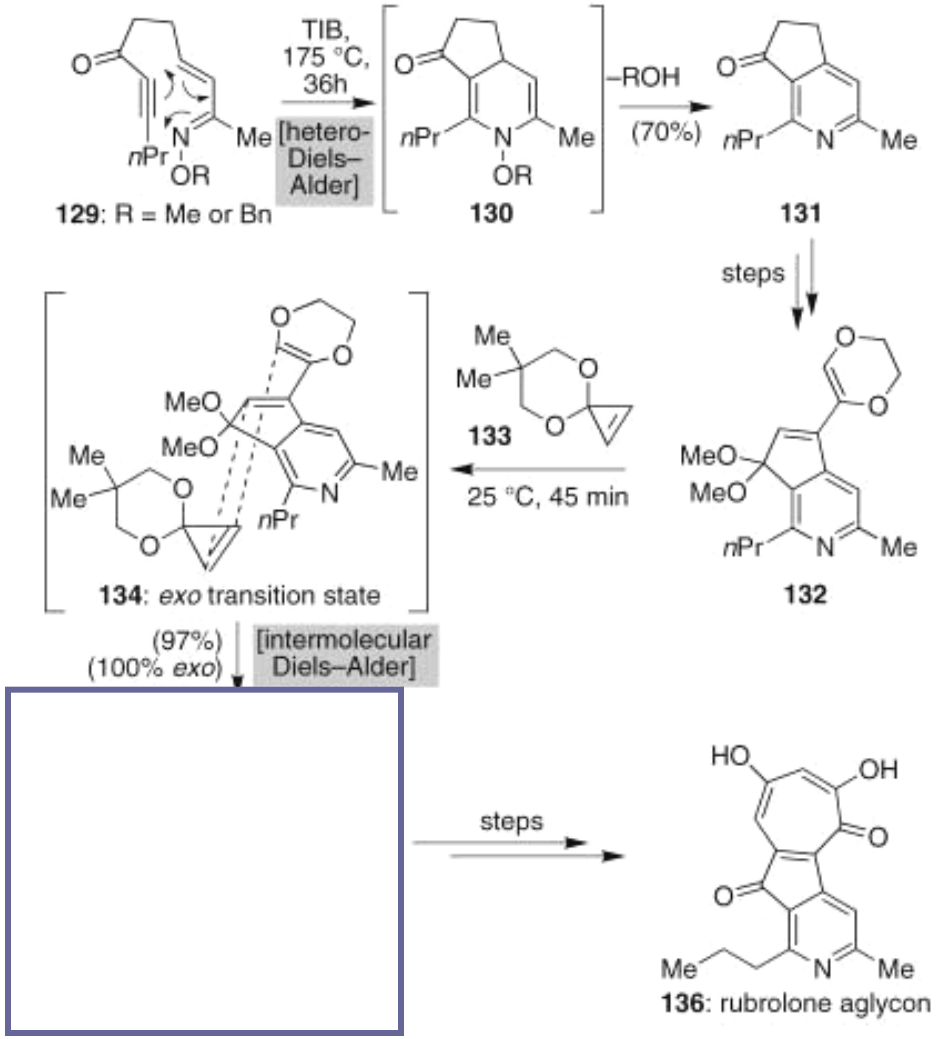
The Diels Alder Reaction in Action

Rubralone: (Dale Boger)

B.S. University of Kansas

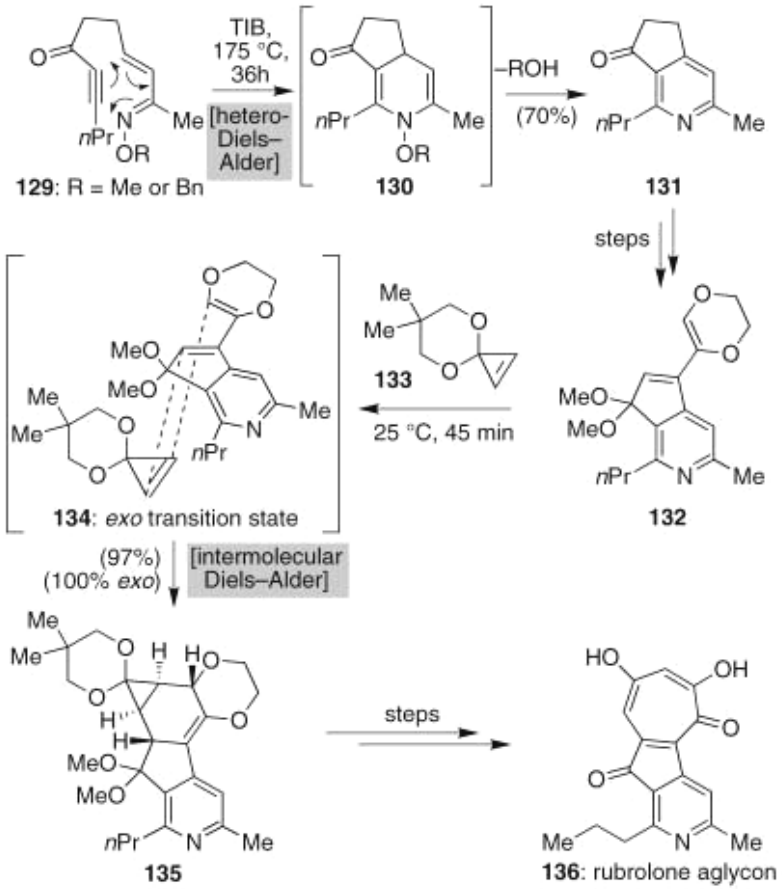
Ph.D. Harvard

Currently at Scripps



The Diels Alder Reaction in Action

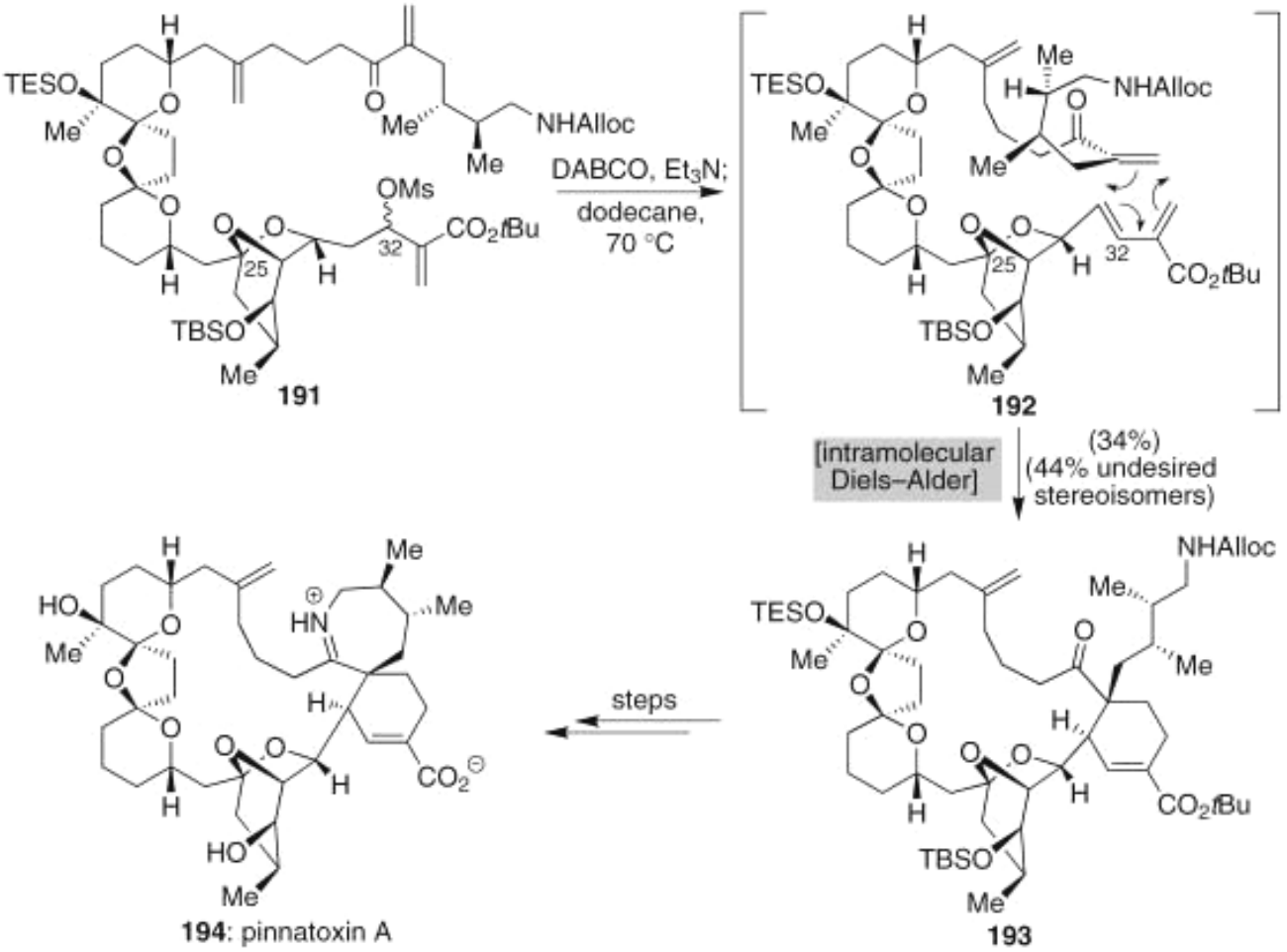
Rubralone: (Dale Boger)



The Diels Alder Reaction in Action

Pinnatoxin: (Kishi)

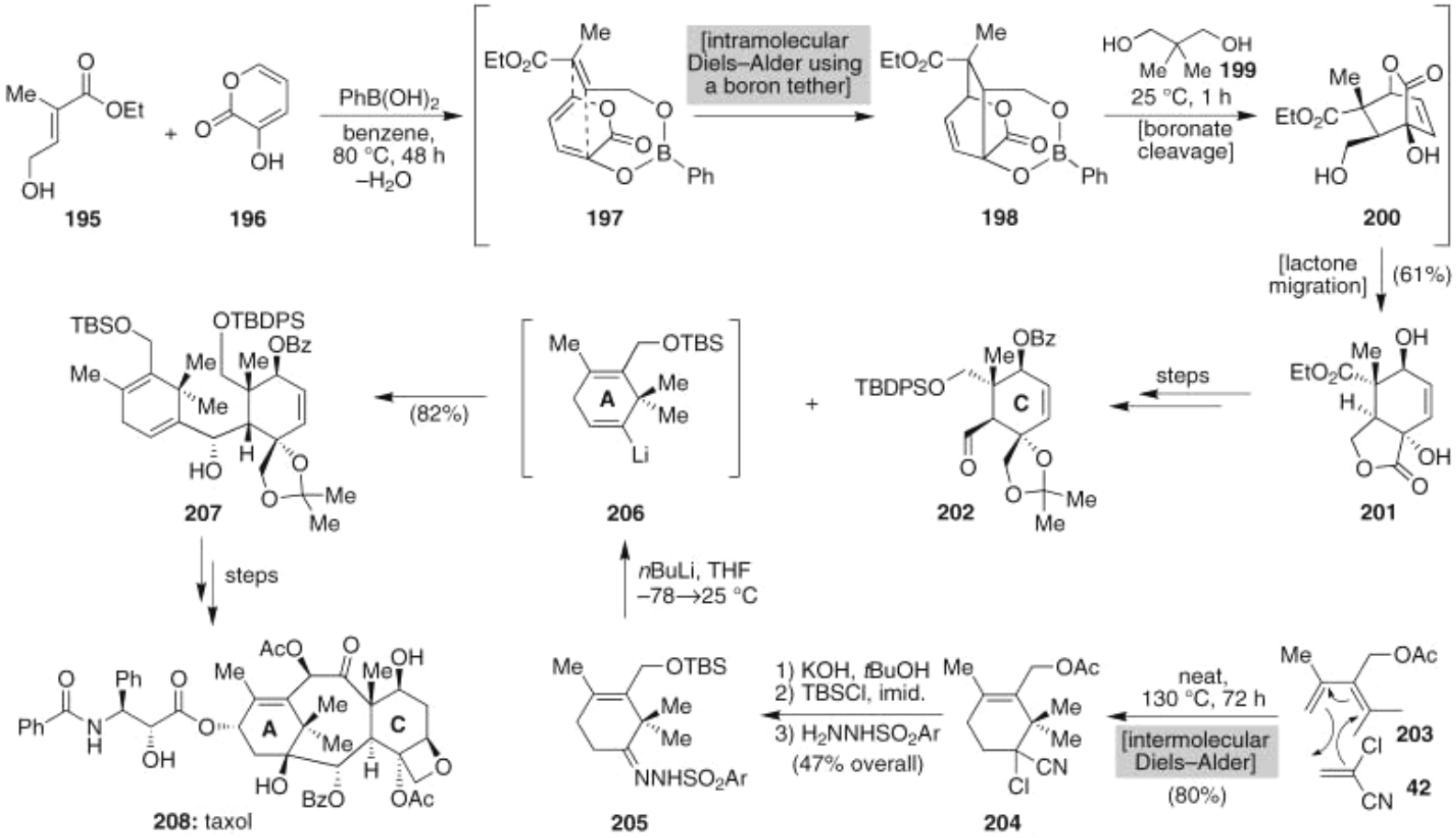
-Antiinflammatory.



The Diels Alder Reaction in Action

Taxol: (K. C. Nicolaou)

-Anticancer



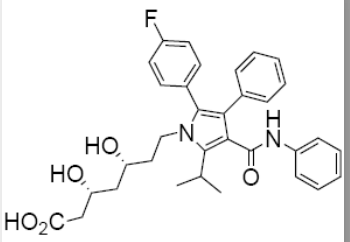
Aromaticity

Special resonance stability inherent in compounds with a specific arrangement of pi electrons.

-Includes benzene and related compounds.

-Aromatic compounds provide the foundation for a number of natural products and industrial chemicals.

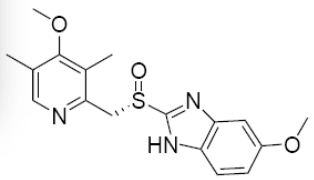
1 Lipitor



2005 Rank:	Company:	2006 Sales:
1	Pfizer	$6.58 Billion

Profile:
An HMG-CoA reductase inhibitor used to lower LDL cholesterol levels.

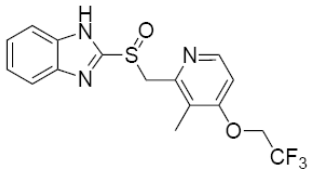
2 Nexium



2005 Rank:	Company:	2006 Sales:
2	Astra-Zeneca	$4.06 Billion

Profile:
A proton pump inhibitor used to treat heartburn and esophagitis.

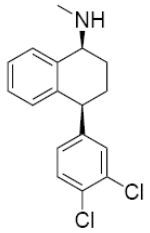
3 Prevacid



2005 Rank:	Company:	2006 Sales:
3	TAP	$3.31 Billion

Profile:
A proton pump inhibitor used to treat gastric reflux disease.

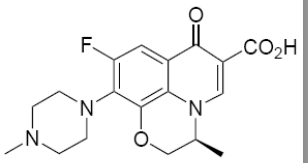
15 Zoloft



2005 Rank:	Company:	2006 Sales:
6	Pfizer	$1.77 Billion

Profile:
A selective serotonin reuptake inhibitor used to treat depression and OCD.

25 Levaquin



2005 Rank:	Company:	2006 Sales:
20	J & J	$1.41 Billion

Profile:
A fluoroquinolone antibiotic used to treat bacterial infections.

The Discovery of Benzene

Michael Faraday (1825)

-Isolated from the condensed gas of street lamps.

-Structure was unknown until 1866 when the accepted structure was initially proposed by Kekule

-Though it contains double bonds, benzene does not behave like many other alkenes.

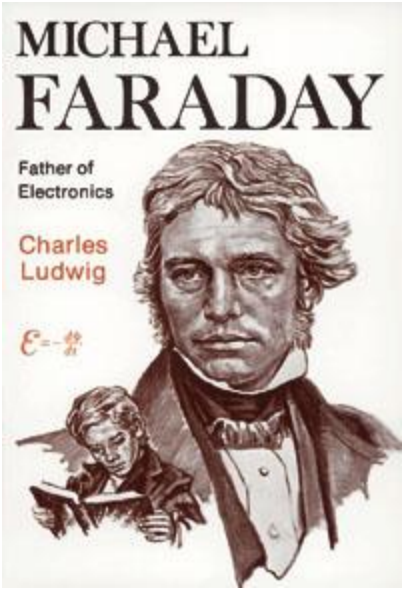
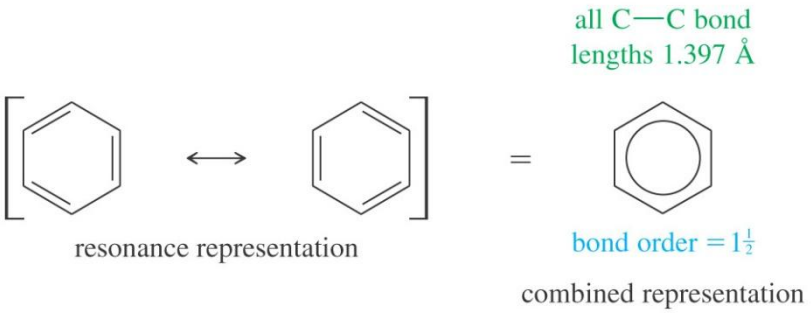
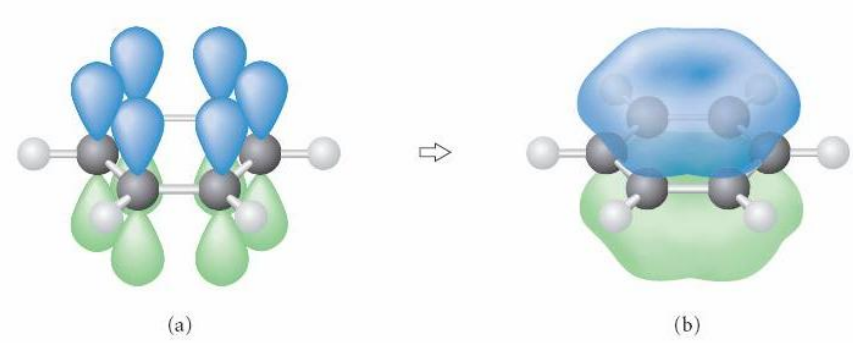


Photo credit: Google Images





Benzene Stability

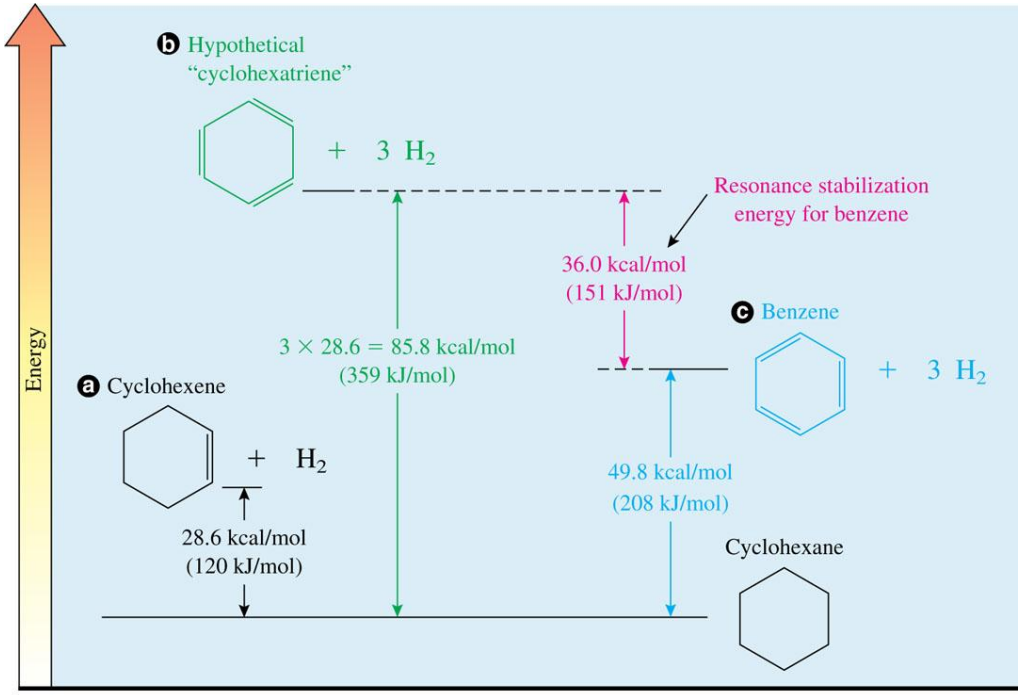
Benzene is resonance stabilized.

-The resonance energy of benzene can be calculated by comparing the energy liberated during a reaction of a benzene molecule and a similar, yet unconjugated system such as cyclohexene.

-Hydrogenation of cyclohexene produces 28.6kcal/mol of heat for one C=C.

-Hydrogenation of benzene is expected to produce 3 x 28.6kcal/mol or 85.8kcal/mol for three C=C.

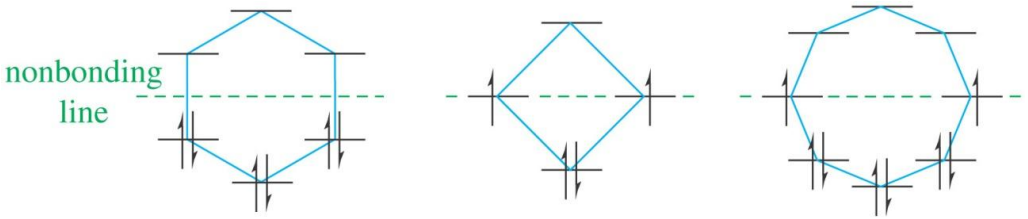
-Actually, only 49.8kcal/mol of energy is produced upon hydrogenation.



(Wade 6th ed.)

A Molecular Orbital Picture of Aromaticity

Considers the use of the polygon rule:



(Wade 6th ed.)

In general, all conjugated systems have one lowest energy molecular orbital, followed by pairs of degenerate molecular orbital at increasingly higher energies.

-If the total number of molecular orbital is even, then there is one highest energy molecular orbital (HOMO) and the molecular orbitals are arranged symmetrically about zero energy.

-Leads to a stable conjugated system that is considered aromatic if the HOMOs are completely filled.

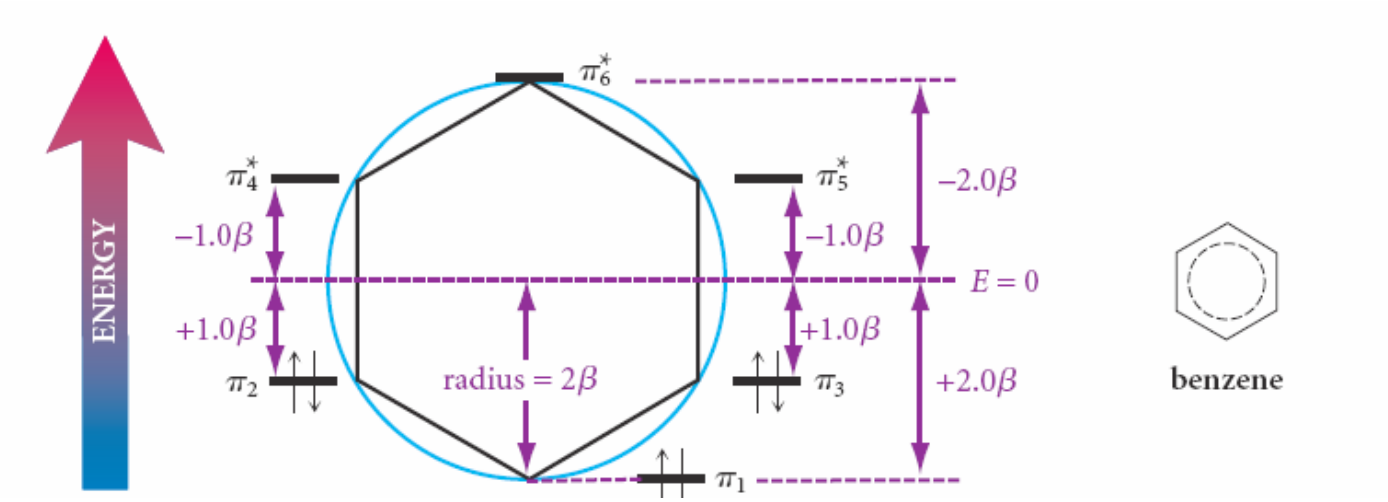
-Leads to conjugated system that is considered anitaromatic if the HOMOs are only half filled.

If the total number of molecular orbitals is odd, then the highest molecular orbital is absent, and the molecular orbitals are no longer symmetrical about zero energy.

-Leads to an unstable conjugated systems that is not considered aromatic.

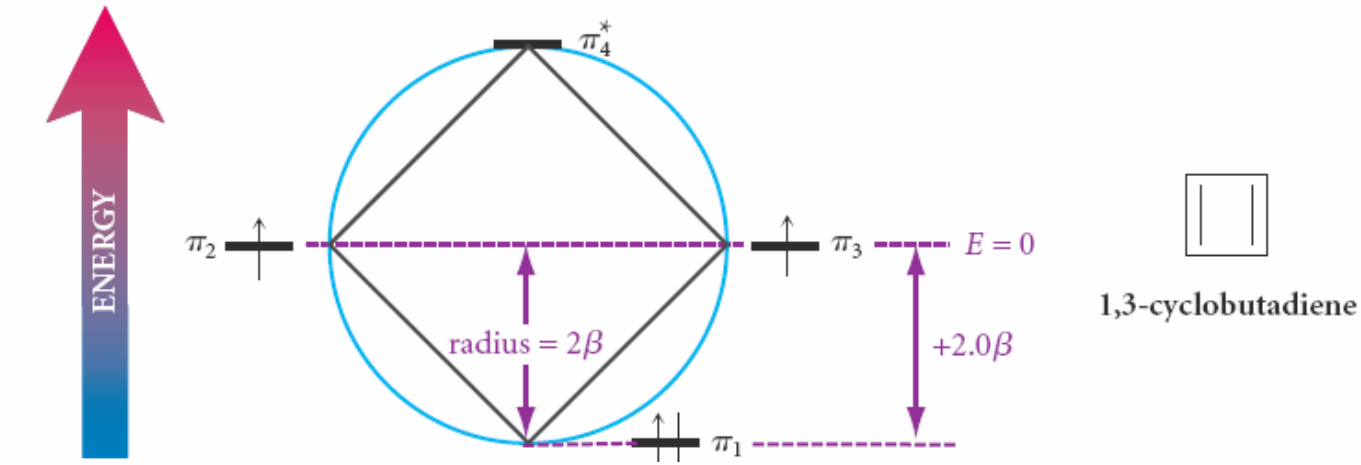
A Molecular Orbital Picture of Aromaticity

Benzene



A Molecular Orbital Picture of Aromaticity

Cyclobutadiene



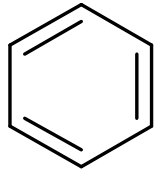
Huckles Rule

Mechanism for determining the aromaticity of a compound based on three rules:

-A compound is aromatic if:

1. The compound is a ring with a series of conjugated p orbitals.
2. The ring system is planar so that the p orbitals overlap in pi fashion, completely around the ring.
3. The system has 4n + 2 pi electrons.

Example: benzene.



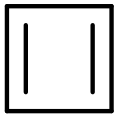
-If a compound does not exhibit aromaticity, it can be either aromatic or non aromatic.

Huckles Rule—Antiaromatic Compounds

If a compound is not considered aromatic, then it can be anitaromatic if the following criteria are met:

1. The compound is a ring with a series of conjugated p orbitals.
2. The ring system is planar so that the p orbitals overlap in pi fashion, completely around the ring.
3. The system has 4n pi electrons.

Example: cyclobutadiene.



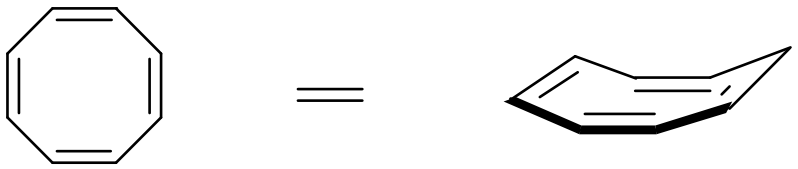
-Antiaromatic compounds are generally highly unstable.

Huckles Rule—Nonaromatic Compounds

If a compound is not considered aromatic, then it can be thought of as non aromatic if the following criteria are met:

1. The compound is a ring with or without a series of conjugated p orbitals.
2. The ring system is not planar.
3. The system has any number of pi electrons.

Example: cyclooctatetraene



-Nonaromatic compounds are generally stable and can be synthesized and readily isolated.

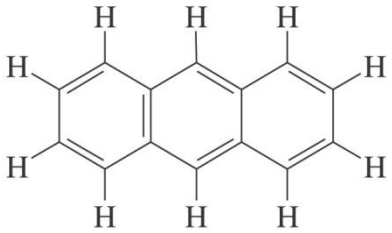
Rules for Polyaromatic Hydrocarbons

Carbon compounds composed of a number of conjugated systems.

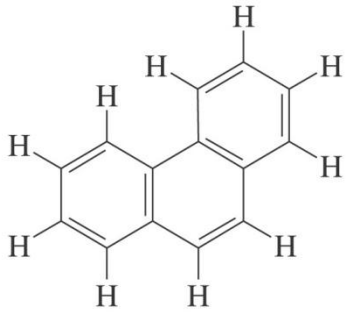
-Generally the systems are fused benzene rings.

-Can be aromatic if the criteria apply.

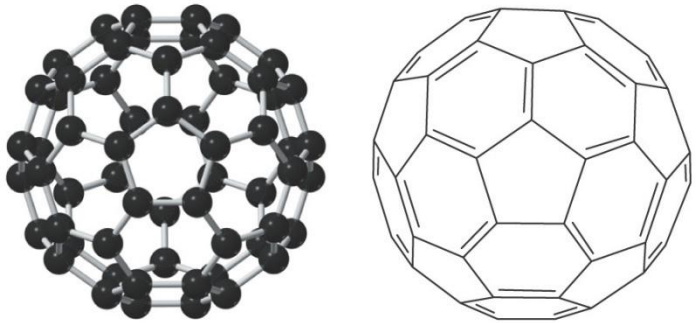
Examples:



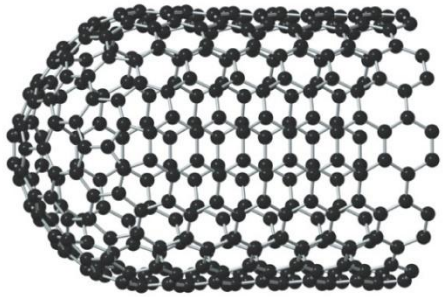
anthracene



phenanthrene



buckyball (C_{60})



carbon nanotube

(Wade 6th ed.)

Rules for Heterocyclic Compounds

Compounds that contain atoms other than carbon such as nitrogen, oxygen, and sulfur.

-Can be aromatic if:

1. The compound is a ring with a series of conjugated p orbitals.
2. The ring system is planar so that the p orbitals overlap in pi fashion, completely around the ring.
3. The system has 4n +2 pi electrons.

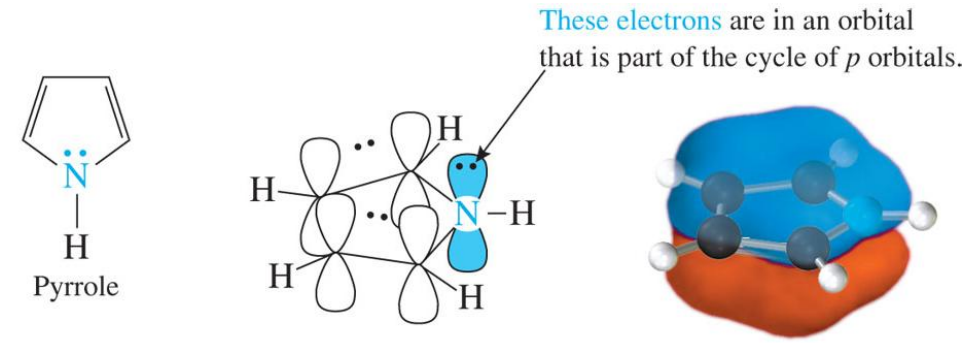
-Special considerations must be given to the heteroatom to determine how many electrons it contributes to the system.

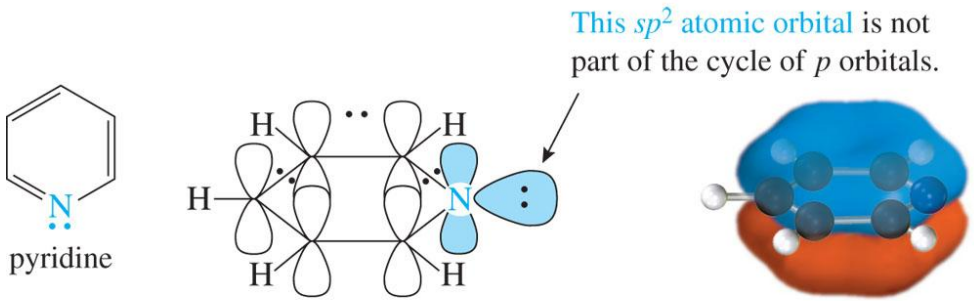
-Determine how many electrons are required to make the bond sp^2 hybridized.

-The remaining electrons count towards the pi system.

Rules for Heterocyclic Compounds

Examples: Pyrrole and pyridine





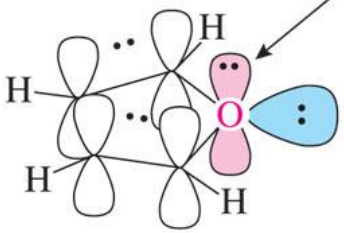
(Hornback 2nd edition)

Rules for Heterocyclic Compounds

Examples: Furan and thiophene

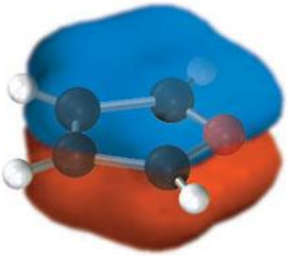
Furan

Thiophene



This orbital is part of the cycle of *p* orbitals, so these two electrons are counted as part of the pi system.

This orbital is perpendicular to the cycle of *p* orbitals, so these two electrons are not counted as part of the pi system.



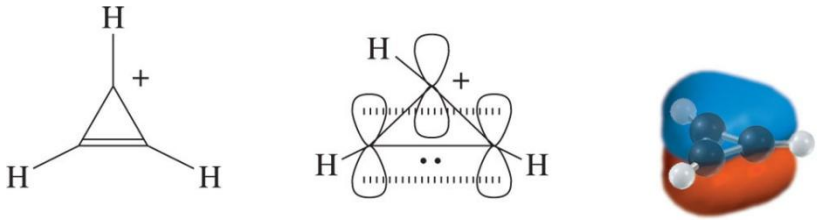
Rules for Ionic Compounds

Ions can be aromatic if they fit the criteria.

-The trick in determining aromaticity is to consider the contribution the charge makes to the overall pi system.

-In general, positive charges contribute no pi electrons to the conjugates system.

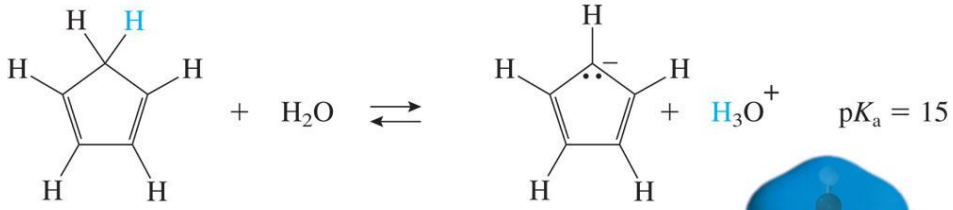
Example: cyclopropenyl carbocation.



The cyclopropenyl carbocation
(Hornback 2nd edition)

-Negative charges contribute two pi electrons to the conjugated system.

Example: cyclopentadiene anion.



Cyclopentadiene

The aromatic cyclopentadienyl anion

(Hornback 2nd edition)

NMR of Aromatic Compounds

Hydrogen's located on aromatic systems generally appear downfield (around 7-8ppm) in comparison to alkenes.

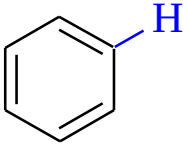
-Occurs because of a ring current.

-Exhibit a paramagnetic ring current.

-Circulating electrons generate a magnetic field that is opposed to the external magnetic field in the center of the ring and parallel to the external magnetic field outside of the ring and in the region where the hydrogens are located.

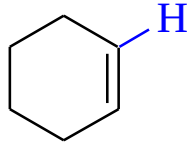
-Ring current exhibited by aromatic compounds is termed diamagnetic.

Example: Benzene



7-8ppm

vs



5-6ppm

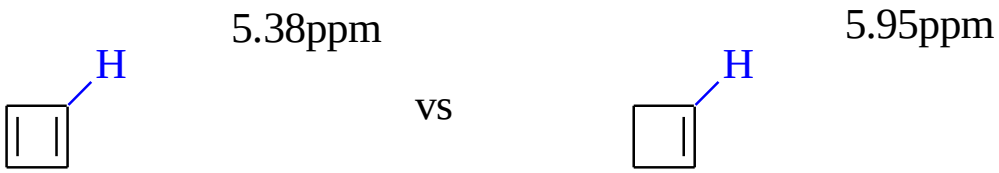
NMR of Anitaromatic Compounds

Hydrogens located on anitaromatic systems generally appear upfield in comparison to alkenes.

-Also exhibit a ring current.

-Ring current is paramagnetic in nature.

Example: Cyclobutadiene

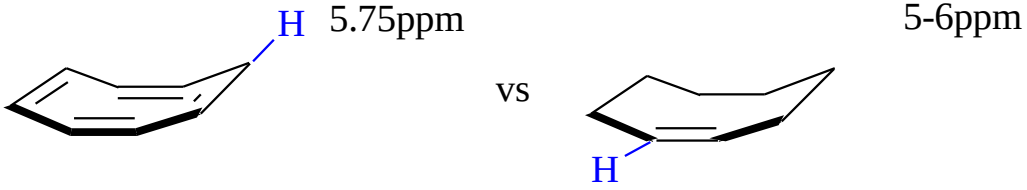


NMR of Nonaromatic Compounds

Hydrogen's located on the double bonds of non aromatic compounds have chemical shifts representative of an alkene.

-No ring current exists.

Example: Cyclooctatetraene

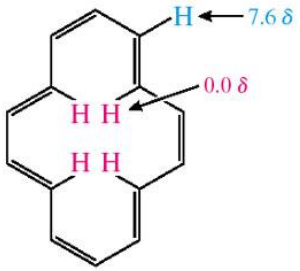


Annulenes

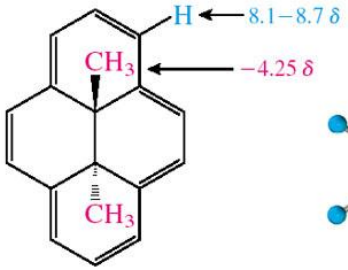
Rings that contain alternating single and double bonds in a single Lewis structure.

-Can be aromatic if the criteria fit.

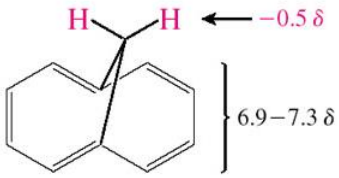
Examples:



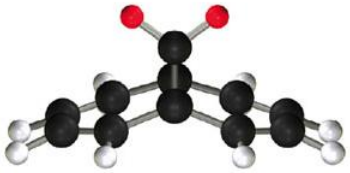
[14]Annulene

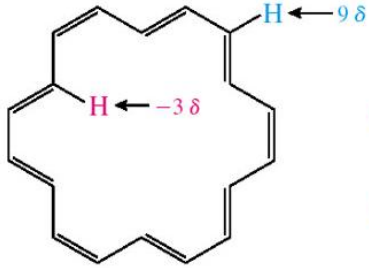


A bridged [14]annulene



A bridged [10]annulene

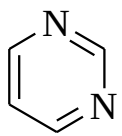




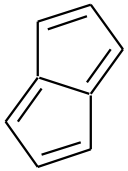
[18]Annulene

(Hornback 2nd edition)

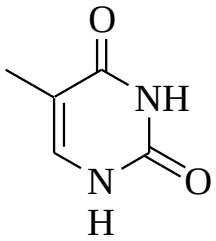
Aromatic, Antiaromatic, or Nonaromatic?



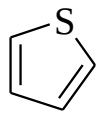
A



B



C



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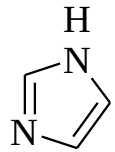

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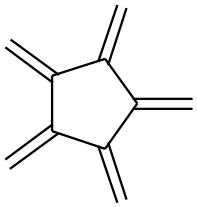

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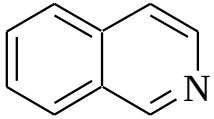

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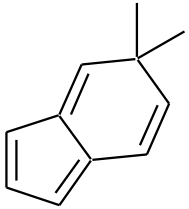

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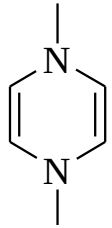

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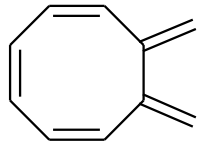

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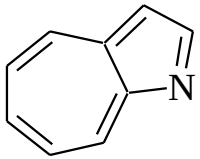
Aromatic, Antiaromatic, or Nonaromatic?



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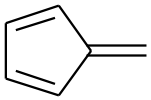

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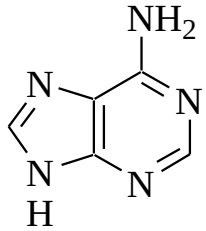

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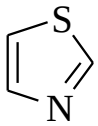

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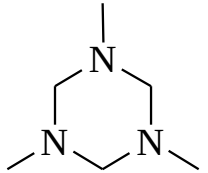

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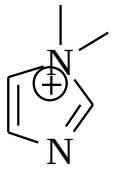

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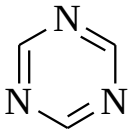

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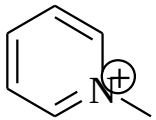

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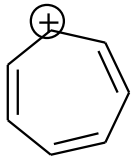

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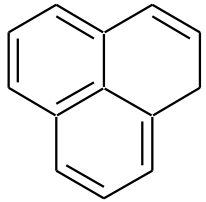

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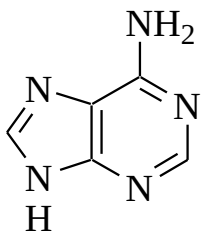
Revisiting Acid-Base Character

Determine the most acidic proton and basic nitrogen on the molecules below:



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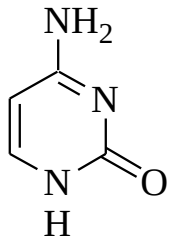

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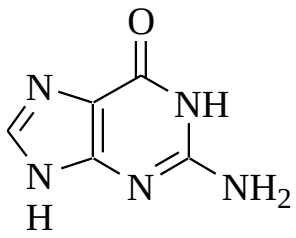

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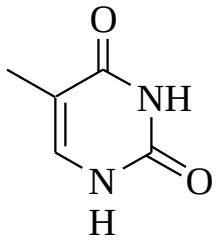

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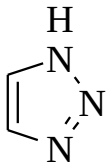

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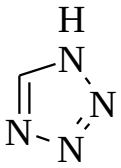

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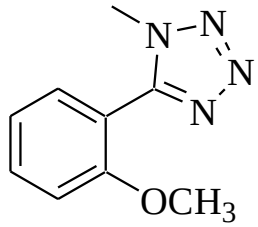

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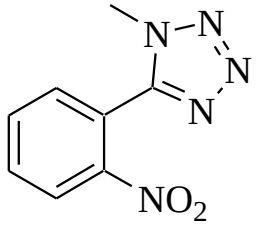

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Chapter 15—Conjugates Systems and Aromatic Compounds

I. Conjugated *vs* Isolated *vs* Cumulated Systems

A. Conjugated systems- systems where double bonds, separated by a single bond, can interact with each other.

-Example: 1,3 pentadiene

B. Isolated systems- systems where double bonds are separated by more than one single bond and can no longer interact with one another.

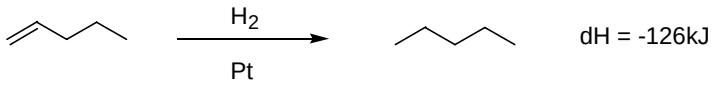
-Example: 1,4 pentadiene

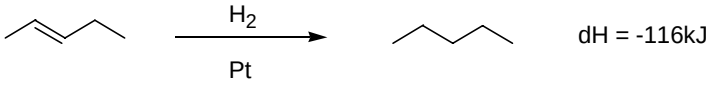
C. Cumulated systems- systems where carbon-carbon double bonds are directly next to one another.

-Example: allene

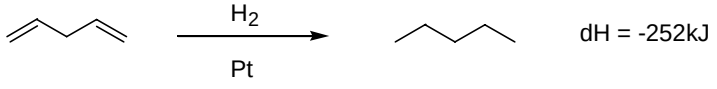
D. Stability- heats of hydrogenation are used to compare the relative stabilities of alkenes.

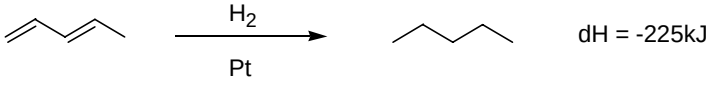
-Examples: monoalkenes





-Examples: dienes





-Conjugated systems are more stable due to resonance energy.

-Summary (Table 15.1)

II. The Molecular Orbital Picture of a Conjugated System

A. Structure and bonding of 1,3 butadiene
-The electrons in the pi system of 1,3 butadiene are delocalized over there bonds instead of two.
-Example: (Figure p. 678, 680)

B. A molecular orbital picture of 1,3 butadiene
-General points to keep in mind;
1. Each p orbital consists of two lobes with opposite phases of the wave function.
2. The orbital's can be arranged in a number of different ways, but the number of pi molecular orbital's must always equal the number of p orbital's in the system.
3. Each molecular orbital can hold two electrons.
4. Most stable molecules have filled bonding molecular orbital's and empty antibonding molecular orbital's.

-1,3 butadiene (Figure 15-1)

-In the planar conformation, 1,3-butadiene can exist as two unique conformers, s-cis or s-trans.
-Example: s-cis *vs* s-trans (Figure 15.2)

-s-trans is more stable due to steric interactions.
-s-trans and s-cis are able to interconvert at room temperature.

III. Allylic Cation's, Anion's and Radical's

Allylic group- molecules that contain allyl groups consisting of a functional group alpha to a carbon-carbon double bond that is usually terminal.
-Examples: allyl bromide, allyl alcohol, allyl benzene
-Cation's, anion's, and radical's generated from allyl substrates are stabilized through resonance.
-General comparison: (Equation 15-20)

-Allylic carbocations are actually about as stable as secondary carbocations.
-Allylic radicals are more stable than tertiary radicals.

-Example: allylic cation (Figure 15-9, p. 704)

IV. Addition Reaction to Conjugated Dienes

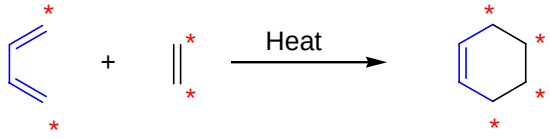
A. Types of Addition Reactions- reactions with conjugated dienes may be 1,2 or 1,4 in nature.
- -1,2 addition- occurs when an electrophile adds across a carbon-carbon double bond.
  - -Example: reaction of 1,3 butadiene with HBr

- -1,4 addition- addition across a conjugated system.
  - -Example: reaction of 1,3 butadiene with HBr

B. Control of 1,2 and 1,4 addition reactions- whether or not a 1,2 or 1,4 addition reaction occurs depends on the temperature of the system.
- -1,2 addition reactions predominate at lower temperatures (the products are said to be the kinetic products).
  - -The 1,2 addition reaction is faster due to a lower activation energy.
  - -Lower temperatures also limit the number of collisions that take place.
- -1,4 addition reactions predominate at higher temperatures (the products are said to be the thermodynamic products).
  - -The 1,4 addition reaction is slower due to a higher activation energy.
  - -The reaction takes place because molecular collisions are more likely and the reverse reaction is likely to occur.
- -Example:

V. The Diels Alder Reaction

A. General Reaction- 4 + 2 cycloaddition reaction between an electron rich diene and an electron poor alkene or alkyne known as a dienophile.



B. Mechanism

C. Considerations

-The reaction is concerted (occurs in one step).

-The transition state requires and appropriate geometry.

-The p orbital's of the diene must be able to overlap with the p orbital's of the dienophile.

-In order for this to occur, the highest occupied molecular orbital (HOMO) of the diene reacts with the lowest unoccupied orbital (LUMO) of the dienophile so that the electrons may flow smoothly from one molecule to the other (Woodward-Hoffman rules).

-The diene must be in an s-cis conformation from proper overlap.

-Example: (Figure 15-8)

-The addition is syn in nature.

-Example:

-Dienophile's containing pi bonds show considerable secondary overlap with the electron rich diene in the transition state resulting primarily in endo products.

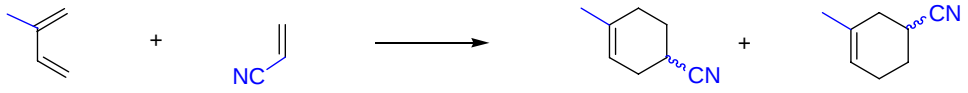
-Example:

-When unsymmetrical reagents are used, mixtures of products are formed, but one tends to predominate.

-The major product is based on the electronics of the dienophile.

-For internally substituted dienes, the 1,4 product predominates.

-Example:



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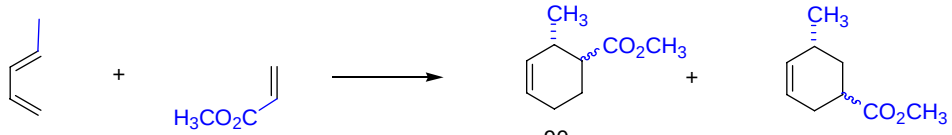
-Key: Think of how the electrons flow.

-For terminally substituted dienes, the 1,3 product predominates.

-Example:



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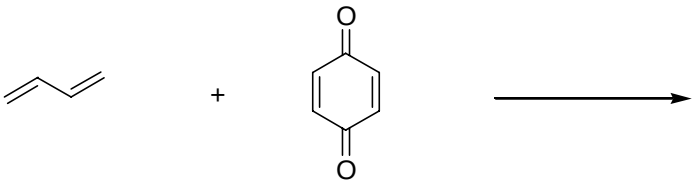
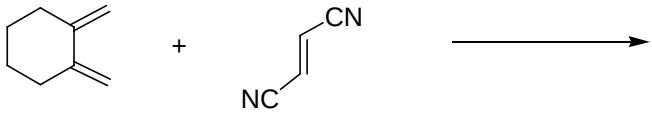
-Key: Think of how the electrons flow.

Practice Problems:

1. 

2. 

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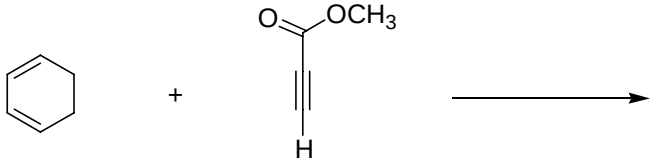
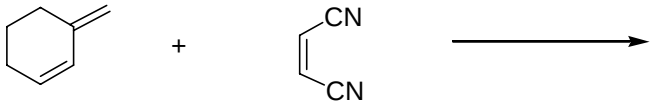
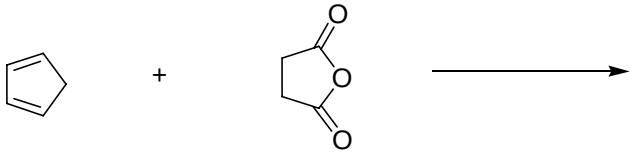
4. 

5. 

6. 

VI. Aromatic Compounds

A. Benzene

-Isolated by Michael Faraday (1825) from the condensed gas of street lamps.

-Structure was unknown until 1866 when the accepted structure was initially proposed by Kekule:

-Though it contains double bonds, benzene does not behave like many other alkenes.

-Benzene is resonance stabilized.

-The resonance energy of benzene can be calculated by comparing the energy liberated during a reaction of a benzene molecule and a similar, yet unconjugated system such as cyclohexene.

-Hydrogenation of cyclohexene produces 28.6kcal/mol of heat for one C=C.

-Hydrogenation of benzene is expected to produce 3 x 28.6kcal/mol or 85.8kcal/mol for three C=C.

-Actually, only 49.8kcal/mol of energy is produced upon hydrogenation.

B. Molecular Orbital Picture of Aromaticity

-Considers the use of the polygon rule:

-In general, all conjugated systems have one lowest energy molecular orbital, followed by pairs of degenerate molecular orbital at increasingly higher energies.

-If the total number of molecular orbital is even, then there is one highest energy molecular orbital (HOMO) and the molecular orbitals are arranged symmetrically about zero energy.

-Leads to a stable conjugated system that is considered aromatic if the HOMOs are completely filled.

-Leads to conjugated system that is considered anitaromatic if the HOMOs are only half filled.

-If the total number of molecular orbitals is odd, then the highest molecular orbital is absent, and the molecular orbitals are no longer symmetrical about zero energy.

-Leads to an unstable conjugated systems that is not considered aromatic.

-Example: Benzene

-Example: Cylobutadiene

C. Huckles Rule- mechanism for determining the aromaticity of a compound based a set of rules.

AROMATIC -a compound is aromatic if:

1. The compound is a ring with a series of conjugated p orbitals.
2. The ring system is planar so that the p orbitals overlap in pi fashion, completely around the ring.
3. The system has 4n + 2 pi electrons.

-Example: benzene.

-If a compound does not exhibit aromaticity, it can be either aromatic or non aromatic.

ANITAROMATIC-a compound is anitaromatic if:

1. The compound is a ring with a series of conjugated p orbitals.
2. The ring system is planar so that the p orbitals overlap in pi fashion, completely around the ring.
3. The system has 4n pi electrons.

-Antiaromatic compounds are generally highly unstable.

Example: cyclobutadiene.

NONAROMATIC-a compound is non aromatic if:

1. The compound is a ring with or without a series of conjugated p orbitals.
2. The ring system is not planar.
3. The system has any number of pi electrons.

-Nonaromatic compounds are generally stable and can be synthesized and readily isolated.

Example: cyclooctatetraene

HETEROAROMATIC SYSTEMS -compounds that contain atoms other than carbon such as nitrogen, oxygen, and sulfur can be aromatic if:

1. The compound is a ring with a series of conjugated p orbitals.
2. The ring system is planar so that the p orbitals overlap in pi fashion, completely around the ring.
3. The system has 4n +2 pi electrons.

-NOTE: Special considerations must be given to the heteroatom to determine how many electrons it contributes to the system.

-Determine how many electrons are required to make the bond sp^2 hybridized.

-The remaining electrons count towards the pi system.

-Examples: pyridine, pyrrole, furan, thiophene

IONS-ions can be aromatic if they fit the criteria.

-The trick in determining aromaticity is to consider the contribution the charge makes to the overall pi system.

-In general, positive charges contribute no pi electrons to the conjugates system.

-Example: cyclopropenyl carbocation

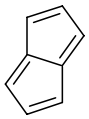
-Negative charges contribute two pi electrons to the conjugated system.

-Example: cyclopentadiene anion

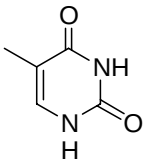
Practice Problems:

Determine whether the following compounds are aromatic, antiaromatic, or nonaromatic.

A

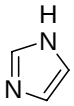


B

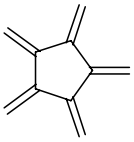


C

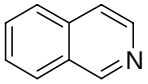
D



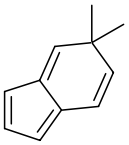
E



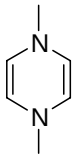
F



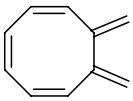
G



H



I

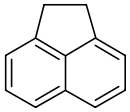


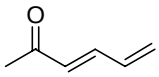
J

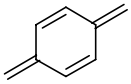
Chemistry 255
Fall 2009
Problem Set 1
Due Date: Wednesday, September 09, 2009

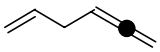
Name:_____

1. Determine whether each of the following molecules contains isolated, conjugated, or cumulated double bonds.

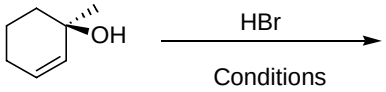
a. 

b. 

c. 

d. 

2. Answer the following questions with respect to the reaction illustrated below between 1-methylcyclohex-2-en-1 and HBr



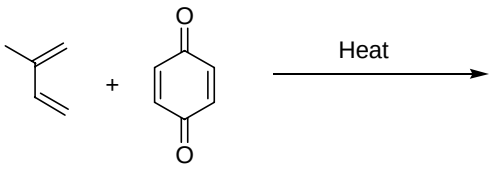
a. Provide a mechanism and draw the major product (the kinetic product) for this reaction when it is conducted at temperatures near -80C.

b. Provide a mechanism and draw the major product (the thermodynamic product) for this reaction when it is conducted at temperatures near 40C.

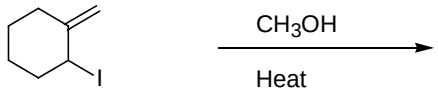
c. Why does the kinetic product predominate at -80C and the thermodynamic predominate at 40C?

3. Provide products for each of the following reactions. Pay careful attention to stereochemistry.

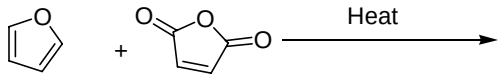
a.



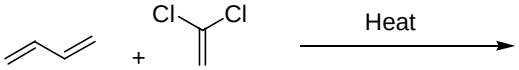
b.



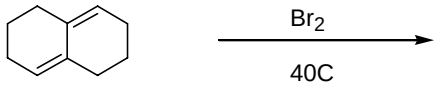
c.



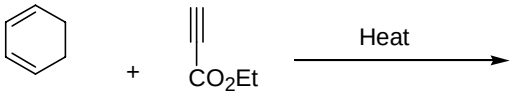
d.



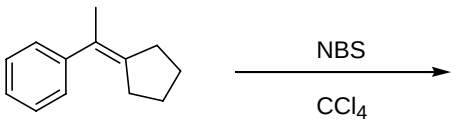
e.



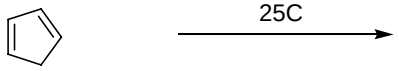
f.



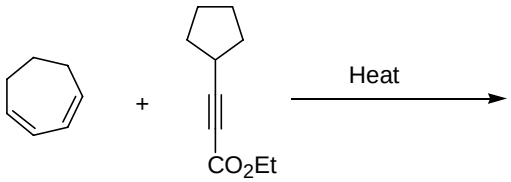
g.



h.

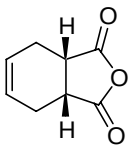


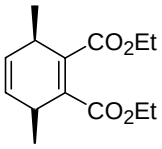
i.

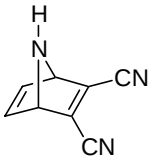


j.

4. What diene and dienophile would be used to prepare the following products below. Pay careful attention to the electronics of the systems you choose.

a. 

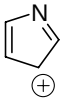
b. 

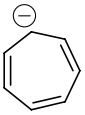
c. 

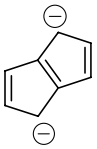
5. Determine whether each of the following compounds is aromatic (A), antiaromatic (AA) or nonaromatic (NA).

a. 

b. 

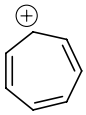
c. 

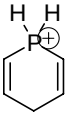
d. 

e. 

f. 

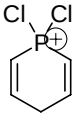
g. 

h. 

i. 

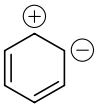
j. 

k. 

l. 

m. 

n. 

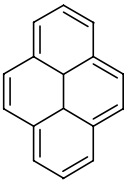
o. 

p. 

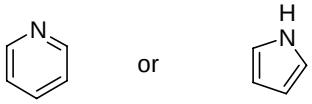
q. 

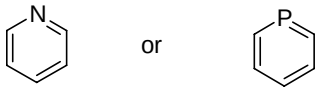
r. 

s. 

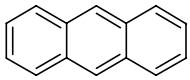
t. 

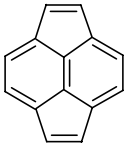
6. Indicate which compound is a stronger base and explain your answer.

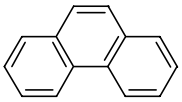
a. 

b. 

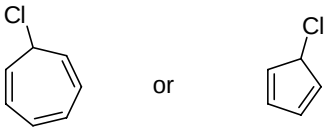
7. For each of the following compounds, which bond is most susceptible to addition of Br_2 in CH_2Cl_2? Briefly explain your answer.

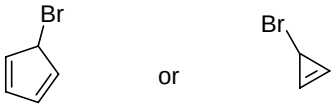
a. 

b. 

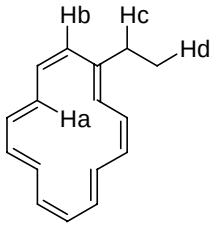
c. 

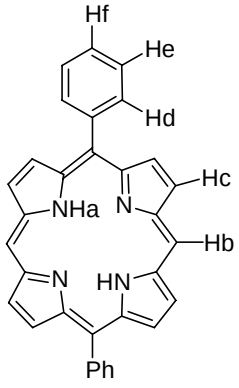
8. Which compound would occur faster in an SN1 reaction? Explain your answer.

a. 

b. 

9. List the protons highlighted on the molecule below in order of highest to lowest chemical shift. Briefly explain your answer.

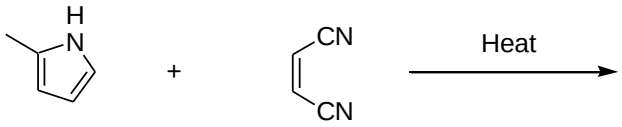
a. 

b. 

Quiz Two
Diels Alder

Name:_____. Score:_____/10

Provide the major product for the reaction below. Pay special attention to stereochemistry.



Chemistry 255
Exam One
Fall 2008

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		5
3		5
4		30
5		10
6		10
7		15
8		5
9		10

There are fourteen pages in this exam including this cover page, a periodic table and three pages of stretching frequencies, chemical shifts, and a table of coupling constants. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

Good Luck ☺

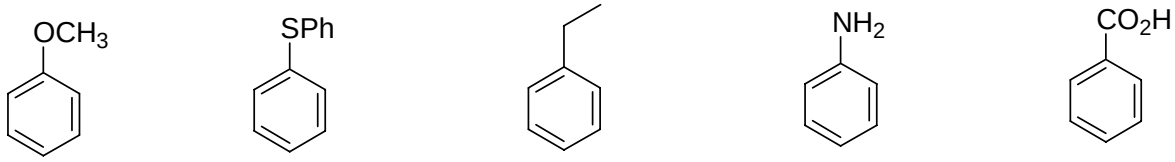
Oh yeah…..and DU = (2C-H-X+N+2)/2

Patience and perseverance have a magical effect before which difficulties disappear and obstacles vanish.

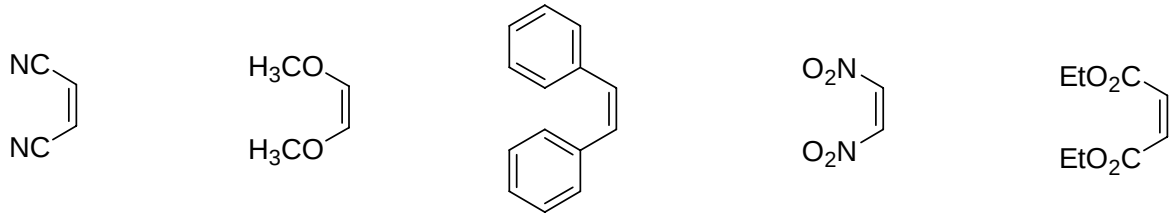
-John Quincy Adams

1. (10 points) In each case circle the correct answer(s). A half of a point will be deducted for each extra answer.

a. Circle the molecule that would react slowest when treated with HNO_3 in H_2SO_4.

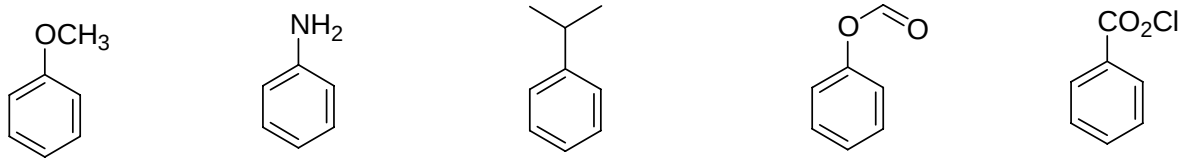


b. Cirlce the dieneophile that would react the fastest in a Diels Alder reaction.

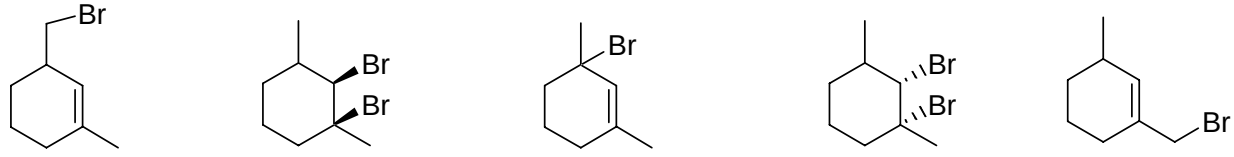


c. Circle the substate that will react the fastest in an SN1 reaction.

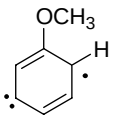
d. Circle starting materials that will undergo a Friedel Crafts acylation.



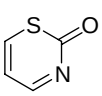
e. Circle the major product formed when 1,3 dimethylcylohexene is brominated with NBS.



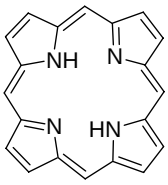
2. (5 points) Determine whether each of the following compounds is aromatic, anitaromatic, or nonaromatic.



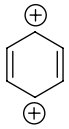
a



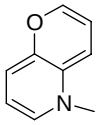
b



c



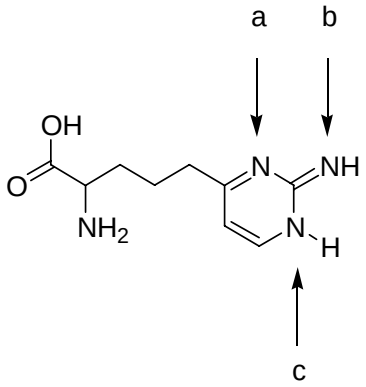
d



e

Compound	Type
A	
B	
C	
D	
E	

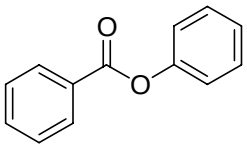
3. (5 points) For the unusual amino acid derivative shown below rank the nitrogen's (a, b, or c) in order of increasing basicity. Briefly explain your answer.



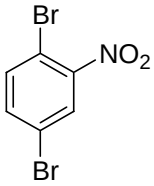
Least Basic----------Most Basic

____ < _____ < _____

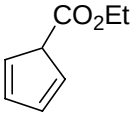
4. (30 points) In each case below, give the major product of the reaction. If there is no reaction write N.R.

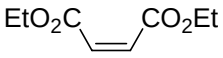
a. 

$\xrightarrow[AlCl_3]{CH_3CH_2Cl}$

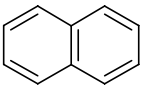
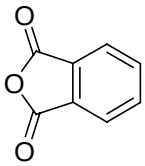
b. 

$\xrightarrow[(1eq)]{NaOH,\ H_2O}$

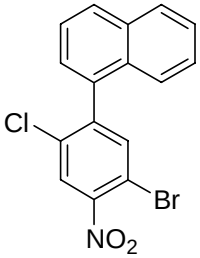
c. 

+ 

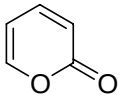
$\xrightarrow[2.\ O_3]{1.\ Heat}$
$\xrightarrow{3.\ H_2O_2}$

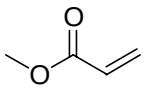
d. 
+ 

$\xrightarrow{1.\ AlCl_3}$
$\xrightarrow{2.\ H_2SO_4\ (dilute)}$

e. 

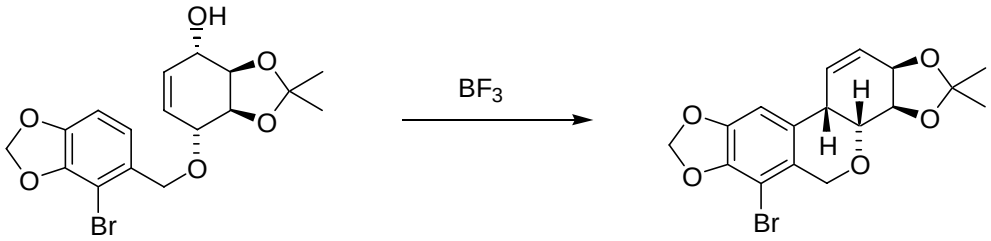
$\xrightarrow{1.\ NaNH_2\ (1eq)}$
$\xrightarrow{2.\ HONO}$
$\xrightarrow{3.\ CuCN}$

f. 

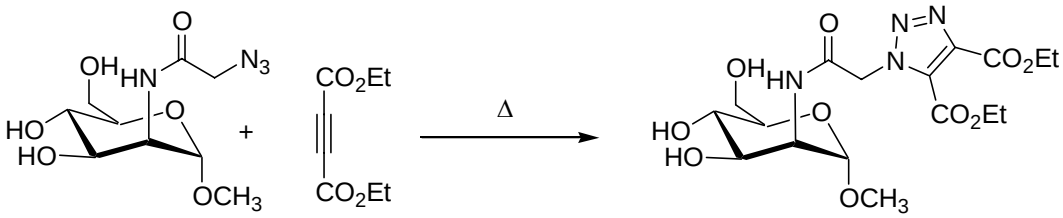


$\xrightarrow{Heat}$

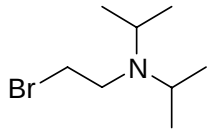
5. (10 points) Electrophilic cyclizations have been used in key steps in the synthesis of steroid. Below is one example where molecule A, upon being treated with boron trifluoride, is converted to molecule B which can then be used to access the final steroid product after several additional reactions. Show a complete mechanism for the formation of B from A, and briefly describe the stereochemistry of the reaction.



6. (10 points) The 1,3 dipolar cycloaddition reaction between an azide and an alkyne to form a 1,2,3-triazole (known as “click chemistry”) has become an important reaction in synthetic organic chemistry. The products of the reaction are stable under physiological conditions and have recently been used to label glycan structures on the surface of diseased cells. Below is an illustrated example of the reaction of an azido sugar derived from mannose and dicarboxyethyl acetylene. Provide a mechanism for the reaction. Hint: You will need to draw the Lewis structure for the azide and follow the electron flow.

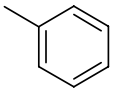


7. (15 points) Detrol LA is a drug marketed by Pfizer that is used to treat overactive bladders. Provide a complete synthesis of Detrol LA using (2-bromo-ethyl)-diisopropyl-amine, toluene, and any other starting materials. Note: This can be done in five to seven steps (or more).

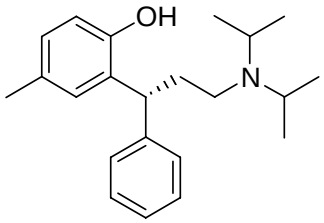


(2-Bromo-ethyl)-diisopropyl amine

and

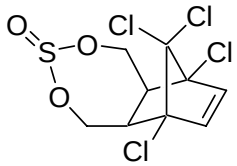


Toluene



Detrol-LA

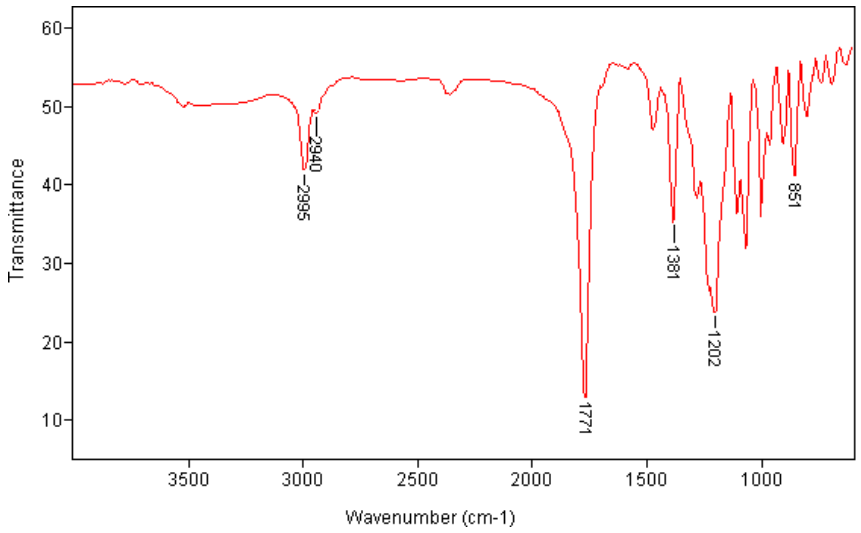
8. (5 points) Endosulfan (shown below) is a pesticide that has been linked to a number of autoimmune disorders. Provide the diene and dienophile that would use in the final step of the synthesis of endosulfan.

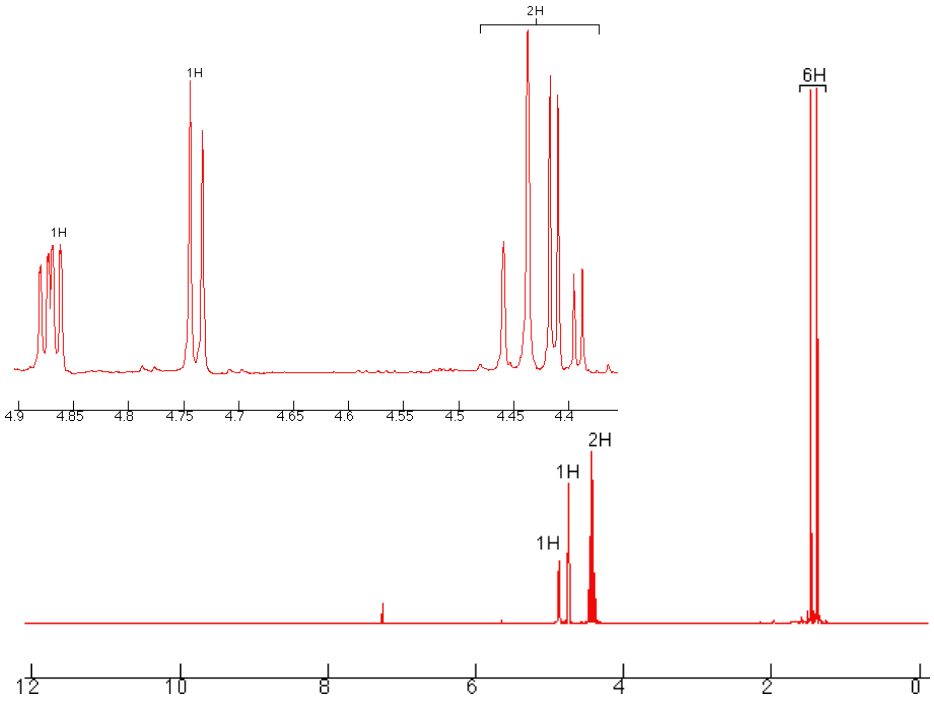


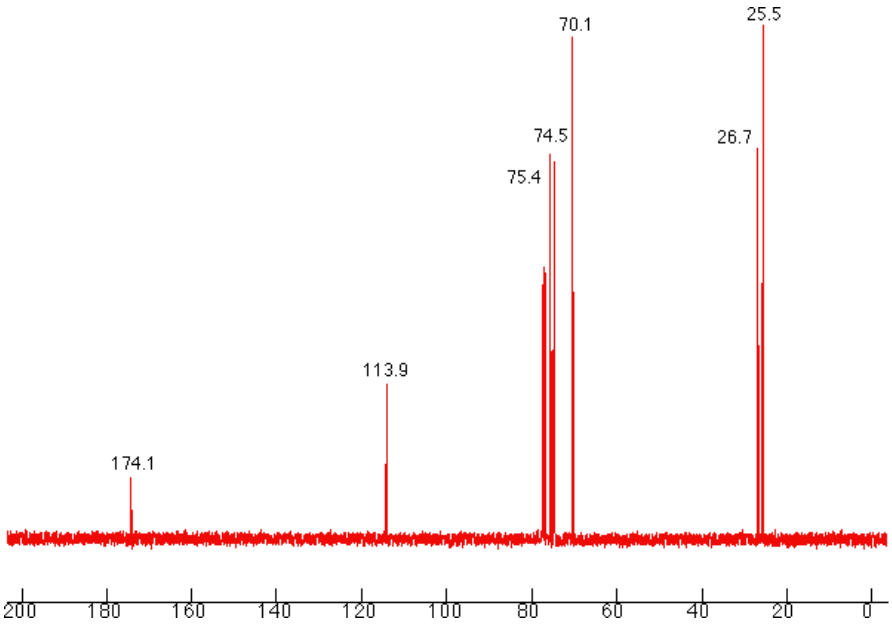
Dienophile

Diene

9. (10 points) Given the following spectral data, provide a reasonable structure or a molecule with the molecular formula $C_7H_{10}O_4$. Be sure to give each spectral component a complete treatment, and provide a reasonable explanation as to how you used the data to arrive at your final answer. If no work is shown, no credit will be given. Place your final answer in the box provided.

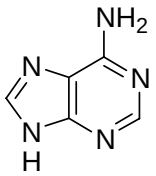


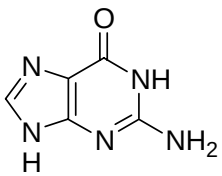


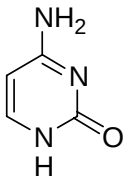


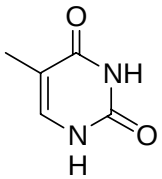
Answer:

BONUS (5 points): Provide names for the common bases below. You must get all of the bases correct to get full credit.

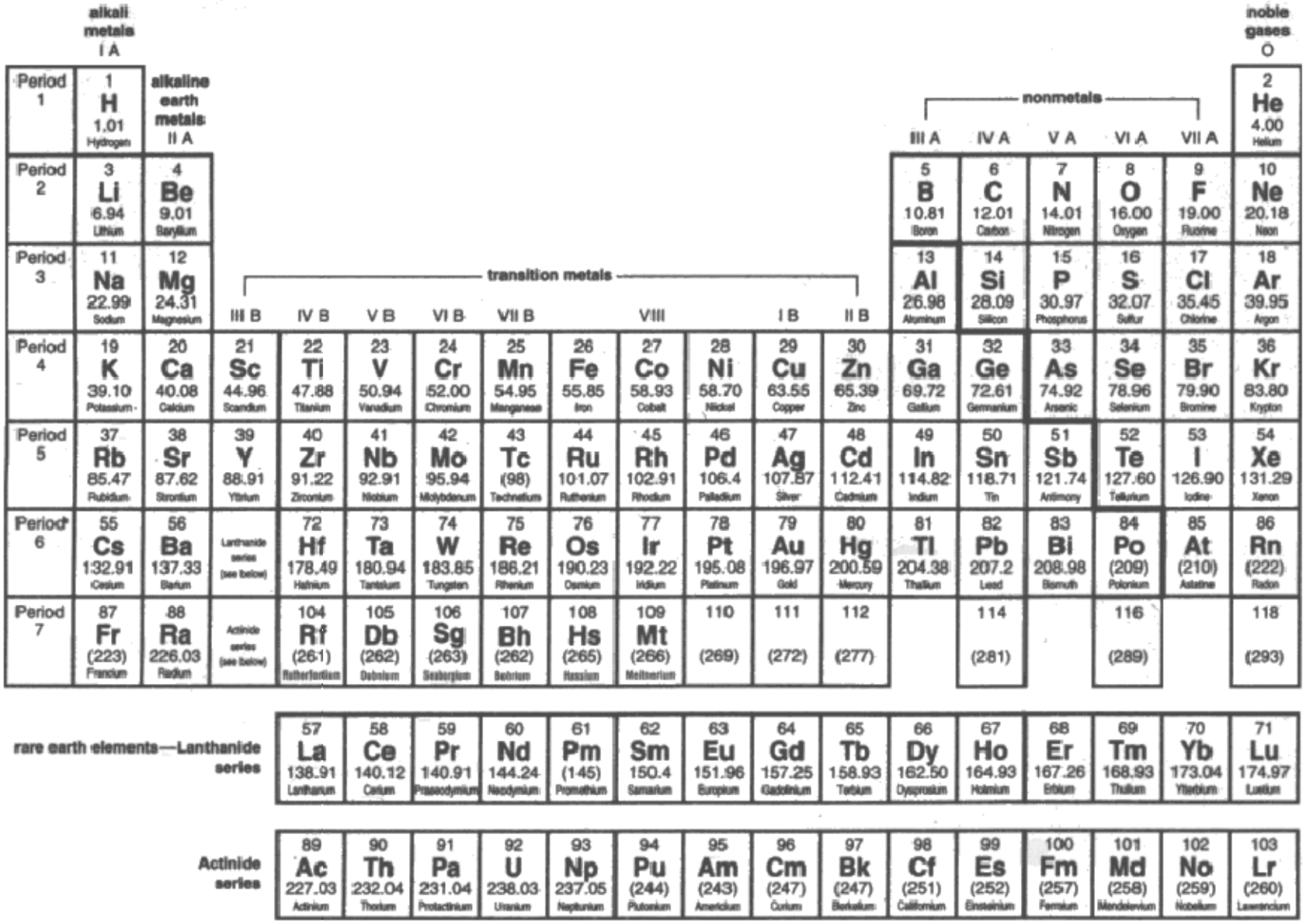




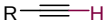
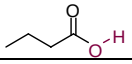
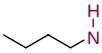
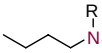
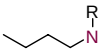
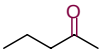
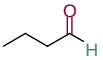
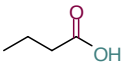
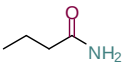
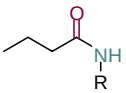
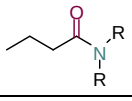
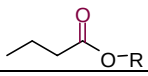
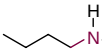
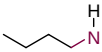
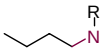
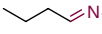




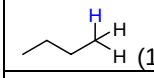
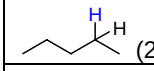
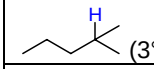
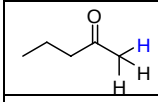
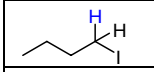
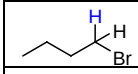
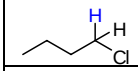
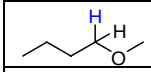
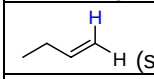
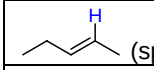
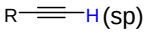
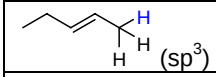
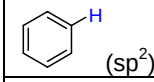
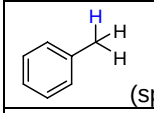
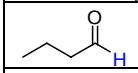
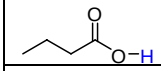
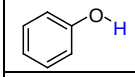
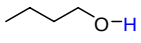
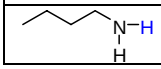
_____ _____ _____ _____


alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
III A
IV A
V A
VI A
VII A
noble gases 0
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
III B
IV B
V B
VI B
VII B
VIII
I B
II B
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

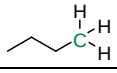
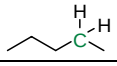
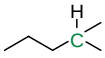
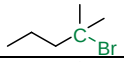
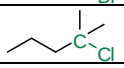
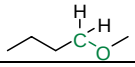
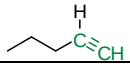
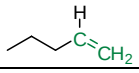
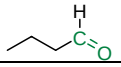
Infrared Stretching Frequencies

Category	Type of Bond	$\tilde{\nu}$ (cm^{-1})	Notes
carbon-carbon		1200	
	 (isolated)	1640-1680	
	 (conjugated)	1620-1640	
	 (aromatic)	~1600	
	 (terminal)	2100-2200	
	 (internal)	2100-2200	may be weak or absent
carbon-hydrogen	 (sp^3)	2800-3000	
	 (sp^2)	3000-3100	
	 (sp)	3300	
oxygen-hydrogen	 (alcohol)	3300	broad
		3000	broad
nitrogen-hydrogen	 (1°)	3300	two sharp spikes
	 (2°)	3300	one sharp spike
	 (3°)	3300	may be weak or absent
carbon-oxygen		1710	may appear ~1785 if strained
		1710	will also see peaks at 2700 and 2800 for the aldehyde C-H stretching
		1710	will also see peaks between 2500-3500 for the O-H stretching
	 (conjugated)	1685-1690	will also see peaks for the R group.
	 (1°)	1640-1680	will also see two spikes around 3300 for the NH stretching
	 (2°)	1640-1680	will also see one spike around 3300 for the NH stretching
	 (3°)	1640-1680	
		~1735	
		~1200	
carbon-nitrogen	 (1°)	1200	will also see two spikes around 3300 for the NH stretching
	 (2°)	1200	will also see one spike around 3300 for the NH stretching
	 (3°)	1200	
		1660	will also see one spike around 3300 for the NH stretching
		1660	
		>2200	

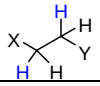
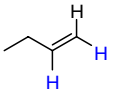
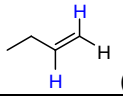
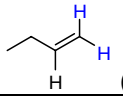
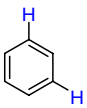
Characteristic ^{1}H NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0.8-1.0	
 (2°)	1.2-1.4	
 (3°)	1.4-1.7	
	2.1	
	3.1-3.3	
	3.4-3.6	
	3.6-3.8	
	3.3-4.0	
 (sp^2)	4.6-5.0	
 (sp^2)	5.2-5.7	
 (sp)	2.5	
 (sp^3)	1.7	
 (sp^2)	6.0-9.5	
 (sp^3)	2.2-2.5	
	9-10	
	10-13	Can be broad and may exchange
	4.5-7.7	Can be broad and may exchange
	0.5-6.0	Can be broad and may exchange
	1.0-5.0	Can be broad and may exchange

Characteristic ^{13}C NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0-35	
 (2°)	0-35	
 (3°)	0-35	
	30-70	
	30-70	
	85-90	
	75-100	
	100-150	
 (sp^2 aromatic)	115-150	
	165-210	May take a while to relax (signals may be weak)

Coupling Constant Values

Type of coupling	J-value (Hz)	Notes
	2-12 (7)	The actual J value depends on the dihedral angle and the nature of the R groups
 (diasterotopic)	12-15	
 (cis)	7-12	
 (trans)	12-15	
 (geminal)	0.5-3	
 (allylic)	3-11	The actual J value depends on the dihedral angle
 (ortho)	6-9	
 (meta)	1-3	

Chemistry 255
Exam Four
Fall 2008

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		25
3		10
4		25
5		20
6(TH)		10

There are eight pages in this exam including this cover page. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

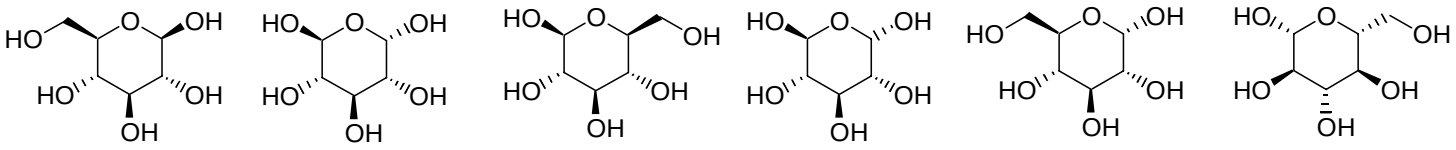
Be sure to follow the directions in answering each of the questions. In working mechanistic based problems you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

Good Luck ☺

What we see depends mainly on what we look for.

-John Lubbock

1. (10 points) Answer the following questions with respect to molecules I through VI:



a. What is the relationship between molecules I and VI?

(i) enantiomers
(ii) diastereomers
(iii) the same compound
(iv) different compounds
(v) none of the above

b. What is the relationship between molecules II and IV?

(i) enantiomers
(ii) diastereomers
(iii) the same compound
(iv) different compounds
(v) none of the above

c. What is the relationship between molecules I and III?

(i) enantiomers
(ii) diastereomers
(iii) the same compound
(iv) different compounds
(v) none of the above

d. What is the relationship between molecules I and V?

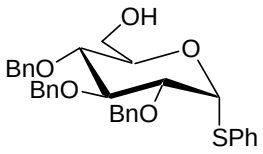
(i) enantiomers
(ii) diastereomers
(iii) the same compound
(iv) different compounds
(v) none of the above

e. Which compound(s) is/are meso?

f. For molecules I, III, V and VI, circle the beta anomers.

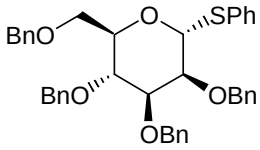
2. (25 points) In each case below, give the major product of the reaction.

a.

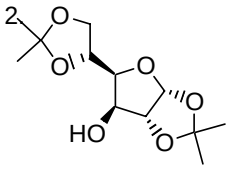


1. DMSO, $(COCl)_2$
   CH_2Cl_2, TEA
2. $CH_2=PPh_3$
   THF

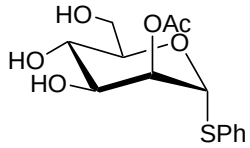
b.



1. NIS, AgOTf
   CH_2Cl_2

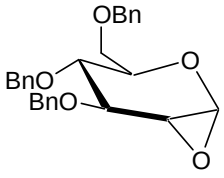


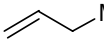
c.



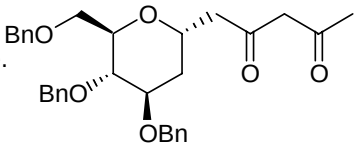
1. $PhCH(OCH_3)_2$
   TsOH, DMF
2. BnBr, NaH
   DMF
3. $NaCNBH_3$, HCl
   Et_2O, THF

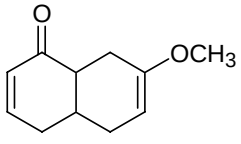
d.



1.  MgBr
   Et_2O
2. BnBr, NaH
   DMF
3. O_3
4. $(CH_3)_2S$

e.

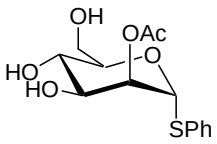




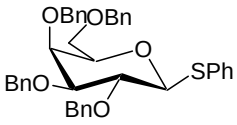
NaOEt. EtOH

3. (10 points) Recently, a novel virosomal formulation of a synthetic oligosaccharide were prepared and evaluated as a vaccine candidate against leishmaniasis. A major component of the vaccine is the tetrasaccharide shown below. Propose a synthesis for this tetrasaccharide using **A**, **B**, as your source of sugars, and any additional reagents.

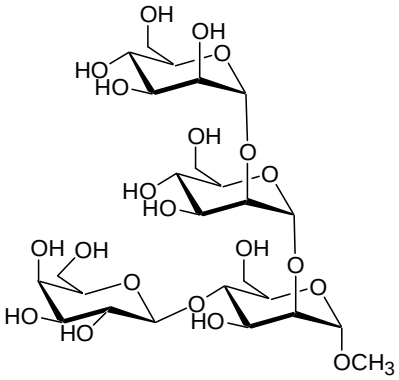
Note: This problem can be done in seven to ten steps using protecting group chemistry we learned in class.



A



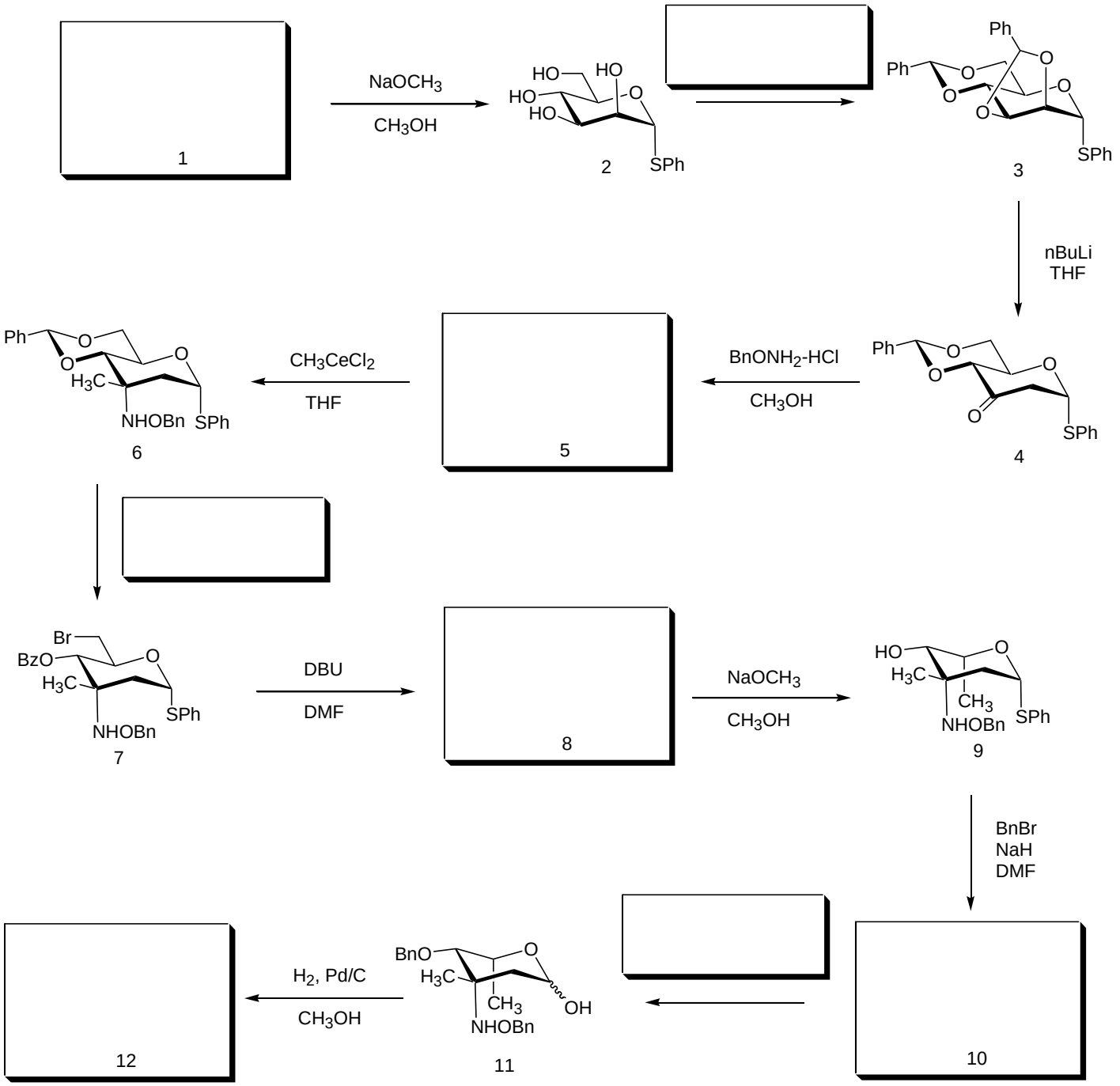
B



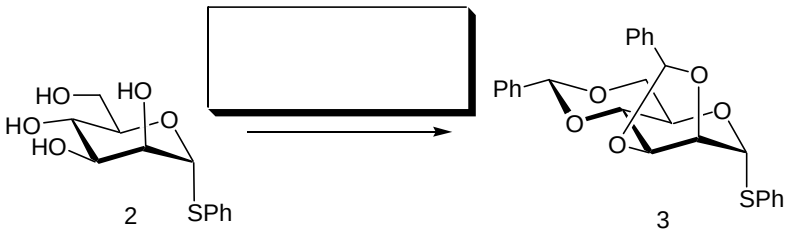
Tetrasaccharide Vaccine Component

4. (25 points) The following questions pertain to the synthesis of vancosamine, the terminal sugar of the glycopeptide antibiotic vancomycin.

a. (10 points) Complete the reaction scheme below by filling in products or reagents as necessary.

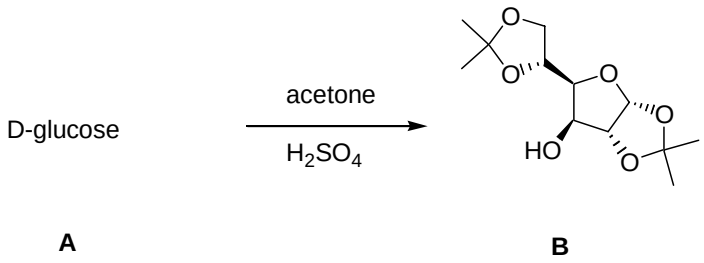


b. (15 points) Provide a mechanism for the conversion of 2 to 3. You need only show the formation of one benzylidene acetal.



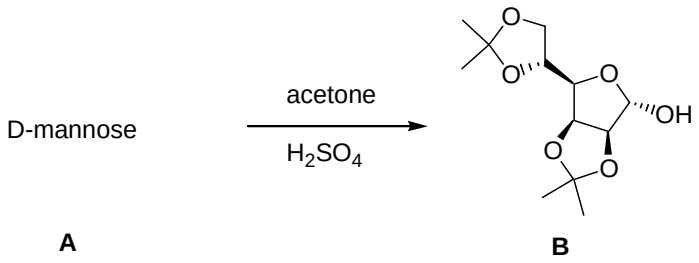
5. (20 points) The following questions pertain to the synthesis of diacetonide sugars which are useful synthons in a number of organic reactions.

a. (10 points) Provide a mechanism for the conversion of D-glucose **A** to the diacetonide **B** under acidic conditions.



A **B**

b. (5 points) The same reaction using D- mannose provides the following results.

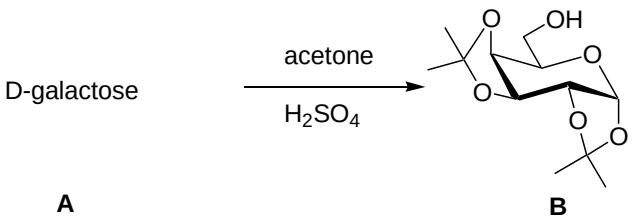


A **B**

Rationalize the differences between the positions of the diacetonide protecting groups.

Hint: Look at the stereochemistry!!

c. (5 points) If similar conditions are used to prepare the diacetonide of D-galactose, the only product detected is the six membered ring. Speculate as to why the five membered ring does not form under these conditions as observed for D-glucose and D-mannose?



A **B**

Chemistry 255
Exam One
Fall 2009

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		5
3		5
4		30
5		10
6		20
7		10
8		10

There are twelve pages in this exam including this cover page, a periodic table and two pages of stretching frequencies, chemical shifts, and a table of coupling constants. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

Good Luck ☺

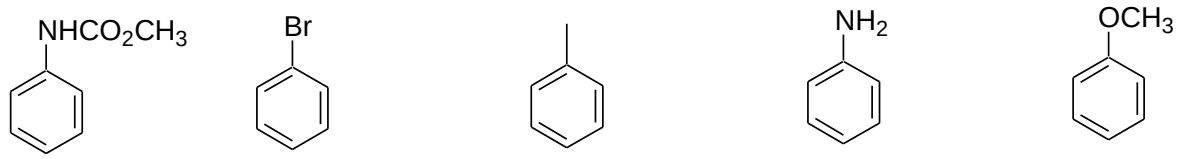
Oh yeah…..and DU = (2C-H-X+N+2)/2

Aerodynamically the bumblebee shouldn't be able to fly, but the bumblebee doesn't know that so it goes on flying anyway.

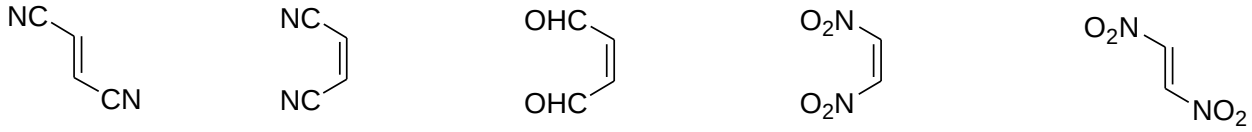
-- Mary Kay Ash

1. (10 points) In each case circle the correct answer(s). A half of a point will be deducted for each extra answer.

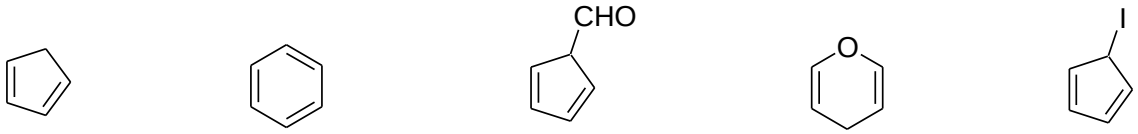
a. Circle the molecule that will serve as activating ortho/para directors when treated with HNO_3 in H_2SO_4.



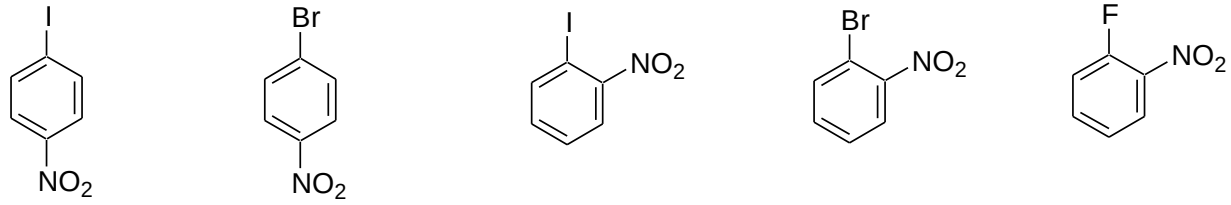
b. Cirlce the dieneophile that would react the fastest in a Diels Alder reaction.



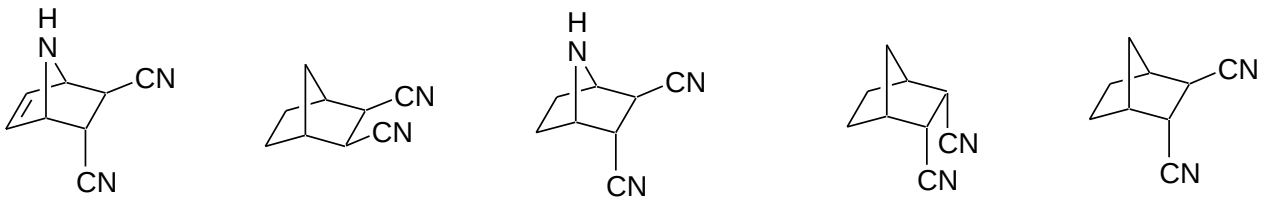
c. Circle the substate that will react the fastest with LDA.



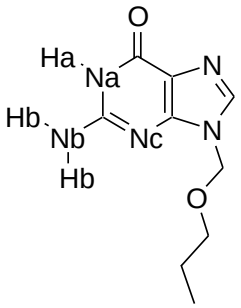
d. Circle the molecule that will undergo an addition elimination reaction the fastest.



e. Circle the major product formed when cyclopentadiene is reacted with trans-1,2-dicyanoethylene.



2. (5 points) The structure of Zoviax, an antiviral used to treat herpes simplex virus, is given below. Please answer the following questions pertaining to this chemical compound.

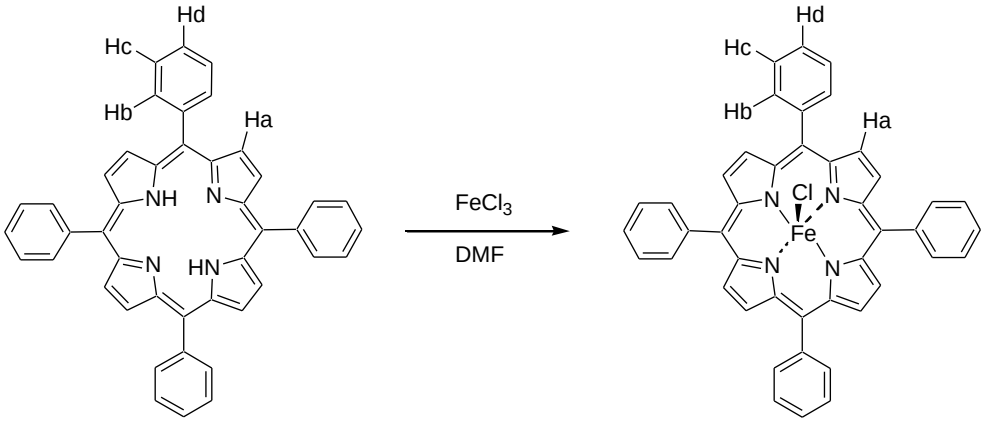


a. Which proton, Ha or Hb, is most acidic and why? (2 points)

b. Which nitrogen, Na, Nb, or Nc, is most basic and why? (2 points)

c. Is the bicyclic Zoviac ring system considered aromatic? Why or why not? (1 point)

3. (5 points) Metalloporphyrins derived from tetraphenylporphyrin (H_2TPP) are often used as catalysts to perform a number of organic transformations including epoxidation and cyclopropanation. Below is a reaction scheme showing the metallation of H_2TPP with iron (III) chloride ($FeCl_3$) to produce iron chloride tetraphenylporphyrin (FeClTPP). The 1H NMR data for each compound is also given below.



H_2TPP: Ha (8.85ppm, s, 8H), Hb (8.28ppm, dd, 8H), Hc,Hd (7.76, m, 12H), NH (-2.79, bs, 2H)
FeClTPP: Hc,Hd (13.32ppm, bs, 12H), Hb (12.21ppm, bs, 8H), Ha (6.42, bs, 8H)

a. Why do Hc, Hb, and Hd shift downfield when the metal is incorporated into the porphyrin center? (2.5 points)

b. Speculate as to why Ha shifts upfield when the metal is incorporated into the porphyrin center. (2.5 points)

c. BONUS: Why do the peaks broaden?

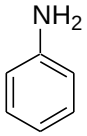
4. (30 points) In each case below, give the major product of the reaction. If there is no reaction write N.R.

a.

CH_3CH_2Cl

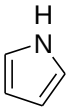
$AlCl_3$

b.



1. Ac_2O, Py
2. 1-chloro-2-methyl propane, $AlCl_3$
3. $NaNO_2$
4. HBF_4

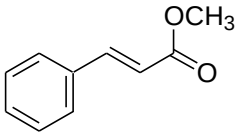
c.



+ $EtO_2C—\equiv—CO_2Et$

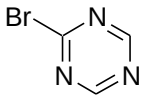
1. Heat
2. H_2, Pt

d.



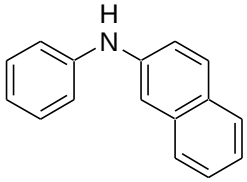
1. $AlCl_3$
2. H_2SO_4 (dilute)

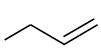
e.



1. NaOH (aq)
2. BnBr, NaH DMF

f.

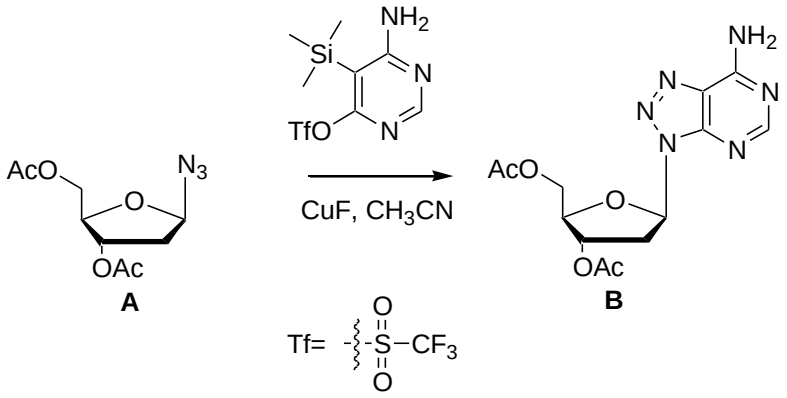




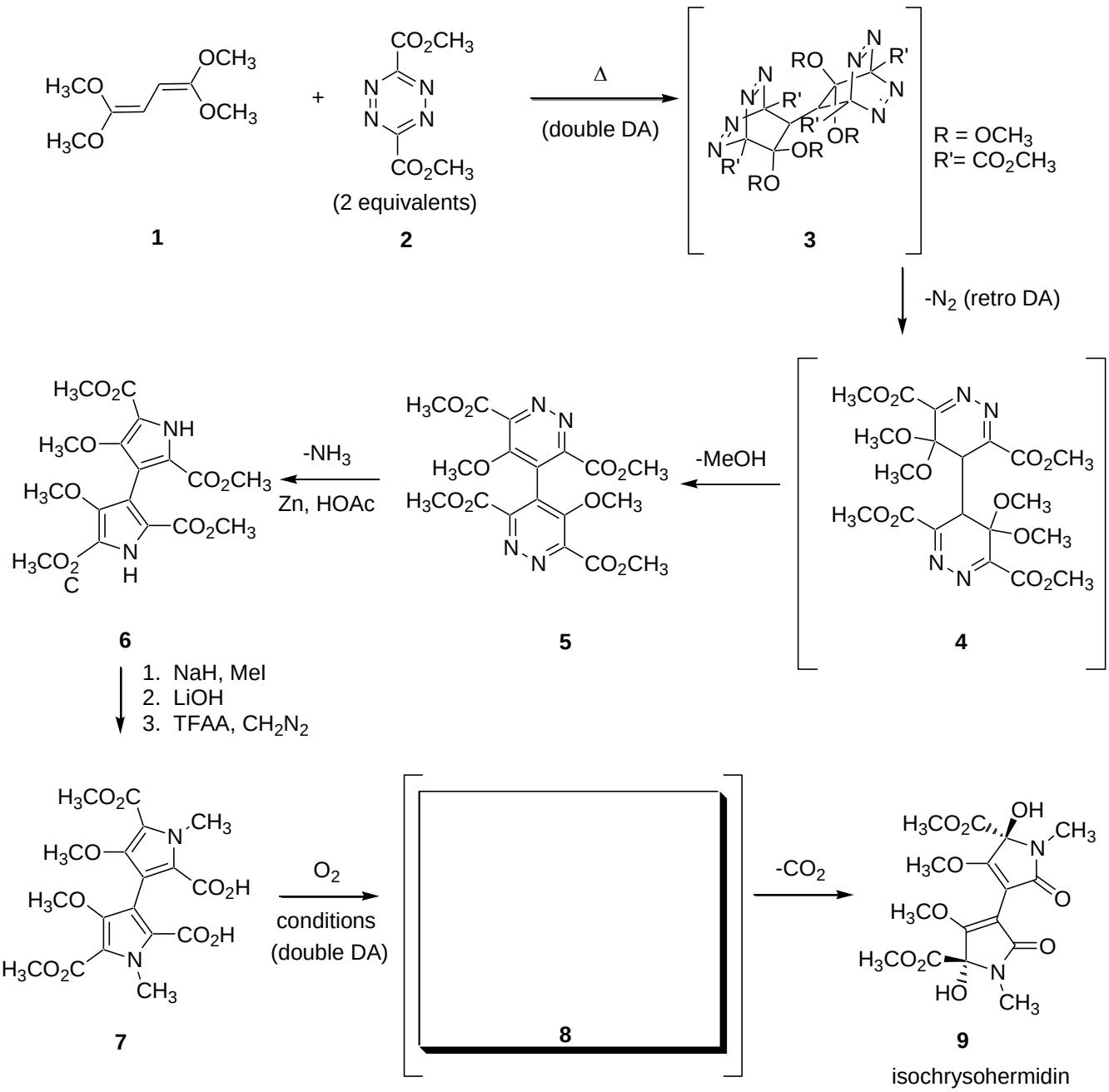
$FeBr_3$

5. (10 points) The 1,3 dipolar cycloaddition reaction between an azide and an alkyne to form a 1,2,3-triazole (known as “click chemistry”) has become an important reaction in synthetic organic chemistry. The products of the reaction are stable under physiological conditions and can be used to study a number of biological systems. Recently, the reaction of sugar azides with TMS/OTf derivatives, as shown below, has proven to be a good method for the generation of nucleoside analogs. These analogs can then be used to study certain disease and for use as pharmaceutics. Show a complete mechanism for the formation of B from A

   Hint: F- acts as a nucleophile.

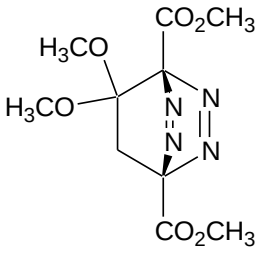


6. (20 points) The synthesis of isochrysohermidin (**9**), a DNA crosslinking agent, was accomplished by Dale Boger and coworkers using almost exclusively Diels-Alder (DA) and retro-Diels-Alder reactions. The synthesis is shown in the scheme below. Answer the questions on the next page, using the scheme below to guide you.



a. In the first step of the reaction, **1** undergoes a double Diels-Alder reaction with **2** to produce intermediate **3**. Provide a mechanism for the Diels-Alder reaction that occurs between **1** and **2**. You may show a single Diels-Alder reaction. (5 points)

b. In the second step, intermediate **3** quickly undergoes a retro-Diels-Alder reaction to produce intermediate **4**. Using the structure provided below, show how this reaction occurs. (5 points)

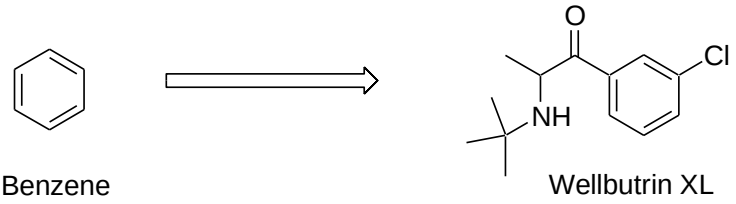


c. What is the driving force for the spontaneous conversion of **4** to **5**. (5 points)

d. Draw the structure of intermediate **8** in the box provided. (5 points)

7. (10 points) Wellbutrin XL is unicyclic, aminoketone antidepressant that has been used as an aid in smoking cessation. Provide a complete synthesis of Wellbutrin XL benzene and other reasonable molecules of four carbons or less.

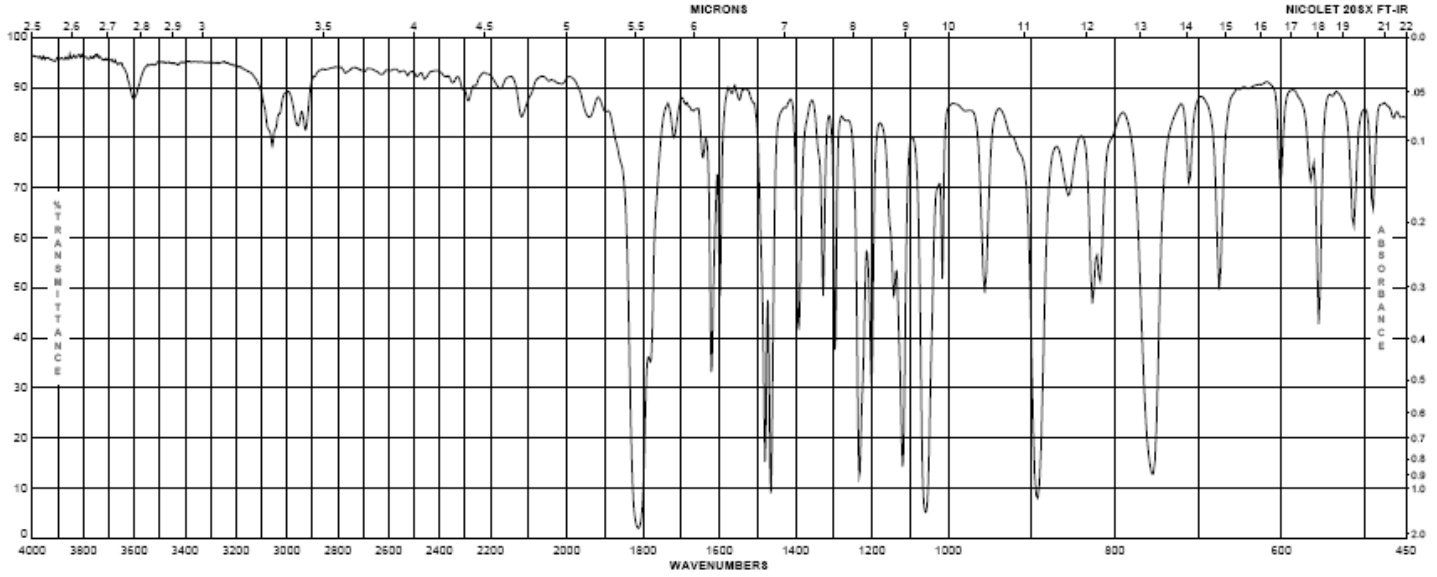
   Note: This can be done in four to seven steps (or more).

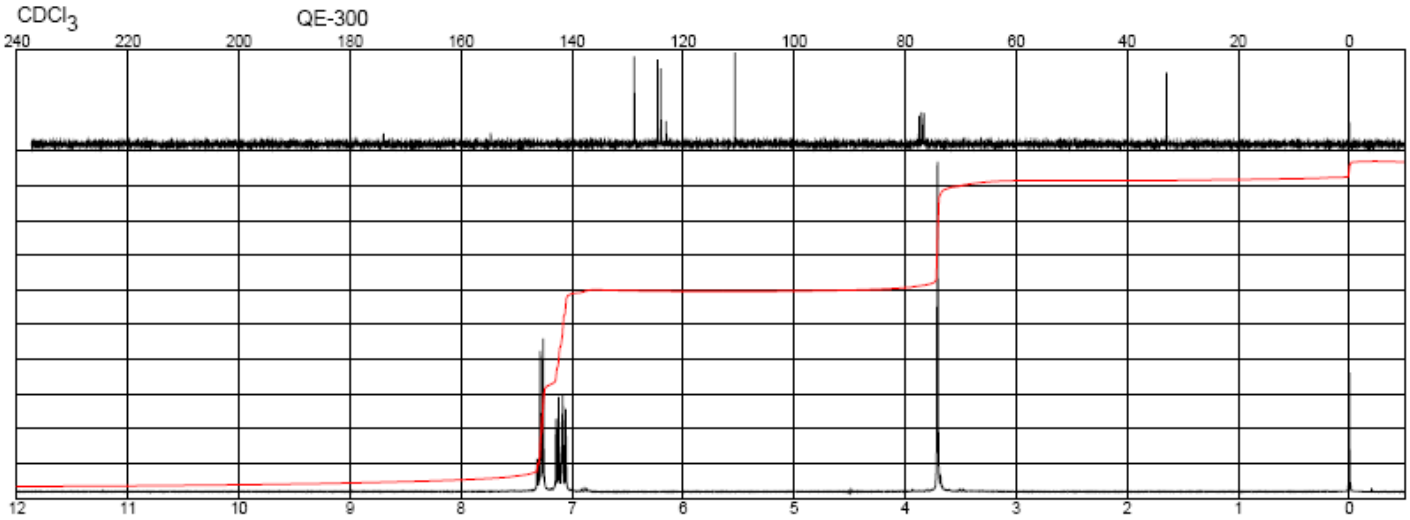


Benzene Wellbutrin XL

8. (10 points) The NMR spectrum below represents a compound with the molecular formula of $C_8H_6O_2$.

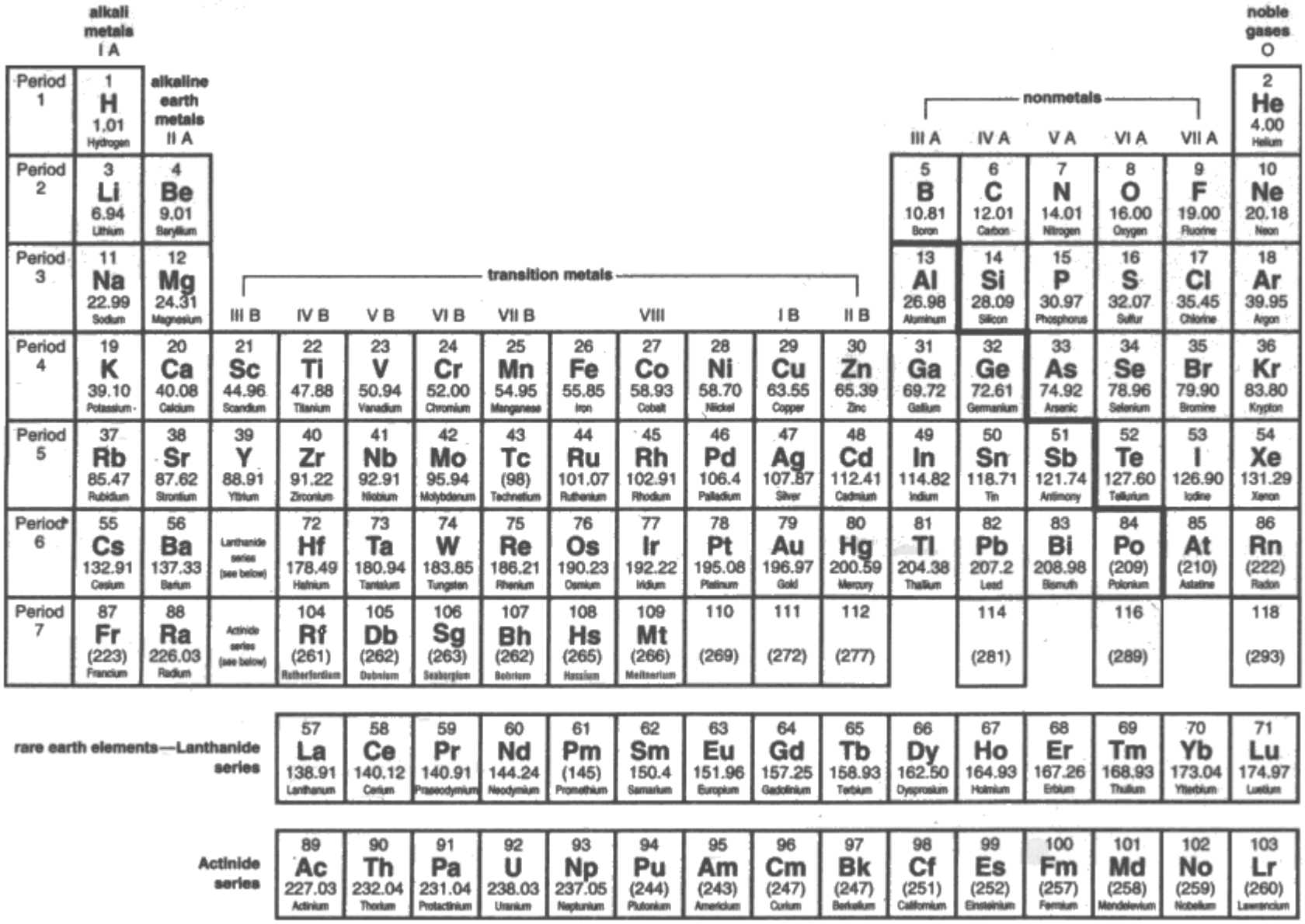
a. Provide a reasonable structure for this compound based on the IR and NMR provided. Show you work and place your labeled (H_a, H_b, H_c etc.) final answer in the box provided.



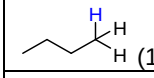
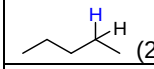
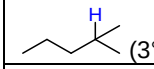
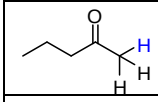
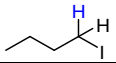
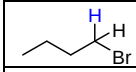
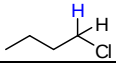
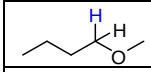
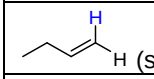
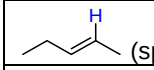
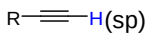
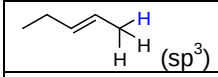
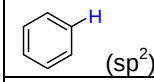
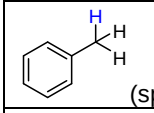
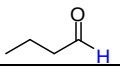
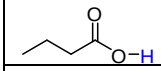
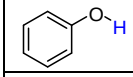
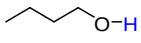
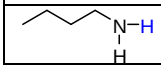


^{1}H NMR signals: 7.21ppm (m, 2H), 7.07ppm (m, 2H), 3.72ppm (s, 2H)
^{13}C NMR signals: 156ppm, 128pmm, 125ppm, 124ppm, 122ppm, 33ppm

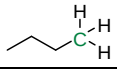
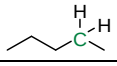
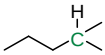
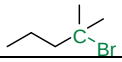
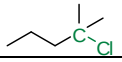
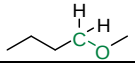
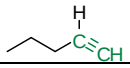
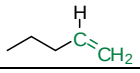
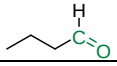
Answer:


alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
noble gases O
III A
IV A
V A
VI A
VII A
III B
IV B
V B
VI B
VII B
VIII
I B
II B
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

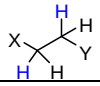
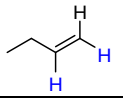
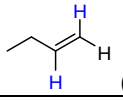
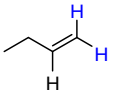
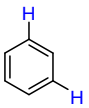
Characteristic ^{1}H NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0.8-1.0	
 (2°)	1.2-1.4	
 (3°)	1.4-1.7	
	2.1	
	3.1-3.3	
	3.4-3.6	
	3.6-3.8	
	3.3-4.0	
 (sp^2)	4.6-5.0	
 (sp^2)	5.2-5.7	
 (sp)	2.5	
 (sp^3)	1.7	
 (sp^2)	6.0-9.5	
 (sp^3)	2.2-2.5	
	9-10	
	10-13	Can be broad and may exchange
	4.5-7.7	Can be broad and may exchange
	0.5-6.0	Can be broad and may exchange
	1.0-5.0	Can be broad and may exchange

Characteristic ^{13}C NMR Chemical Shifts

Type of Bond	Chemical Shift (ppm)	Notes
 (1°)	0-35	
 (2°)	0-35	
 (3°)	0-35	
	30-70	
	30-70	
	85-90	
	75-100	
	100-150	
 (sp^2 aromatic)	115-150	
	165-210	May take a while to relax (signals may be weak)

Coupling Constant Values

Type of coupling	J-value (Hz)	Notes
	2-12 (7)	The actual J value depends on the dihedral angle and the nature of the R groups
 (diasterotopic)	12-15	
 (cis)	7-12	
 (trans)	12-15	
 (geminal)	0.5-3	
 (allylic)	3-11	The actual J value depends on the dihedral angle
 (ortho)	6-9	
 (meta)	1-3	

Chemistry 255
Exam Four
Fall 2009

Name:_____ Student ID:_____

Score:_____/100 **Grade**: A A- B+ B B- C+ C C- D+ D D- F

Number	Points Earned	Points Possible
1		10
2		25
3		20
4		10
5		10
6		15
7(TH)		10

There are ten pages in this exam including this cover page and a periodic table. Please check to make sure that all of the pages are here before you begin and be sure to return all of these papers when you are finished. Use a pen with blue or black ink for the entire exam. You may choose to use a pencil, but no regrades will be considered once the exam has been returned.

Be sure to follow the directions in answering each of the questions. In working mechanistic based problems you must show all of your work. No credit will be given unless all work is clearly shown and the method of solution is logically correct.

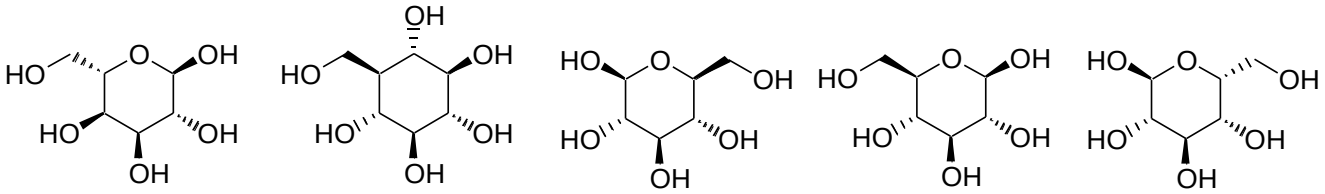
Good Luck ☺

At the end of your life, you will never regret not having passed one more test, not winning one more verdict, or not closing one more deal. You will regret time not spent with a husband, a friend, a child, or a parent.

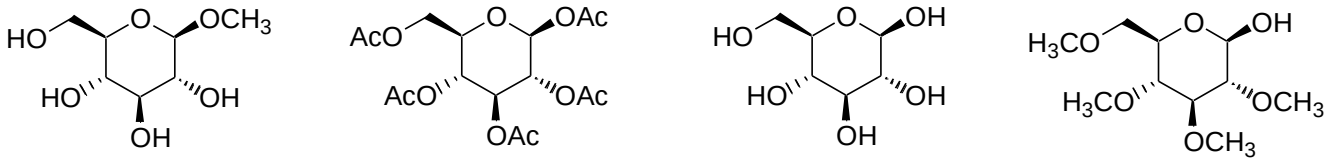
--Barbara Bush

1. (10 points) In each case circle the correct answer(s). A half of a point will be deducted for each extra answer.

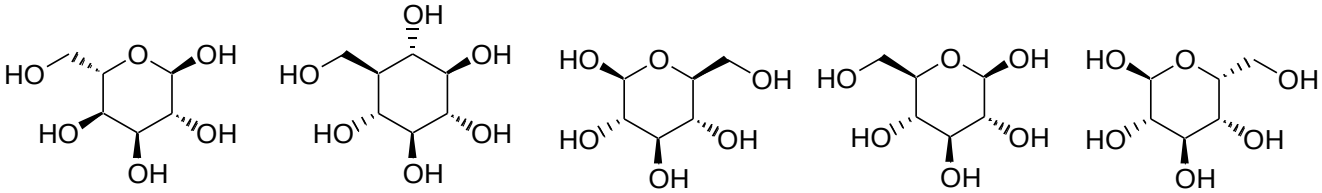
a. Circle all the D-sugars.



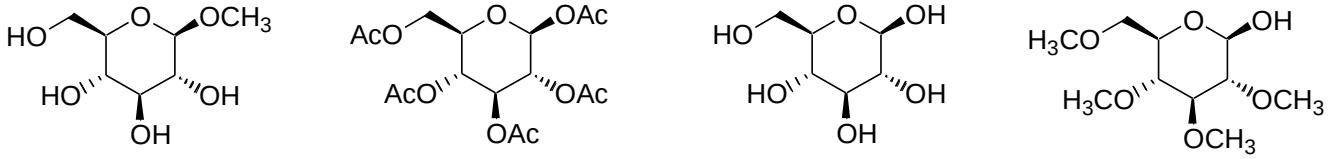
b. Circle all of the reducing sugars.



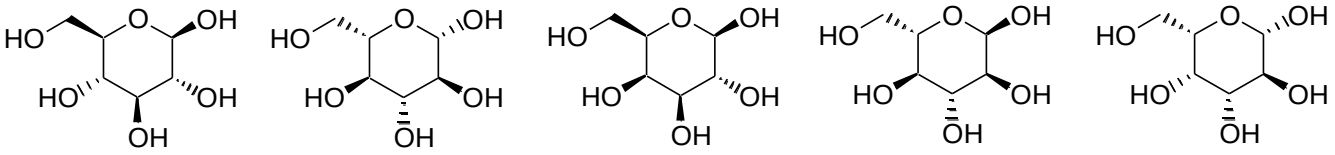
c. Circle all the L-sugars.



d. Circle the alpha anomers.

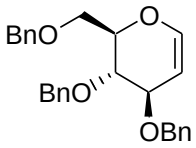


e. Circle the enatiomer of β-D-galactopyranose.



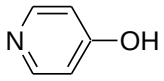
2. (25 points) In each case below, give the major product of the reaction. Play close attention to stereochemistry.

a.

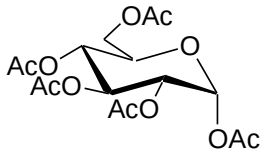


1. DMDO, CH_2Cl_2
2. LiSEt, THF

3. Ac_2O, py
4. NIS, AgOTf

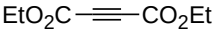


b.

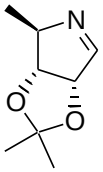


1. HBr, AcOH
2. NaN_3, DMF

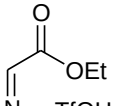
3.



c.



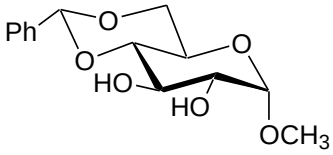
1.



TfOH

2. Dibal-H, THF

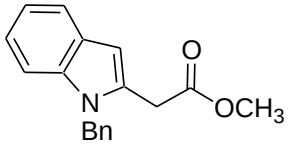
d.



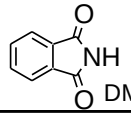
1. BnBr, NaH
   DMF

2. $LiAlH_4$, $AlCl_3$
   Et_2O, CH_2Cl_2

e.



1. HBr. AcOH
2.



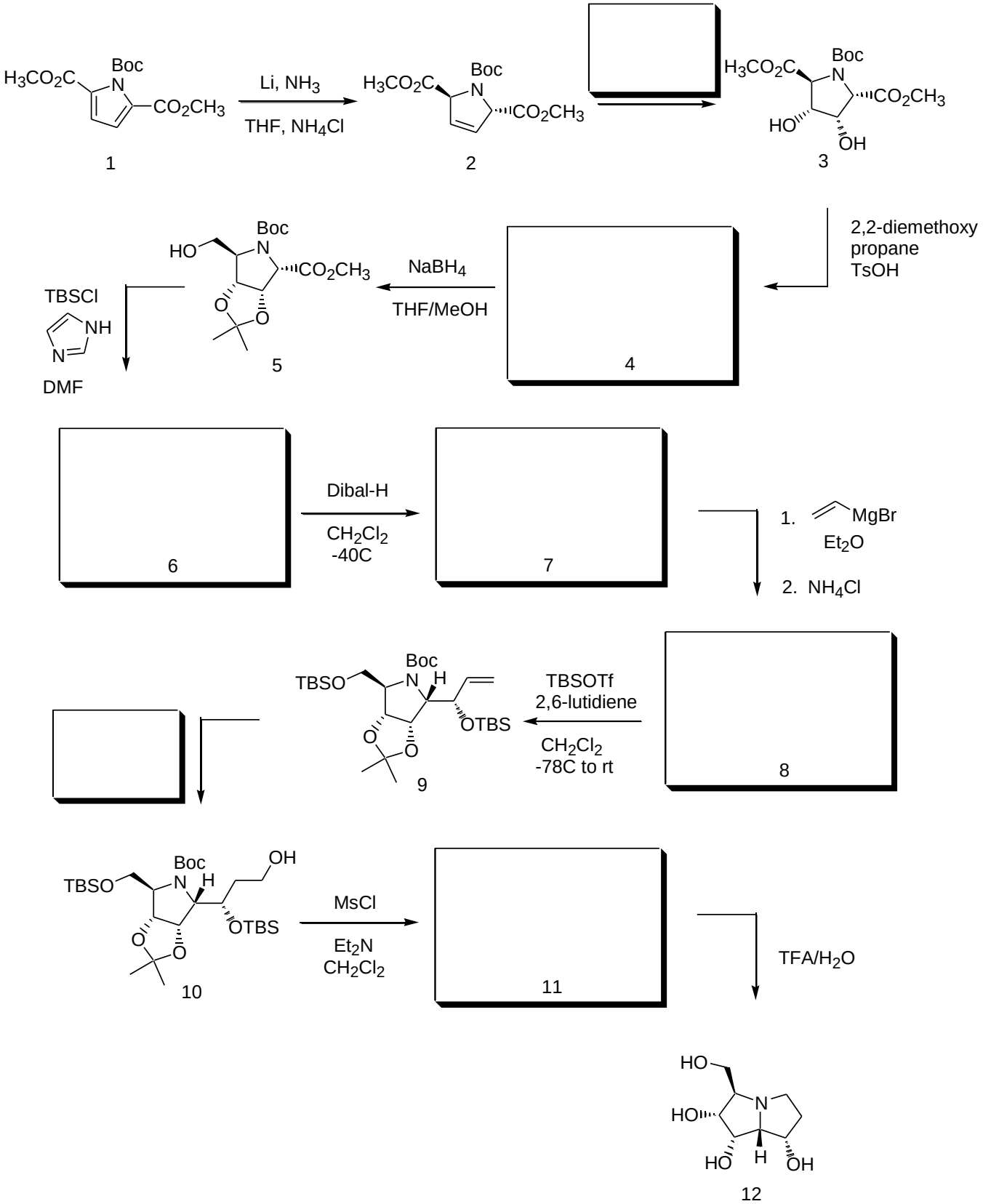
DMF

3. KOH
4. H_2, Pt/C

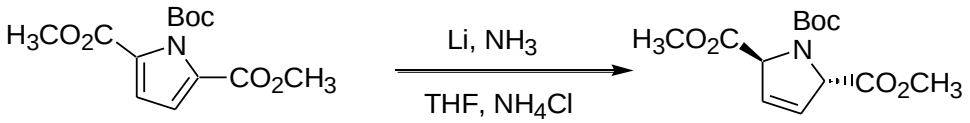
Bonus (5 points) What is the common name of the product of letter E?_____

3. (20 points) 1-Epiaustraline is a polyhydroxylated pyrolizidine effective against a number of glycosidsases.

a. (15 points) Complete the reaction scheme below by filling in products or reagents as necessary.

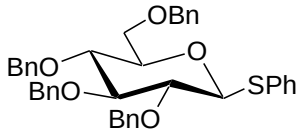


b. (5 points) Provide a mechanism for the conversion of 1 to 2.
HINT: Birch Reduction!

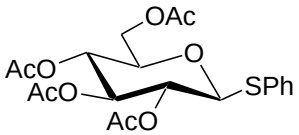


1 2

4. (10 points) The "armed/disarmed" concept is a important in assessing the reactivity of carbohydrates. For example, of the two molecules below (**A** and **B**), one reacts much faster in a glycosylation reaction than the other.



A

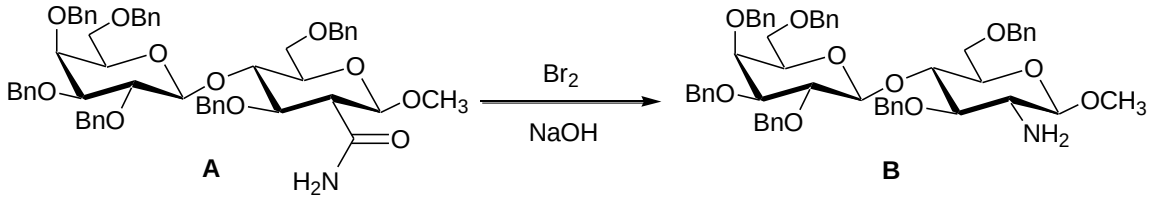


B

a. Circle the molecule you would expect to rate fastest based on an oxonium ion intermediate. (5 points)

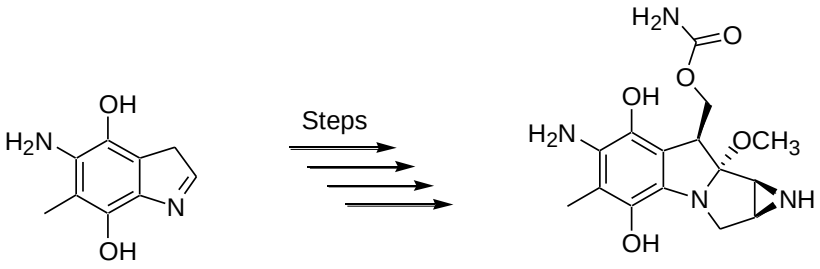
b. Briefly describe the reasoning for your choice. (5 points)

5. (10 points) The Hoffman elimination has been proposed as one method to make 2-amino sugars. Propose a complete mechanism for the conversion of **A** to **B**.



6. (15 points) The biologically active form of mitomycin C, shown below, is known to have both antibiotic and antitumor properties. Mitomycin C cross-links guanine residues of the DNA double helix generating kinks and bends in the helix.

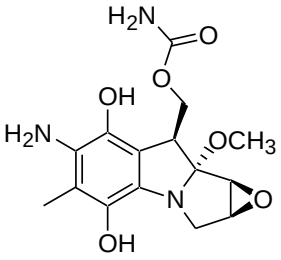
   a. Propose a synthesis of mitomycin C from 5-amino-6-methyl-3*H*-indole-4,7-diol and any other reasonable reagents. <u>You must also show the preparation of the aziridine in your synthesis</u>. (10 points)



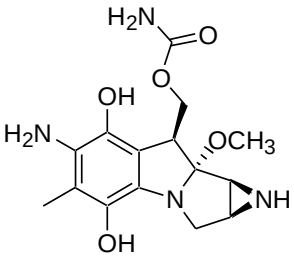
5-Amino-6-methyl-3*H*-indole-4,7-diol

Mitomycin C

b. The biologically active form of mit"oxy"mycin C, a derivative of mitomycin C is shown below. In reactivity studies, mit"oxy"mycin C is less reactive than mitomycin C. Further studies have shown that this has to do with the fact that the aziridine of mitomycin C, which is directly involved in the crosslinking, is more reactive than the epoxide of mit"oxy"mycin C. Briefly explain why the aziridine ring is more reactive than the epoxide ring. Be specific. Ring strain is the answer. Why does the aziridine ring experience more strain? (5 points)

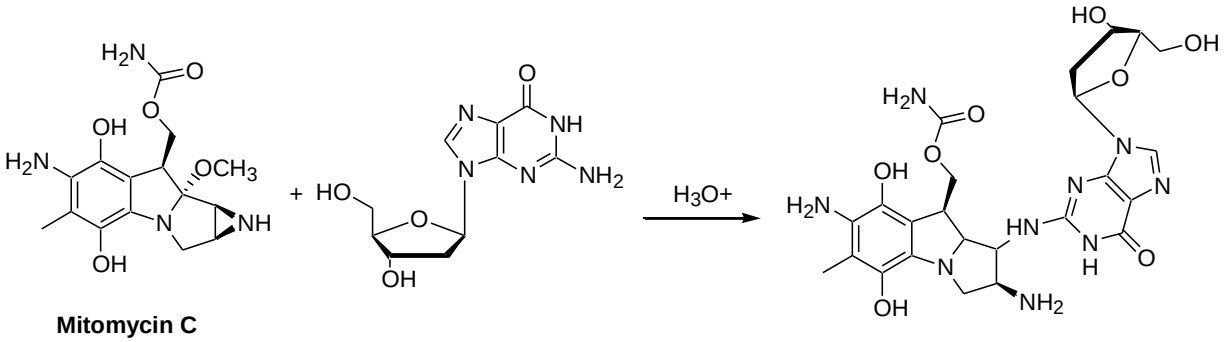


Mit"oxy"mycin C

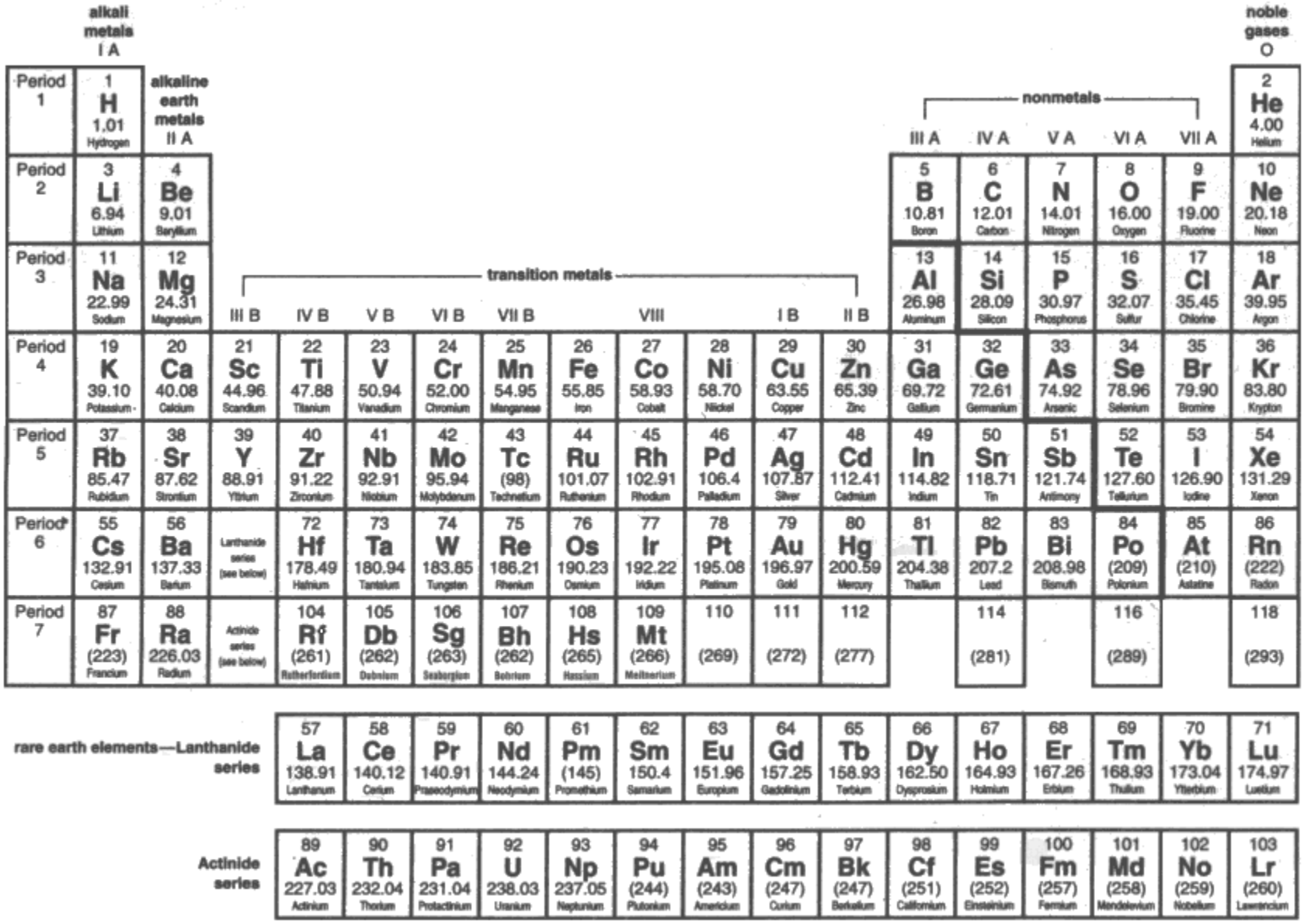


Mitomycin C

Bonus (10 points): On a separate sheet of paper, show how mitomycin C binds to guanine under acidic conditions. The overall reaction is shown below for your convenience.



Mitomycin C


alkali metals I A
alkaline earth metals II A
transition metals
nonmetals
noble gases O
III B IV B V B VI B VII B VIII I B II B
III A IV A V A VI A VII A
Period 1
Period 2
Period 3
Period 4
Period 5
Period 6
Period 7
1 H 1.01 Hydrogen
2 He 4.00 Helium
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
13 Al 26.98 Aluminum
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.96 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc (98) Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.4 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.74 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Cesium
56 Ba 137.33 Barium
Lanthanide series (see below)
72 Hf 178.49 Hafnium
73 Ta 180.94 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Tl 204.38 Thallium
82 Pb 207.2 Lead
83 Bi 208.98 Bismuth
84 Po (209) Polonium
85 At (210) Astatine
86 Rn (222) Radon
87 Fr (223) Francium
88 Ra 226.03 Radium
Actinide series (see below)
104 Rf (261) Rutherfordium
105 Db (262) Dubnium
106 Sg (263) Seaborgium
107 Bh (262) Bohrium
108 Hs (265) Hassium
109 Mt (266) Meitnerium
110 (269)
111 (272)
112 (277)
114 (281)
116 (289)
118 (293)
rare earth elements—Lanthanide series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.96 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinide series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu (244) Plutonium
95 Am (243) Americium
96 Cm (247) Curium
97 Bk (247) Berkelium
98 Cf (251) Californium
99 Es (252) Einsteinium
100 Fm (257) Fermium
101 Md (258) Mendelevium
102 No (259) Nobelium
103 Lr (260) Lawrencium

Exam Four Take Home Problem

The mechanism on the following page should take you approximately twenty minutes to complete, however you may use all the time you need.

Here are the following guidelines concerning this problem:

1. You may use any written or electronic resource you find to complete this problem on the following page. If you have used materials beyond those distributed in this course, please reference them in your final work.

2. You are not to discuss in any way any part of this question with anyone other than Nicole Snyder until all of the exams have been submitted for grading. This means that you may not consult with other students, professors, parents, former high school mentors etc. until I post a note stating that all exams have been collected.

3. This question is due no later than **10:00pm on Thursday, December 03, 2009**.

A violation of these guidelines is a violation of the honor code and will result in an automatic zero on the fourth exam. In addition, the student or student(s) found in violation will be subject to disciplinary action.

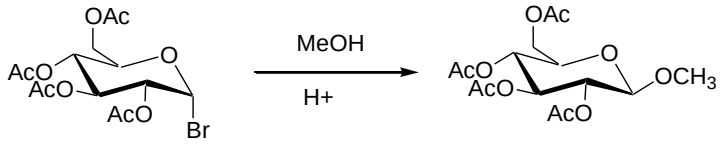
Please sign this paper before submitting your final work as a hard copy **directly to me**.

Good luck and let me know if you have any questions or concerns.

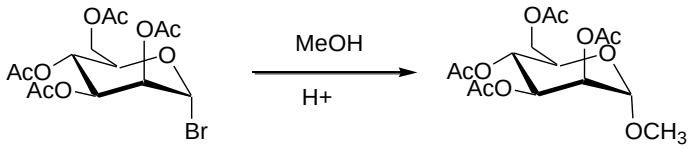
I, _____, certify that I have referenced any sources I used to complete this exam beyond those distributed to me as part of this course. I, _____, also certify that the answers contained in this exam are my own work, and that I have neither accepted help from any other student, professor, parent, or high school mentor, nor have I have given advice to any other student in the course regarding the material in this exam. I, _____, understand that violating these guidelines is a violation of the honor code and if I am found to have violated the honor code, I will be subject to disciplinary action.

Signed_____ Date: ___/___/___

(10 points) The reaction of 2,3,4,6-tetraacetyl-α-D-bromo glucopyranoside (A) in acidic methanol gives exclusively 2,3,4,6-tetraacetyl-β-D-*O*-methyl glucopyranoside, while 2,3,4,6-tetraacetyl-α-D-bromo mannoyranoside (B) under the same reaction conditions gives exclusively 2,3,4,6-tetraacetyl-α-D-*O*-methyl mannopyranoside. Explain this phenomenon <u>using mechanisms</u> to support your answer.



A



B

HINT: The acetate group at C2 is directly involved in the mechanism. Draw this group out.

Teaching Materials—Chemistry 371

Research Methods in Chemistry 371

Course Syllabus Fall 2009

Instructors Prof. Bradley M. Wile — Office: Science Center 1060, x4402
Prof. Nicole I. Snyder — Office: Science Center 1073, x4742

Laboratory Tuesday & Thursday, 1–4 PM Science Center 1052
No unsupervised lab work is ever permitted!
Class Tuesday 12-1 PM before the regular lab session begins (SCCT 1050)

Course Content

In this course the class will focus on a single project for the entire semester. The project involves the preparation and characterization of a group of coordination complexes using porphyrins as ligands. The project will be broken into three different sections. In the first section, you will synthesize porphyrin ligands, use these to make metal complexes and then characterize these complexes with a variety of techniques (IR, NMR, UV-vis, Raman, magnetic susceptibility). In the second section of the course you will study the electrochemical, ligand binding, and reaction properties of the complexes that you have made. In the final third of the semester you will use the tools that you have learned to design and carry out your own independent project.

Graded Work

For each section you will complete a written report and make an oral presentation on your work. For the second and third sections of the course you will also write a proposal outlining your objectives.

Proposals should be 3-5 pages and briefly outline important background literature on that particular aspect of the project and succinctly outline the goals that you hope to achieve in your lab work. Proposals must also contain a list of chemicals and lab apparatus (other than the usual beakers, hot plates, etc.) you will need to carry out the project.

The reports should be written in the style of a journal article, and should include introductory material, an experimental section, and a results and discussion section along with a conclusion. The final report for the course should include all the synthetic work and the characterization for your independent project.

All laboratory reports (typed and properly referenced) are due according to the attached schedule. Reports that are late by one week will be lowered one full letter grade.

Oral reports will be ten to twelve minutes in length, summarizing the important aspects of your written reports.

Grading Scheme

Oral and Written Report I: Porphyrin Metal Complexes	20%
Proposal I: Reactivity Studies	5%
Oral and Written Report II: Reactivity Studies	25%
Proposal: Independent Project	10%
Oral and Written Report III: Independent Project	30%
Laboratory Technique*	10%

*includes an assessment of your laboratory technique and the quality of your work in the lab, including effort, neatness, waste disposal, safety, initiative, and improvement over the semester.

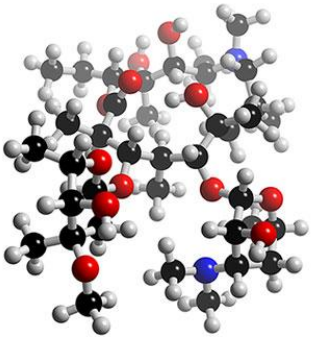
Departmental Seminars

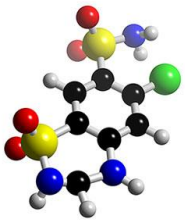
Attendance at all Chemistry Department Seminars is required as part of your class work. Seminars are usually scheduled at 3:00 on Friday afternoons.

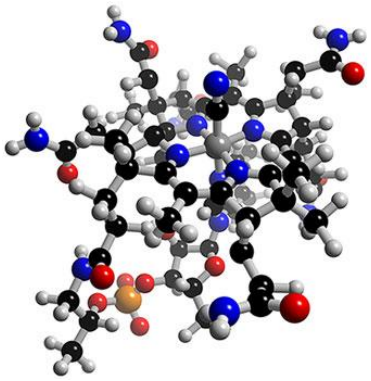
Schedule for Laboratory and Group Meetings

Week of	Tuesday Lab	Thursday Lab	Tuesday Discussion Topic
Aug. 23	**No Class**	Orientation to the Project & Preparation of TPP	**No Class**
Aug. 30	Synthesis & Characterization of a Porphyrin	Synthesis & Characterization of a Porphyrin	Reading a Scientific Paper
Sep. 06	Synthesis & Characterization of a Porphyrin	**Proposal Due** Synthesis & Characterization of a Porphyrin	Searching the Chemical Literature
Sep. 13	Synthesis & Characterization of a Porphyrin	**Oral Reports** on Complex Synthesis	Ligand Field Theory
Sep. 20	Reactivity Studies	Reactivity Studies	Physical Methods for the Characterization of Metal Complexes
Sep. 27	**Report I Due** Reactivity Studies	Reactivity Studies	Coordination Chemistry of Porphyrin Complexes
Oct. 04	Reactivity Studies	Reactivity Studies	Coordination Chemistry of Porphyrin Complexes
Oct. 11	**Proposal Due** Reactivity Studies	**No Class**	Anaerobic Synthesis Techniques
Oct. 18	Reactivity Studies	**Oral Reports** on Reactivity Studies	"Cantor's Dilemma"
Oct. 25	**Report II Due** Independent Projects	Independent Projects	Group Meeting
Nov. 01	Independent Projects	Independent Projects	Group Meeting
Nov. 08	Independent Projects	Independent Projects	Group Meeting
Nov. 15	**No Class**	**No Class**	**No Class**
Nov. 22	Independent Projects	Independent Projects	Group Meeting
Nov. 29	Independent Projects	Independent Projects	Group Meeting
Dec. 06	**Oral Reports** on Independent Projects	**Report III Due** Cleanup	

Teaching Materials—Chemistry 380







Organic Chemistry II
Chem 255
Course Syllabus—Fall 2008
August 28, 2008-December 12, 2008

Instructor Information:

Nicole L. Snyder, Ph.D. (Call me Nicole)

Office: SCCT 1072
Lab: SCCT 1068
Office Hours: Tuesday: 8:00am until 10:00am
Wednesday: 3:00pm until 5:00pm
Thursday: 10:00am until 12:00pm
Sunday: 7:00pm until 9:00pm
….and also by appointment
Office Phone: (315) 859-4742
Email: nsnyder@hamilton.edu

Course Objectives:

The second semester of organic chemistry is designed to introduce you to a number of key organic reactions that are used in the synthesis of molecules as simple as Splenda and as complex as calicheamicin. We will begin this course by quickly reviewing a number of topics covered in the first semester, including acid-base chemistry, molecular spectroscopy, and many of the key substitution and elimination reactions you learned before you left for the summer. It is my hope that our initial discussions will help lay the foundation for developing a strong approach to the study of chemical reactions and organic synthesis.

As the semester progresses, we will build upon the concepts you learned in the first semester of organic chemistry, and apply them to many new reactions including pericyclic reactions, carbonyl chemistry, and amine and amide reactivity. Some of the reactions we learn will be named after the organic chemist(s) who discovered them and will have interesting back stories. Other reactions will have significant implications in the synthesis and preparation of pharmaceutical compounds that make our everyday lives easier. When possible, I will make every attempt to connect this material to the current literature, and will highlight the scientists invested in the project and illustrate key reactions that have been used to prepare synthons of chemical or biochemical interest.

In the last few weeks of the course we will discuss how the chemistry you have learned applies to biological systems. We will begin with a systematic approach to the organic and biosynthetic pathways used to prepare complex carbohydrates and proteins that play an important role in our everyday lives. We will learn how these molecules come together at the molecular level to form complex interactions that have vast implications on several processes going on in your body as you read this introduction. We will also explore how several of the compounds we have discussed throughout the course interact with biomolecules to either inhibit or aid in their activity.

Your success in this course will depend heavily on your ability to think mechanistically about the reactions we learn in this course, and apply them to the synthesis of simple and complex molecules and molecular systems. In many cases, the work you do will seem like a large puzzle. The reactions you have learned will be the pieces of the puzzle, and you will need to carefully fit the pieces together to form the final product. Many of you will ultimately find this to be a very rewarding experience, but will probably be frustrated with the process initially. Please do not be discouraged. Your hard work and efforts in this course will eventually pay off, and you will learn skills in this course that will last you a lifetime.

Throughout our journey through the second semester of organic chemistry, I will serve as your guide. I promise to work hard each day to help make sure you understand the material, and you are always welcome to drop by, email, or call me with any questions or concerns you may have. I hope that in the end, you will look back on this experience with fond memories of your time spent learning how the world around you works from a molecular perspective.

*Images taken from chem3D.com database

Course Requirements:

Students taking Chemistry 255 are required to gain access to the course and lab textbooks, a model kit, and a laboratory notebook. More information on each of these items is provided below.

I. *Textbook*—I will assign readings and problems from the textbook and other resources that I feel are necessary to convey a particular topic. The required textbook for this class is **"Organic Chemistry" (6th edition) by Leroy G Wade.** Please make sure that you are using the sixth edition for this course.

II. *Model set*—Access to a molecular model set for organic chemistry is essential to this course and is therefore required. Prentice Hall molecular model sets are available at the bookstore and online. There are also a number of other good (and cheap) molecular model sets out there. Please see me if you would like some suggestions.

III. *Laboratory textbook and notebook*—Laboratory handouts will be handed out in class and posted on Blackboard. A required supplementary text for the laboratory, **"The Organic Chem Lab Survivial Manual" (7th edition) by Zubrick et. al.**, can be purchased in the college bookstore. You should be able to produce a copy of the lab (printed from the web) and the text during your assigned laboratory period. In addition, you will be required to purchase and maintain a Freeman Laboratory Notebook specifically for use in the laboratory portion of the course. A handout for keeping the laboratory notebook will be provided during your designated laboratory period and requirements may vary by instructor.

Class Format:

Each week I will provide a general overview of the material that we will be covering in class. I have proposed the following schedule that is subject to change based on Holidays and other important school related functions:

Monday:	-Homework and molecule of the week distributed
	-General lecture
Tuesday:	-Evening review session 7:30pm until 9:30pm
***Wednesday*:**	-Homework problems from previous week collected and select problems discussed
	-General lecture
	-In class problem session
Thursday:	-Homework graded and returned outside my office door by 9:00pm
***Friday*:**	-Quiz
	-Molecule of the week collected.
	-General lecture

Class Policies:

It is expected that all students in this section of Chemistry 255 will take note of the following policies:

I. *Attendance*—Attendance is strongly encouraged. If you need to miss a class for any reason, please contact me in advance so that I can arrange to have missed work delivered to you in a timely fashion. Also note that class participation is considered for 10% of your final grade (see below). Frequently missing class can and will negatively impact your grade.

II. *Class and lab participation*—Every member of the class is expected to participate in lecture and in lab. This means that you must verbally interact with me during class by answering questions. You must also interact with your fellow classmates during group discussions, lab, and through the use of Blackboard when appropriate. Class participation is worth 10% of the final grade. Failing to participate will result in the reduction of your final grade in the course.

III. *Late work or missed assignments*—Work that is not completed on time will be marked late unless arrangements are made **in advance** to turn the work in at an alternate time other than the due date. In addition, ten percentage of the point total for the assignment will be deducted for each day the assignment is late. If the assignment is more than five days past due, no credit will be given.

IV. *Academic honesty*— Each individual is expected to follow the academic conduct code (Honor Code) set forth by Hamilton College. The honor code will be strictly enforced in this course and there will be no warnings.

V. *Learning disabilities*— In accordance with the Americans with Disabilities Act, any student who has a documented learning disability will be provided with reasonable accommodations designed to meet his/her needs. Before any such assistance can occur, it is the responsibility of the student to see that documentation is on file with the appropriate individual. Please see me as soon as possible to discuss any need for accommodations.

Student Evaluation:

You will be evaluated in this course on a regular basis. The basis for course evaluation is provided below with explanations:

Quizzes:	5%
Problem Sets:	5%
Molecule of the Week:	10%
Lab Reports:	25%
Hourly Exams:	30%
Final Exam:	10%
Named Reaction Summary	5%
Class Participation:	10%

I *Quizzes*—There will be a short five point quiz given the Friday of each week unless an exam is scheduled for the Thursday of that week. Each quiz is designed to test your knowledge of the material covered in the previous week's classes. Overall, there will be ten quizzes scored for a total of 50 points or 5% of the final grade.

II. *Problem Sets*—Problem sets will be assigned at the beginning of each week and will correspond to the material that we will be covering throughout that week. Problem sets will be collected and graded on a weekly basis, generally the following Wednesday of the week the assignment was made. Each assignment must be turned in on time and will be worth ten points. Ten problem sets will be counted towards your final grade. This comprises 50 points or 5% of the final grade.

III. *Molecule of the Week*—Every week you and several of your classmates will be assigned a small molecule and will be asked to provide a short synthesis (usually less than ten steps) of the compound you have been assigned. Molecules will be distributed on Monday, and will be collected and graded at the end of the week. You are strongly encouraged to work with your classmates on this assignment and there will be at least fifteen to twenty minutes of class time per week to work in groups on your assigned molecule. Each assignment must be turned in on time and will be worth ten points. Ten assignments will be counted towards your final grade. This comprises 100 points or 10% of the final grade.

IV. *Lab reports*—Completing lab is essential to understanding organic chemistry. Throughout the term we will complete a total of eleven experiments that relate to material covered in lecture. These assignments are designed to help you maximize your lecture experience and should be thought of as a supplement to your in class lecture. Lab assignments will be graded separately by your individual lab instructor and will count towards 25% of the final grade. Please see your laboratory instructor for details about the grading of laboratory assignments.

Note: Failure to turn in two or more lab reports will constitute and automatic failure of the course!

V. *Hourly exams*—There will be four scheduled 100 point hourly exams given throughout the semester. The dates for these exams are given below:

Exam I—Thursday, September 25, 2008 (7:00pm-9:00pm)
Exam II—Thursday, October 23, 2008 (7:00pm-9:00pm)
Exam III—Thursday, November 13, 2008 (7:00pm-9:00pm)
Exam IV—Thursday, December 04, 2008 (7:00pm-9:00pm)

In most cases, each exam will reflect material that we have covered in class up to the week prior to the exam. At the end of the semester, I will drop the lowest exam score. The remaining three exams will count for 300 points or 30% of the final grade. Exams will not be curved and **you must take all four exams** in order for me to drop your lowest exam score.

VI. *Final Exam*—There will be one final exam worth 100 points or 10% of the final grade. The exam will be cumulative. **The final exam is schedule for Tuesday, December 16, 2008 from 2:00pm until 5:00pm.** More details will be provided as we approach the final exam period.

VII. *Named Reactions Summary*—You will be required to complete a short 3-5 page summary (including figures) on a named reaction that you will select during the first few weeks of the course. Details for the content of this summary will be discussed during the first day of class. The final paper will be due **no later than midnight on December 07, 2008**. This assignment is worth 50 points or 5% of the final grade.

VII. *Class participation*—Frequent participation in class is required and counts for 10% of the final grade. There are two major ways to participate in class: (i.) during lectures; and (ii.) during group problem sessions. Throughout the lecture, students will be asked to provide answers to problems that are presented. Initially, I will ask for volunteers. If there are no volunteers, I will call on a student at random. The student that is chosen will have the opportunity to consult with another classmate or two before answering the question. During group problem sessions, students will be asked to work on a particular problem or set of problems. After a given period of time, each group will be asked to choose a group member to represent the group and present the groups solution to their assigned problem(s). Class participation will count for 100 points or 10% of the final grade.

Schedule of Events:

Class	Monday	Wednesday	Friday
Week One **August 26th** **Introduction and Review**	**-No Class**	**-No Class**	- Introduction - Quick review of key reactions - Introduction to synthesis
Week Two **September 01** **Conjugated Systems**	- Epoxidation (Handout) - Chapter 15 (15.1-15.4)	- Chapter 15 (15.5-15.9)	- **QUIZ I (Review)** - **Molecule of the Week Due** - Chapter 15 (15.10-15.11)
Week Three **September 08** **Conjugated Systems and Aromatic Compounds**	- Chapter 15 (15.10-15.11) and supplement	- **PROBLEM SET I DUE (Chapter 15)** - Chapter 16	- **QUIZ II (Chapter 15)** - **Molecule of the Week Due** - Chapter 17 (17.1-17.4)
Week Four **September 15** **Aromatic Compounds**	- Chapter 17 (17.5-17.9)	- **PROBLEM SET II DUE (Chapter 16)** - Chapter 17 (17.10-17.11)	- **QUIZ III (Chapter 16)** - **Molecule of the Week Due** - Chapter 17 (17.12-17.14)
Week Five **September 22** **Ketones and Aldehydes**	- Chapter 19 (19.11, 19.18)	- **PROBLEM SET III DUE (Chapter 17. 19.18)** - Chapter 18 (18.1-18.8) - **Review for Exam I (Chapters 15-17, 19.18, 19.11)**	- Chapter 18 (18.9-18.12)
Week Six **September 29** **Ketones and Aldehydes**	-Chapter 18 (18.13-18.14)	- **Named Reaction Selection Due** - Chapter 18 (18.15-18.17)	- **QUIZ IV (Chapter 18 though 18.14)** - **Molecule of the Week Due** - Chapter 18 (18.18-18.21)
Week Seven **October 06** **Amines**	- Chapter 19 (19.1-19.10, 19.12-19.13)	- **PROBLEM SET IV DUE (Chapters 18-19)** Chapter 19 (19.15-19.17)	- **QUIZ V (Chapter 18 and 19 through 19.13)** - **Molecule of the Week Due** - Chapter 19 (19.19-19.21)
Week Eight **October 13** **Carboxylic Acids and Carboxylic Acid Derivatives**	- Chapter 20 (20.1-20.10)	- Chapter 20 (20.11-20.15) - **QUIZ VI (Chapter 20—Take Home)**	**No Class-Fall Break**
Week Nine **October 20** **Carboxylic Acid Derivatives**	- Chapter 21 (21.1-21.5) - **QUIZ VI Due** - **Molecule of the Week Due (Take Home)**	- **PROBLEM SET V DUE (Chapter 20)** - Chapter 21 (21.6-21.9) - **Review for Exam II (Chapters 18-20)**	- Chapter 21 (21.10-21.16)
Week 10 **October 27** **Enolates**	- Chapter 22 (22.1-22.4)	- **PROBLEM SET VI DUE (Chapter 21)** - Chapter 22 (22.5-22.6)	- **QUIZ VII (Chapter 21)** - **Molecule of the Week Due** - Chapter 22 (22.7-22.8)
Week 11 **November 03** **Enolates**	- Chapter 22 (22.9-22.11)	- **PROBLEM SET VII DUE (Chapter 22 through 22.11)** - Chapter 22 (22.12-22.14)	- **QUIZ VIII (Chapters 21 through 22.11)** - **Molecule of the Week Due** - Chapter 22 (22.15-22.17)
Week 12 **November 10** **Enolates**	- Chapter 22 (22.18-22.19)	- **PROBLEM SET VIII DUE (Chapter 22 through 22.19)** - **Review for Exam III (Chapter 21-22)**	- Chapter 23 (23.1-23.8)
Week 13 **November 17** **Carbohydrates**	- Chapter 23 (23.9-23.12 and supplement)	- **Draft of Named Reaction Due** - Chapter 23 (23.18-23.19)	- **QUIZ IX (Chapter 23 through 23.12)** - **Molecule of the Week Due** - Chapter 23 (23.20-23.24 and supplement)
Week 14 (November 24)	**No Class-Holiday**	**No Class-Holiday**	**No Class-Holiday**
Week 15 **December 01** **Carbohydrates and Amino Acids**	- **Draft of Named Reaction Returned** - Chapter 24 (24.1-24.4)	- **PROBLEM SET IX DUE (Chapter 23 and 24 through 24.4)** - Chapter 24 (24.5, 24.7, 24.11) - **Review for Exam IV (Chapters 22-23)**	- Chapter 24 (24.8-24.9, 24.13)
Week 16 **December 08** **Amino Acids and Review**	- **Named Reaction Summary Due 12/07/08 by 12:00am** - Additional reactions (Handout)	- **PROBLEM SET X DUE (Chapter 24)** - **Review for Final**	- **QUIZ X (Chapter 24)** - **Molecule of the Week Due** - **Review for Final**

Chemistry 380—Fall 2008
Artistic Presentation

The purpose of this assignment is to introduce you to different ways of presenting scientific information. Many of you are accustomed to writing papers and laboratory reports, both of which are excellent method of disseminating practical and interesting material. However, sometimes a more visual approach to the material is desirable. In my experience, artistic presentation and subsequent interpretation can substantially improve a student's ability to learn difficult material. This project will help you explore your creative side through a work of art that reflects the nature of the material we are covering in Chemical Immunology. Your ability to present your work, and interpret the work of others will benefit your understanding of many aspects of this course.

Though it is hard to provide a distinct definition of art, the following list gives some common characteristics of what might be considered a successful work of art:

I. Work that is created with the intention of evoking an understanding or an attempt at understanding a specific topic.
II. Work that confers a particularly appealing or aesthetically satisfying structure or form on a particular topic.
III. Work that encourages the discussion of a specific topic through interpretation.
VI. Work that communicates on many different levels of appreciation.

Forms of Artistic Expression:

The following are suggestions for projects you might consider undertaking. These are only suggestions and you are not limited to the following list.

I. Drawings, paintings, or sculptures representing individuals or topics discussed in the course.
   -Examples include drawings, sketches, cartoons, paintings, and sculptures (paper or electronic) of individual persons, molecular structures, proteins, enzymes, and/or biological systems that are covered in the course.
II. A literary work of fiction or non-fiction that focuses on a particular aspect of the course.
   -Examples include poems or short stories that highlight important people associated with the field of carbohydrate chemistry or important molecular interactions (i.e. a protein and a carbohydrate never meant to be together, suddenly find themselves bonding in harms way).
III. A ten minute film or skit representing an important aspect of the material (you may involve other people who are not affiliated with the course if you choose to do this type of a project).
   -Examples might include a short film or skit that describes a particular immunological interaction (i.e. a movie that involves several bacterium taking over a fictional town called Glycopolyis and the eradication of those bacteria using a pharmaceutical reagent).
IV. A collage of original photographs or published photographs on the subject matter.
   -Examples include photographs of individuals, x-ray crystal structures, or journal article covers highlighting the importance or relevance of important biomolecules of importance in the world today.
V. Crafts using natural media including wood, clay, glass, textiles and metal.
   -Examples include glass blowing, stained glass, mosaics, pottery, cross stitching, crocheting, rug hooking, weaving, knitting, leather work, scrapbooking, origami, papier-mâché, wood carving and wood working of individual persons, molecular structures, proteins, enzymes, and/or biological systems discussed in the course.

As a guideline you should put a minimum of eight to ten hours into your project over the course of the next two months, and your project should cost under $20.00.

Time Frame:

Topic—Please choose a topic and form of presentation. Please submit a brief description of your project by Friday, September 12, 2008. Your submission should include a note about the form of your artistic presentation (see examples above) and any resources you might need in order to properly display your work.

Individual Meetings—Please make an appointment to meet with me individually during the week of September 22, 2008 to discuss your potential project. I will post a sign-up sheet on Blackboard the week of September 15th.

Abstract—A short abstract of 500 words or less is due on or before Friday, October 10, 2008. I will be putting together a book of abstracts for presentation day so no late submissions please.

Presentations—Artistic presentations are scheduled for the week of October 27, 2008. Please be prepared to present your work in class and give a five minute description of your work (longer if you are doing a video or skit).

Evaluations—Your work will be evaluated through self-assessment of your own work, as well as an assessment from your peers and the course instructor (me!). The quality of your work of art, and your ability to effectively discuss your work will be an important part of the overall evaluation. We will discuss specific criteria as the presentation period approaches.

Chemistry 380—Fall 2008
Individual Research Presentation
Synthesis and Biological Evaluation of an
Immunologically Relevant Pharmaceutical Reagent

During the final half of this semester, each of you will work on a short paper and presentation that describes the total synthesis and discusses the biological evaluation of an immunologically relevant pharmaceutical reagent. In general, this project is meant to teach you how synthetic organic chemistry is applied to the rational design and synthesis of molecules that are used to combat infectious diseases and immune disorders. This project will also teach you how to approach and utilize the chemical and biochemical literature effectively in addition to learning how to write and present in a style that is adequate for scientific communication.

I have set forth some guidelines for the required paper and presentation in the following text. Please remember that these guidelines should be strictly adhered to but you are also free to contribute additional ideas and/or resources that you think are appropriate

.

Choosing a Target Molecule:

You can choose to present the total synthesis of any molecule that interests you as long as it has been used to treat an infection or immunological disorder. However, you will want to keep in mind that no two persons in the class can choose to present work on the same molecule. Therefore, you want to get your choices in to me as soon as possible.

There are literally hundreds of published total syntheses of natural (and unnatural!) products that are used to treat infections and immune disorders. The key here is that you want to choose a compound for which there has been at least one total synthesis reported in the past ten years (1998 to present). You should also make sure that the synthetic is not too obscure, and you should narrow your topic to something that is manageable so that you don't have too much information to digest. That said, you will also want to make sure that you choose a compound that has been investigated thoroughly enough to accumulate the amount of information necessary to meet the requirements set forth for this project, including biological relevance.

Outlining the Paper and Presentation:

Once you have chosen a topic you should begin to outline your short paper and presentation keeping in mind that the following items should appear somewhere in your discussion:

1. A short description of the main individual(s) (principle investigators) responsible for the work you are presenting (0.5-1 page/1-2 slides)
   -You should include a brief biography of the individual(s) credited with the total synthesis and/or the elucidation of the mode of activity of the pharmaceutical reagent you are studying (i.e. Where are they from? How did they decide to study chemistry, biochemistry, or immunology? Where were they employed at the time the synthesis and/or biological evaluation took place? Where are they now etc).
   -You should discuss any other important contributions of the individual(s) associated with your pharmaceutical reagent to the physical and life science fields (detailed accounts here are not necessary).
2. Present the total syntheses for your compound. (1-2 pages/2-4 slides)
   -Describing the chemistry in detail for each step of the reaction.
   -Highlight any problems or significant accomplishments in the synthesis of your pharmaceutical reagent.
   -Discuss any mechanisms that are not straightforward.
3. Include a complete and thorough discussion of the biological activity of the molecule. (1-2pages/2-4 slides)
   -Discuss the target audience for your pharmaceutical compound (i.e. What infection or immunological disorder is being targeted?).
   -Discuss the pharmacological activity of the drug with an emphasis on the role the reagent plays in alleviating an immune response at the molecular level.

Preparing the Paper and Presentation:

The following guidelines should be followed when putting together the rough and final drafts of your paper for submission:

***Title Page*:** You should include a title page with your name and the course information typed and centered about half way down the page. You may be creative with the title page if you would like.

Abstract: You should include a **graphical** abstract (300 words or less) with a title and name typed and centered at the top of the page. You may be creative with your titles if you would like. (30 points)

Body: Your paper should contain a title page and the body should be two to three pages in length (excluding references). Your presentation should be ten minutes in length. Both the paper and presentation should be well organized, and should include any relevant figures, schemes, and tables. All of your work should be referenced appropriately. (60 points)

References: References should include the following: (10 points)

1. One current (2000 or later) journal article that describes the total synthesis of your molecule.
2. At least three references total with at least two from primary sources including scientific journals from chemistry, biochemistry, biology, physics, or other disciplines.
3. Proper citation using either end notes or footnotes using superscript Arabic numerals. References should be made in proper format according to the *ACS Style Guide*. Recent examples may be found in the *Journal of the American Chemical Society* (JACS) as well as the *Journal of Organic Chemistry* (JOC). To review the *ACS Style Guide* please drop by my office. Copies of JACS and JOC can be found in the library.

You should use the material in your paper to put together your presentation. The presentation itself will be worth 50 points.

Due Dates:

Please keep the following deadlines in mind when putting together your paper and presentation:

Topics*:* Your topic is due no later than Friday, September 26, 2008 by 5:00pm and should be submitted to me via Blackboard with the main references that you are planning to present.

Outline*:* A complete outline is due no later than Friday, October 31, 2008 by 5:00pm and should be submitted to me via Blackboard as an MS Word file or PDF.

Graphical Abstract*:* The graphical abstract is due on Friday, December 05, 2008 by midnight and should be submitted to me via Blackboard as an MS Word file.

Final Paper*:* The final abstract and completed paper is due by midnight on December 07, 2008. Papers should be submitted both electronically (MS Word or PDF) and via hard copy. Late papers will be accepted and will receive a reduction in the overall points as outlined in the class syllabus.

Presentations*:* A formal presentation of your work will be presented during the last week of class. Presentations should be between five and seven minutes. The presentation voted as "best in the class" will receive a special award.

Evaluations—Your work will be evaluated through self-assessment of your own work, as well as an assessment from your peers and the course instructor (me!). We will discuss specific criteria as we approach the presentations.

CHEMICAL IMMUNOLOGY
CHEMISTRY 380
FALL 2009

Instructor Information:

Nicole L. Snyder, Ph.D. (Call me Nicole)

Office: SCCT 1072

Lab: SCCT 1068

Office Hours: Monday: 10:00am until 11:00am
Wednesday: 3:00pm until 5:00pm
Thursday: 8:00am until 9:00am
Sunday: 5:00pm until 6:00pm
….and also by appointment

Office Phone: (315) 859-4742

Email: nsnyder@hamilton.edu

Course Objectives:

This course is designed to introduce you to the exciting field of chemical immunology. The course itself will be a mix of chemistry, biochemistry, and biology, and will be divided into two overlapping modules. The first module will provide a basic overview of immune system with an emphasis on the important interactions that take place between macromolecules from a molecular perspective. I will do my best to zoom in on key interactions that occur between the proteins, carbohydrates and other biomolecules involved in many normal and abnormal immune processes. This portion of the course will rely heavily on the course text *Cellular and Molecular Immunology* by Abdul K. Abbas, Andrew H. Lichtman, and Shiv Pillai.

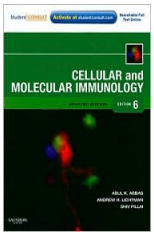
The second module of the course will focus on the pathology and treatment of infectious diseases and immune disorders. We will use actual case studies to highlight the pathology of these diseases and disorders, and will use a number of resources to rationalize the molecular basis for the clinical manifestations that are observed in the cases we study. As part of this module we will focus on vaccine development and rational drug design, including the synthesis of some of the more interesting pharmaceutics on the market. This portion of the course will rely heavily on *Molecules and Medicine* by E. J. Corey, Barbara Czako, and Laslo Kurti, the current literature and handouts which will be provided as the course progresses.

Throughout the course I will serve largely as a guide. While I have provided some structure for our journey, I hope that we can consider the "Schedule of Events" largely fluid. As you can imagine there is an abundance of material on many of the topics we will cover, and it would be impossible (and unadvisable) to try to cover everything in a one semester course. That said, if we discuss a topic that you would like to see expanded, I am happy to spend more time and go into greater detail with the material that pertains to that topic. Likewise, if there are topics that you do not see on the syllabus that you would like to see covered, please let me know and I will do my best to include the material in the course.

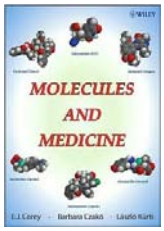
Finally, it is my sincere hope that you find this course and the material we cover as interesting as I do, and that by the end of the semester you will look back on this experience with fond memories of your time spent learning how the world around you works from an immunological perspective. Here's to a great semester!

*Image adapted from: Liu, F.-T.; Rabinovich, G. A. *Nat. Rev. Cancer* **2005**, *5*, 29-41.

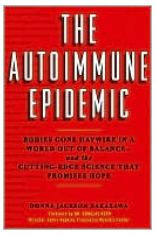
Required Texts:



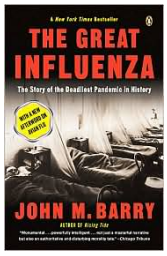
1. Cellular and Molecular Immunology (2009)
   By: Abul K. Abbas, Andrew H. Lichtman and Shiv Pillali
   ISBN: 9781416031239



2. Molecules and Medicine (2007)
   By: E. J. Corey, Barbara Czako and Laslo Kurti
   ISBN: 9780470227497

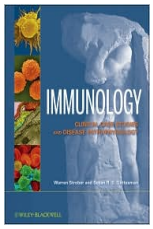


3. The Autoimmune Epidemic (2008/9)
   By: Donna Jackson Nakazawa
   ISBN: 9780743277754

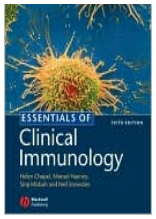


4. The Great Influenza (2005)
   By: John M. Barry
   ISBN: 9780143036494

Optional Texts:



5. Immunology: Clinical Case Studies and Disease Pathophysiology (2009)
   By: Warren Strober and Susan R. S. Gottesman
   ISBN: 9780471326595



6. Essentials of Clinical Immunology (2007)
   By: Helen Chapel, Mansel Haeney Siraj Misbah and Neil Snowden
   ISBN: 9781405127615

Helpful Web Resources:

1. The Journal of Immunology
   http://www.jimmunol.org/
2. Nature: Immunology
   http://www.nature.com/ni/index.html
3. Annual Review of Immunology
   http://arjournals.annualreviews.org/loi/immunol?cookieSet=1
4. Immunology
   http://www.blackwellpublishing.com/imm_enhanced/
5. The Antibody Resource Page
   http://www.antibodyresource.com/
6. Microbiology and Immunology Online—University of South Carolina School of Medicine
   http://pathmicro.med.sc.edu/book/immunol-sta.htm
7. The Biology Project—Arizona State University
   http://www.biology.arizona.edu/immunology/immunology.html

Notes on Student Assessment:

You will be evaluated in this course on a regular basis. The basis for course evaluation is provided below with explanations:

Midterm (Take Home)	20%
Final (Take Home)	20%
Homework	15%
Artistic Presentation	15%
Original Research Proposal	15%
Class Participation	15%

Midterm: The midterm will focus on the first half of the course. The midterm will be take-home and the questions will be taken from the homework (see below). The midterm will be administered on Tuesday, October 13, 2009 and will be due by 5:00pm on Friday, October 23, 2009.

Final: The final will focus on the second half of the course. The final will be take-home and the questions will be taken from the homework (see below). The final will be given on Tuesday, December 08, 2009 and will be due by 10:00pm on Tuesday, December 15, 2009 by 10:00pm.

Homework: Each week you are expected to write one question based on the reading for that week. This question should be representative of a ten to twenty point essay question that would appear on an exam (midterm or final). You are expected to provide a one and a half to one page answer (typed) with references (if necessary) to the question you submit. Your question should remain confidential and you will be required to submit you work with the following statement:

> I, _____, certify that I have referenced any sources I used to complete this assignment beyond those distributed to me as part of this course. I,_____, also certify that the work in this assignment is my own work, and that I have neither accepted help from any other student, professor, parent, or high school mentor, nor have I have given advice or information to any other student in the course regarding the material in this assignment. I, _____, understand that violating these guidelines is a violation of the honor code and if I am found to have violated the honor code, I will be subject to disciplinary action.
>
> Signed_____Date: ___/___/___

You will be graded on your ability to create an original, challenging question and reasonable answer to your own question in your own words. I will select one question from each student in the class for both the midterm and the final exams. Homework questions will be due by Friday at 5:00pm and must be submitted through Blackboard.

Artistic Presentation: As part of this course you are required to put together an artistic presentation that in some way reflects the nature of the topics we are covering. Examples of work include poems, short stories, collages, paintings, or sculptures. The grading for this particular portion of the course will depend heavily on the effort you put forward. Five percent of your grade will come from the self evaluation of your own project. Another five percent of your grade will be determined by a peer assessment of your work. The final five percent of your grade is based on the instructor's assessment of your work. A detailed handout for this project will be distributed during the first week of classes.

Original Research Proposal: You will work in groups of four to five this semester to put together an eight to ten page original research proposal that addresses a major immunologic disease and a proposed treatment for the disease (i.e. H5N1). Your project will give a detailed description of the disease from an immunological perspective and a plausible mode of treatment (design and synthesis/preparation of an immunologic and cellular/animal study). Five percent of your grade will come from a combination of self assessment and an assessment of your contributions to the project by your group members. Another five percent of your grade will be determined by an outside reviewer who will asses and provide feedback on your work. The final five percent of your grade is based on the instructor's assessment of your work. A detailed handout and sample proposal for this project will be distributed in the first week of classes.

Class Participation: Frequent participation in required. Everyone must participate in class each week in some way. Failure to participate will result in a lowering of the overall grade. The more you participate, the better your grade will be!

Student with Disabilities: In accordance with the Americans with Disabilities Act, any student who has a documented learning disability will be provided with reasonable accommodations designed to meet his/her needs. Before any such assistance can occur, it is the responsibility of the student to see that documentation is on file with the appropriate individual(s). Please see me as soon as possible to discuss any need for accommodations.

Schedule of Events:

Week of…	Topic(s)
August 23, 2009	**Introduction—**Syllabus and other materials.
August 30, 2009	**Introduction to the Immune System** Text Readings: CMI—Chapters 1 through 3; MM—pages 2-34 and 112-120; AE—pages 1-75
September 06, 2009 *and* *September 13, 2009*	**Antibodies, Antigens, and their Interactions: An Overview** Text Readings: CMI—Chapters 4 through 7; MM—pages 2-34 and 112-120; AE—pages 76-120 ***Artistic presentation topic due via email on Friday, September 18, 2009 by midnight***
September 20, 2009 *and* *September 27, 2009*	**Immunological Memory, Lymphocyte Development, Immunological Tolerance and Vaccination** Text Reading: CMI—Chapters 8 through 11; MM—pages 2-34 and 112-120; AE—pages 126-166 ***Research groups assigned on Friday, September 25, 2009***
October 04, 2009 *and* *October 11, 2009*	**Effector Mechanisms** Text Readings: CMI—Chapters 12 through 15; MM—pages 130-172; AE—pages 167-220 Case Studies: (Handouts) 1. Bacterial: rheumatic fever, septicemia, syphilis, tuberculosis, and leprosy 2. Fungal: ringworm, athletes foot, oral thrush 3. Viral: Epstein-Barr, herpes, hepatitis, measles, Dengue, Marburg, certain cancers, and HIV 4. Parasitic: malaria, leishmaniasis, shistosomiasis, trypanosomiasis, and toxoplasmosis ***Artistic presentation abstract due via email on Friday, October 09, 2009 by midnight***
October 18, 2009	**Autoimmune Diseases** Text Readings: CMI—Chapter 18; MM—pages 122-126; AE—pages 221-263 and discussion Case Studies: (Handouts) 1. Accepted: myasthenia gravis, multiple sclerosis, diabetes mellitus type 1, celiac disease, inflammatory bowel disease, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, Guillain-Barre syndrome (GBS), Graves' disease, Addison's disease, Sjorgren's syndrome, Goodpasture's syndrome 2. Suspected: Chagas disease, endometriosis, and schizophrenia ***Midterm exam distributed on Tuesday, October 13, 2009 (due October 23, 2009 by 5:00pm)*** ***Original research proposal Topic Due on Wednesday, October 14, 2009 by 4:00pm***
October 25, 2009	**Allergies, and Hypersensitivity** Text Readings: CMI—Chapter 19; MM—pages 38-54; GI—pages 1-166 Case Studies: (Handouts) 1. Mastocytosis: cutaneous, systemic 2. Anaphylaxis: acute, type I 3. Asthma: intermittent, mild, moderate, persistent 4. Vasculitis: Kawasaki disease, Wegener's granulomatosis, Churg-Strauss Syndrome, Takayasu's arteritis, Henock-Schonlein purpura, cryoglobulinemia, giant cell arteritis (temporal) ***Midterm exam due on Friday, October 23, 2009 by 5:00pm***
November 01, 2009	**Artistic Presentations** ***Outline for group original research proposals due via email on Friday, October 30, 2009 by midnight***
November 08, 2009	**Immunodeficiency** Text Readings: CMI—Chapter 20; GI—pages 167-228 Case Study: (Handouts) 1. Bruton's disease 2. Chronic granulomatous disease 3. Chediak-Higashi syndrome 4. Severe Combined immunodeficiency Disorder (SCID) 5. Common Variable Immunodeficiency Disorder (CVID) 6. Acquired Immune Deficiency Disorders (AIDS)
November 15, 2009	**Transplantation Immunology** Text Readings: CMI—Chapter 16; MM—pages 122-126 (review); GI—pages 229-296 Case Study: (Handouts) 1. Allotransplantation 2. Xenotransplantation 3. Blood and bone marrow transfusions
November 22, 2009	**No Class—Holiday**
November 29, 2009 *and* *December 06, 2009*	**Cancer Immunology** Readings: CMI-Chapter 17 and Handouts; MM—pages 184-198; GI—pages 297-447 Case Study: (Handouts) 1. B-cell neoplasms: chronic lymphocytic leukemia, small lymphocytic lymphomas, prolymphocytic leukemia, follicular, mantle cell, MALT, nodal, marginal. and splenic lymphomas, hairy cell leukemia, Burkitt's lymphoma, plasmacytoma, lymphomatoid granulomatosis 2. T/NK-cell neoplasms: prolymphocytic leukemia, large granular lymphocytic leukemia, NK cell leukemia, peripheral T-cell lymphoma, mycosis fungoides, Sezary syndrome, primary cutaneous lympho-proliferative disorder, anaplastic lymphoma, extranodal NK/T cell lymphoma, other T-cell lymphomas 3. Hodgkins lymphoma: nodular sclerosis, mixed cellularity, lymphocyte rich and depleted 4. CNS cancers: neuroepithelial, cranial and paraspinal nerve, meninges ***Final exam distributed on Tuesday, December 08, 2009 (due Tuesday, December 15 , 2009 by 10:00pm)***

Chemistry 380—Fall 2009
Artistic Presentation

The purpose of this assignment is to introduce you to different ways of presenting scientific information. Many of you are accustomed to writing papers and laboratory reports, both of which are excellent method of disseminating practical and interesting material. However, sometimes a more visual approach to the material is desirable. In my experience, artistic presentation and subsequent interpretation can substantially improve a student's ability to learn difficult material. This project will help you explore your creative side through a work of art that reflects the nature of the material we are covering in Chemical Immunology. Your ability to present your work, and interpret the work of others will benefit your understanding of many aspects of this course.

Though it is hard to provide a distinct definition of art, the following list gives some common characteristics of what might be considered a successful work of art:

I. Work that is created with the intention of evoking an understanding or an attempt at understanding a specific topic.
II. Work that confers a particularly appealing or aesthetically satisfying structure or form on a particular topic.
III. Work that encourages the discussion of a specific topic through interpretation.
VI. Work that communicates on many different levels of appreciation.

Forms of Artistic Expression:

The following are suggestions for projects you might consider undertaking. These are only suggestions and you are not limited to the following list.

I. Drawings, paintings, or sculptures representing individuals or topics discussed in the course.
   -Examples include drawings, sketches, cartoons, paintings, and sculptures (paper or electronic) of individual persons, molecular structures, proteins, enzymes, and/or biological systems that are covered in the course.
II. A literary work of fiction or non-fiction that focuses on a particular aspect of the course.
   -Examples include poems or short stories that highlight important people associated with the field of carbohydrate chemistry or important molecular interactions (i.e. a protein and a carbohydrate never meant to be together, suddenly find themselves bonding in harms way).
III. A ten minute film or skit representing an important aspect of the material (you may involve other people who are not affiliated with the course if you choose to do this type of a project).
   -Examples might include a short film or skit that describes a particular immunological interaction (i.e. a movie that involves several bacterium taking over a fictional town called Glycopolyis and the eradication of those bacteria using a pharmaceutical reagent).
IV. A collage of original photographs or published photographs on the subject matter.
   -Examples include photographs of individuals, x-ray crystal structures, or journal article covers highlighting the importance or relevance of important biomolecules of importance in the world today.
V. Crafts using natural media including wood, clay, glass, textiles and metal.
   -Examples include glass blowing, stained glass, mosaics, pottery, cross stitching, crocheting, rug hooking, weaving, knitting, leather work, scrapbooking, origami, papier-mâché, wood carving and wood working of individual persons, molecular structures, proteins, enzymes, and/or biological systems discussed in the course.

As a guideline you should put a minimum of eight to ten hours into your project over the course of the next two months, and your project should cost under $20.00.

Time Frame:

***Topic*—**Please choose a topic and form of presentation. Please submit a brief description of your project by Friday, September 18, 2009. Your submission should include a note about the form of your artistic presentation (see examples above) and any resources you might need in order to properly display your work.

***Individual Meetings*—**Please make an appointment to meet with me individually during the week of September 27, 2009 to discuss your potential project. I will post a sign-up sheet on Blackboard the week of September 15th.

***Abstract*—**A short abstract of 500 words or less is due on or before Friday, October 09, 2009. I will be putting together a book of abstracts for presentation day so no late submissions please.

***Presentations*—**Artistic presentations are scheduled for the week of November 01, 2009. Please be prepared to present your work in class and give a five minute description of your work (longer if you are doing a video or skit).

***Evaluations*—**Your work will be evaluated through self-assessment of your own work, as well as an assessment from your peers and the course instructor (me!). The quality of your work of art and your ability to effectively discuss your work will be an important part of the overall evaluation. We will discuss specific criteria as the presentation period approaches.

Chemistry 380—Fall 2009
Original Research Proposal
Preparation and Biological Evaluation of an
Immunologically Relevant Pharmaceutical Reagent

During the final half of this semester you will work in groups of four to five to prepare an original research proposal that describes the preparation of and biological evaluation of an immunologically relevant pharmaceutical reagent. In general, this project is meant to teach you how synthetic chemistry is applied to the rational design and preparation of molecules that are used to combat infectious diseases and immune disorders. This project will also teach you how to approach and utilize the chemical and biochemical literature effectively, in addition to learning how to write and present in a style that is adequate for scientific communication.

I have set forth some guidelines for the required proposal in the following text. Please remember that these guidelines should be strictly adhered to but you are also free to contribute additional ideas and/or resources that you think are appropriate.

Choosing a Target:

The best way to choose a target is to first choose a disease or disorder for which there is currently no effective treatment. For example, glioblastoma multiforme (GBM) is the most aggressive type of primary brain tumor in humans and accounts for half of all primary brain tumor cases. The median survival time from the time of diagnosis is just over one year, with only one in every twenty patients surviving for more than three years. The most common methods for the treatment of GBM are palliative and include surgery, radiation therapy, and chemotherapy. More recently, a number of tumor-pulsed dendritic cell vaccines have been developed for the treatment of patients with GBM. While these vaccines have improved the prognosis for many patients with GBM, by nature they remain patient specific and are highly constrained by the time required to obtain peptide antigen from individual tumor cell lines. Therefore, a more effective and tumor specific, rather than patient specific therapeutic is needed to treat GBM. In this particular case the disease has been identified, and the need for a more effective pharmaceutical compound has been established. Once you have identified a disease or disorder, then you can begin to get creative!

Your goal as a group is to come up with a novel treatment for the disease you chose to study. The pharmaceutic you chose to study will most likely be one of three flavors:

(i) A specific antigen (carbohydrate, peptide, protein, lipid etc.) that has been isolated and found to be associated only with the pathogen responsible for the disease or with the diseased cell itself. Preparation of the compound and conjugation to an appropriate adjuvant/carrier protein may provide a potential treatment for the disease or disorder.

(ii) The pharmaceutic is based off of a compound already in use. The current compound has been rendered ineffective in many cases due to resistance, but an analog of the drug may prove successful in overcoming resistance while maintaining a biological profile similar to the original pharmaceutic.

(iii) A compound has been identified as a natural product capable of eliciting a favorable immune response. Unfortunately, the compound can only be harvested from an organism that is found in limiting quantities and therefore must be synthesized. Your goal in this case will be to prepare, analyzed and test the compound for biological activity.

A few points to consider… First, you want to choose a disease or disorder that has been relevant for at least the past ten years (2000 to present), and it will be easier to work through the details of your proposal if you have a lead on a potential mode of treatment. Second, you should also narrow your topic to something that is manageable so that you don't have too much information to digest in order to come up with a plausible treatment plan. That said, you will also want to make sure that you choose a disease or disorder that has been investigated thoroughly enough to accumulate the amount of information necessary to meet the requirements set forth for this project.

Outlining the Proposal:

Once you have chosen a topic you should begin to outline your proposal keeping in mind that the following sections should appear somewhere in your document:

1. Introduction (0.5 pages)
   -The introduction is essentially an extended abstract that presents and provides a brief explanation of the scientific problem and a general overview of the researcher(s) plans to address the problem.
   -Note that the specific goals of the research project should be as clear and concise as possible and the question(s) that the researcher(s) are trying to address should be explicit.
2. Background and Significance (1-3 pages)
   -The background and significance section is a more detailed version of the introduction and should be used to state the problem and significance of the proposed research in reasonable detail. The reviewer should be left with the impression that the research outlined in the proposal is very important and will have a significant impact in the field.

-As a general rule, this section should provide information on the pathology and immunology of the disease you are planning to study, in addition to any treatment methods that are currently available. If current treatment methods are available, the advantages and disadvantages of some of the more relevant examples should be highlighted.

3. Design and Preparation of a Target Pharmaceutic (2-3 pages)

-This design aspect of this section depends on whether you are choosing to prepare an pharmaceutic based on a known target (i.e. a specific antigen that has been isolated), by modifying a drug that is currently on the market but no longer effective (i.e. mutations have occurred that have rendered the drug ineffective), or by preparing a known compound that has not been synthesized before (i.e. a compound recently discovered in the *Journal of Natural Products* that has anticancer properties) or the known synthesis is inefficient. In either case you should provide specific details as to why you believe the pharmaceutic you are proposing is a potential solution to the scientific problem presented in the background and significance section. You should also provide a retrosynthetic analysis for the preparation of your pharmaceutic.

-The preparation section should provide a detailed synthetic plan for the preparation of your target pharmaceutic.

4. Biological Studies (2-3 pages)

-The biological studies section should provide a brief list of the techniques you plan to employ to evaluate your compound. You cannot use human models for this proposal so you will need to find a suitable cellular or animal model for your studies.

-You should also be clear about the information that you are trying to extract from each of the studies you plan to carry out and should explicitly state how each study answers the question(s) you are trying to ask.

5. References (unlimited)

-References should be made in proper format according to the *ACS Style Guide*. Recent examples may be found in the *Journal of the American Chemical Society* (JACS) as well as the *Journal of Organic Chemistry* (JOC). To review the *ACS Style Guide* please drop by my office. Copies of JACS and JOC can be found in the library.

To help guide you, I have provided you with a recent proposal that I will be submitting to the Humboldt Fellowship program for my studies abroad in Germany during my sabbatical year. This proposal is confidential and should not be shared with anyone outside of Chemical Immunology and Immunopharmacology.

Due Dates:

***Research Groups**:* Research groups will be assigned no later than Friday, September 25, 2009 by 5:00pm. The goal is to generate groups with one individual from each of the four concentrations represented (chemistry, biochemistry, biology, and neuroscience).

***Topics**:* Your topic is due no later than Wednesday, October 13, 2009 by 4:00pm and should be submitted to me via Blackboard with a list of three main references you are planning to use.

***Outline**:* A complete outline is due no later than Friday, October 30, 2009 by midnight and should be submitted to me via Blackboard as an MS Word file or PDF.

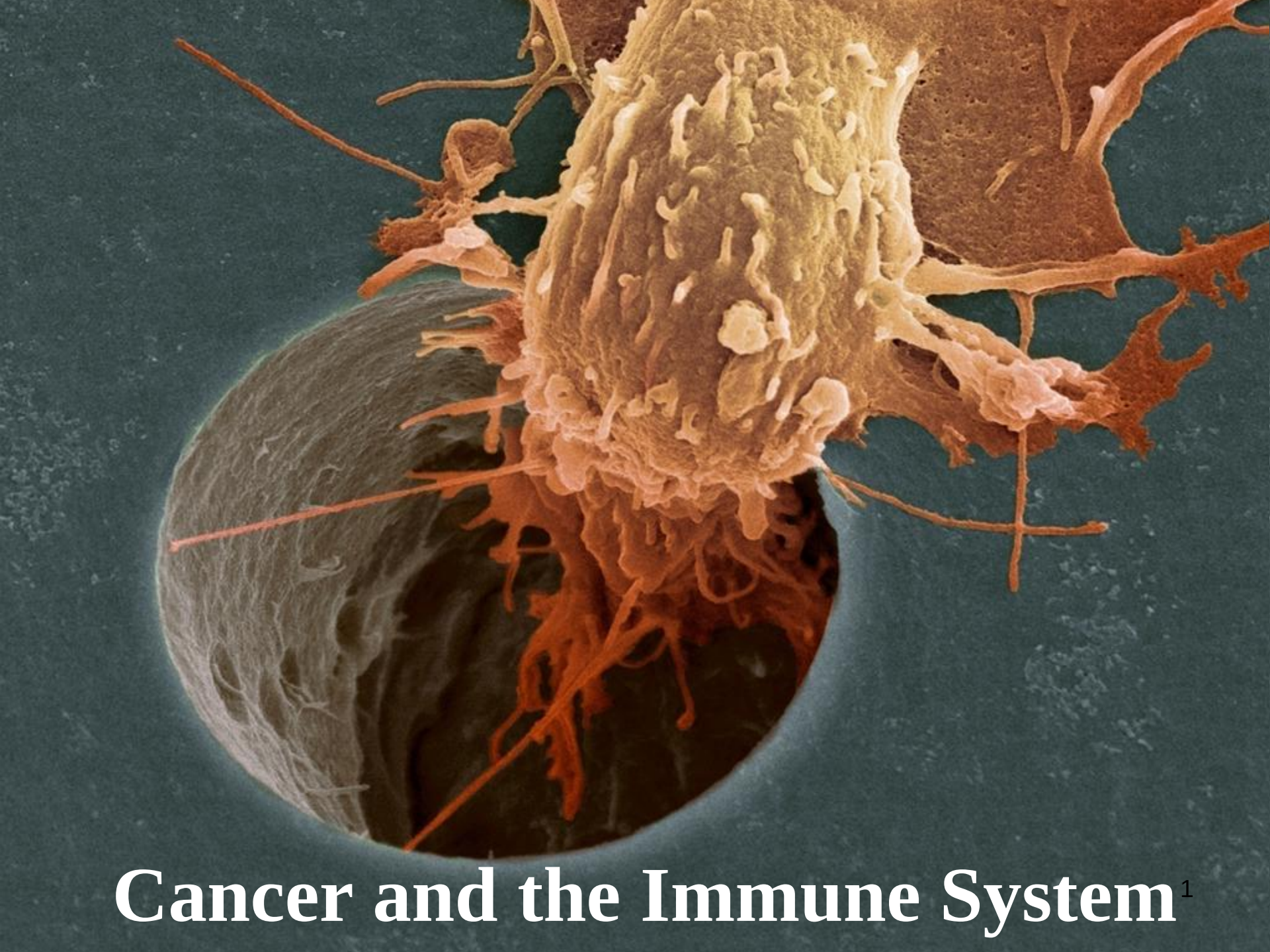
***Final Proposal**:* The final proposal will be due the week of November 29, 2009. Proposals should be submitted both electronically (MS Word or PDF) and via hard copy. Late proposals will be accepted and will receive a reduction of 10% per day late from the total points awarded.

Evaluation of Your Proposal:

Your final grade will be based on a self evaluation, an evaluation of your individual contributions to the proposal by your group members, assessment by a qualified outside reviewer, and an instructor assessment broken down as follows:

Self Evaluation:	3% (30 points)
Group Evaluation:	2% (20 points)
Outside Reviewer Assessment:	5% (50 points)
Instructor Assessment:	5% (50 points)

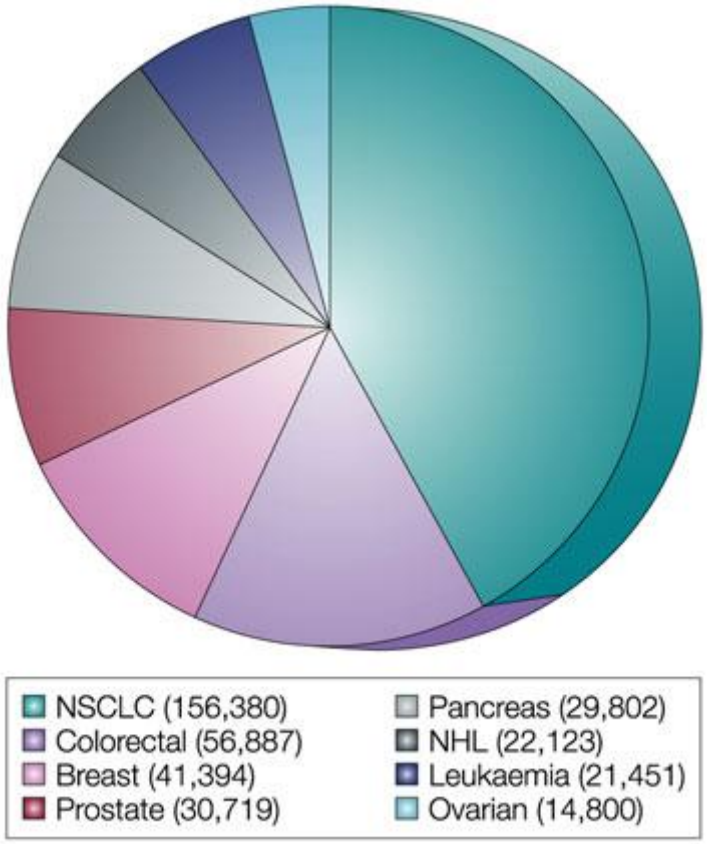
The outside reviewers will be faculty colleagues from institutions across the country and around the World. Your names and other identifying information will be removed from the proposal and the review will be blind. Reviewer comments will be shared with the entire group.



Cancer and the Immune System

Incidence

Cancer is a leading cause of death in the United States.



Number of Deaths Per Year
Image Credit: Farmer , G. *N. Rev.Drug Disc.***2004**, 547.

Types of Tumors (Neoplasms)

Benign-

Malignant-

Metastisis-

Tumor Growth and Metastasis

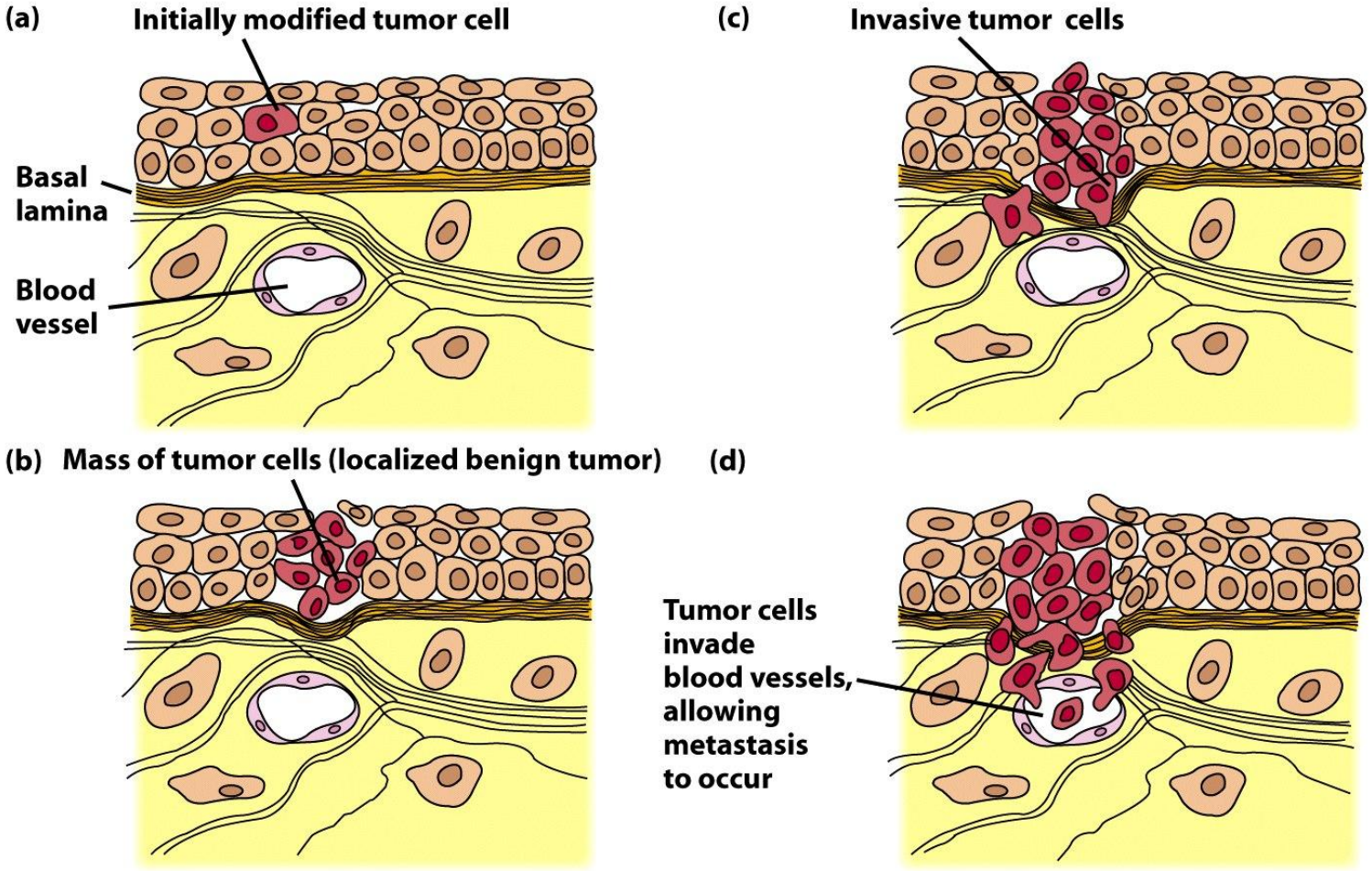


Image Credit: Kuby 6th ed.

Types of Malignant Tumors

Carcinomas (80%)-

Lymphomas (9%)-

Sarcomas (1%)-

Malignant Transformations

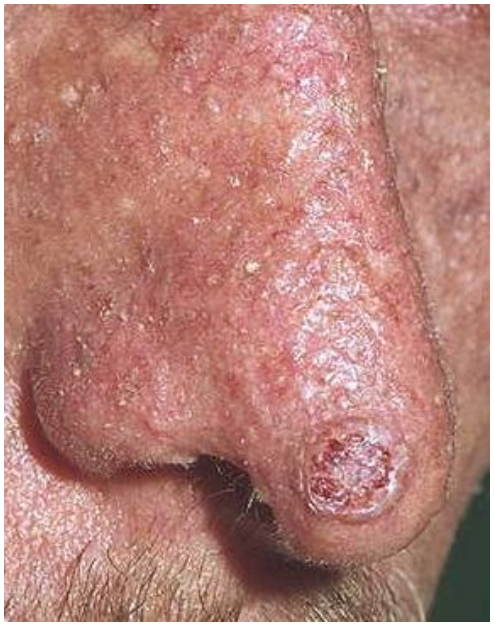
Healthy cells are transformed through two major routes:

1. Genetic Mutations
2. Viral infections

Image Credits: Google Images

Mutations

Xeroderma pigemtosa-defect in UV-specific endonuclease



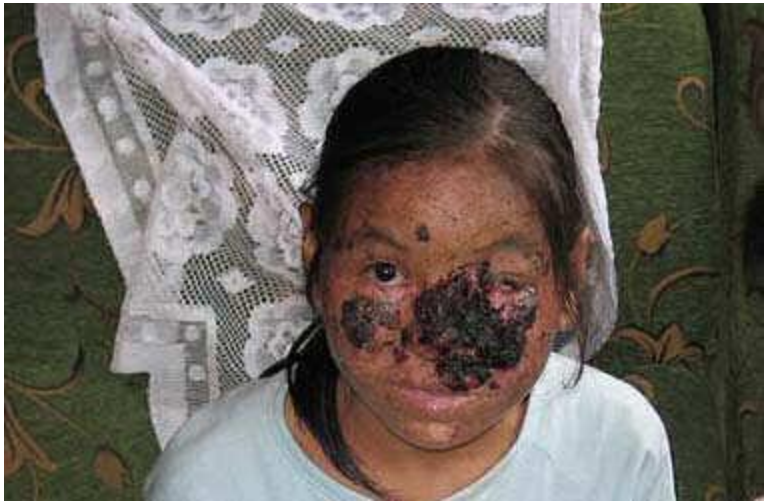


Image Credits: Google Images

Viruses Play a Role

T-cell lymphoma is associated with the Human T-cell Leukemia Virus-1 (RNA).

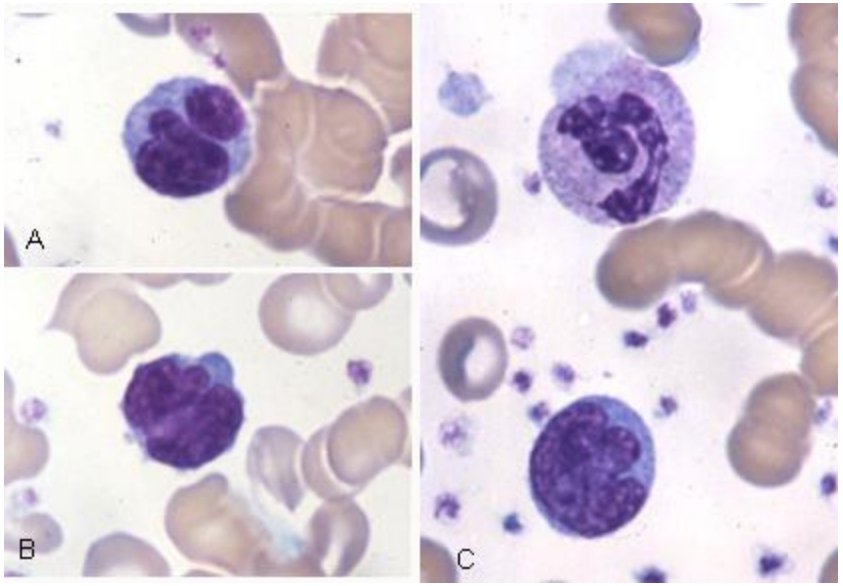


Image Credits: Access Medicine.com

Viruses Play a Role

Kaposi's Sarcoma is linked to Herpesvirus-8 (RNA).

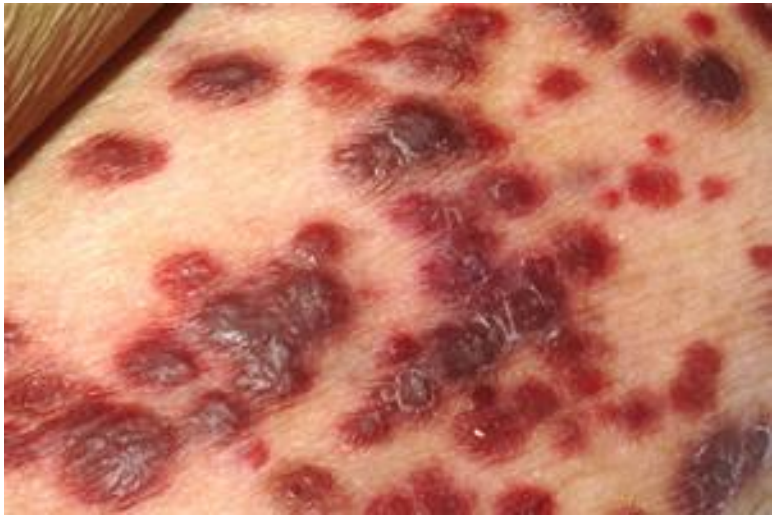


Image Credits: CancerQuest.org

Viruses Play a Role

Cervical carcinoma is linked the several serotypes of the human papillomavirus (DNA).

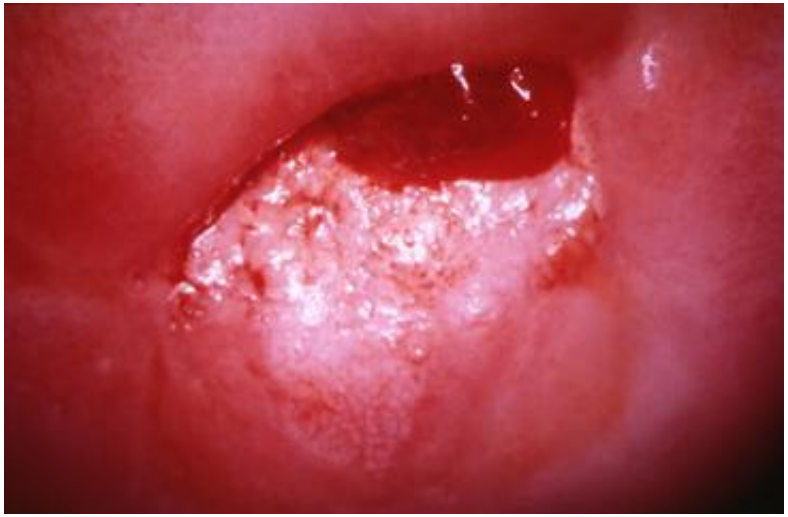


Image Credits: Medscape

Viruses Play a Role

Liver carcinoma is linked to the hepatitis B virus (DNA).

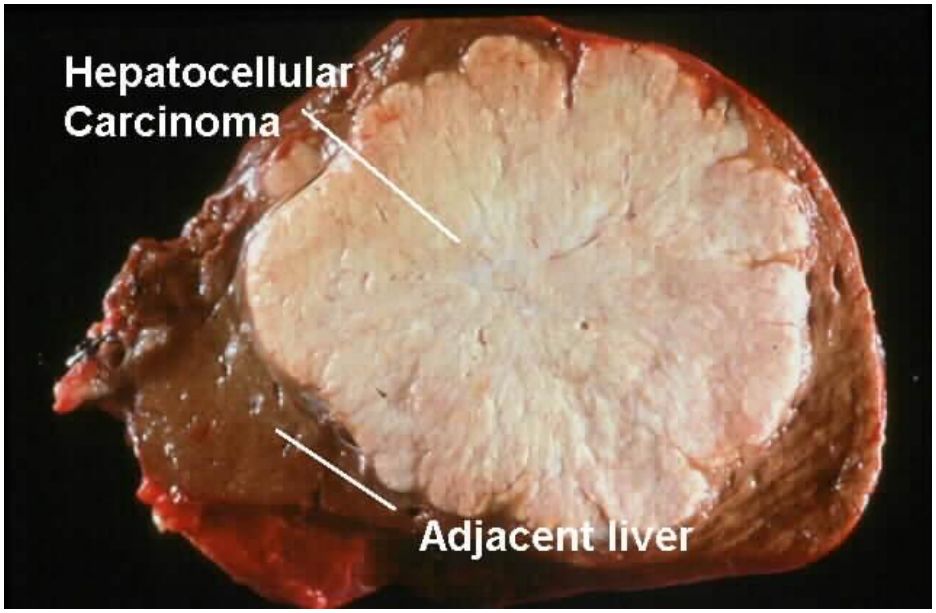
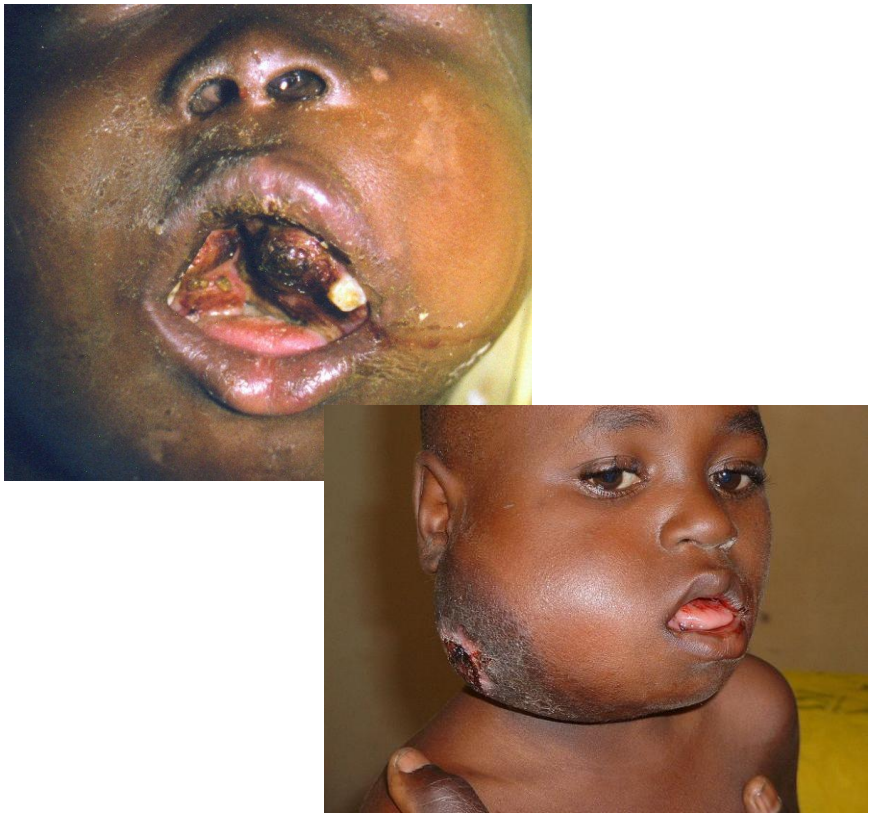


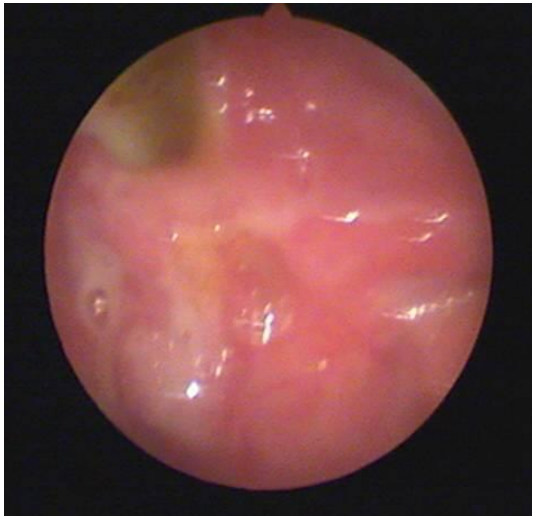
Image Credits: OSU Pathology

Viruses Play a Role

Epstein-Barr Virus is linked to Burkitt's lymphoma in African populations and nasopharyngeal carcinoma in Asian populations (DNA).



Burkitts Lymphoma
Image Credits: Wikipedia



Nasopharyngeal Carcinoma
Image Credits: Google Images

RNA vs DNA Viruses

Based on the Baltimore Classification System:

There are seven different replication strategies based on this system (Baltimore Class I, II, III, IV, V, VI, VII):

1. dsDNA (adeno, herpes, pox)
2. ssDNA (parvo)
3. dsRNA (reo)
4. (+)ssRNA (picorna, toga)
5. (-)ssRNA (orthomyco, rhabdo)
6. ssRNA-RT (retro)
7. dsDNA-RT (hepadna)

RNA viruses replicate in the cytosol (unless they are retroviruses).

-Retroviruses transcribe the RNA into DNA vis reverse transcriptase.

-Oncogenes can be transcribed into the hosts DNA.

-Example: Rous sarcoma virus (V-Src-protein kinase).

DNA viruses replicate in the nucleus.

-Use the cells machinery—especially polymerases to replicate.

-The cell is usually forced to undergo replication.

Oncogenes

Suggested by Howard Temin (1971).

-Temin thought the genes were not unique to viruses and could be found in healthy cells (proto-oncogenes/ cellular ongocognes).

-Cellular and viral oncogenes are highly conserved.

-What does this suggest?

Most cellular oncogenes encode for growth controlling proteins.

Cellular proliferation and death must be balanced!

Howard Temin
Image Credits: Wikipedia

Cancer Associated Genes

Three categories of genes:

1. Genes that induce cellular proliferation.

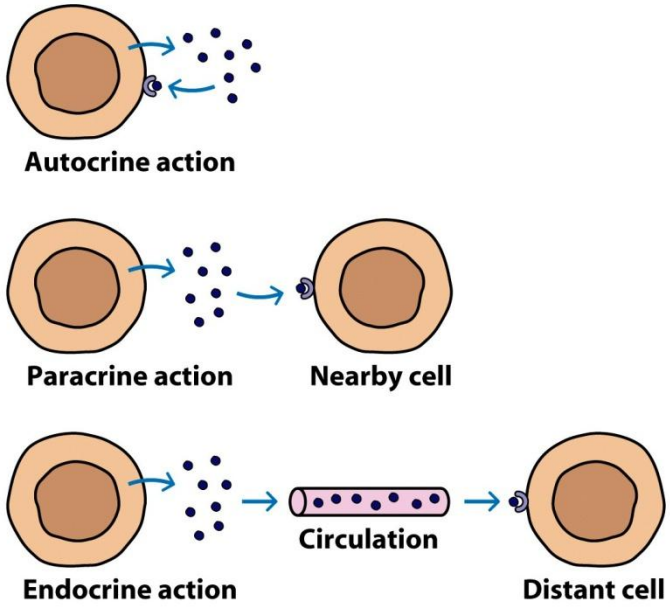


Photo credit: Kuby 6th edition

2. Tumor suppressor genes (inhibit cellular proliferation).
3. Genes that regulate programmed cell death.

Cancer Associated Genes

Functional classification of cancer-associated genes

Type / name	Nature of gene product
CATEGORY I: GENES THAT INDUCE CELLULAR PROLIFERATION	
Growth factors	
sis	A form of platelet-derived growth factor (PDGF)
Growth factor receptors	
fms	Receptor for colony-stimulating factor 1 (CSF-1)
erbB	Receptor for epidermal growth factor (EGF)
neu	Protein (HER2) related to EGF receptor
erbA	Receptor for thyroid hormone
Signal transducers	
src	Tyrosine kinase
abl	Tyrosine kinase
Ha-*ras*	GTP-binding protein with GTPase activity
N-*ras*	GTP-binding protein with GTPase activity
K-*ras*	GTP-binding protein with GTPase activity
Transcription factors	
jun	Component of transcription factor AP1
fos	Component of transcription factor AP1
myc	DNA-binding protein
CATEGORY II: TUMOR SUPRESSOR GENES, INHIBITORS OF CELLULAR PROLIFERATION*	
Rb	Suppressor of retinoblastoma
p53	Nuclear phosphoprotein that inhibits formation of small-cell lung cancer and colon cancers
DCC	Suppressor of colon carcinoma
APC	Suppressor of adenomatous polyposis
NF1	Suppressor of neurofibromatosis
WT1	Suppressor of Wilm's tumor
CATEGORY III: GENES THAT REGULATE PROGRAMMED CELL DEATH	
bcl-2	Suppressor of apoptosis

*The activity of the normal products of the category II genes inhibits progression of the cell cycle. Loss of a gene or its inactivation by mutation in an indicated tumor-suppressor gene is associated with development of the indicated cancers.

Image Credit: Kuby 6th ed.

Proto-oncogene Conversion

Proto-oncogenes can be converted to oncogenes.

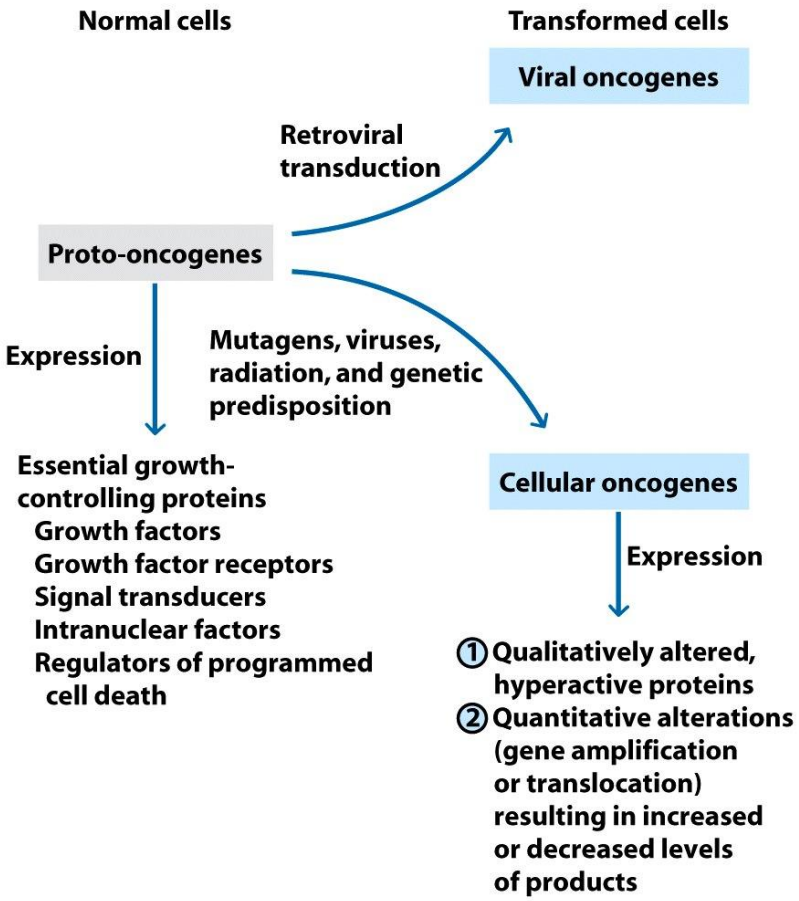


Image Credit: Kuby 6th ed.

Cancer Cells are Immortal

HeLa cells (cervical) are still used for studies today!

-Henrietta Lacks (HeLa) who died on October 04, 1951.

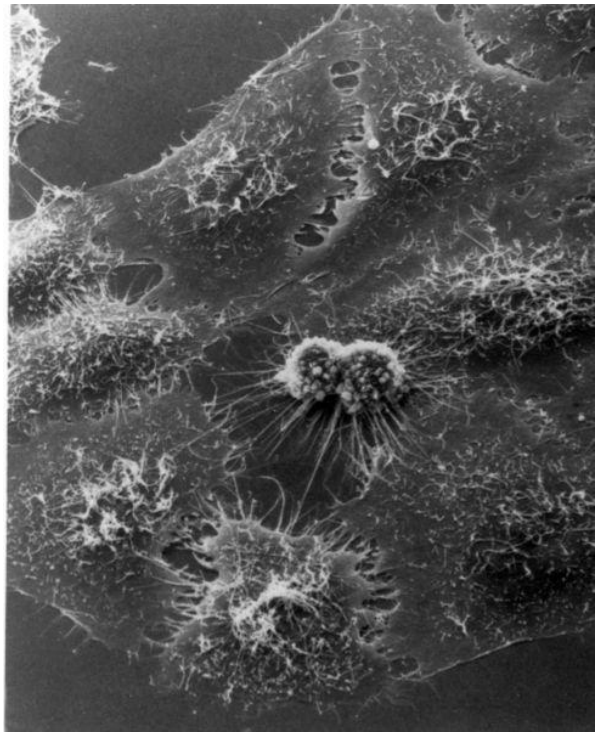


Image Credits: Wikipedia

Tumors of the Immune System

Lymphomas and leukemia's are associated with the immune system.

- -Lymphomas proliferate as solid tumors in lymphoid tissue (bone marrow, lymph nodes, thymus).
  - -Include Hodgkin's and non-Hodgkin's lymphomas.
- -Leukemia's proliferate in blood and lymph.
  - -Classified as acute or chronic.

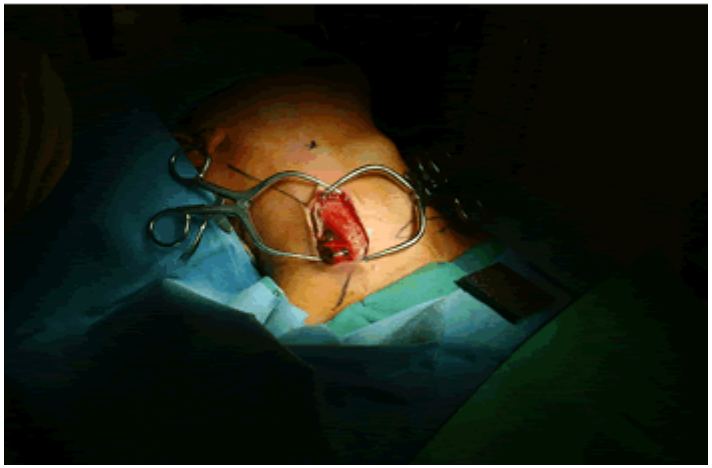


Image Credits: ThyroidCancer.com

Hodgkins' vs Non-Hodgkins Lymphoma

Hodgkins Lymphoma-spreads from one lymph node group to another.

-Characterized by the presence of RS cells (giant B-cells).

-Bimodal age distribution group (15-35 and above 55).

-Four different stages:

I. Involvement of a single lymph node region (I) or single extralymphatic site.

II. Involvement of two or more lymph node regions on the same side of the diaphragm or one lymph node region and a contiguous extralymphatic site

III. Involvement of lymph node regions on both sides of the diaphragm, which may include the spleen and/or limited contiguous extralymphatic organ or site

IV. Stage IV is disseminated involvement of one or more extralymphatic organs

Non-Hodgkins Lymphoma-very aggressive.

-Many different subtypes.

-Can affect both B and T-cells.

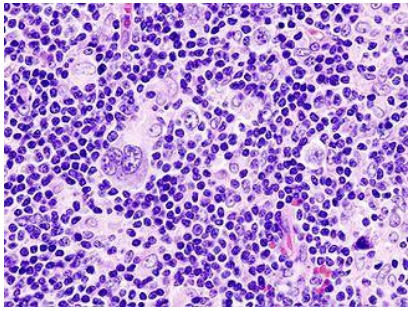


Image Credits Wikipedia

Leukemia

Involve translocation of the c-myc gene.

Acute-appear suddenly and progress rapidly.

- -Arise in less mature cells.
- -Include acute lymphocytic (ALL) and myelogenous leukemia (AML).
- -Can develop at any age.

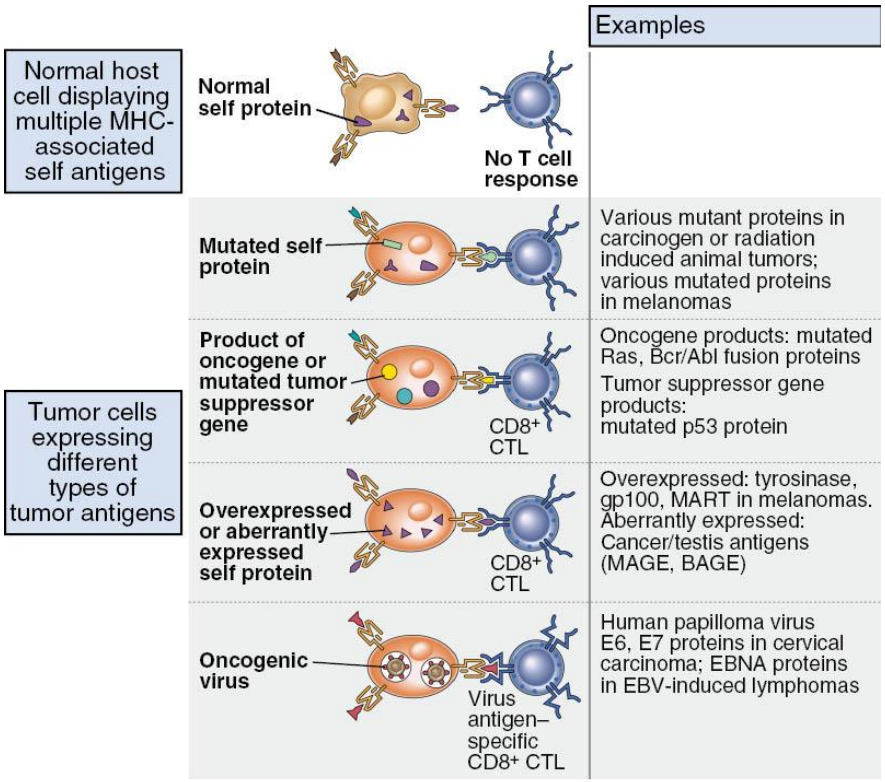
Chronic- less aggressive and develop slowly (patients are barely symptomatic).

- -Arise in more mature cells.
- -Include chronic lymphocytic (CLL) and myelogenous leukemia (CML).

Tumor Antigens

Two types of tumor antigens:

1. Tumor-specific transplantation antigens- antigens that are unique to tumor cells and do not occur on normal cells in the body.
2. Tumor-associated transplantation antigens- antigens that are not unique to tumor cells.



Tumor Antigens

Type of antigen	Examples of human tumor antigens
Products of oncogenes, tumor suppressor genes	Oncogenes: Ras mutations (~10% of human carcinomas), p210 product of Bcr/Abl rearrangements (CML), overexpressed Her-2/neu (breast and other carcinomas)
	Tumor supressor genes: mutated p53 (present in ~50% of human tumors)
Mutants of cellular genes not involved in tumorigenesis	p91A mutation in mutagenized murine mastocytoma; various mutated proteins in melanomas recognized by CTLs
Products of genes that are silent in most normal tissues	Cancer/testis antigens expressed in melanomas and many carcinomas; normally expressed mainly in the testis and placenta
Products of overexpressed genes	Tyrosinase, gp100, MART in melanomas (normally expressed in melanocytes)
Products of oncogenic viruses	Papillomavirus E6 and E7 proteins (cervical carcinomas)
	EBNA-1 protein of EBV (EBV-associated lymphomas, nasopharyngeal carcinoma)
	SV40 T antigen (SV40-induced rodent tumors)
Oncofetal antigens	Carcinoembryonic antigen (CEA) on many tumors, also expressed in liver and other tissues during inflammation
	Alpha-fetoprotein (AFP)
Glycolipids and glycoproteins	GM_2, GD_2 on melanomas
Differentiation antigens normally present in tissue of origin	Prostate-specific antigen
	Markers of lymphocytes: CD10, CD20, Ig idiotypes on B cells

Abbreviations: CML, chronic myelogenous leukemia; CTL, cytotoxic T lymphocyte; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; Ig, immunoglobulin; MART, melanoma antigen recognized by T cells.

Tumor Specific Antigens

Identified on tumors induced with chemical or physical carcinogens, and on some virally induced tumors.

-The immune system usually eliminates tumor cells bearing large numbers of antigens.

-Cells with low levels of antigen generally go undetected.

Examples:

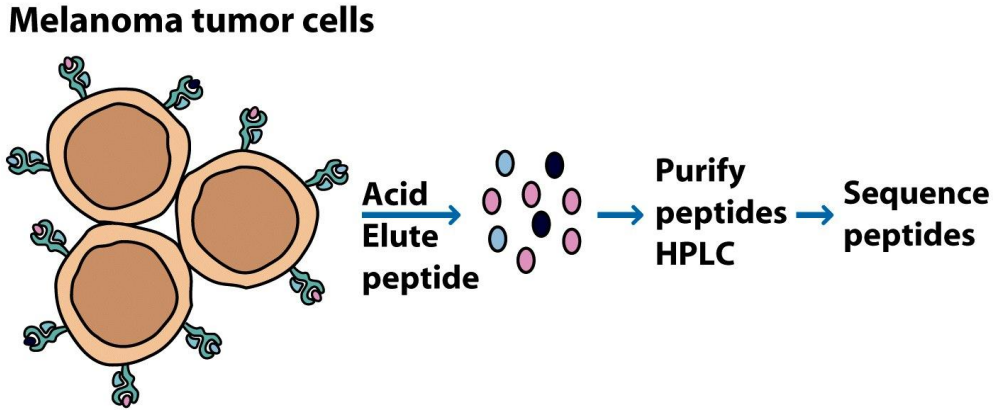
1. Methylcholanthrene
2. UV light.

Immune responses specific to one tumor does not protect against the other tumor and unfortunately small modifications to tumor antigens often occur with chemical or physical carcinogens.

Tumor Specific Antigens

Two methods are used to identify tumor specific antigens:

1. Acid digestion and HPLC analysis.



2. Preparation of cDNA libraries.

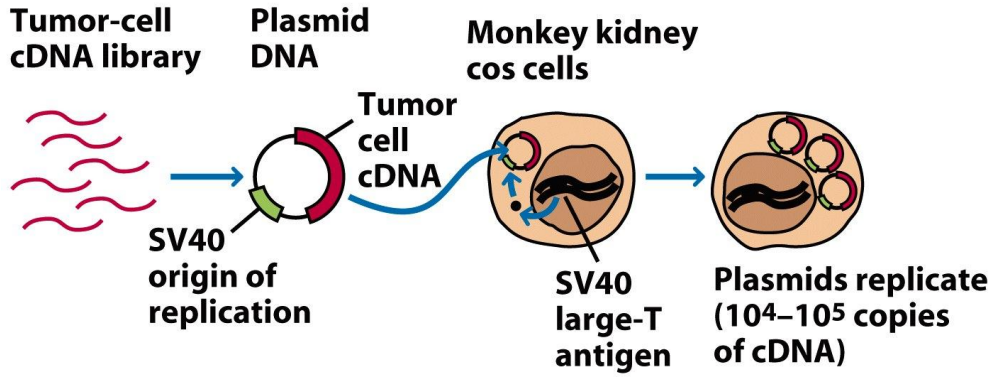


Image Credits: Kuby 6th ed.

Tumor Specific Antigens

Virally induced tumor specific antigens created upon exposure to a virus are all similar in nature.

Examples:

1. Burkitts lymphoma cells express a nuclear antigen of the Epstein-Barr virus.
2. HPV E6 and E7 proteins are found in over 80% of the invasive cervical cancers.

What are the implications of this?

Tumor Associated Antigens

Unfortunately most tumor antigens are not unique to tumor cells.

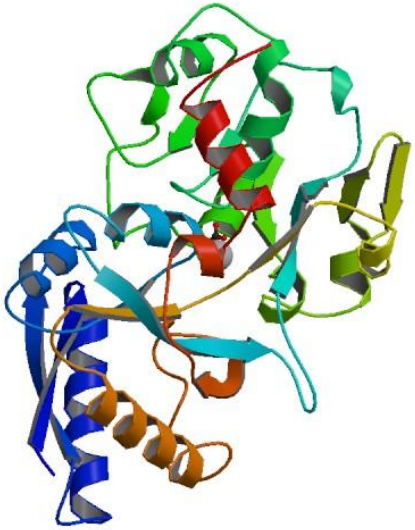
For oncogene proteins the level of expression is what counts!

Most are proteins expressed on fetal cells and over expressed on adult cells:

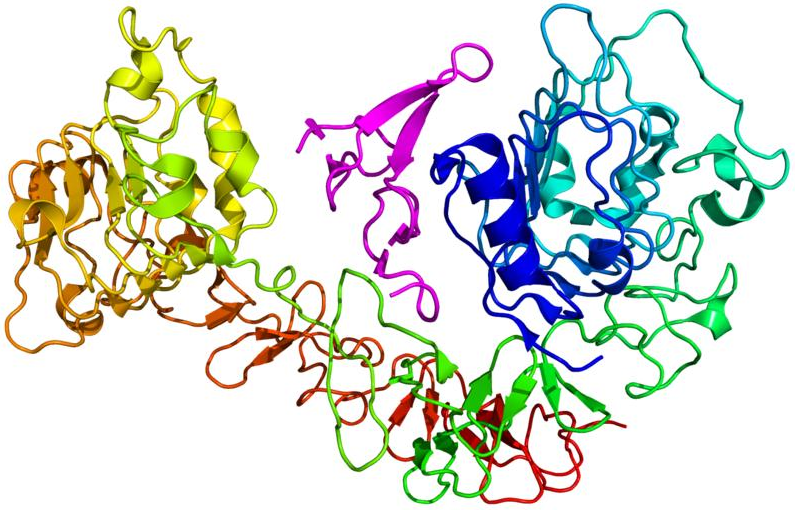
Example: growth factors

-Transferrin growth factor p97- aids in the transport of iron into cells (6 to 60x).

-Epidermal growth factor receptor (100x).



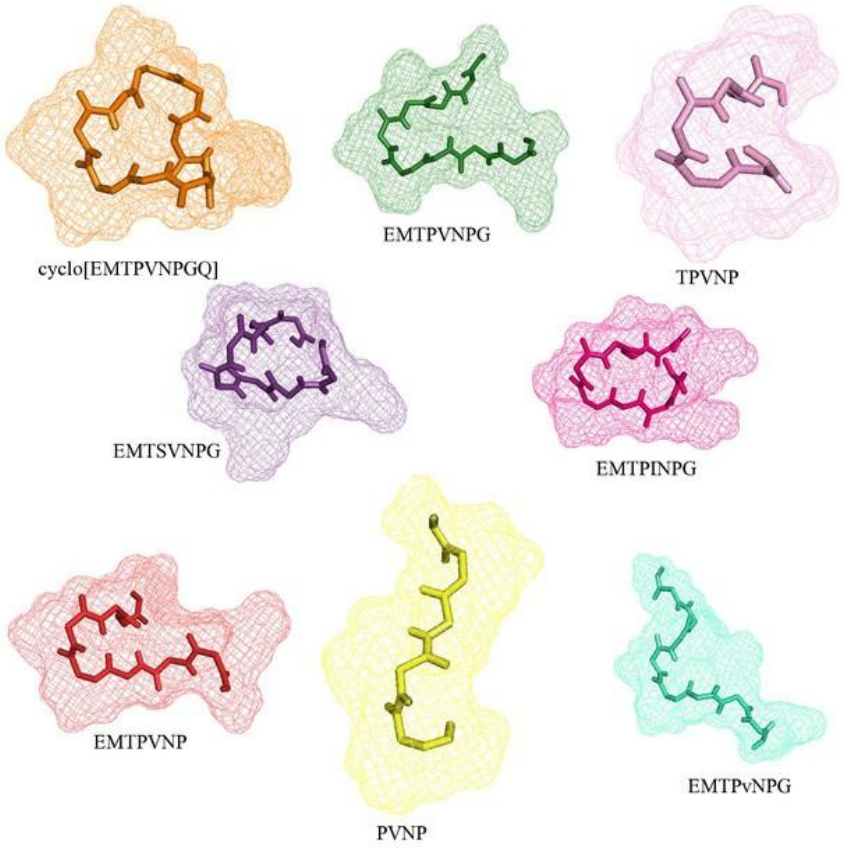
Transferrin



Epidermal Growth Factor Receptor

Tumor Associated Antigens

Alpha-fetoprotein (AFP)- associated with liver cancer.



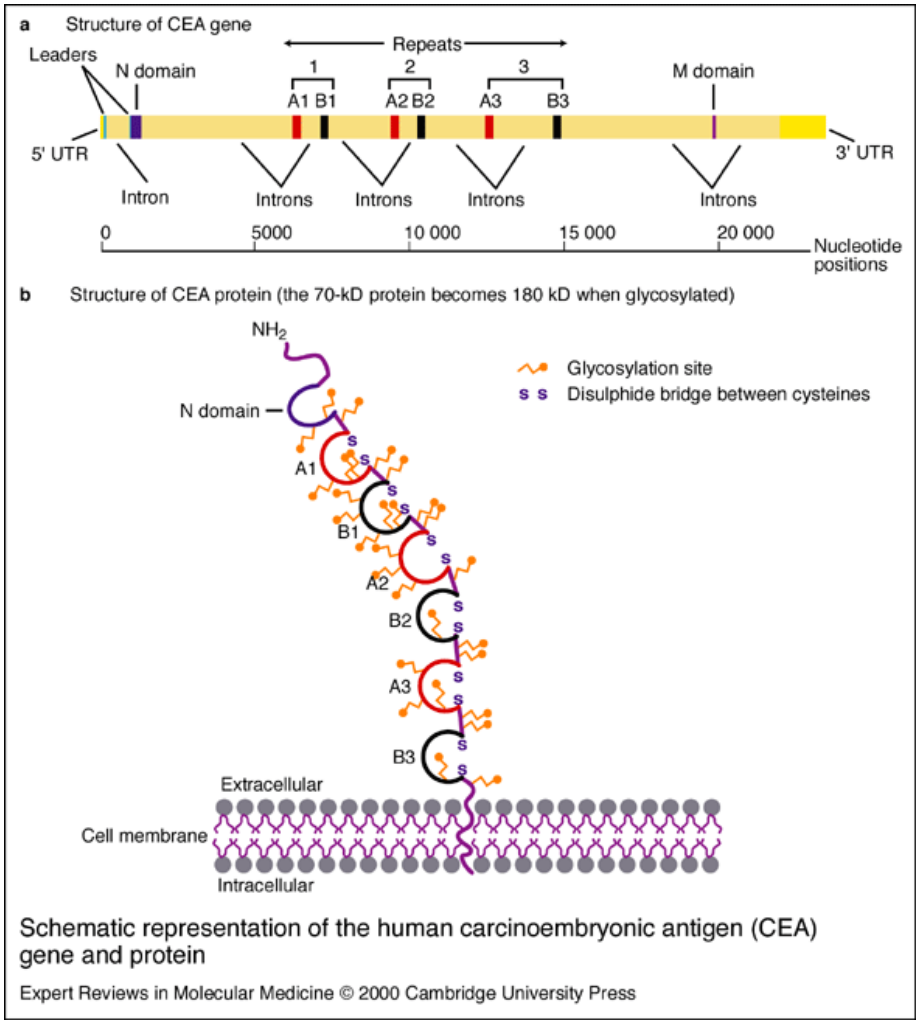
"Computational Design and Experimental Discovery of an Anti-estrogenic Peptide Derived from Alpha-Fetoprotein" Karl N. Kirschner, Katrina W. Lexa,* Amanda M. Salisburg,* Katherine A. Alser,* Leroy Joseph, Thomas T. Andersen, James A. Bennett, Herbert I. Jacobsen, and George C. Shields, J. Am. Chem. Soc. 129 (2007) 6263-6258.

Disease	No. of patients tested	% of patients with high AFP or CEA levels*
AFP > 400 μg/ml		
Alcoholic cirrhosis	NA	0
Hepatitis	NA	1
Hepatocellular carcinoma	NA	69
Other carcinoma	NA	0
CEA > 10 mg/ml		
Cancerous		
Breast carcinoma	125	14
Colorectal carcinoma	544	35
Gastric carcinoma	79	19
Noncarcinoma malignancy	228	2
Pancreatic carcinoma	55	35
Pulmonary carcinoma	181	26
Noncancerous		
Alcoholic cirrhosis	120	2
Cholecystitis	39	1
Nonmalignant disease	115	0
Pulmonary emphysema	49	4
Rectal polyps	90	1
Ulcerative colitis	146	5

*Although trace amounts of both AFP and CEA can be found in some healthy adults, none would have levels greater than those indicated in the table.

Image Credit: Kuby 6th ed.

Tumor Associated Antigens



Schematic representation of the human carcinoembryonic antigen (CEA) gene and protein

Disease	No. of patients tested	% of patients with high AFP or CEA levels*
AFP > 400 μg/ml		
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Hepatitis	NA	1
Hepatocellular carcinoma	NA	69
Other carcinoma	NA	0
CEA > 10 mg/ml		
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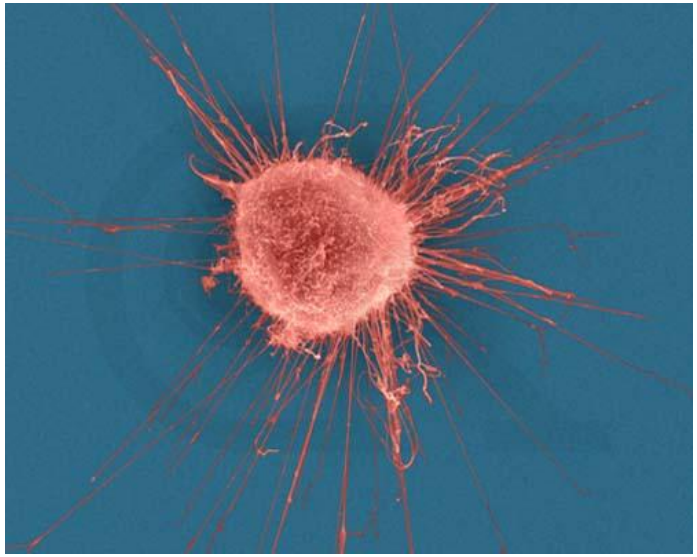
*Although trace amounts of both AFP and CEA can be found in some healthy adults, none would have levels greater than those indicated in the table.

Image Credit: Kuby 6th ed.

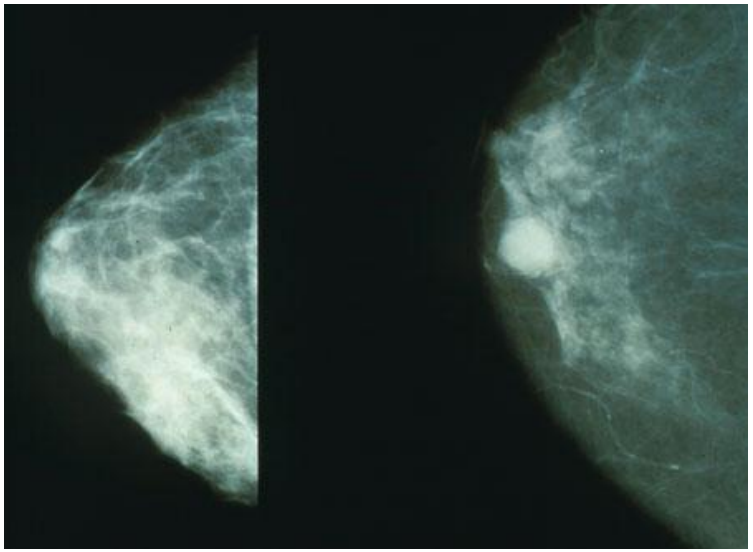
Neu Protein

Expressed in high levels by anti-cancer cells.

-Anti-Neu monoclonal antibodies can be used to treat breast cancer.



Breast Cancer Cell
Image Credit: Google Images



Mammogram Showing Breast Cancer
Image Credit: national Cancer Institute

Melanoma's

Five oncofetal type antigens have been identified:

1. MAGE-1 (40%)
2. MAGE-3 (75%)
3. BAGE
4. GGE-1
5. GAGE-2

Additional differentiation antigens have been identified including:

-Tyrosinase
-gp100
-Melan-A
-MART-1
-gp75

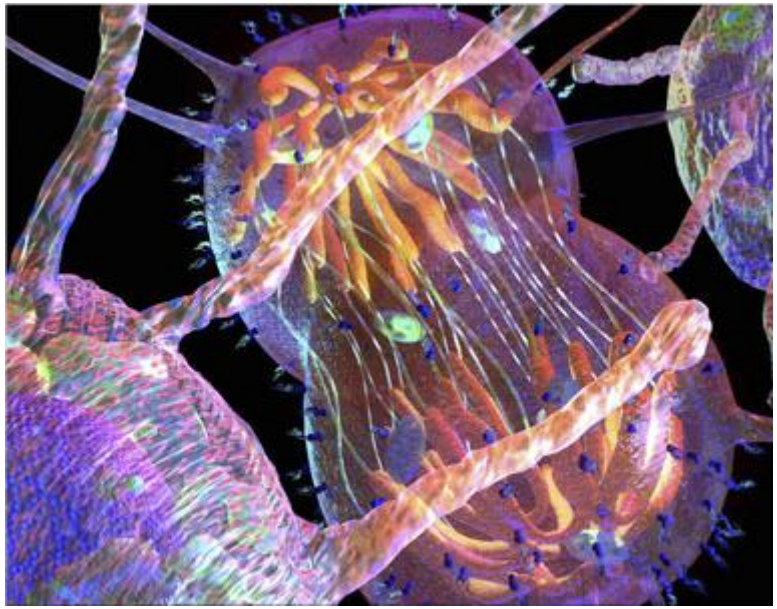


Image Credit: Google Images

The Role of Natural Killer Cells

Natural Killer Cells and macrophages target and kill cancer cells.

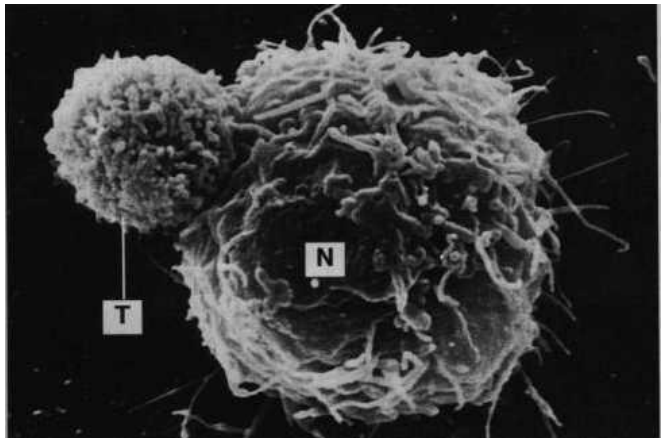
-They are not limited by decreased MHC expression.

-These cells can bind antibody-coated tumor cells leading to ADCC.

-Macrophages use lytic enzymes, ROS, RNS, and TNF-a to kill cancer cells.

Example: Chediak-Higashi Syndrome

-A decrease in the number of NK cells leads to certain types of cancer.



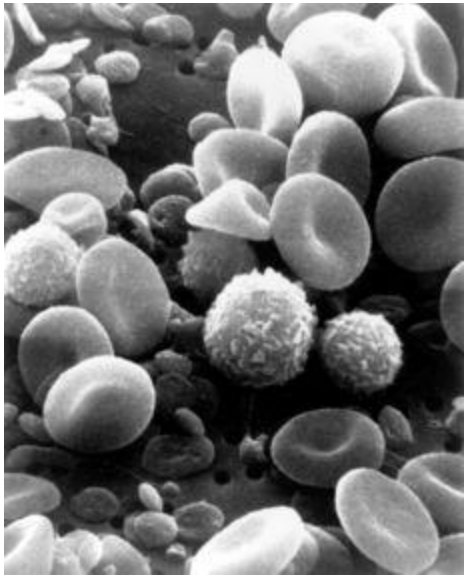
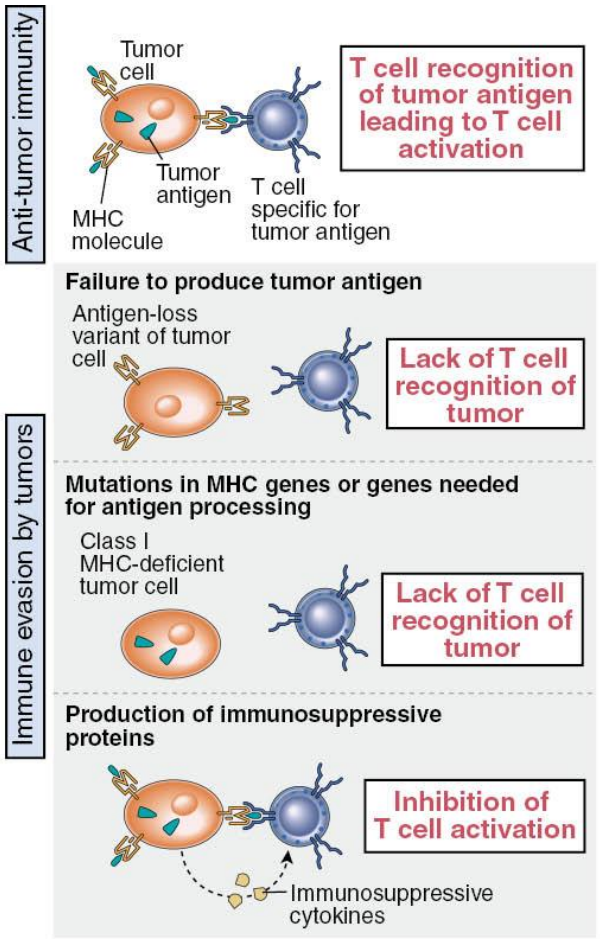


Image Credits: Google Images

Tumor Evasion

Cancer cells evade the immune system in four major ways:

1. Anti-tumor antibodies can enhance tumor growth.
   - -Specific for the antigen.
2. Antibodies can modulate tumor antigens.
   - -Known as antigenic modulation.
3. Tumor cells express low levels of class I MHC.
   - -Inhibits or restricts binding of CD8.
   - -Can have an impact on autoimmunity.
4. They may provide poor co-stimulatory signals.
   - -Decreased IL-2 production due to the lack of the production of B7 on APC and CD28 on T-cells.



Therapeutics-Cytokine Based Immunotherapy

Cytokine	Tumor rejection in animals	Inflammatory infiltrate	Immunity against parental tumor (animal models)	Clinical trials
Interleukin-2	Yes; mediated by T cells	Lymphocytes, neutrophils	In some cases of renal cancer, melanoma	Renal cancer, melanoma
Interleukin-4	Yes	Eosinophils, macrophages	No long-lasting immunity in human trials	Melanoma, renal cancer
Interferon-γ	Variable	Macrophages, other cells	Sometimes	
TNF	Variable	Neutrophils and lymphocytes	No	
GM-CSF	Yes	Macrophages, other cells	Yes (long-lived T cell immunity)	Renal cancer
Interleukin-3	Sometimes	Macrophages, other cells	Sometimes	

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.

Therapeutics-Mab's

Monoclonal Antibodies

-Currently eight monoclonal antibodies are used to treat cancer.

Monoclonal antibodies approved for the treatment of human cancers

Name	Trade name	Used to treat	Year approved
Rituximab	Rituxan	Non-Hodgkin's lymphoma	1997
Trastuzumab	Herceptin	Breast cancer	1998
Gemtuzumab ozogamicin*	Mylotarg	Acute myelogenous leukemia (AML)	2000
Alemtuzumab	Campath	Chronic lymphocytic leukemia (CLL)	2001
Ibritumomab tiuxetan*	Zevalin	Non-Hodgkin's lymphoma	2002
Tositumomab*	Bexxar	Non-Hodgkin's lymphoma	2003
Cetuximab	Erbitux	Colorectal cancer, head and neck cancers	2004 2006
Bevacizumab	Avastin	Colorectal cancer	2004

*Conjugated monoclonal antibodies

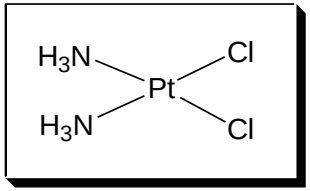
Image Credit: Kuby 6th ed.

-Guided missile approaches using monoclonal antibodies is also a hot area of research.

Cisplatin

Pharmaceuticals

Antitumor drug discovered serendipitously in 1845.



Binds to DNA to form 1,2-, and 1,3-intrastrand and interstrand cross-links disrupting replication and protein production.

Used to treat testicular, ovarian, cervical, head, neck esophageal and nonsmall cell lung cancers.

Several side effects, and intrinsic and acquired resistance limit the efficacy of the drug.

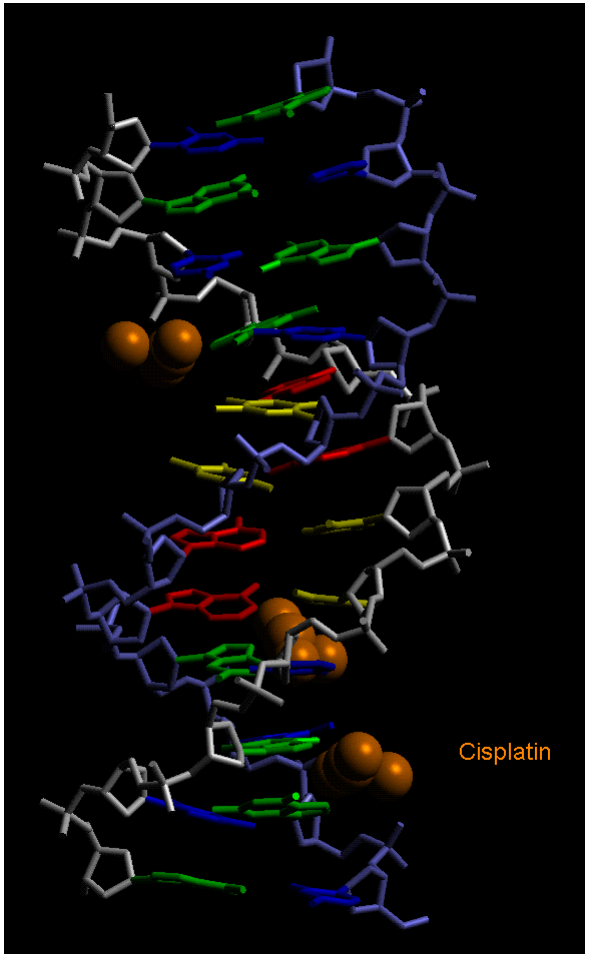


Image Credit: Google Images

Cisplatin

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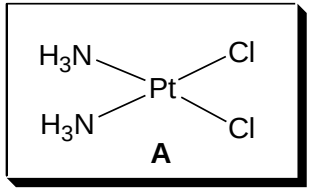
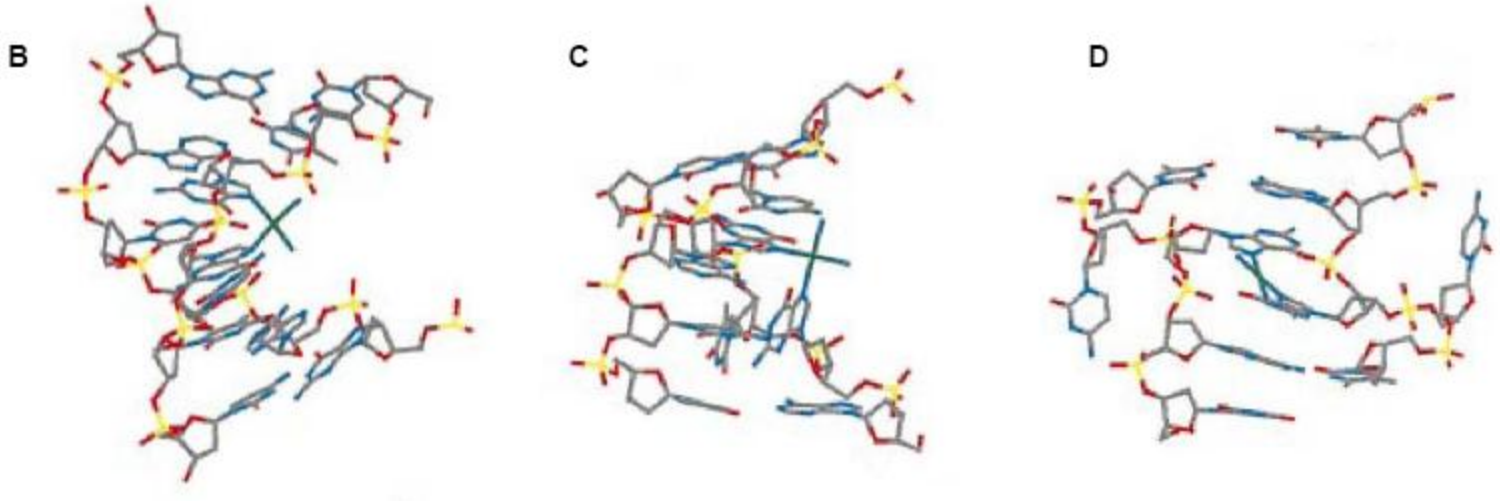
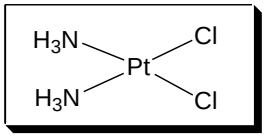


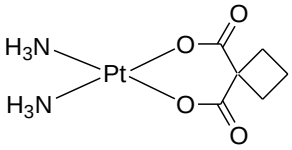


Cisplatin (A) and cisplatin bound to DNA via 1,2-intrastrand (B), 1,3-intrastrand (C), and interstrand (D) crosslinking. Figure adapted from Lippard et. al. *Chem. Rev.* **1999**, *99,* 2467-2498

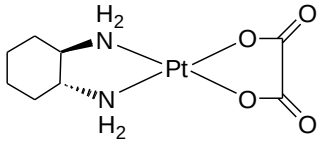
Cisplatin Derivatives



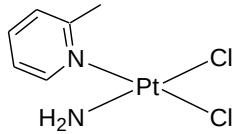
Platinators:



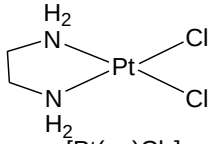
Carboplatin



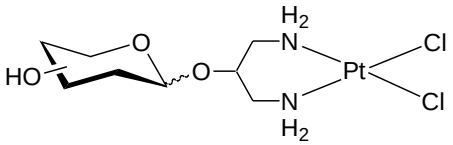
Oxaliplatin



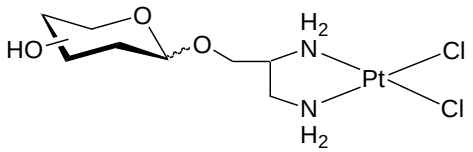
$[PtCl_2(NH_3)(2\text{-picoline})]$



$[Pt(en)Cl_2]$

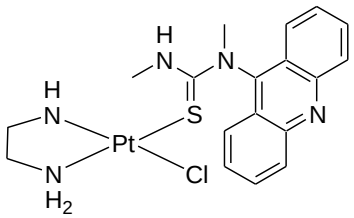


$[PtCl_2(2\text{-(Sugar)-pn})]$

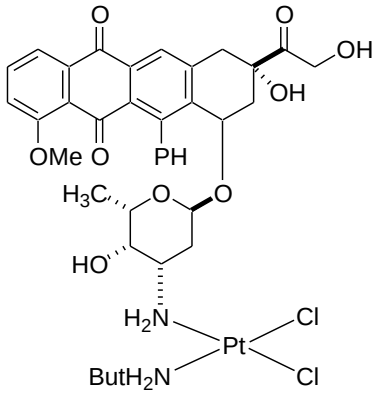


$[PtCl_2(1\text{-(Sugar)-pn})]$

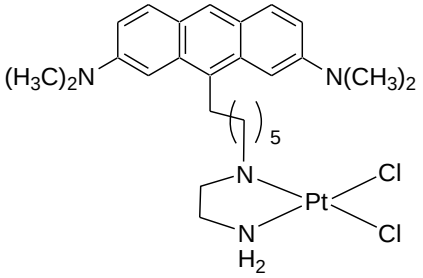
Platinators and Intercalators:



Platinum Acridine Thiourea Conjugates



Platinum-Doxorubicin Conjugate

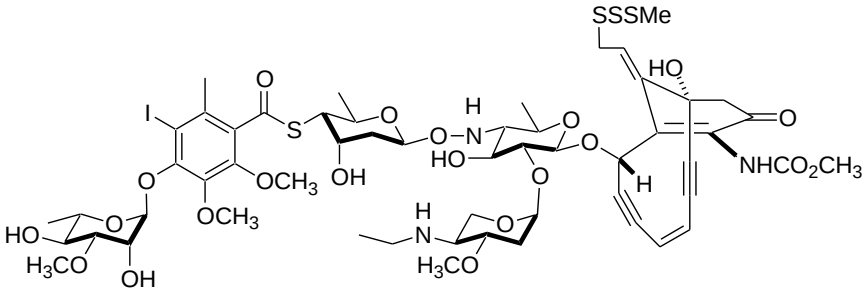


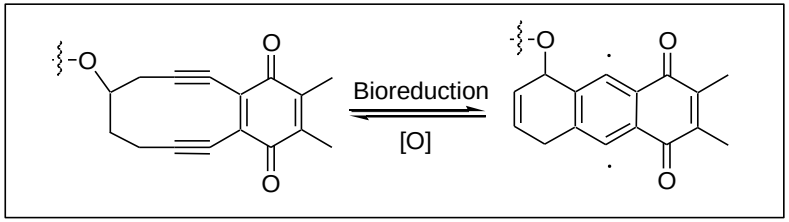
Platinum Acridine Orange

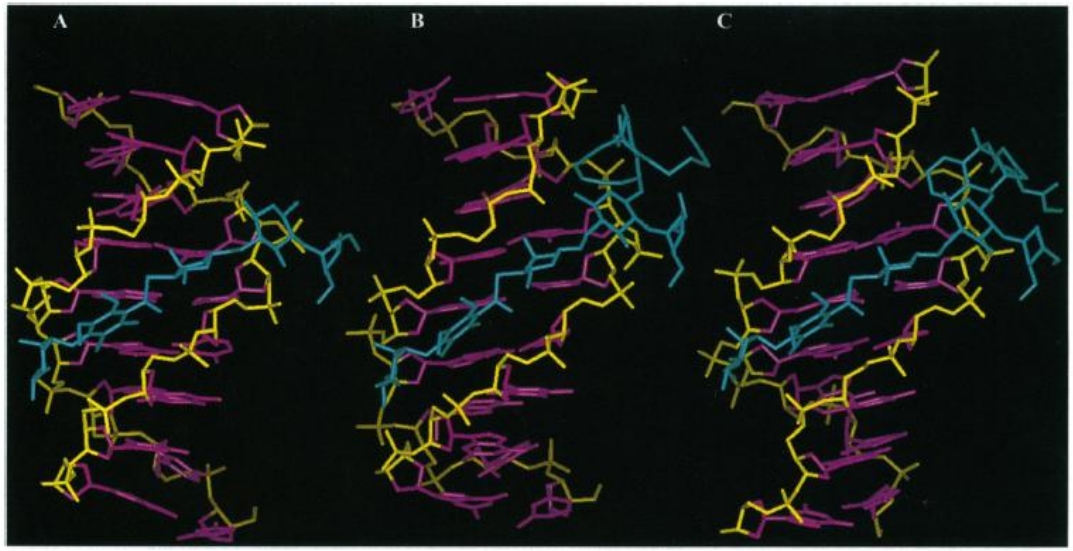
Calicheamicin

Carbohydrate binds in the major grove of DNA

The warhead undergoes Bergman Cyclization and intercalates.







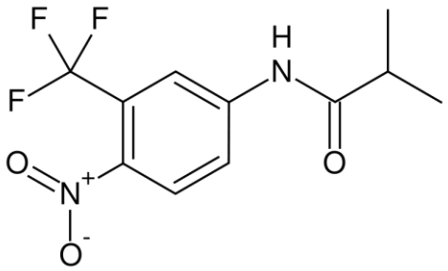
Proc. Natl. Acad. Sci. **1995,** *92*, 10506-10510.

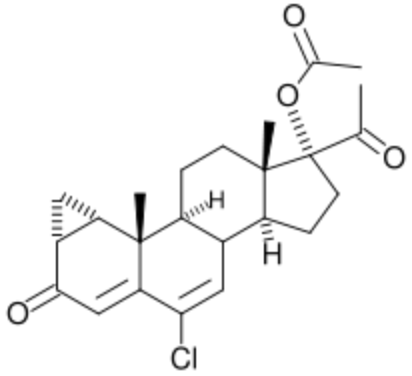
Therapeutics

Pharmaceuticals

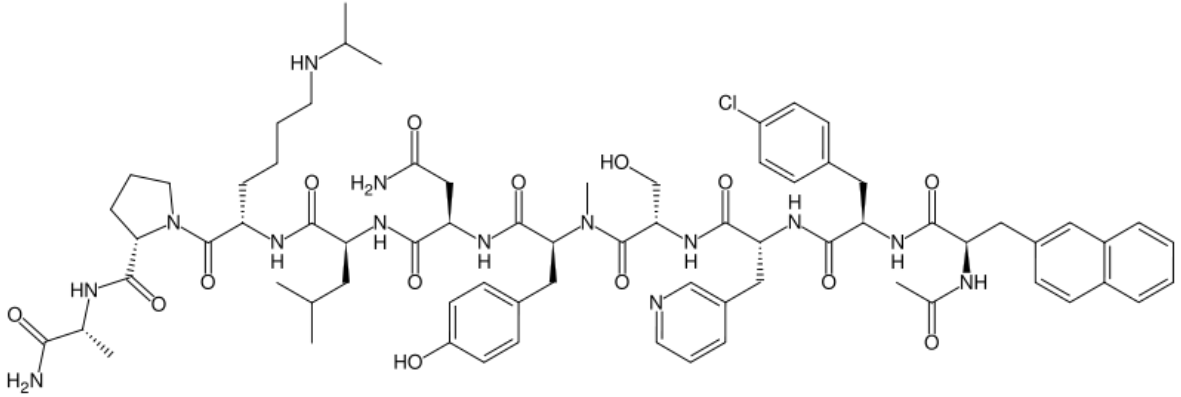
-Prostate Cancer

-Antiandrogen drugs (flutamide/cyproterone)- competitive inhibitor of testosterone





-Gonadotropin Releasing Hormone Antagonists (Aberelix) -compete



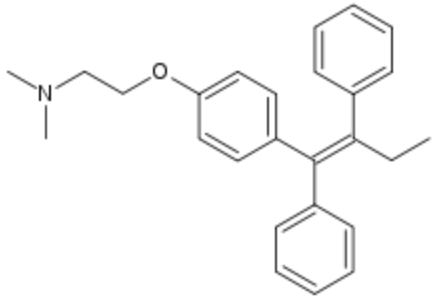
Therapeutics

Pharmaceuticals

-Breast Cancer

Tamoxifen

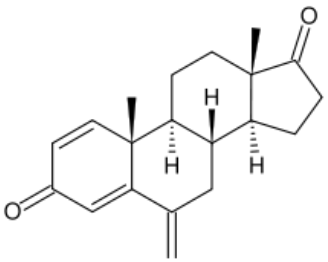
-Selective estrogen receptor modulator.

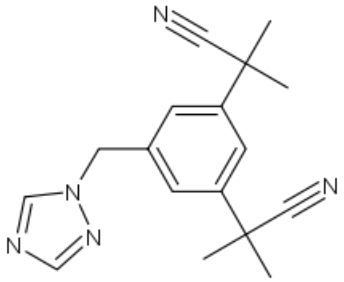


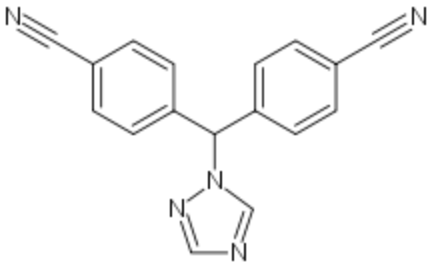
Aromasin's

-Prevent the formation of steroids estradiol (from testosterone) and estrone (from Androstenedione).

-Can be steroidal (exemstane) or nonsteroidal (anastrazole/letrazole).







Vaccines!

Type of vaccine	Vaccine preparation	Animal models	Clinical trials
Killed tumor vaccine	Killed tumor cells + adjuvants	Melanoma, colon cancer, others	Melanoma, colon cancer
	Tumor cell lysates + adjuvants	Sarcoma	Melanoma
Purified tumor antigens	Melanoma antigens	Melanoma	Melanoma
	Heat shock proteins	Various	Melanoma, renal cancer, sarcoma
Professional APC-based vaccines	Dendritic cells pulsed with tumor antigens	Melanoma, B cell lymphoma, sarcoma	Melanoma, non-Hodgkin's lymphoma, prostate cancer, others
	Dendritic cells transfected with genes encoding tumor antigens	Melanoma, colon cancer	Various carcinomas
Cytokine- and costimulator-enhanced vaccines	Tumor cells transfected with cytokine or B7 genes	Renal cancer, sarcoma, B cell leukemia, lung cancer	Melanoma, sarcoma, others
	APCs transfected with cytokine genes and pulsed with tumor antigens		Melanoma, renal cancer, others
DNA vaccines	Immunization with plasmids encoding tumor antigens	Melanoma	Melanoma
Viral vectors	Adenovirus, vaccinia virus encoding tumor antigen ± cytokines	Melanoma, sarcoma	Melanoma

Abbreviations: APC, antigen-presenting cell.

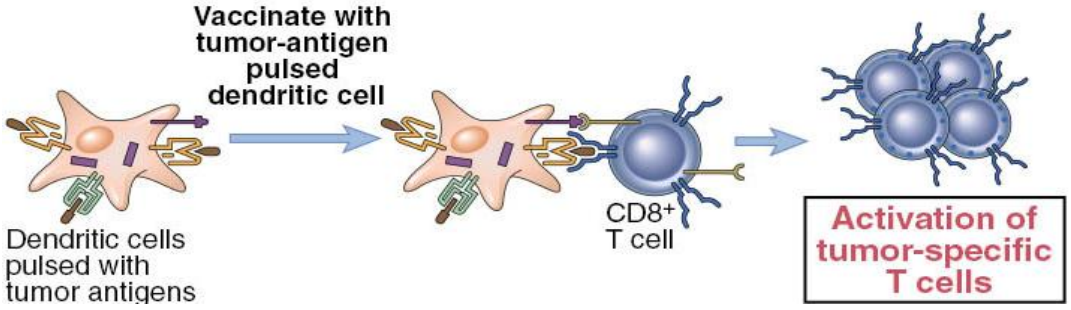
Dendritic Cell Vaccines

-Dendritic cells pulsed with autologous antigen derived peptides have been shown to increase the median survival time of the patient with GBM.

-Yu and coworkers demonstrated that autologous tumor specific peptide-pulsed dendritic cells could be used to enhance cytotoxic T-lymphocyte responses resulting in the targeting and destruction of tumor cells. (*Cancer Res.* **2001**, *61*, 842-847)

-Yamanaka and Yu also showed that autologous tumor lysate consisting of undefined tumor-associated antigens could also be used to generate a similar immune response without requiring the time needed to extract and purify tumor specific peptides. (*Br. J. Cancer* **2003**, *89*, 1172-1179 and *Cancer Res.* **2004**, *64*, 4973-4979)

-Wu and coworkers described a vaccine based on dendritic cells raised against autologous glioma cell lysate EGFRvIII which has been effective for the treatment of GBM through phase II clinical trials. (*J. Neurooncol.* **2006**, *76*, 23-30)



Dendritic Cell Vaccines

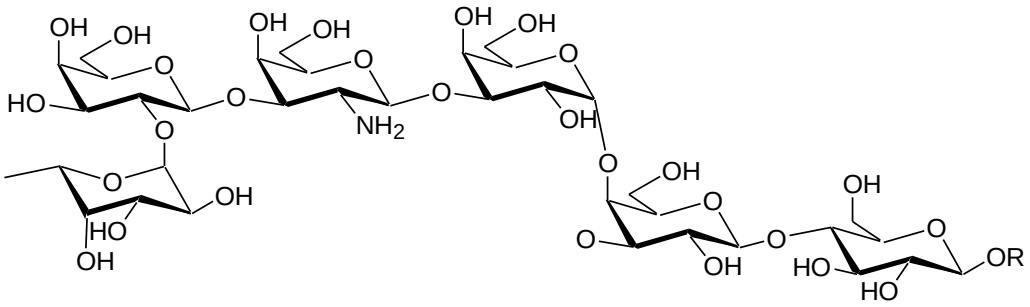
So what is the problem?

-Patient specific and are constrained by the time required to obtain antigen specific peptides.
-Nonspecific antigens from the tumor lysates of GBM patients has decreased the overall time that it takes to prepare these vaccines, but nonspecific antigen also increases the risk of developing an uncontrolled autoimmune response.
-The EGFRvIII vaccine has been highly effective, tumor cells obtained from patients with recurrent GBM no longer expressed EGFRvIII, rendering the vaccine useless for the continuous treatment of GBM.
-Personal nature of these vaccines makes them relatively expensive and impractical for mainstream therapy.

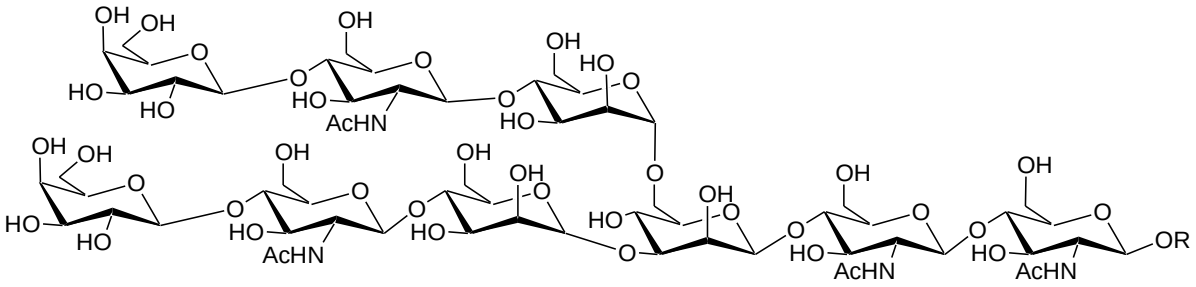
Therefore, a more effective and tumor specific, rather than patient specific vaccine is needed to treat GBM.

Carbohydrate-Based Vaccines!

Globo-H-Hexasaccharide



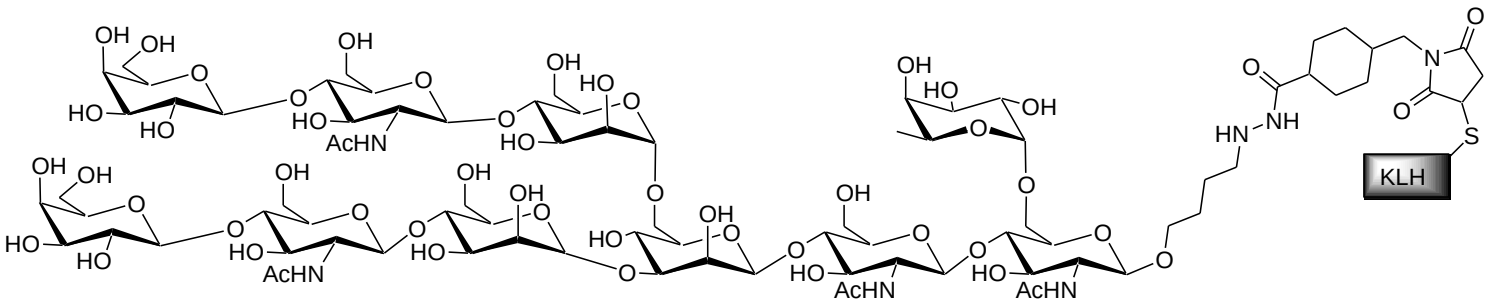
Prostate Specific Antigen



Carbohydrate-Based Vaccines!

GBM!

-Gliomas are aggressive primary brain tumors.
-GBM is the most aggressive form of glioblastoma and is characterized by the presence of pseudopalisading necrosis and hyperplasic blood vessels.
-Patients with GBM often exhibit seizures, although other symptoms such as difficulties in speech, vision problems, and decreased sensation will often occur earlier but generally go unnoticed.
-Patients afflicted with GBM usually die within two years of diagnosis.



Exam I—Chemical Immunology and Immunopharmacology
Fall 2008

Please answer **fifteen** of the following sixteen questions. Each question is worth ten points, and one of fifteen questions you answer can be your own question. You do not need to rewrite the answer to your own question, simply write "Free" or "10/10" in the box next to your question. If you are not sure which question is your own since I have modified a few of your questions to make them easier (yes many of you wrote very difficult questions) please email me and I will let you know.

This exam should take you approximately three to four hours to complete, but you may use all the time you need.

Here are the following guidelines concerning this exam:

1. You may use any written or electronic resource you find to complete these questions. If you have used materials beyond those distributed in this course, please reference them in your final work.

2. You are not to discuss in any form any part of this exam with anyone other than Nicole Snyder until all of the exams have been submitted for grading. This means that you may not consult other students (especially the students in the course who have written these questions), professors, parents, former high school mentors etc. until I post a note stating that all exams have been collected.

3. **Everyone** must answer questions 1 and 10.

4. This exam is due no later than **midnight on October 24, 2008**.

A violation of these guidelines is a violation of the honor code and will result in an automatic zero on the exam and the student or student(s) found in violation will be subject to disciplinary action.

Please sign this paper before submitting your final work as a hard copy **directly to me**.

Good luck and let me know if you have any questions or concerns.

I, _____, certify that I have referenced any sources I used to complete this exam beyond those distributed to me as part of this course. I, _____, also certify that the answers contained in this exam are my own work, and that I have neither accepted help from any other student, professor, parent, or high school mentor, nor have I have given advice to any other student in the course regarding the material in this exam. I, _____, understand that violating these guidelines is a violation of the honor code and if I am found to have violated the honor code, I will be subject to disciplinary action.

Signed_____ Date: ___/___/___

1. ____/10 During the inflammatory response stage of our immune response to an antigen, extravasation must occur in order for cells to leave the blood stream and enter the site of infection. The process of extravasation involves three main stages: rolling, activation and firm attachment, and transendothelial migration. During the process of rolling, endothelial cells express P and E selectins (cell adhesion molecules) which circulating neutrophils bind using the sialyl Lewis^x antigen (shown below in Figure 1) on the surface of the neutrophil.

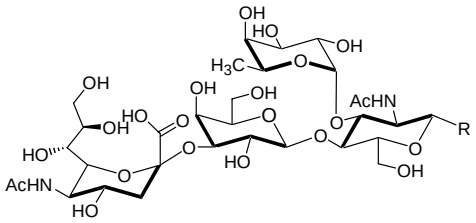
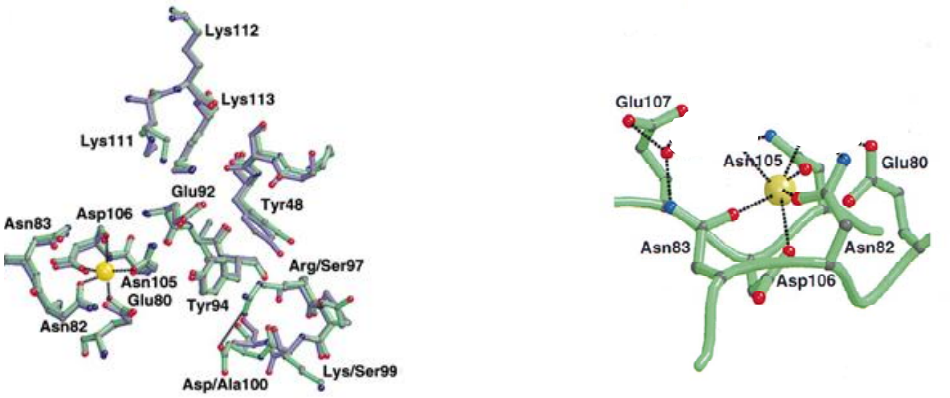


Figure 1: Sialyl Lewis^x antigen.

(a) The binding domain of a selectin is shown in Figure 2A below. In what way would you expect the sialyl Lewis^x antigen to bind to the selectin shown? Show the bonding pattern in the partial structure in Figure 2B below.

Hint: The calcium ion is a critical part of the bond and the amino acid residues are key.



A **B**

Figure 2: Selectin binding domain (A) and partial structure (B).

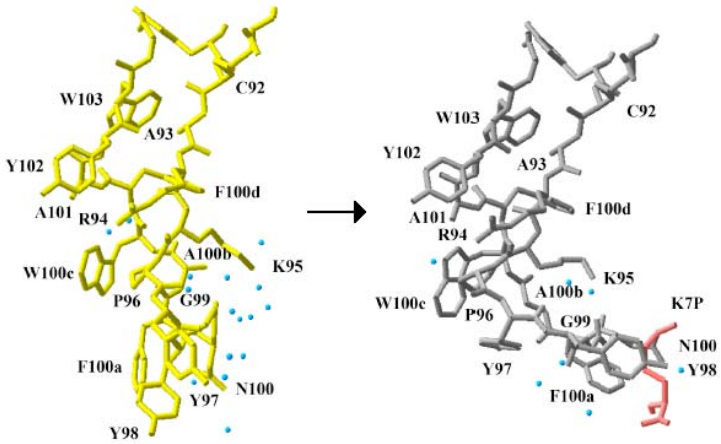
(b) What kind of bonding is occurring, and why is this bond not strong enough to firmly attach the neutrophil to the endothelial cells?

2. ____/10 We have learned that macrophages will ingest pathogens or apoptotic cells, however this "eat-me" signal (which calls the macrophages on apoptotic cells) is not very well understood. Please elaborate on what is known about this mechanism.

Hint: Phosphatidylserine is important!

3. ____/10 A common symptom experienced by people who have respiratory infections is a change in nasal mucus. Nasal mucus is usually a clear, fluid substance that serves as an important part of the defense system of the human body. However, in response to some infections, the body produces excess mucus. During this period, mucus often becomes thick and discolored, usually a beautiful yellow or green hue. Explain why mucus production is increased and why it becomes discolored during an infection in terms of the immune response.

4. ____/10 One example of loop moiety found on the extracellular portion of the TcR/CD3 complex of a helper T-Cell is shown below. This moiety binds directly to a peptide being presented by an MHC molecule on an APC. A significant conformational change occurs in the loop when it binds to the peptide. Please answer the questions below with respect to the structures in Figure 3.



A **B**

Figure 3: Extracellular portion of the TcR/CD3 complex of a helper T-Cell

(a) If the loop is originally stabilized by water molecules shown in blue below, deduce a possible stabilization mechanism for the new conformation of the molecule. Consider Tyr97 and Lys 95 specifically.

(b) The change from conformation A to B results in a compaction of the loop moiety. What molecular interactions might result from this loop compaction?

Hint: There are two other loops that are part of the TcR, and they are not involved in direct binding to the peptide.

5. ____/10 Patients that present an immunodeficiency disease where CD4 T-cells are defective, also show the inability to produce one or more antibody classes. Discuss how a problem with CD4 T-cells could cause a problem in antibody class production.

6. ____/10 HIV (Human Immunodeficiency Virus) is a virus that is unique in the sense that it attacks human immune cells and turns them into viral factories. The main target of the HIV infection is the helper T-cell. Leonard and Roy describe HIV's mechanism of binding to helper T-cells in the review provided on Blackboard (*Current Medicinal Chemistry*, 2006, *13,* 911-934). Based on their description of how HIV enters the cell, and your knowledge of T-cells, comment on why inhibiting the chemokine protein CCR5 instead of inhibiting gp120 and CD4 would be advantageous.

Note: You can find all the information you need in the first few pages, don't read the whole thing.

7. ____/10 Answer the following questions with respect to dendritic cells:

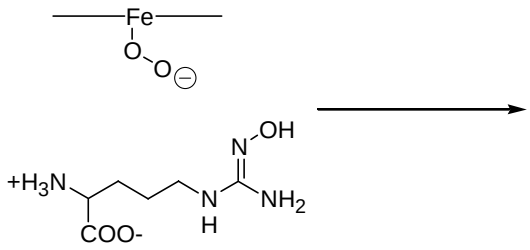
(a) What are dendritic cells (DCs)?

(b) How do DCs activate cytotoxic T-cells (Tc), and why are they particularly good activators of these cells? Be specific.
Note: There are two ways in which CD8 T-cells are activated by DC's

8. ____/10 *Yersinia Pestis*, the cause of bubonic plague, collects in the lymph nodes, spreads through the blood, and eventually causes fatal sepsis. One of the reasons for its high virulence is that it has adapted a response to nitric oxide-derived reactive nitrogen species. Answer the following questions with respect to these processes.

(a) What is the structure of NO?

(b) Provide a mechanism for the production of nitric oxide by nitric oxide synthase, starting from the L-arginine derivative shown below.



(c) Nitric oxide primarily kills bacteria, which are then dealt with by PMNs. What are PMNs and why might these two be linked? Furthermore, why would these adaptations for the innate immune response make *Y. Pestis* so deadly?

9. ____/10 Some bacteria (example *Mycobacterium* spp.) are capable of evading the natural killing process of a phagocyte. How are the enzymes SOD and catalase used by bacteria in an attempt to avoid death by the phagocyte? Be specific. You answer should include the chemical reactions that take place.

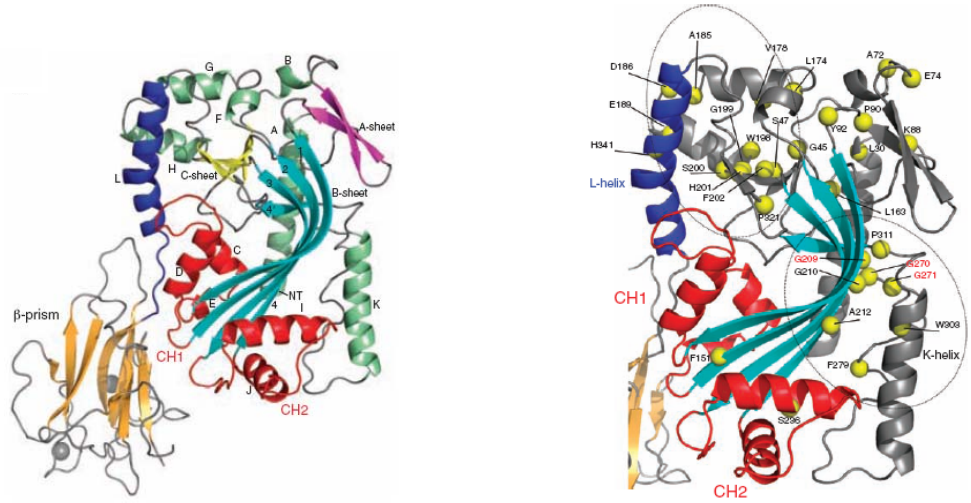
10. ____/10 Ever since JP stopped taking steroid supplements following a traumatic psychological breakdown, his immune system has not been functioning properly. In the past couple of days, JP has felt lumps in his tonsils, and realized that something must be wrong. Knowing his swelling lymphatic system probably is not a good thing, he decided to go to Urgent Care to get his blood examined. Following a blood test, JP found out that he has an extraordinarily high concentration of the membrane attack complex (MAC) in his system. MAC complexes form through the complement pathways in JP's innate immune system. C5b, which can be reached by any of the three pathways, activates C6, C7, C8, and C9 sequentially to form the C5b6789 complex, the MAC.

Fearing JP's health would deteriorate past its current condition, the doctor prescribed a daily dose of the drug AntiBigMac, which contains a large quantity of CD59, a protein that should be widely expressed on JP's cell surfaces but seemingly missing. The doctor informed JP of the unfortunate fact that in the medical profession, AntiBigMac is sometimes referred to as AntiBowelMovement, with obvious side effects. Upon seeing JP's seemingly unwillingness to take the drug, the doctor told JP that his life may depend on his continued intake of the drug. "Negligence can be fatal," said Doc.

Answer the following questions with respect to the case study above.

(a) How does MAC function and why is it potentially dangerous to have high concentrations of it in the immune system for prolonged periods of time?

(b) MAC and perforin share a common monomer domain known as the MACPF domain shown in Figure 4 below.



A **B**

Figure 4. The MACPF domain found in both the membrane attack complex and perforin. A shows the structure of the entire MACPF domain. B shows a larger version of the functional portion of the monomer, with the highly conserved regions of the molecule circled in black.

The type of pore complex formed form these monomers can be seen in Figure 5. The B-prism, which consists of mostly hydrophobic amino acids, is the original portion of the domain to be inserted into the membrane of the target cell, but this action is not what forms the large pore that is used for cell lysis. Postulate a possible mechanism for pore formation.

Hint: The CH1 and CH2 alpha helices contain half hydrophilic and half hydrophobic amino acids.

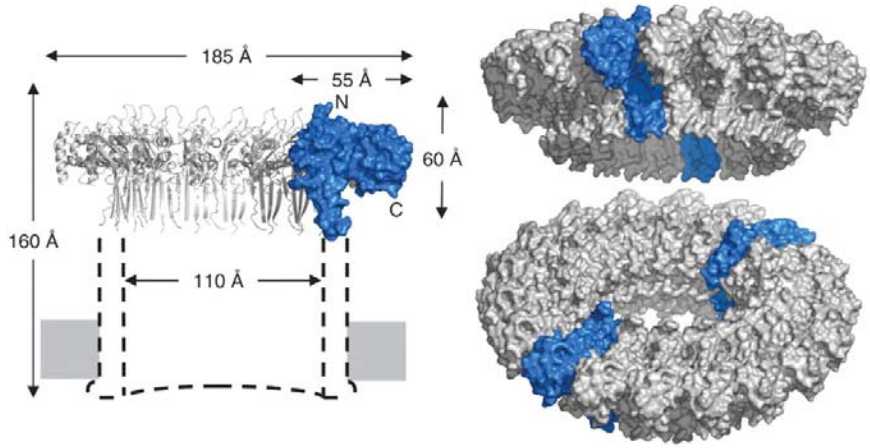


Figure 5. Predicted model for an MAC or porforin pore formed to initiate lysis of a target cell. Monomers containing the MACPF domain bind together to form a circular protein which extends through the membrane of the target cell to form a pore. An example of what would be a single monomer is highlighted in blue.

(c) What is CD59 and how may it help JP with his current condition?

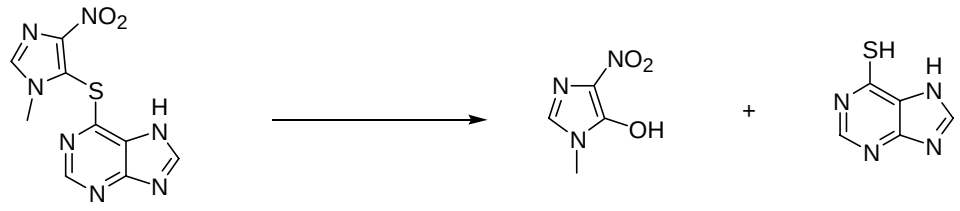
11. ____/10 Answer the following questions with respect to apoptosis.

(a) Apoptosis is an integral cellular process of the immune system. Yet, clean up of apoptotic cells is just as important as the death program itself. Please identify the two "professional" clean-up cells and the general characteristics of these cells that allow them to perform their job, as well as a list of "non-professional" cleaners.

(b) One difficulty found in studying the phagocytosis of apoptotic cells is that dying cells have rarely been observed *in vivo*. Why is this observation actually beneficial in terms of our health?

(c) There are a multitude of receptors involved in the phagocytosis of apoptotic cells. One main mode of studying these receptors is through inhibition, by presenting ligands and monoclonal antibodies to phagocytes or apoptotic cells and seeing if uptake is inhibited. Complete inhibition has never been achieved experimentally, furthering the number of different receptors involved. The nematode *C. elegans* has shown the ability to phagocytose apoptotic cells but being the simple organism it is, it does not have a developed immune system. How does this information lead to an evolution explanation for the number of receptors used in the phagocytosis?

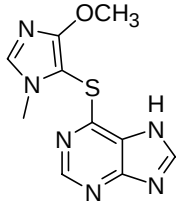
12. ____/10 Although YM protected himself from plasticizers by buying a metal thermos, his long showers and pleather fashion cravings exposed him to many neurotoxins. With these toxins and other environmental toxins in his system YM began to feel ill. Dr. B's prognosis was that YM had developed a mild form of Lupus. After searching the literature Dr. B decided to treat YM with azathioprine which breaks down in the stomach into two different compounds as shown in Scheme 1 below.



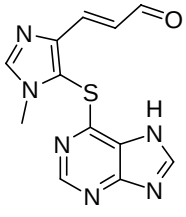
Scheme 1: The breakdown of azathioprine.

(a) Propose a mechanism for this breakdown.
Hint: Remember what is in your stomach.

(b) Unfortunately, the terrible American economy has forced Dr. B to use one of the cheap foreign azathioprine analogs GoodForYou ® and MakeYouGood ® (shown below). Only one of these drug analogs works propose a mechanism explaining why.
Hint: These drugs break down in similar fashion as azathioprine.



Good For You



Make You Good

13. ____/10 Microbes vary in size shape and form. Bacteria for example can be classified by the varying structure of their membranes. Bacteria, for the most part, universally share the presence of peptidoglycans on the surface of the plasma membranes. The only difference being that gram-negative bacteria versus gram-positive bacteria have a layer of lipopolysaccharides covering the peptidoglycans. Beta lactams have been used extensively to treat gram positive bacterial infections. However, resistance to beta lactams has been building, and recently scientist have started to using drug cocktails to treat bacterial infections. One example of this method is augmentin. Answer the following questions with respect to augmentin treatment.

(a) What are the major components of augmentin and how do these components work together synergistically to prevent cell wall biosynthesis?

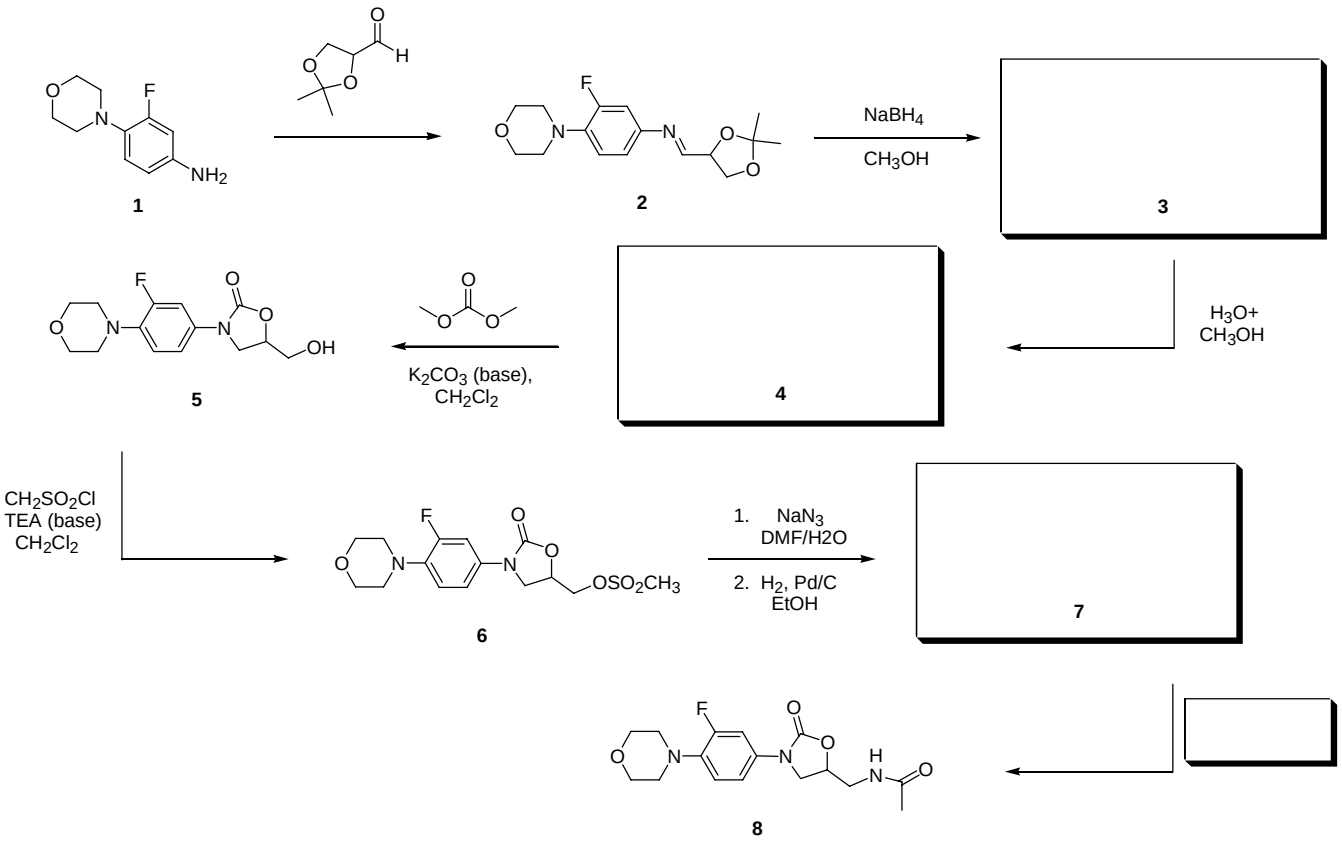
(b) One of the components of augmentin deals with the inhibition of beta lactamases. What type of inhibition does this component use to prevent cell wall biosynthesis?

(c) By introducing multiple drugs in bacterial treatment, how have scientists theoretically decreased the prevalence of resistant strains? Also how can the use of multiple drugs in treatment benefit the long-term health of patients?

14. ____/10 Why is it about the structure of beta lactams that allow them to inhibit bacterial cell wall biosynthesis? Be specific and approach your answer from a molecular perspective.

15. ____/10 Linezoid is an antibiotic that is active against many strains of multi-drug resistant Gram (+) bacteria such as MRSA. Part of the class of antimicrobial oxazolidinones, linezoid is synthetically made via relatively simple process starting with (S)-glyceraldehyde acetonide, a readily available starting material. A partial scheme (Scheme 2) for this synthesis is shown below.

(a) Fill in the blanks in the synthetic pathway below by providing either the product of a reaction step or the reagents as indicated.



Scheme 2. Synthesis of linezoid.

(b) Provide mechanism for the conversion of 1 to 2.

(c) Provide a mechanism for the conversion of 4 to 5.

16. ____/10 As stated in *The Autoimmune Epidemic*, mercury poisoning is becoming more prevalent, and may be responsible for a large number of autoimmune diseases. Presently, there are a few drugs on the market that can be used to treat mercury poisoning. Unfortunately, none of these drugs are completely effective, and most come with severe side-effects. One of the drugs currently used for the treatment of mercury poisoning is dimercaptosuccinic acid (DMSA). Answer the following questions with respect to DMSA.

(a) DMSA is a chelating agent which works to remove mercury stores in the body. Draw the structure for this drug,

(b) Describe how the chelation process works to rid the body of mercury poisons.

Exam II—Chemical Immunology and Immunopharmacology
Fall 2008

Please answer **fifteen** of the following eighteen questions. Each question is worth ten points, and one of fifteen questions you answer can be your own question. You do not need to rewrite the answer to your own question, simply write "Free" or "10/10" in the box next to your question. If you are not sure which question is your own since I have modified a few of your questions to make them easier (yes many of you wrote very difficult questions) please email me and I will let you know.

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2. You are not to discuss in any form any part of this exam with anyone other than Nicole Snyder until all of the exams have been submitted for grading. This means that you may not consult other students (especially the students in the course who have written these questions), professors, parents, former high school mentors etc. until I post a note stating that all exams have been collected.

3. **Everyone** must answer questions 6, 10, 12 and 18.

4. This exam is due no later than **5:00pm on Thursday, December 11, 2008**.

A violation of these guidelines is a violation of the honor code and will result in an automatic zero on the exam and the student or student(s) found in violation will be subject to disciplinary action.

Please sign this paper before submitting your final work as a hard copy **directly to me**.

Good luck and let me know if you have any questions or concerns.

I, _____, certify that I have referenced any sources I used to complete this exam beyond those distributed to me as part of this course. I, _____, also certify that the answers contained in this exam are my own work, and that I have neither accepted help from any other student, professor, parent, or high school mentor, nor have I have given advice to any other student in the course regarding the material in this exam. I, _____, understand that violating these guidelines is a violation of the honor code and if I am found to have violated the honor code, I will be subject to disciplinary action.

Signed_____ Date: ___/___/___

1. ____/10 Mycophenolic acid (MPA) is a noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) and is used for the treatment of Dengue fever and several autoimmune diseases. Please answer the following questions with respect to MPA.

(a) Draw the structure of mycophenolic acid.

(b) What is the importance of inhibiting IMPDH?

(c) Using the active site below in Figure 1, show how mycophenolic acid binds to inosine monophosphate dehydrogenase. Please use blue or red ink to indicate the hydrogen bonds.

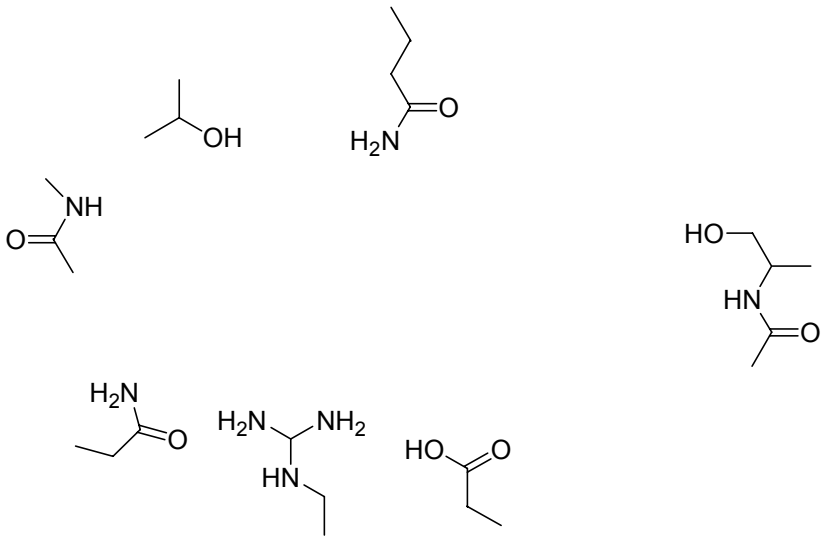


Figure 1. Inosine monophosphate dehydrogenase active site.

2. ____/10 Vaccinia virus and variola virus (smallpox) come from the same family of pox viri. Please answer the following questions with respect to these two viruses.

(a) Because of their homologous structure, live vaccinia is used to vaccinate people against the variola virus. What type of immunity and vaccination is this?

(b) Is there a biological disadvantage to using the vaccinia virus?

(c) Since 1971, production of the smallpox vaccine has stopped. Why are individuals no longer vaccinated against smallpox?

(d) Smallpox innoculation was formerly accomplished through exposing scabs of one individual to the variola virus from the pus or scabs of others already infected. It was found that people who were inoculated using this method had a much milder response to the disease, with a 0.5-2% mortality rate as opposed to the normal 20-30% mortality rate. Explain this phenomenon (think vectors) and why this method is less desirable than vaccination using variola virus.

3. ____/10 The spread of cat scratch disease to humans exemplifies one of the many ways in which bacteria transcend the body's physical barriers to infection. As mentioned during lecture, the disease is often spread to humans by cats carrying the bacteria *Bartonella henselae*. Usually, a bite or scratch from an infected cat does the trick. The following scenario serves as a case study of sorts:

> A Hamilton College student spotted a cat on Martin's Way as she was walking back to her room from the science center one night. Thinking nothing of it, she stopped to pet the very sweet and affectionate feline. She walked away with a few small scratches from the cat's sharp claws (the cat was, of course, pawing for attention), but thought nothing of it.
>
> By the time she returned to her room, the areas that had been scratched were swollen, red, itchy and blotchy. Her eyes were red and swollen shut, and she experienced constricted breathing. The symptoms had dwindled but not disappeared nearly twelve hours later, so she decided to seek medical attention (She of course, probably should have done so sooner). The student was diagnosed with cat scratch disease and prescribed augmentin and prednisone.
>
> The student took the medications as prescribed, taking the first doses of each roughly at the same time. Soon after, she noticed that she was developing a rash on her back, legs, face, and neck. As it turns out, the student had an allergic reaction to the amoxicillin component of augmentin. However, the rash disappeared shortly after she took her third dose of prednisone (which preceded her second dose of augmentin).
>
> The rash did not reappear the next day despite the fact that she continued with her second dose of augmentin, but the student was concerned about having another allergic reaction. She expressed her concerns to the Health Center at her follow-up visit, and was instructed to stop taking augmentin. She was prescribed cephalexin as a replacement.

Please address the following questions regarding the above scenario:

(a) Briefly discuss the functional class(es) of augmentin and prednisone, and propose why they were originally prescribed.

(b) Postulate as to why the rash went away despite the fact that the student continued to take augmentin.

(c) Finally, briefly explain what type of drug cephalexin is and propose why it was chosen as a replacement for augmentin in response to the student's allergic reaction.

4. ____/10 There have been recent, somewhat disturbing, television commercials advertising a European brand of over the counter medicine. This medicine, called Sinupret, is claimed to be able to "build immunity." How might Sinupret work and is the claim that it builds immunity valid?

5. ____/10 One of the more common syndromes associated with allergies is asthma. Asthma is a form of allergic reaction triggered by a variety of sources. The main symptom of asthma is shortness of breath caused by the narrowing of the airways. Currently, there are many drugs being used to treat asthma, both short-term, to quickly relieve the symptoms, and long-term drugs, to be used daily for long periods of time. One of the current long-term drugs, Serevent Diskus (generic name: salmeterol), is a part of a group of asthma drugs called long-acting β-2 agonists. Answer the following questions with respect to Serevent Diskus.

(a) What is the chemical structure of this drug?

(b) How does this drug function in the treatment of asthma?

(c) What is it about the structure of Serevent Discus that makes it particularly effective for this type of treatment? Be specific.

6. ____/10 The following questions pertain to cyclophosphamide, shown in Figure 2 below.

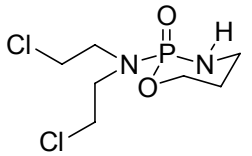
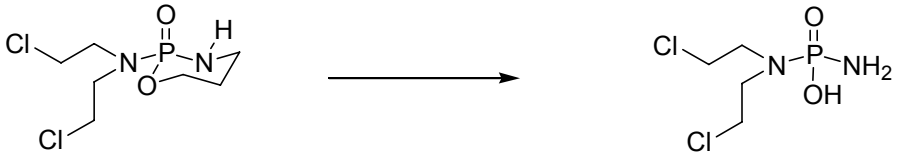


Figure 2. Cyclophosphamide.

(a) What autoimmune disease is this drug used to treat, and what is the main problem with the use of this drug?

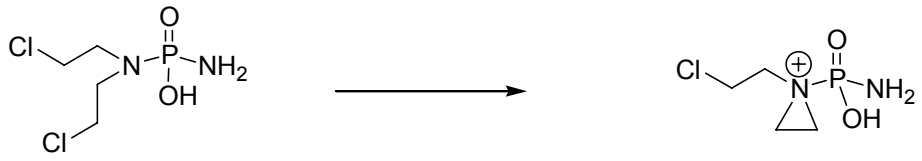
(b) Cyclophosphamide prevents DNA replication by alkylating DNA. However, the active form of the drug is a derivative of the molecule known as phosphoramide mustard (Figure 2) . Investigate the how the body reacts with cyclophosphamide, and show mechanistically how phosphoramide mustard is generated from cyclophosphamide.



Cyclophosphamide Phosphoramide Mustard

Figure 2. Conversion of cyclophosphamide to phosphoramide mustard.

(c) Alkylation by the phosphoramide mustard goes through an aziridine intermediate as shown in Figure 3. Provide a possible mechanism for the formation of the aziridine intermediate, and briefly discuss how this alkylation occurs.



Phosphoramide Mustard

Aziridine Intermediate

Figure 3. Conversion of phosphoramide mustard to an aziridine intermediate.

7. ____/10 The following structure (Figure 4) is retinol, the most common dietary form of vitamin A.

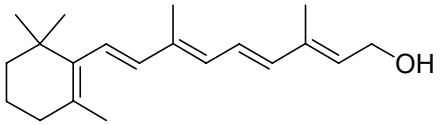


Figure 4. Retinol

Answer the following questions with respect to retinol.

(a) What is the significance of retinol to the immune system? In answering this question, elaborate on problems that arise when there is a deficiency of this compound.

(b) What is Lutz-Lewandowsky epidermodysplasia verruciformis and how can retinol be used as a treatment for this disease?

8. ____/10 Avoiding contact with an allergen can usually prevent an allergic response. If someone with an allergy to a certain allergen accidently comes into contact with it, there are measures to prevent anaphylactic shock. One such measure is the use of adrenaline, which can reverse the effects of anaphylaxis. Adrenaline binds to the β-adrenergic receptor, which sends a cascade effect signaling smooth muscle relaxation. Picture below in Figure 5 is a top view of adrenaline in the binding site.

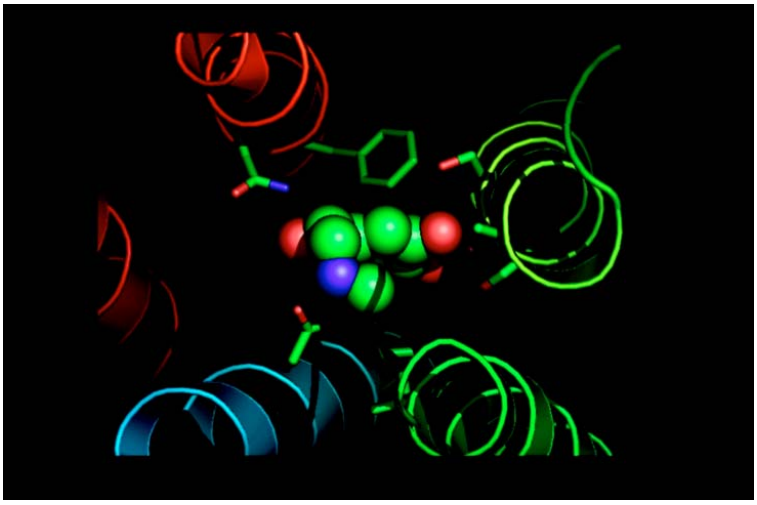
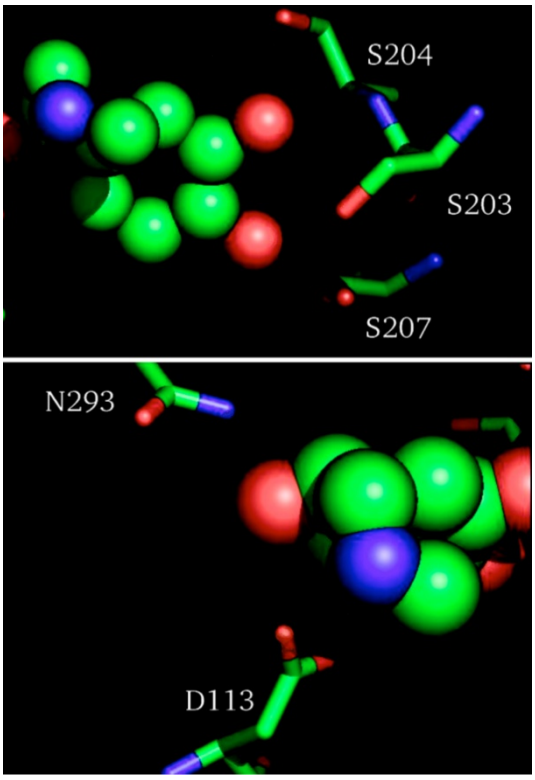


Figure 5. Binding of adrenaline to the β-adrenergic receptor.

Answer the following questions with respect to this binding event:

(a) Predict the hydrogen bonding that occurs in the binding of adrenaline using the images below.



(b) Once adrenaline binds to the β-adrenergic receptor an event is triggered within the cell, and the concentration of a certain compound increases. What is this compound and what is its role in the cell? (Hint: ATP is the precursor to this molecule.)

9. ____/10 Mast cells are a huge part of the hypersensitivity response. IgE molecules bind to the FcεR1 receptor of mast cells leading to the release of secretory granules. The contents of these granules include histamine, heparin, cytokines, and proteases. The surface crystal structure of mouse mast cell protease (mMCP-6) from the tryptase family of proteases is shown in Figure 6 below. This protease's active form is a tetramer.

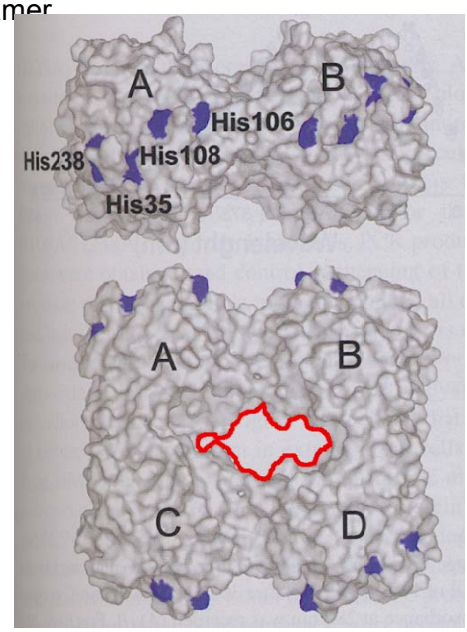


Figure 6. mMCP-6

Answer the following questions with respect to mMCP-6:

(a) What about its active site (red) makes it of particular interest to scientists developing protease inhibitors?

(b) One of the possible methods for stopping this proinflammatory is to inhibit its formation. It is known that this molecule requires heparin and a slightly acidic pH (~6.0) to form. Why does this make sense based on the above structure?

10. ____/10 Recently, a new class of potential HIV drugs was discovered by researchers. This class of drug not only targets one viral protein, it targets two! The general scaffold of this drug can be seen below.

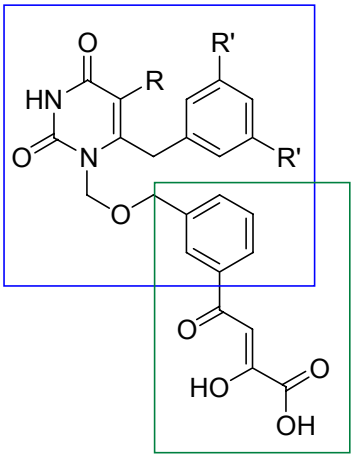


Figure 7. New "hybrid" HIV pharmaceutic.

Please answer the following questions with respect to this pharmaceutic:

(a) What enzyme do you think the blue moiety inhibits? You should be able to determine this answer from material presented in the lecture.

(b) What enzyme do you think the green moiety inhibits? You may need to look a little harder for the answer to this one.

(c) The green moiety contains and enol. Based on what you learned in organic chemistry, you would think this molecule would exist in the keto form. Speculate why the enol form predominates here.

(d) What advantages do you think dual inhibitors carry over drugs that only inhibit one enzyme? Frame your answer in the context of viral targets

(e) Down-on-his-luck researcher Noah Deeah wrote a grant proposal to the NIH, requesting money to synthesize an analog of this inhibitor, seen below in Figure 8.

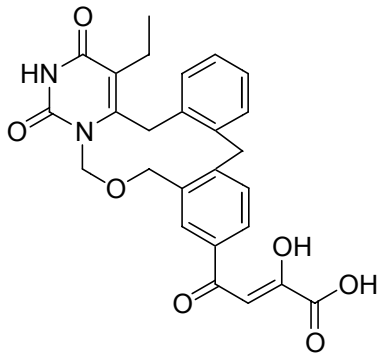


Figure 8. HIV inhibitor analog

He argued that the methylene tether between the two aryl groups, and the corresponding rearrangement of the diketoacid group would enable him to better mimic the green inhibitor, and would thus increase potency against the second enzyme (from part (b) above). Explain why the NIH flatly denied his appeal. (Hint: a picture of the original drug bound to the first enzyme (from part (a) above) is seen below in Figure 9.)

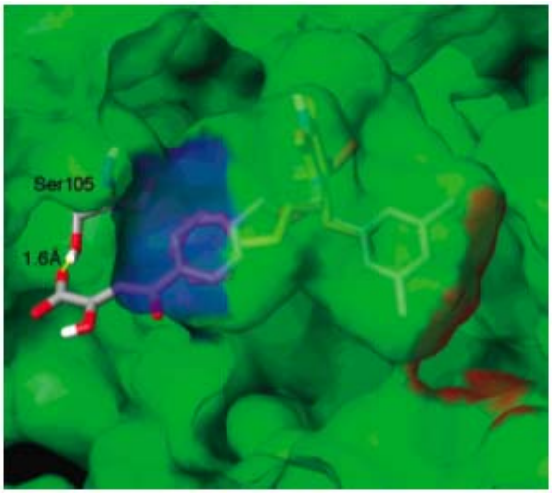


Figure 9. Deeah's analog bound to enzyme "a"

11. ____/10 For each of the following mini case studies below, provide the following:

(a) A complete diagnosis.
(b) A diagnostic medical test that could be run to determine if the diagnosis in (a) is correct.
(c) A treatment plan for each patient.

Case 1: Tommy Lafferty

Tommy, a young infant, is brought to the hospital due to a high fever, hyperhidrosis (abnormal increased perspiration) and jaundice. Examination of the Tommy's mouth shows oral ulcerations and periodontal disease. Further examination of the Tommy's body showed patchy distribution of non-pigmented skin. Tommy's parents have blonde hair, but the doctor notices that Tommy's hair is a lighter, silvery-blonde color[1].

(a) Diagnosis:

(b) Diagnostic:

(c) Treatment:

Case 2: Kevin Johnson

Kevin, a 2 month old child, is brought to the hospital when his parents discovered a large lump under Kevin's armpit. This lump had occurred before, but spontaneously disappeared within a few days. Along with the abscess, Kevin has come down with pneumonia, has persistent diarrhea, and eczema.

(a) Diagnosis:

(b) Diagnostic:

(c) Treatment:

12. ____/10 Compound A, shown below in Figure 10, is a potential inhibitor of an enzyme that is required for the proliferation of a terrible viral infection. The virus has incredible adaptive properties and this enzyme has already evolved to produce several drug resistant mutants to elude elimination from the host body. A mixture of drugs usually works well to keep the infection under control.

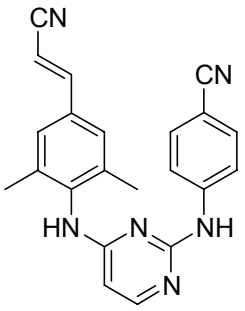


Figure 10. Compound A

The movie uploaded on Blackboard shows this potential drug in the binding pocket of the enzyme. The first seven seconds of the movie shows the molecule (green) in the binding pocket of the wild-type enzyme (white). The next section of the movie (from seven to seventeen seconds) shows in orange the region where drug-resistant strains mutations occur. The movie then compares the way compound A binds to the mutated and the wild-type enzymes. Compound A in the mutant enzyme has a conformation (yellow) that is different from the conformation it presents within the wild-type binding pocket. The final seconds show a view from a different angle, giving a complete visual presentation of the conformational changes of compound A from binding the wild-type enzyme to binding the mutant.

Answer the following questions with respect to the video:

(a) What aspect of the mutant makes it resistant to many of the current treatments on the market that are specific to this enzyme?

(b) What is special about compound A that allows it to inhibit both the mutant and wild forms of the enzyme?

13. ____/10 In class, we learned that sodium cromoglycate (shown below in Figure 11) is one possible treatment for an allergic reaction. How does the drug work to help treat the allergic reaction? What does calcium have to do with this binding process?

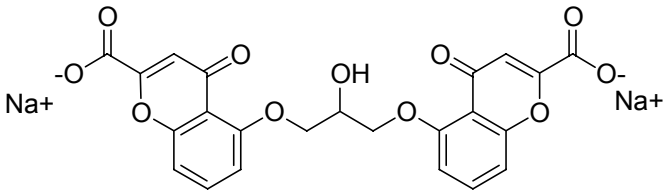


Figure 11. Sodium cromoglycate

14. ____/10 Urushiol, the chemical in poison ivy that causes contact dermatitis, is also found in the stem and skin of mangos. When a mango is plucked from the tree, a sap oozes from the severed stem that contains a form of urushiol similar to that found in poison ivy.

A group of 17 American youth traveled to Israel where, as part of a summer camp, they are employed in mango picking. While picking mangos, all the American youth developed contact dermatitis, but their fellow Israeli campmates did not. The American youth came from a part of the US where poison ivy sensitivity is endemic. All individuals, American and Israeli, had no previous exposure to mango stem sap.

Based on this information, explain in immunological depth, the observed American youth's allergy to mango at first exposure.

15. ____/10 Answer the following questions with respect to cell proliferation.

(a) During cell proliferation, there are certain checkpoints. Why are these checkpoints important and what will happen if this surveillance system fails?

(b) Tumor suppressor genes (TSG) encode proteins that inhibit uncontrolled cell growth. Oncogenes encode proteins that promote loss of cell cycle control, inhibition of apoptosis, and malignancy in cells. p53 is an example of a TSG that encodes a transcription factor that activates expression of genes involved in the cell cycle. In the case that an oncogene suppresses wild type p53, how can p53 activity be induced in cancer cells? Be specific.

16. ____/10 As the human immune response to cancer has become better understood, there has been increasing interest in developing cancer vaccines. One type of cancer vaccine is a dendritic cell-based vaccine. Answer the following questions with respect to dendritic cell-based vaccines.

(a) What does this kind of vaccine entail?

(b) Recently, the use of dendritic cells in prostate cancer vaccine preparations has been reported. What are the two main methods used to prepare the dendritic-cell based vaccines for prostate cancer? Which method was preferable and why?

(c) What are some challenges faced in creating a therapeutic vaccine for cancer patients?

17. ____/10 A characteristic feature of Chediak-Higashi Syndrome and other forms of albinism is very pale, white skin. There is tyrosinase-positive albinism and tyrosinase-negative albinism.

(a) What is the role of tyrosinase and how can it cause albinism?

(b) Why do people with tyrosinase-positive albinism still have pale skin?

(c) Is Chediak Higashi Syndrome a tyrosinase-positive or tyrosinase-negative disease? Give a plausible explanation for this, taking into consideration the mutations in lysosomal trafficking regulators.

18. ____/10 Many natural products can undergo rearrangement reactions, such a Bergmann and Myers-Saito cylcizations to produce reactive oxidative species. These free radical species are useful for cleaving DNA and are potent anticancer agents.

(a) Usually Bergmann cyclizations require high temperatures; however, certain substituted compounds such as calicheamicin (Figure 12) can form reactive species under physiological conditions. Show the mechanism to produce the reactive radical species starting from calicheamicin.

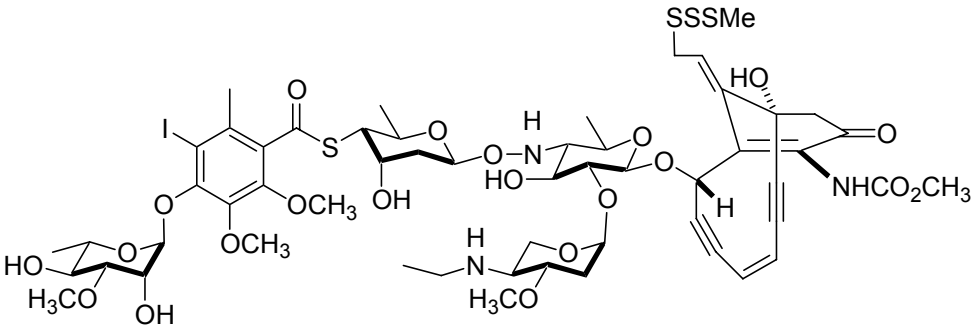


Figure 12. Calicheamicin

(b) Another compound shown to activity against tumor cells is neocarzinostatin (Figure 13), which forms the reactive oxidative species through the Myers-Saito cyclization. Show the mechanism to produce the reactive radical species using R-SH as a nucleophile.

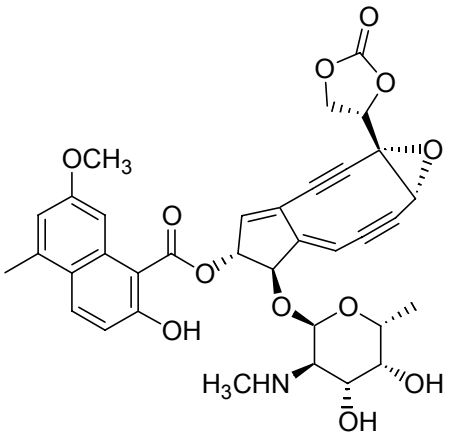


Figure 13. Neocarzinostatin

(c) Briefly describe how calicheamicin and neocarzinostatin function as pharmaceutics.

Exam I—Chemical Immunology and Immunopharmacology
Fall 2009

Please answer **fifteen** of the following twenty-five questions. Each question is worth ten points, and one of fifteen questions you answer can be your own question. You do not need to rewrite the answer to your own question, simply write "Free" or "10/10" in the box next to your question. If you are not sure which question is your own since I have modified a few of your questions to make them easier (yes many of you wrote very difficult questions) please email me and I will let you know.

This exam should take you approximately three to four hours to complete, but you may use all the time you need.

Here are the following guidelines concerning this exam:

1. You may use any written or electronic resource you find to complete these questions. If you have used materials beyond those distributed in this course, please reference them in your final work.

2. You are not to discuss in any form any part of this exam with anyone other than Nicole Snyder until all of the exams have been submitted for grading. This means that you may not consult other students (especially the students in the course who have written these questions), professors, parents, former high school mentors etc. until I post a note stating that all exams have been collected.

3. **Everyone** must answer questions 3, 13, 15 and 23.

4. This exam is due no later than **midnight on Friday, October 23, 2009**.

A violation of these guidelines is a violation of the honor code and will result in an automatic zero on the exam and the student or student(s) found in violation will be subject to disciplinary action.

Please sign this paper before submitting your final work as a hard copy **directly to me**.

Good luck and let me know if you have any questions or concerns.

I, _____, certify that I have referenced any sources I used to complete this exam beyond those distributed to me as part of this course. I_____, also certify that the answers contained in this exam are my own work, and that I have neither accepted help from any other student, professor, parent, or high school mentor, nor have I have given advice to any other student in the course regarding the material in this exam. I, _____, understand that violating these guidelines is a violation of the honor code and if I am found to have violated the honor code, I will be subject to disciplinary action.

Signed_____ Date: ___/___/___

1. (/10) At a fundamental level the immune system of all vertebrates can be divided into two types of immunity, innate and adaptive.

   a. Briefly describe the difference between innate and adaptive immunity.

   b. Plants possess a more limited immune system. Unlike humans, plants lack an adaptive immune system that contains macrophages, antibodies, and complements. However, plants do possess an innate immune system with comparable receptors, known as “resistance proteins” to the human immune system. Compare and contrast the resistance proteins found in plants to toll-like receptors utilized in the human immune system.

2. (/10) Human defensins are known not only for their antimicrobial activity against both Gram positive and Gram negative bacteria, viruses, unicellular parasites, and yeast, but also for their chemotactic properties for $CD4^+$ T-cells and immature dendritic cells.

   a. There are currently two models (Figure 1) postulating the permeabilization of cells by defensins:

      (i.) *Carpet model*: Molecules densely surround the cell surface and cause necrosis

      (ii.) *Pore model*: Molecules form multimeric solvent-permeable pores in the cell membrane, causing leakage

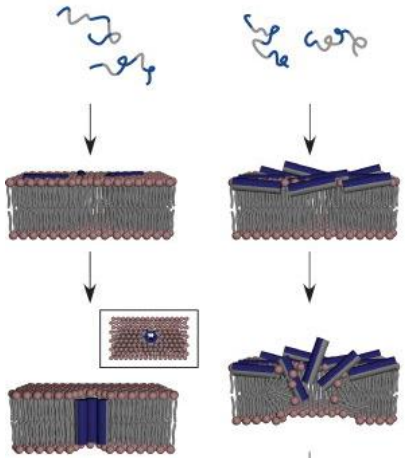


Figure 1. Pore model (left) and carpet model (right) for permeabilization by defensins.

How might the structure of β-defensins contribute to their ability to bind to and eliminate microbe targets based on these models? Think about the conformation of β-sheets and alpha-helices, for example.

   b. Based on your knowledge of both the structure and function of human defensins, how might increased salt concentrations affect the antimicrobial activity of defensins?

   c. Why is a defensin more likely to target a prokaryotic cell membrane than a mammalian cell membrane?

3. (/10) P, E and L type selectins are vital for the normal inflammatory immune response. The sialyl Lewis^x ligand is responsible for loosely bonding with these selectins to allow neutrophil migration along blood vessel endothelia. Consider the binding interaction between E-selectin and the sialyl Lewis^x ligand (shown below in Figure 2), two of the molecules involved in leukocyte rolling.

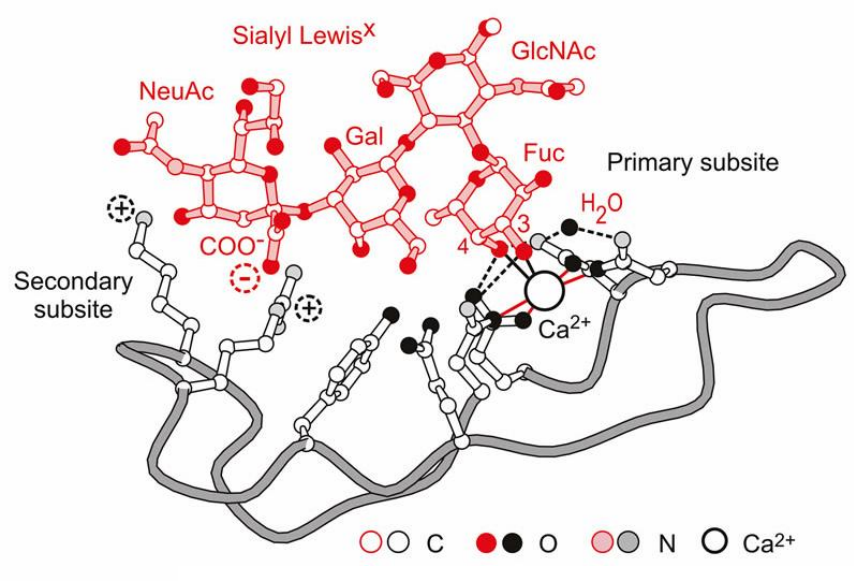


Figure 2. Sialyl Lewis^x binding E-selectin

a. How would the interaction change if the following changes were made:
   i. The hydroxyl groups on the C3 and C4 carbons of the terminal fucose were replaced with amino groups?

   ii. The terminal sialic acid was replaced with glucose?

b. If it is possible to strengthen or weaken the ligand/E-selectin binding interaction, why would or why would this not be favorable?

Unfortunately the sialyl Lewis^x ligand shows essentially no different affinity between the selectin types – thus making receptor specific studies and targeted anti-inflammatory therapies difficult to implement. Recently a set of novel oligonucleotide ligands have been identified that show a high degree of specificity for L-type selectin. Figure 3 shows the binding activity of an L-specific ligand (**A**) before and after the addition of EDTA.

c. Knowing that EDTA is commonly used as a chelating agent, explain the dramatic drop in activity of **A** following the addition of EDTA. Based on the data, also explain the relative importance of ion-dipole bonding versus other bonding types for the activity of **A**. The other ligand shown (**B**) is an unreactive control molecule.

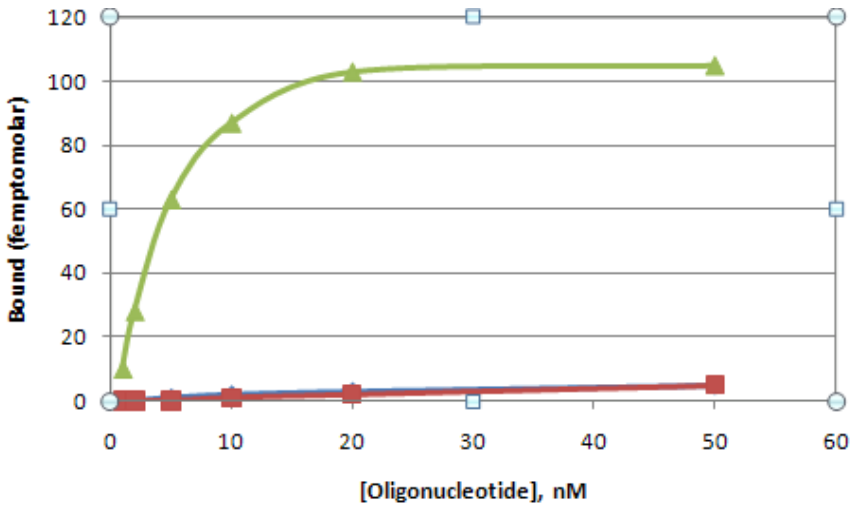


Figure 3. Graph of oligonucleotide concentration versus the amount bound to L-selectin (Green = **A**, Red = **A** + EDTA, Blue = **B**)

4. (/10) Glucocorticoids are steroid hormones that bind to the glucocorticoid receptor which is responsible for the regulation of genes involved in development, metabolism, and immune response. For instance, Cortisol (Figure 4)has been used as a treatment for rheumatoid arthritis since it reduces the immune response in patients.

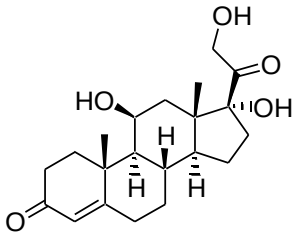


Figure 4. Cortisol

a. Glucocorticoids downregulate the expression of numerous cytokines, lymphokines, and growth factors by destabilizing the mRNAs and blocking transcription. Describe how the inflammatory response would be affected by downregulation of interleukins and interferons.

b. Since glucocorticoids can block the immune response, the total synthesis of glucocorticoids and/or their steroid backbone can lead to new drugs that can be used to treat auto-immune diseases, such as rheumatoid arthritis. In 1976, a research group developed a pathway for the synthesis of 11α-Hydroxyprogesterone (Figure 5).

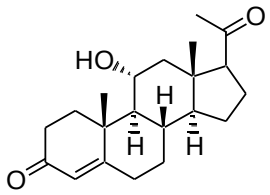
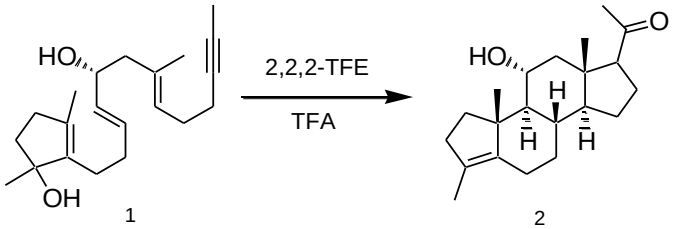


Figure 5. 11α-Hydroxyprogesterone

In the synthesis of 11α-Hydroxyprogesterone, intermediate I was stirred with trifluoroacetic acid and 2,2,2-trifluoroethanol to obtain intermediate II. Show the mechanism for this reaction without paying attention to stereochemistry.



1 2

5. (/10) An important part of the innate immune response is the ingestion and killing of microbes by macrophages. The word macrophage comes from the Greek, meaning big eaters, from *macros*, “large” and *phagein*, “eat”.

   a. What is the role of a macrophage in both the innate and adaptive immune systems and how are they activated?

   b. Describe the process of ingestion by macrophages from phagocytosis to the fusion of the phagosome with the lysosome.

   c. Discuss the enzymes and chemicals that phagocytes use to kill microbes.

   d. In what way might a macrophages be involved in an autoimmune disease? Briefly discuss.

6. (/10) Dendritic cells are crucial in determining the type of effector T-cell that mediates an immune response. Often being described as the link between innate and adaptive immunity, dendritic cells function through a combination of CLRs (C-type lectin receptors) and TLRs (Toll-like receptors).

   a. Describe how CLRs and TLRs work together to mediate an immune response.

   b. Describe the significance of CLRs in maintaining homeostatic control within the immune system.

7. (/10) Antibody cleavage, creating the two fragments Fab and Fc, is induced through the use of the enzyme papain. The active site of papain consists of a catalytic triad composed of the amino acids cysteine-25, histidine-159, and asparagines-158 (Figure 6). One of these amino acids is often neglected while writing the mechanism of the bond cleavage reaction because its role is mainly to maintain orientation. What amino acid mutation would provide a catalytic triad system that could perform this reaction more efficiently? Elaborate on how this new amino acid would enhance the reaction by providing the "new" mechanism incorporating this amino acid residue.

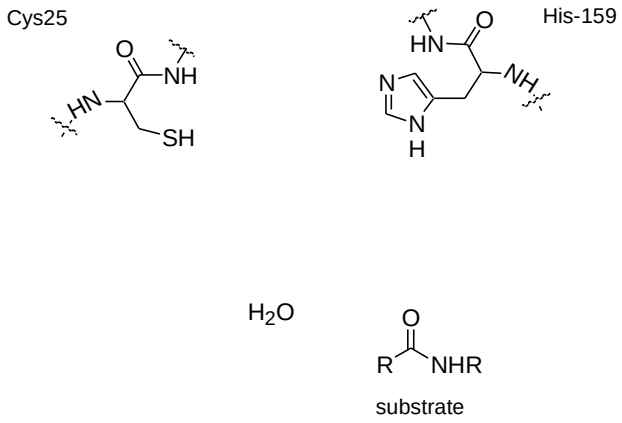


Figure 6. The active site of papain.

8. (/10) Some harmful bacteria such as *N. gonorrhoeae, N. meningitides,* and *H. influenza* contain IgA proteinases that are very substrate specific to IgAs from humans, some gorilla and apes. The enzymes cleave specifically at Pro-Ser or Pro-Thr regions. The two isotypic forms of IgA in human are IgA1 and IgA2 as shown in Figure 7 below.

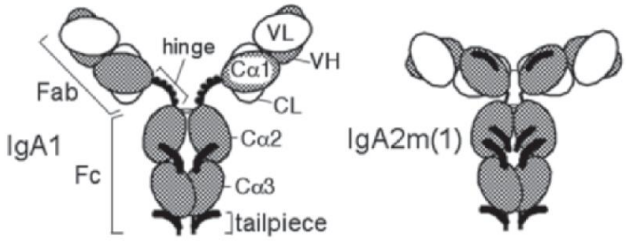


Figure 7. The structure of IgA1 and IgA2.

a. What is the major difference between the two isotypic forms of IgA differ in structure?

b. Given what we know about general cleavage of antibody, which of the IgA antibody do you think will be more resistant to the bacteria described earlier? Briefly explain your answer.

c. Continuing from b, which of the two isotypic form would have more Pro-Ser and Pro-Thr regions? Briefly explain your answer.

9. (/10) While many antibodies cause the destruction and/or removal of pathogens indirectly, certain antibodies, known as neutralizing antibodies (NAbs) are capable of effecting the deactivation of some pathogen directly. Provide four mechanisms through which NAbs cause the neutralization of viruses.

   Hint: the mechanism varies depending on which stage of replication the virus is undergoing when the NAb binds to it.

10. (/10) According to Abbas, Lichtman and Pillai, “Although the affinity of [many individual monovalent interactions] may be high, the overall avidity may be relatively low.” This is stated in reference to the binding of antibodies to antigens on a foreign body’s surface. We know that the avidity of antigen binding is not necessarily a result of strong individual affinities, and so polyvalent interactions are often favored in the broad sense. Explain thermodynamically why this may be the case. At higher temperatures, would you expect to see more avidity for mono or polyvalent reactions? (Hint: remember that $\Delta G = \Delta H - T\Delta S$!)

11. (/10) In 1986, Richard Lerner made the startling discovery that antibodies could behave like enzymes and catalyze reactions *in situ*. Specifically, he showed that antibodies generated against phosphonate esters (Figure 8) greatly increased the rate of hydrolysis of the corresponding esters.

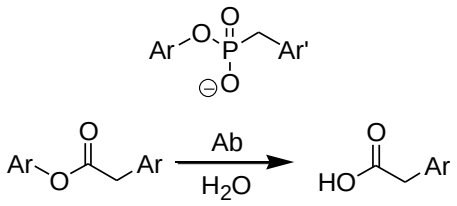


Figure 8. Example of ester hydrolysis by anti-phosphonate ester antibody.

a. How is the structure and function of an active site on an enzyme different from the binding site of an antigen?

b. How can an antibody catalyze a reaction, and how specifically does this antibody accomplish that goal?

c. Suppose you wanted to catalyze the methanolysis of 2-chloropropane. Propose a compound to generate catalytic antibodies for this reaction, and explain what structural considerations went into designing your antibody-generator. Hint: What is the nature of this reaction and the transition state of the reaction?

d. Now you want to catalyze the reaction of sodium methoxide with 2-chloropropane in DMF at cold temperatures. Propose a compound to generate catalytic antibodies for this reaction, and explain what structural considerations went into designing your antibody-generator. Hint: What is the nature of this reaction and the transition state of the reaction?

e. An upstart pharmaceutical company called Smiley Fun Co. has drafted you to design an antibody system that will both bind to specific antigens on tumor cells and catalyze the transformation of a prodrug into a nasty cytotoxic drug they have just developed. What are some restraints of this form of drug delivery, and what ideas do you have for facilitating it?

12. (/10) The major histocompatibility complex (MHC) plays a major role in immunological presentation and distinguishing self from non-self. Answer the following questions with respect to the MHC.

a. Compare and contrast the two different types of MHC, Class I MHC and Class II MHC. Be sure to include a reasonable treatment of the general structure and function of each.

b. T-cell receptors (TcR) bind to MHC and are ultimately involved in pathogen clearance. What are three levels of protein-protein interaction that appear to be critical to antigen presentation by MHC and TCR recognition? While all of the interactions are critical, which interactions would be considered the most critical?

c. Class I MHC is expressed on nearly all cells and present antigenic peptide components processed inside the cell, including self-molecules. Class II MHC presenting cells act differently, generally presenting only non-self molecules. Briefly explain why class II MHC molecules do not generally bind self-molecules.

13. (/10) Consider a system containing assembled Class II MHC and two peptide strands A and B. Determine a kinetics scheme for this system and find the concentration of the MHCA complex in terms of the other components. Assume all reactions are first order and the system is at equilibrium. Qualitatively assign values for the rate constants and describe how the expression reflects the experimental results for antigen competition.

14. (/10) Little Freddy loves hot dogs, so he was psyched to find out that when his favorite MHC product (Class II) binds to peptides, it looks kind of like a hot dog! Class II MHC molecules consist of two glycoproteins (an alpha chain and a beta chain) and are found on B-cells, monocytes, and macrophages. Class II MHC molecules bind to peptides (of 10-30 residues) on T-cells.

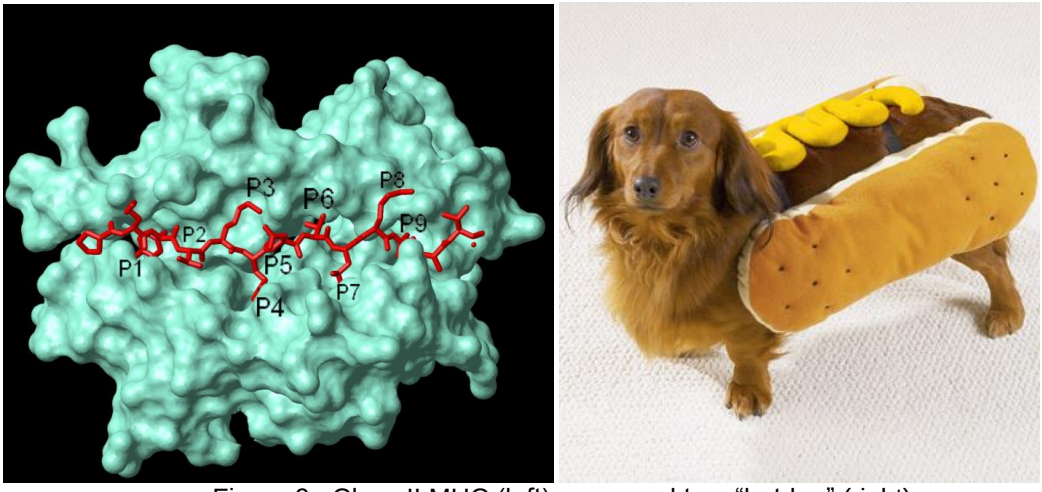


Figure 9. Class II MHC (left) compared to a "hotdog" (right).

a. Class II MHC molecules bind promiscuously (though Little Freddy doesn't know what that means). How does the structure of the binding region of these molecules help achieve this promiscuity?

b. To bind his hotdog to the bun, Little Freddy uses syrup. However, to bind a peptide to MHC II, different forces are needed. Five anchors on MHC II are used to keep peptides bound: P1, P4, P6, P7, and P9. Arg plays a role in the binding at P1. Phe, Pro, Leu, Ile, and Val play a role in binding at P4. Asn plays a role in binding at P6. Tyr and Trp play a role in binding at P7. Finally, Asp and Arg play a role in binding at P9. Describe the main forces used (hydrogen bonding, ionic forces, etc) when a peptide binds to MHC for each pocket. Name an amino acid for each that would suffice for binding in each anchor. Note: P9 binds differently than P1.

Site	**Class II MHC Amino Acid at Site**	**Potential Forces Used For Bonding**	**Potential Complementary Amino Acid(s)**
P1	Arg		
P4	Phe, Pro, Leu, Ile, Val		
P6	Asn		
P7	Tyr, Trp		
P9	Asp, Arg		

15. (/10) As we learned in lecture ubiquitination is a vital process in the larger scheme of protein degradation and antigen presentation. We know the role it plays, but not much about the protein Ubiquitin (Ub). Please answer the following questions with respect to ubiquitination.

a. Research and report three major characteristics of the Ubiquitin (Ub).

b. A series of enzymes is required to mediate the attachment of Ub to an abnormal protein. There are three major types of enzymes in this series known as enzymes E1, E2, and E3. Based on the Figure 10 below, describe the specific role of each enzyme.

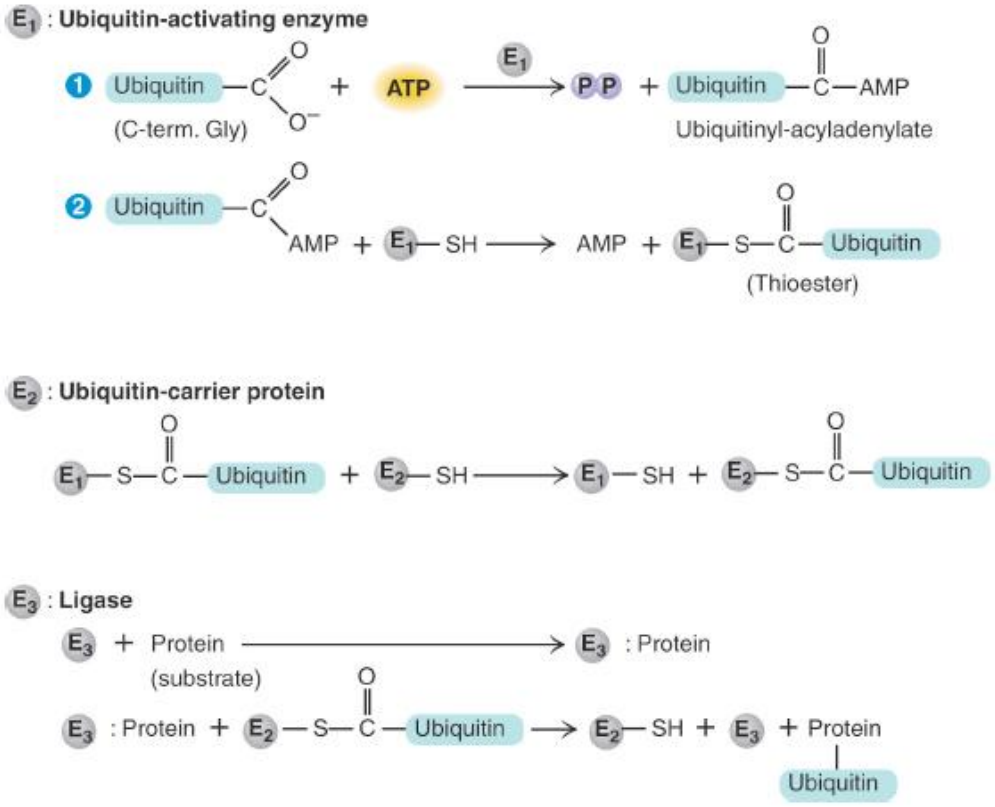


Figure 10. Ubiquitination by Ub using E1, E2 and E3.

c. The start and end of the Ub-Protein conjugation – after Ub has been modified to its reactive state by E1 Ub-activating enzymes - is seen in Figure 11 below. Propose a mechanism that explains the reaction scheme.

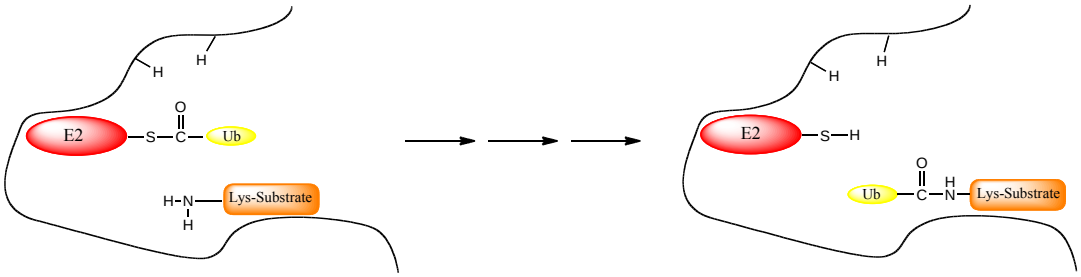
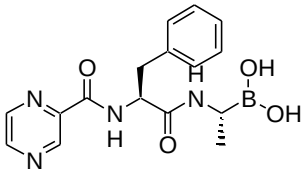
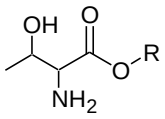


Figure 11. Ubiquitination.

16. (/10) Proteasomes are very complex molecules found in a cell's cytosol and in the endoplasmic recticulum. They are responsible for the proteolysis of many different proteins and are involved in antigen processing. Proteasomes also aid in some very important cellular processes, particularly the ubiquitination pathway. The proteasome active site, an N-terminal threonine residue, exists within the inner core of the proteasome. Certain proteasome inhibitors have been synthesized to target and inhibit this threonine residue, the most important being Bortezomib (Figure 12). Bortezomib was the first proteasome inhibitor clinically tested in humans.



(a)

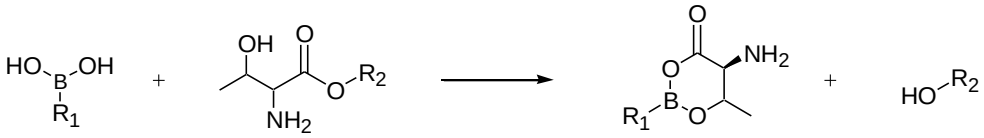


(b)

Figure 12. Bortezomib (a) and an N-terminal threonine residue (b).

a. What clinical purpose would a proteasome inhibitor serve?

b. Knowing that the boronic acid functional group is integral in the reaction between Bortezomib and the threonine residue, propose a mechanism for the following reaction:



c. What properties of the boronic acid group make Bortezomib so important in this reaction?

d. If the boron were substituted with a silicon atom, would the reaction still be successful? Why or why not?

17. (/10) The process for class II MHC-antigenic peptide presentation is complex. The beginning of the formation of the class II MHC molecule starts in the endoplasmic reticulum (ER) with the association of the alpha and beta chains supported by chaperones, such as calnexin. Once the alpha and beta chains are associated with each other, they are held together by the invariant chain (I_i) that keeps the molecule from binding to self-peptides in the ER. The class II MHC is then transferred to the class II vesicle (CIIV) where it undergoes proteolysis to for the class II-associated invariant chain peptide (CLIP). Within the CIIV, the molecule HLA-DM removes CLIP and allows the class II MHC to bind to the broken down antigentic peptide, which is then transferred to the cell surface and is expressed. Answer the following questions with respect to the process described above:

    a. What is the major molecule involved in the proteolytic degradation of I_i into the CLIP?

    b. What molecule is this similar to that we have talked about in class?

    c. Please provide a mechanism for this proteolytic degradation.

18. (/10) This is question covers the biology and maintenance of naïve (resting) and activated lymphocytes.

a. Why do naïve lymphocytes not survive as long as activated lymphocytes (lymphoblasts)? In answering this question, describe the transition from naïve to activated lymphocyte states.

b. How are naïve lymphocyte levels in the body maintained?

c. What is X-linked severe combined immunodeficiency disease (SCID) and how is this disease related to lymphocyte development? Hint: IL-7 plays a role!

19. (/10) In experiments demonstrating that antigen- presenting cells (APCs) must cleave protein antigens in order to present the peptides to a T cell and elicit a response, APCs failed to elicit T cell responses when fixed with glutaraldehyde before antigen digestion. If, however, antigenic proteins were artificially digested with trypsin after fixation, the APCs were able to present these antigenic protein fragments and elicit a T cell response.

a. Propose a mechanism for how trypsin cleaves a peptide (Hint: water plays a role).

b. You have a number of different trypsin molecules, each with a mutation in which the Ser-195 amino acid is replaced with tyrosine, threonine, or cysteine. Rate these mutants from most active to least active, and provide a reasoning for this order.

c. Trypsin has an optimal pH of about 8. Indicate what steps of the tryptic cleavage mechanism would be less likely to occur under an acidic pH.

d. The aspartate residue (Asp-189) is very important to the ability of trysin to cleave peptides at specific locations. Rate the relative ability of mutants with this residue replaced by glutamate, lysine, serine, or alanine to discriminate where to cleave peptides as does the native enzyme, and explain your rational.

20. (/10) Assess the following statements and determine whether they are true or false. If true, explain why or if false, change and add to the statement so it's true.

a. T or F An M cell is a memory cell that can be found in the gastro-intestinal tract specializing in food antigens.

b. T or F Without the presence of antigens, naïve lymphocytes would not proliferate and could only survive 2-3 weeks.

c. T or F Naïve T-cells interact directly with a foreign microbe at the site of infection, developing into mature, specific T-effector cells.

d. T or F Naïve CD4 T-cell interactions with dendritic cells in lymph nodes are crucial to the development of CD8 T-cells.

e. T or F Leukemia is considered an auto-immune disease.

21. (/10) Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder, which affects different parts of the body and produces symptoms such as painful joints, fever and fatigue (all extreme). We know these facts about SLE from reading about the toxic waste sites in Buffalo, New York (1980s) in the novel, *The Autoimmune Epidemic*. Antimalarial medicines are often administered to help treat patients suffering from SLE. Answer the following questions pertaining to antimalarials.

    a. Draw the structures of hydroxychloroquine, chloroquine and quinacrine, three well know antimalarial medications.

    b. Chloroquine is a common antimilarial drug that is known to have anti-inflammatory effects, and is often used for the treatment of SLE. Studies have shown that chloroquine is an inhibitor of antigen processing by the exogenous pathway. Give two possible explanations for this observation.

22. (/10) Trichloroethylene (TCE) is an industrially used solvent, but has been found to induce several autoimmune diseases. One of these is hypersensitivity dermatitis. Answer the following questions with respect to TCE.

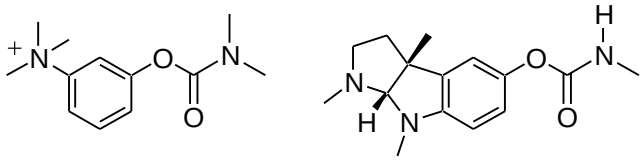
a. What are the effects of exposure to TCE?

b. Some individuals more susceptible to hypersensitivity dermatitis after exposure to TCE than others. Provide a plausible molecular explanation for this observation.

23. (/10) The following questions pertain to Myasthenia, an autoimmune disorder that affects motor function.

a. Based on your knowledge of autoimmune disorders and motor control, what type of receptors must myasthenia gravis affect in order to have crippling effects on the motor system? Give a brief description of how this system works.

b. Acetylcholine is broken down by acetylcholine esterase. A common treatment for myasthenia gravis is an acetylcholine esterase inhibitor. The theory being that by preventing the breakdown of released acetylcholine, the communication between the nerve and muscle will be increased. Two acetylcholine esterase inhibitors, neostigmine and physostigmine, are shown below. What are the main differences between these two molecules? (think polarity) In treating this autoimmune disorder, it is important to only affect the peripheral nervous system. Which one of these drugs is specific to the PNS and why?



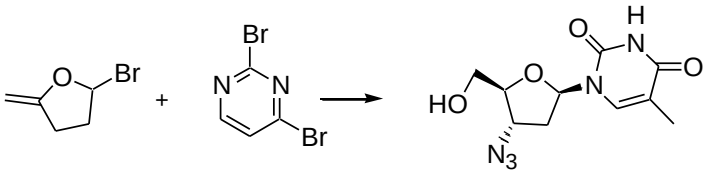
(a) (b)

Figure 13. Neostigmine (a) and Physostigmine (b).

c. Myasthenia gravis is an interesting autoimmune disorder because of the fact that the antibodies do not damage tissue or cause an inflammation response. Briefly describe how the immune system is thought to attack the acetylcholine receptors.

24. (/10) Reverse transcriptase inhibitors and protease inhibitors were highlighted as the two classes of drugs primarily used to treat HIV. In class, we spoke about retrovir, also known as AZT, which is shown below:

a. Provide a synthesis for retrovir below using the starting materials provided. Compare and contrast your synthesis to the original synthesis of AZT.



b. A recent study has revealed that over 76 % of patients undergoing HIV treatment have resistance to one or more anti-retroviral drugs. This is partly due to the rapid formation and propagation of numerous HIV variants due to the tendency for mutation in the HIV genome. Briefly discuss how AZT functions as a reverse transcriptase inhibitor, and propose a mechanism for resistance to AZT.

c. Discuss how resistance mechanism to a transcriptase inhibitor would differ from that of a protease inhibitor.

25. (/10) Congratulations! You have been appointed as Senior Biochemical Researcher for PharmaCore, one of the largest and most prestigious pharmaceutical companies in the world. As the youngest Senior Biochemical Researcher, you have a reputation to build. It is assumed that you will carry on the company's quest for excellence. For your first project, you are required to find a synthesis of the anti-HIV drug Indinavir (Crixivan™). This drug has been on the market for a while, but the company desires to have an edge over the competition by reducing the costs of production. Also, our biochemical mathematicians have predicted that the addition of a methyl group at the α-position next to the pyridine, and a slight change in stereochemistry will increase its protease inhibitor activity. The starting reagents are provided below. You are expected to provide a full, detailed synthesis, showing all the steps and making sure the stereochemistry is correct. You can use any other reagents you want, but you must use all of the starting materials at least once in your synthesis. Make haste and remember, the company expects nothing less than excellence. Good Luck!

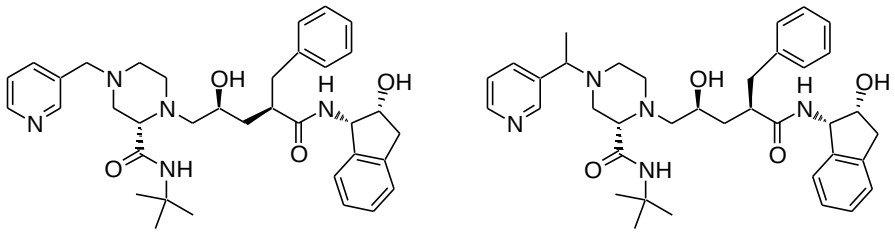
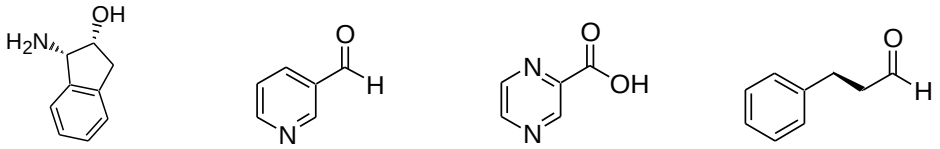


Figure 14. Indinavir (Crixivan™) and Indinavir 2.

Starting Reagents:



Exam II—Chemical Immunology and Immunopharmacology
Fall 2009

Please answer **fifteen** of the following twenty three questions. Each question is worth ten points, and one of fifteen questions you answer can be your own question. You do not need to rewrite the answer to your own question, simply write "Free" or "10/10" in the box next to your question. If you are not sure which question is your own since I have modified a few of your questions to make them easier (yes many of you wrote very difficult questions) please email me and I will let you know.

This exam should take you approximately three to four hours to complete, but you may use all the time you need.

Here are the following guidelines concerning this exam:

1. You may use any written or electronic resource you find to complete these questions. If you have used materials beyond those distributed in this course, please reference them in your final work.

2. You are not to discuss in any form any part of this exam with anyone other than Nicole Snyder until all of the exams have been submitted for grading. This means that you may not consult other students (especially the students in the course who have written these questions), professors, parents, former high school mentors etc. until I post a note stating that all exams have been collected.

3. **Everyone** must answer questions 3, 7, 8 or 12, 16 and 23.

4. This exam is due no later than **5:00pm on Friday, December 18, 2009**.

A violation of these guidelines is a violation of the honor code and will result in an automatic zero on the exam and the student or student(s) found in violation will be subject to disciplinary action.

Please sign this paper before submitting your final work as a hard copy **directly to me**.

Good luck and let me know if you have any questions or concerns.

I, _____, certify that I have referenced any sources I used to complete this exam beyond those distributed to me as part of this course. I, _____, also certify that the answers contained in this exam are my own work, and that I have neither accepted help from any other student, professor, parent, or high school mentor, nor have I have given advice to any other student in the course regarding the material in this exam. I, _____, understand that violating these guidelines is a violation of the honor code and if I am found to have violated the honor code, I will be subject to disciplinary action.

Signed_____ Date: ___/___/___

1. ____/10 We have discussed how the invasion of pathogens can lead to the inflammatory response which leads to numerous effects including swelling to isolate foreign substances from further contact with body tissues. This response, however, can sometimes be detrimental to the body and anti-inflammatory drugs are necessary to counter its effects. Several compounds, including ibuprofen, flurbiprofen, fenoprofen, ketoprofen, carprofen, and naproxen are part of class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs). These compounds are widely used to treat the effects of the inflammatory response such as pain and edema.

a. Please draw structures for each of these compounds in Table 1 below.

Table 1. Examples of NSAIDs.

Compound	**Structure**	**Compound**	
ibuprofen		flurbiprofen	
fenoprofen		ketoprofen	
carprofen		naproxen	

b. How are these compounds structurally similar, and what is it about the structure of these compounds that give rise to their anti-inflammatory properties? Be specific.

2. ____/10 Little Freddy has found his calling. After attending a Slayer concert, Little Freddy knew that he had to make a career as a death metal guitarist. So, Little Freddy saved up the money he made from his 9-5 job, told his boss to "screw off" (Little Freddy was taught not to swear), and bought himself a ESP electric guitar. Rock on, Little Freddy. Rock on. Unfortunately, Little Freddy got caught up in the rock star lifestyle and began to get sick. Fortunately, Death Metal Freddy has Death molecules in his immune system. These death receptors, also known as Fas receptors can be found on some antigen presenting cells. After the APC binds its class I MHC to a CD8 T cell TCR, the Fas ligand is presented on the T cell and binds to the Fas receptor on the APC, leading to apoptosis.

a. How does the binding of Fas receptor to Fas ligand lead to cell death?

b. Lpr/lpr mice produce low levels of Fas protein and gld/gld mice have interferences in the binding of the Fas ligand to the Fas receptor. What complications can arise from these mutated mice?

c. Interestingly, Fas does NOT play a role in the apoptosis of cells infected with HIV-1. What is a possible reason for the Fas resistance of directly infected cells?

3. ____/10 A patient suffering from a rare genetic disorder is unable to produce a protein involved in the complement response.

a. Compare and contrast the three different pathways in which complement can be activated.

b. What is the outcome of complement activation?

c. Given the following observations, identify the affected protein then explain how the patient's condition leads to each symptom.

1. The patient is able to generate a functional, but weak, complement response following infection by a pathogen.
2. The patient's response to pathogens with surfaces rich in mannose is unusually weak.
3. Mast cell and basophil degranulation is atypically slow in the patient.
4. Clearance of activated complement complexes is somewhat faster in the patient.

4. ____/10 The process of phagocytosis and subsequent destruction of the ingested cell is vital to the human immune system. Reactive oxygen species (ROS) are believed to be one active ingredient used to destroy phagocytized bacteria.

a. What are the proposed ways in which ROS results in the destruction of the microbe? Be succinct.

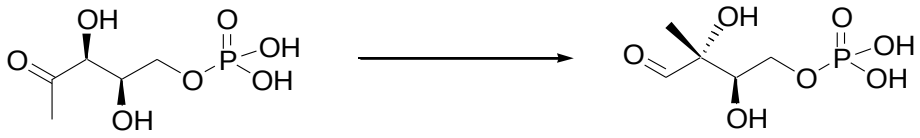
b. To reduce oxygen molecules into superoxides, a few major players are necessary: flavocytochrome, NADPH, and the cytosolic complex of p47-phox, p67-phox, p40-phox, and p21rac. Briefly describe this process.

c. The disease Chronic Granulomatous Disease (CGD) results in a deficiency of superoxide molecules, reducing the effectiveness of phagocytic cells. CGD causes mutations of key amino acids that directly affect the electron transport chain process. One such mutation is $Arg_{54} \rightarrow$Ser, changing the potentials in the flavocytochrome from -225 and -265 mV to -300mV. Explain why this is detrimental.

d. Another mutation is $Pro_{156} \rightarrow$ Gln. Explain the result of this mutation.

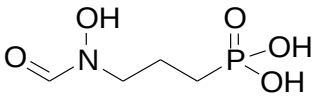
5. ____/10 The DOXP pathway is an important metabolic pathway that leads to the formation of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These molecules are important in many processes including protein synthesis, cell membrane maintenance, hormones, and glycosylation. Inhibiting this pathway in bacteria and parasites can make for an effective treatment.

a. One of the important steps in this pathway in *E. coli* is shown below. This occurs within the active site of D-1-deoxyxylulose-5-phosphate reductoisomerase. Show a mechanism for this conversion.



b. In this enzyme, the substrate is bound into the active site through coordination bonds at a metal cation. Where does this cation bind?

c. Using your answer from (b), explain how the antibiotic fosmidomycin, shown below, inhibits this pathway.



6. ____/10 Defensins and protegrins belong to a class of peptides that contain several intramolecular disulfide bonds that stabilize a conformation containing amphipathic β sheets. They are believed to exert antimicrobial effects primarily through membrane disruption and inducing leakage. A growing field of research has been to design analogs of naturally occurring peptides that are similar in antimicrobial activity and with low toxicities towards host cells. One such peptide under investigation is an 18-residue linear peptide with three hexameric KIGAKI repeats, $(KIGAKI)_3$-NH_2. In attempt to increase the antimicrobial effects and selectivity of this peptide, $(KIGAKI)_3$-NH_2 has been subjected to various mutations (Table 2). To determine of the effectiveness of the peptides, the minimum inhibitory concentration (MIC) was measured, and hemolysis of red blood cells (% hemolysis) was used in determining the peptides' effects on mammalian cell integrity (Table 3). Please answer the following questions based on the data presented in these two tables.

Table 2. Amphipathic β sheets generated.

Peptide	Amino Acid Sequence
KIGAKI	K I G A K I K I G A K I K I G A K I– NH_2
P_8-KIGAKI	K I G A K I K **P** G A K I K I G A K I– NH_2
P_{18}-KIGAKI	K I G A K I K I G A K I K I G A K **P**– NH_2
KIAGKIA	K I A G K I A K I A G K I A K I A G K I A– NH_2

Table 3. Biological evaluation of Amphipathic β sheets.

Peptide	MIC (*μ*g/mL)		% Hemolysis
	E. coli	*S. aureus*	
KIGAKI	2	8	20
P_8-KIGAKI	6	16	10
P_{18}-KIGAKI	2	8	10

a. Define the terms MIC and % hemolysis. As part of your answer, discuss the strengths and limitations of these two methods when used as a measure of antimicrobial activity.

b. From the observed data above, what can you conclude about the mutations and their effects of the activity of the peptide?

c. When considering effective methods in administering antimicrobial peptides, one suggestion is to change all of the amino acids of the peptide from its L-configuration to its D-configuration. Why might this be a useful method in increasing the effectiveness of the peptide?

7. ____/10 Augmentin is an antibiotic that is used to treat a variety of antimicrobial infections. Augmentin consists of amoxicillin, an antibiotic similar in structure to penicillin, and clavulanic acid, a beta lactamase inhibitor.

a. Draw the structure of amoxicillin and clavulanic acids in the Table 4 below and discuss the benefit of using these two compounds in combination to fight bacterial infections.

Table 4. Amoxicillin and clavulanic acid.

Amoxicillin	
Clavulanic acid	

b. Explain the how amoxicillin and clavulanic acid function using curved arrow notation to support your answer.

c. Monobactam drugs such as aztreonam and ezetimibe (Figure 1) are often administered with amoxicillin treatments to prevent bacterial resistance from developing. These molecules serve the same role as clavulanic acid. Using curved arrow notation to support your answer, rank aztreonam and ezetimibe in order of reactivity with β-lactamases assuming the only determining factor here is electronics (i.e. sterics does not play a role). Briefly discuss your answer.

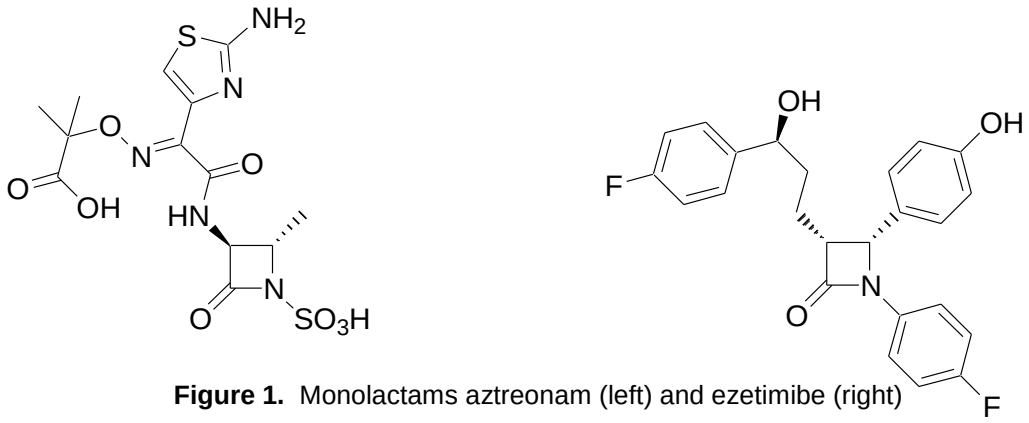
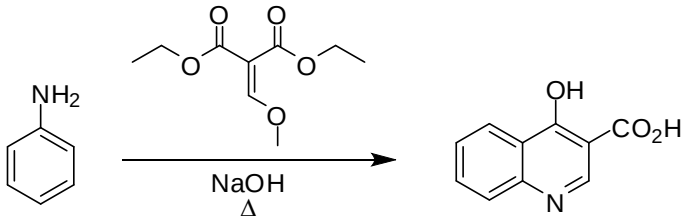


Figure 1. Monolactams aztreonam (left) and ezetimibe (right)

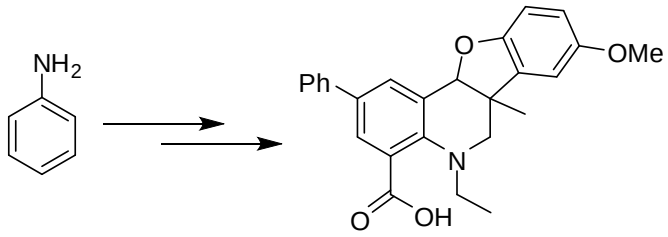
8. ____/10 This question concerns the synthesis, properties, and functional group activity of the quinolones, important antibiotics with the general structure below.

a. There are numerous methods for making quinolones, including the Gould-Jacobs Reaction shown below. Provide a mechanism for this reaction.



b. Show mechanistically how one would obtain the quinolone from the hydroxybenzopyridine product of the Gould-Jacobs reaction using ethyl bromide?

c. After eating some pills he found on the floor at the last board meeting, the CEO of the new pharmaceutical company Smiley Fun Co. had a hallucination in which he learned that the novel N-substituted pterocarpan shown below was a potential antibiotic. He has now charged you with synthesizing it from aniline, using only commercially available reagents, or else lose your job. Since you have several babies at home to feed, you start cracking on a synthesis. Good luck! (Hint: Start by making the corresponding quinolone of aniline, and then use the reactivity of the quinoline's various functionalities to your advantage. This can be done in around 14 steps and with only two cross-couplings).



d. Why might he be wrong about his purported panacea? (Hint: Compare the functional groups to those of known quinolone antibiotics)

9. ____/10 In class we discussed superantigens, with a specific focus on toxic shock syndrome and the toxic shock syndrome toxin-1 (TSST-1) produced by *Staphylococcus aureus*.

a. How does TSST-1 function as a superantigen?

b. Clindamycin (Figure 2) is an *anti-Staphylococcal* drug used to treat Toxic Shock Syndrome. Below is the structure of clindamycin and its derivative, lincomycin (Figure 2). How do these compounds function? That is, what bacterial cell process is inhibited by clindamycin?

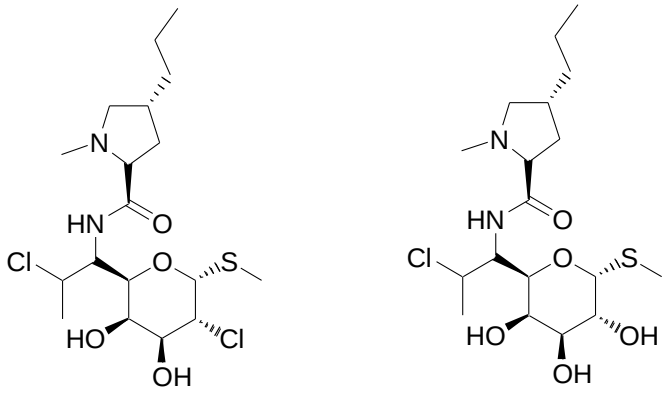


Figure 2. Clindamycin (left) and lincomycin (right).

c. There is one small structural difference between these two compounds. What is it, and what advantage does this structural difference give clindamycin over lincomycin?

d. There are many ways to synthesize clindamycin from lincomycin. One method involves reacting lyncomycin with *N*-chlorosuccinimide and triphenylphosphine. Why is chlorine substitution specific for the 2-OH? Provide a mechanism to support your answer.

e. Below are the unique and shared structural features of TSST-1, a variation of *Staphylococcal* exotoxin (SEC 3) and a variation of *Streptococcal* pyrogenic exotoxin (SPE A) (Figure 3). These three superantigens were administered to rabbits intravenously, orally, and vaginally. Because TSST-1 was the only of the three that caused an effect vaginally, it was concluded that this was the only antigen that was able to cross epithelial cell walls. What are the major differences in the shapes of TSST-1 and SEC/SPEA and how do these structural variances provide TSST-1 with the unique ability to cross epithelial cell walls?

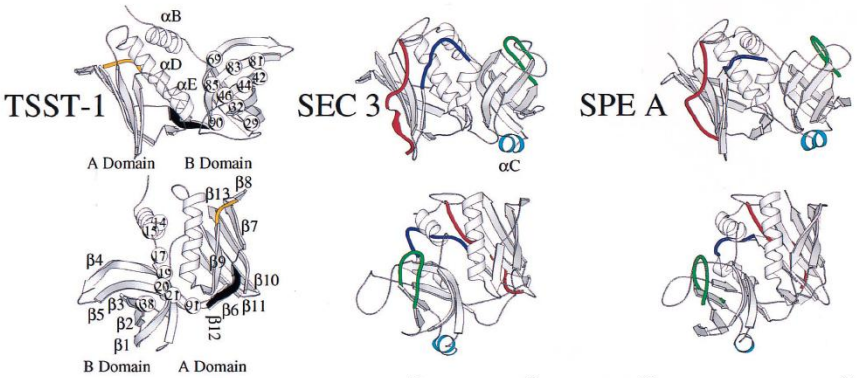


Figure 3. Structures of TSST-1, SEC-3 and SPE A.

10. ____/10 The gram-positive bacterium *Streptococcus pyogenes* group A is a human pathogen that is known to cause mild to severe infections of the skin and throat including rheumatic fever, strep throat, scarlet fever and necrotizing fasciitis. In order to cause infection, the bacteria must colonize the oropharynx or external skin.

a. Briefly describe how the bacteria are able colonize in the host and evade phagocytosis.

b. Some of the most harmful strains of *S. pyogenes* were recently found to produce a protease called SpyCEP (Strep. pyogenes cell envelope protease). How does this protease promote the survival of the bacteria, and how could this finding affect future treatment for this infection?

c. The elucidation of the gene mutation that results for the coding of SpyCEP has been a relatively recent development. What specific event is believed to give rise to this mutation?

11. ____/10 The following questions correspond to the fungal infection *Candida albicans*.

a. Briefly discuss three innate immune system mechanisms of defense against fungal infections caused specifically by *Candida albicans.*

b. Knowing that the virulence of *Candida albicans* is due to its ability to transition from yeast cells to filamentous hyphae, it has been shown that *C. albicans* infections can be suppressed by certain chemicals that specifically prevent this transition. Which of the molecules in Figure 4 might you expect would have an effect on the morphological state of *C. albicans* (i.e. prevent filamentation)?

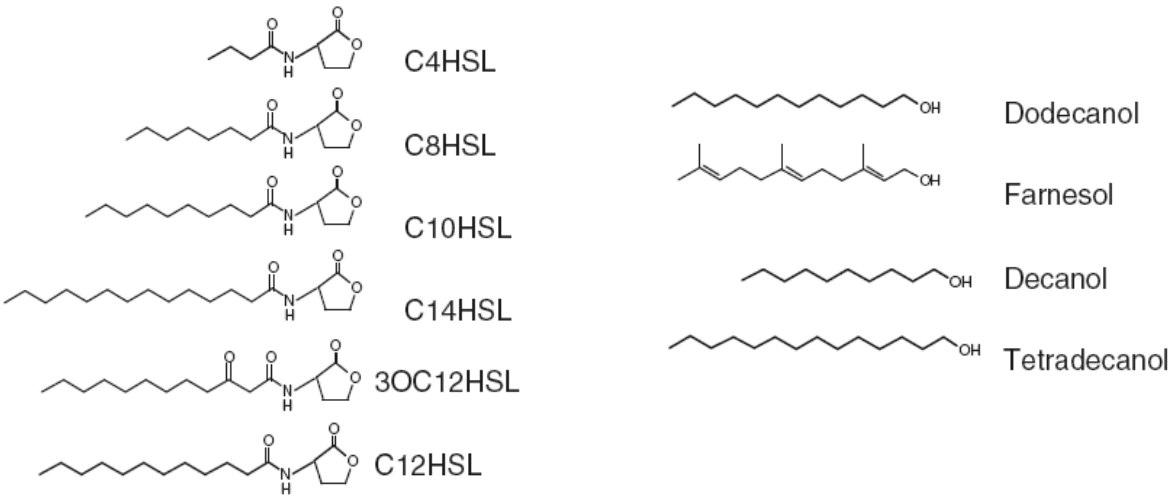


Figure 4. Potential *C. albicans* suppressors.

c. Where might these effector molecules originate, and what do they have in common?

12. ____/10 You have done a fantastic work by successfully synthesizing *Indinavir 2* as you last project for PharmaCore. The management and your coworkers are all pleased with your work, and you have been assigned another project to complete.

a. This time, you must provide a full synthesis for the *modified version* of mycophenolic acid (Figure 5) using benzene, 3-methyl-hex-2-ene-1,6-diol, and any other reagents with no more than three carbons (excluding solvents and catalysts).

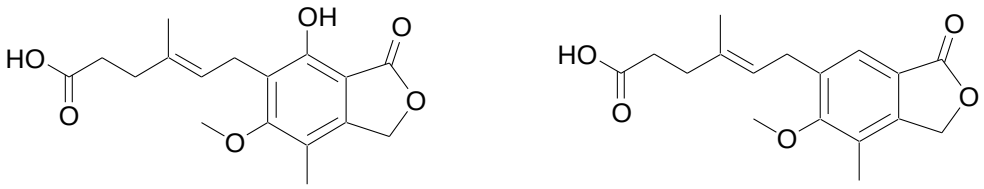


Figure 5. Mycophenolic acid (left) and modified mycophenolic acid (right).

b. Based on what you learned in class, would you expect the modified version to be more or less effective than the original version of mycophenolic acid? Be specific.

13. ____/10 John is a college graduate who was working in Zaire as a provisional teacher in high-poverty areas. After working in Zaire for 2 years, he decided to come back to the U.S. to continue on his graduate studies. Just before coming back to the U.S., John had a brief contact with one teacher that have been feeling ill for a couple of days for no particular reason. Unknown to John, that teacher had contracted a very exotic disease and was showing early symptoms of the disease. Back in the U.S., John had also started to show similar symptoms, such as fever, intense weakness, muscle pain, headache, and sore throat. John went to his doctor but the doctor had no idea of the severity of John's condition. Instead, John's doctor told him to get some rest. But John's condition only deteriorated, John vomited frequently, had diarrhea, and frequent external bleeding. John then visited another doctor, who knew John's conditions and subjected him to an intensive oral rehydration therapy under quarantine. After recovering, John's new doctor told him that he had contracted a hemorrhagic fever causing disease known as Ebola. Answer the following questions with respect to this disease.

a. The Ebola viral protein VP35 (Figure 6) is a multifunctional protein that serves in the RNA polymerase complex, as well as a structural/assembly factor, and a suppressor of IFN responses. VP35 contains two highly conserved sequences of basic amino acid residues. Yet, only one sequence binds to dsRNA, which is located near the c-terminus. Why does dsRNA only bind here?

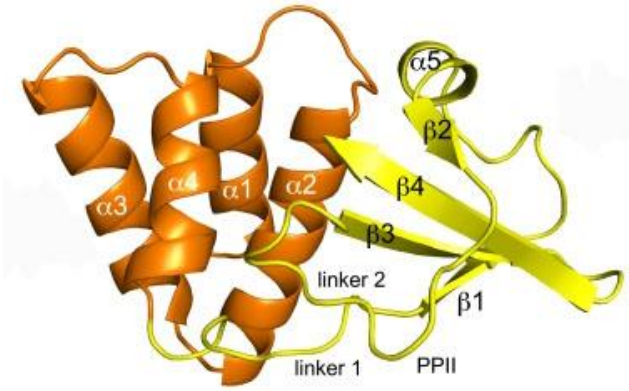


Figure 6. Ebola VP35.

b. The wild type amino acid sequence (shown below) is mutated to give rise to mutants I through III. How would you expect these mutations to affect the key interactions that occur with dsRNA?

Wild Type = P R A C Q K S L R P

Mutant I = P R A C Q K S L A P

Mutant II = P E D C Q E S A D P

Mutant III= P R L C R K S L R K

14. ____/10 The following question focuses on the ability of methyl- β cyclodextrin (MβCD) to inhibit the entry of flaviviruses, such as West Nile virus and dengue virus, into host cells.

a. Explain how the physical and chemical structure of MβCD leads to its ability to initiate this inhibition of flavivirus entry.

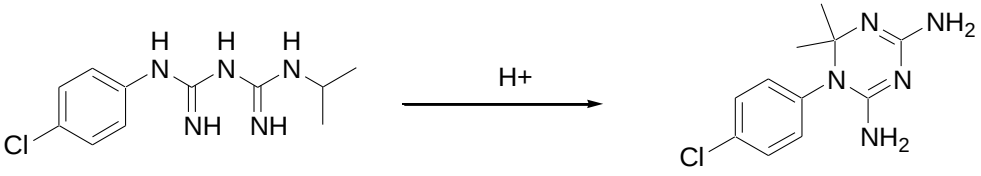
b. Explain why methyl- β cyclodextrin would be a poor choice as a potential therapeutic agent for the treatment of viruses such as West Nile virus and dengue virus?

15. ____/10 A single malarial infection often lasts for a long time, sometimes as long as 480 days. Malarial infections are often persistent and are characterized by dormant stage and active stage where recrudescence, periodic peaks when the patients have high fever, will occur.

a. Despite long exposure to the infection, why does our immune system have such a hard time controlling it? Or, what kinds of characteristics allow the parasite to initiate a chronic stage of malaria?

b. Why is it crucial for the parasite to survive for such a long period in the human body? Why would this characteristic be more crucial for parasites vs. bacteria?

c. The antimalarial drug malarone is composed of two compounds: proguanil and atovaquone. Proguanil is only effective against *P. falciparum* in its metabolized state, cycloguanil. However, proguanil in its unmetabolized state enhances the effectiveness of atovaquone. How do cylcoguanil and atovaquone work to inhibit *P. falciparum?* As part of your answer, please use curved arrow notation to show how proguanil cyclizes to cycloguanil. The general conversion is shown below:



16. ____/10 Chloroquine and quinine are commonly used as anti-malarial drugs. Answer the following questions with respect to chloroquine and quinine.

a. Draw the neutral structures of chloroquine and quinine in Table 5 below.

Table 5. Neutral chloroquine and quininie.

Chloroquine	
Quinine	

b. For chloroquine, $pKa_1 = 8.3$ and $pKa_2 = 10.2$. For quinine, $pKa_1 = 5.07$ and $pKa_2 = 9.07$. Use this information to determine the forms of chloroquine and quinine at physiological pH and place these structure in the Table 6 below.

Table 6. Chloroquine and quininie at physiological pH.

Chloroquine	
Quinine	

c. Using the information in b, briefly describe how each compound functions as an anitmalarial?

d. Amodiaquine (Figure 7), a derivative of chloroquine, was designed to combat strains of malaria resistant to chloroquine. Propose a synthesis of this molecule starting from 4-bromo-7-chloroquinoline and benzene.

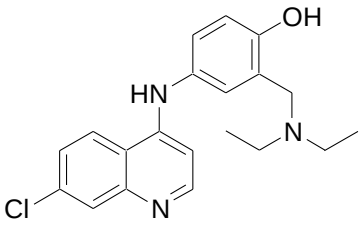


Figure 7. Amodiaquine.

e. Based on your answer in b and the structure of amodiaquine, would you expect the charge of amodiaquine at physiological pH to be greater than, equal to, or less than chloroquine? Briefly explain your answer.

17. ____/10 The following questions deal with the role of Duffy antigen in *Plasmodium vivax* invasion of red blood cells and in normal physiological function.

a. Recently scientists have identified and characterized a 140 kDa Duffy binding protein expressed by *Plasmodium vivax* that plays an integral role in the parasites invasion of erythrocytes. As the name suggests, Duffy binding protein binds to Duffy antigens on the surface of RBCs allowing the parasitic cells to adhere to and invade the host blood cells. Explain how knowledge of Duffy binding protein's structure could lead to treatments for *P. vivax* infection.

b. It has been noted that Duffy antigen receptor for cytokines (DARC) has a great deal of structural similarity to many G-coupled chemokine receptors. However, DARC binds to a much wider variety of chemokines than most G-coupled chemokine receptors and is not coupled to the G-protein signaling pathway. Given this information, propose a likely function for DARC *in vivo*.

18. ____/10 Read the following case study and provide the following in Table 7: a complete diagnosis, a diagnostic medical test that support you diagnosis, and a treatment plan for the patient, Jacob Long.

Patient Name: Jacob Long
Age: 32
Weight: 80kg
Date: February 4, 2009
Notes: Patient complaining of intense abdominal pain and muscle weakness, as well as bloody stool. Blood pressure has risen significantly within the last year (patient history). Enlargement of his spleen and large intestine is also noted. Patient recently spent Christmas (2008) in southwest Laos in a forest meditation monastery reconnecting with nature. Patient reports daily bathing in the local river until his skin began to itch and strange bumps began to appear on his extremities. Upon return to the US, he had a period of unexplainable fever, coughing and diarrhea. The patient did not receive medical attention at the time.

Table 7. Jacob Long diagnosis, testing and treatment.

Diagnosis	
Diagnostic Test	
Treatment Plan	

19. ____/10 Lopinavir is a compound used to treat HIV.

a. Provide the structure of lopinavir below.

b. Lopinavir functions by inhibiting cleavage of the “gag-pol” substrate. What is this substrate and why is important to the life cycle of the virus. Be specific.

c. Show the mechanism for the cleavage of the Gag-Pol substrate.

d. Based on your answer in c, discuss how lopinavir functions as a drug. Use structures and/or mechanisms to support your answer.

20. ____/10 Eric Lewis has a polymorphism in a gene that codes for a receptor protein which makes the protein completely inactive! He has had infections frequently throughout his life and childhood and his lymph nodes are poorly developed. However he has not run across an infection he could not handle. He has a high level of IgM and low levels of IgA or IgG.

For each of the receptor proteins in Table 8 below, describe their function and whether it is possible Eric Lewis has a polymorphism in the gene coding for the receptor protein based on what you know about his condition from the short passage above.

Table 8. Molecules for consideration in Eric Lewis's condition.

Molecule	**Function**	**Polymorphism Possible? Why or why not?**
CD4		
CD8		
CD28		
CD154		

21. ____/10 Answer the following questions with respect to D-penicillamine.

a. Draw the structure of D-penicillamine below.

b. D-penicillamine is a chelating drug that is used to treat Wilson's disease and rheumatoid arthritis. Unfortunately, it has the side effect of inducing autoimmunity in some individuals. Discuss the uses of this drug and its method of action in both Wilson's disease and rheumatoid arthritis.

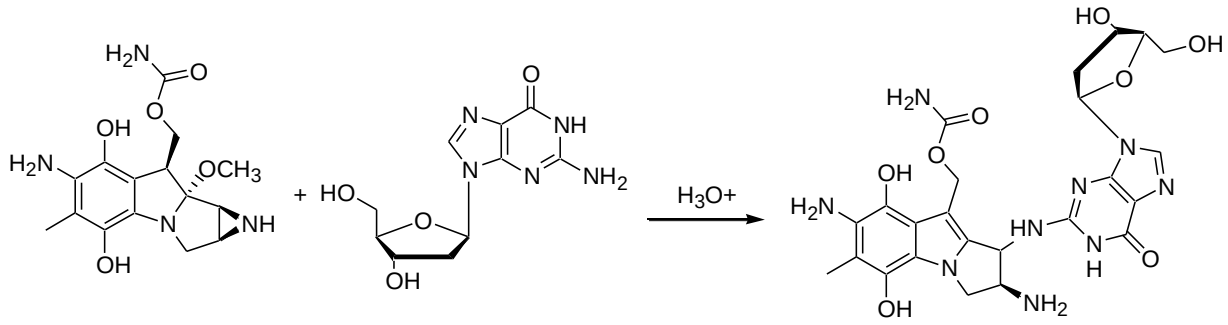
c. What autoimmune responses have been linked to D-penicillamine?

d. D-penicillamine has been shown to react with aldehydes on the surface of macrophages to form a thiazolidine. Provide a mechanism for this reaction using curved arrow notation, and briefly discuss how the reaction between D-penicillamine and macrophages plays a potential role in the side effects of the drug.

22. ____/10 Antifugals are vital in controlling every day fungal infections such as ringworm, jock-itch, and athlete's foot. Two examples of antifungals that aid in their treatment are amphotericin B and nystatin. Both of these drugs are examples of the polyene class, which bind to ergosterol, changing the composition of the cell membrane which ultimately causes cell destruction. Recently, studies have also highlighted the importance of antifungals, such as amphotericin B and nystatin, on cancer patients. Please explain why antifungals are so important to cancer patients, some of the major challenges of these drugs, and a current method in which amphotericin B and nystatin are distributed.

23. ____/10 The biologically active form of mitomycin C, shown below, is known to have both antibiotic and antitumor properties. Mitomycin C cross-links guanine residues of the DNA double helix generating kinks and bends in the helix.

a. Using curved arrow notation, show how mitomycin C binds to guanine under acidic conditions. The overall reaction is shown below for your convenience.



mitomycin C

b. The biologically active form of mit"oxy"mycin C, a derivative of mitomycin C is shown below in Figure 8. In reactivity studies, mit"oxy"mycin C is less reactive than mitomycin C. Further studies have shown that this has to do with the fact that the aziridine of mitomycin C, which is directly involved in the crosslinking, is more reactive than the epoxide of mit"oxy"mycin C. Briefly explain why the aziridine ring is more reactive than the epoxide ring. Be specific. Ring strain is the answer. Why does the aziridine ring experience more strain?

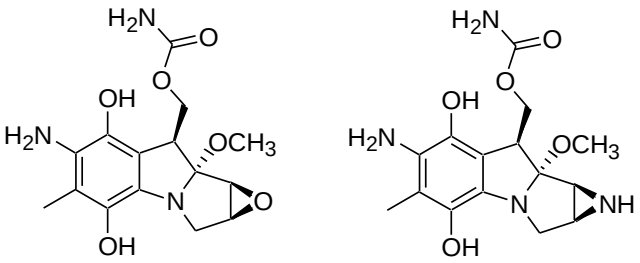


Figure 8. Mit"oxy"mycin C (left) and mitomycin C (right).

Immunology

An Artistic Presentation

October 29, 2008 and October 31, 2008

Science Center 2017

12:00-12:50

The Presenters

in alphabetical order

Maxwell Akuamoah-Boateng..... Hypersensitivity Type I: A Stop Motion Silent Film

Katherine Alser..... The Monster Macrophage: A Children's Tale

Alexa Ashworth..... CD3 Scrapbook

Sarah Bertino..... The Journey of a Pathogen from Infection to Destruction

Jessica Dietz..... Antibody and Antigen Model

Sydney Fasulo..... Antigen Presenting Macrophage

David Hamilton..... Personal Encounters with Autogens

Victoria Jenkins..... Circular Amplification Loop Flipbook

Amy Klockowski..... Hypersensitivity Type I: A Stop Motion Silent Film

Chris Lorenc..... Lord of the Immune System: The Battle of Measles

Yuqi Mao..... The Cytotoxic Battle Within: What Really Goes on Under the Skin

Divij Mathew..... ROCKY VI Antigen!! Cell No!

Trevor Pedrick..... Advanced Electron Micrographs of Various Pathogens

Jared Pienkos..... The Cytotoxic Battle Within: What Really Goes on Under the Skin

Ben Saccamano..... Lego Pixelation of Dendritic Cells

Ryan Seewald..... Small Pox Table

Julianee Tylko..... Imma Immunoglobin's Life as an Upperclassman at Antibody University

Benjamin Van Arnam..... The Cytotoxic Battle Within: What Really Goes on Under the Skin

**Photo Credit: NIAD

Amy Klockowski and Maxwell Akuamoah-Boateng
Hypersensitivity Type 1: A Stop-motion Silent Film

The story will follow the life of Max Akuamoah-Boateng, beginning with how he acquired his peanut-specific IgE antibodies, to the horrors that follow his accidental ingestion of one of Amy Klockowski's delicious peanut butter cookies. The movie will be shot in individual still frames, pieced together to form a fluid film. All immunological components: T helper cell, B cell, plasma cells, IL4, IgE, mast cells, ect, will be created using common household items. Accompanying the film will be a truth poster about the dangers and prevalence of food allergies in the United States.

Katherine Alser
The Monster Macrophage: A Children's Tale

Our story begins with a young, healthy epithelial cell going about its daily routine in the lung-tissue neighborhood. One day, the cell encounters a stranger. Being a particularly friendly and vibrant cell, the young epithelial decides to try to make friends. The stranger appears to have its own friends though, and is more interested in mingling with cells in a different part of town – one that has recently had a serious mucosal disruption. The healthy cell is left rather dejected and confused because most cells in the area seemed to have been pretty nice so far. Suddenly, the healthy cell sees the group of strangers being engulfed by huge monsters! The cell is quite frightened, and though communication with neighboring cells has recently been disrupted, it seeks out answers about this monster. Eventually, the cell finds out that the strangers it tried to make friends with were really rude, troublesome pathogens that were hurting other healthy cells. As it turns out, the monster was just doing its job, a macrophage disposing of riff raff in order to keep the neighborhood safe and its occupants alive and well.

Alexa Ashworth
CD3 Scrapbook

A lot can be learned about a person just by looking at a scrapbook of their life. Clippings, notes, and pictures can all be mounted into an album and kept to represent an individual through art. In this project, CD3 proteins will be represented through a scrapbook. T-cell receptors are associated with a group of proteins with signaling functions called CD3 proteins. There will be four different CD3 proteins represented: gamma (γ), delta (δ), epsilon (ε), and zeta (ζ). These proteins together form the CD3 complex, which initiates intracellular signaling pathways when T-cell receptors recognize an antigen. Through this scrapbook, we will be able to learn what these proteins look like (their structures), their associates (what they are able to bind to), and most importantly their work (their function in the cell and in association with the T-cell receptor).

Sarah Bertino
The Journey of a Pathogen from Infection to Destruction

The story will begin with an infection in the upper respiratory tract from one of the common cold viruses. The pathogen will journey into the body, detailing its invasion tactics. Once infection has occurred, the immune system begins to mount a defense, which will be described in detail using fun-interactive methods. The story will dramatically unfold as the body battles against the invader until it has been destroyed.

Jessica Dietz
Antibody and Antigen Model

The ability for antibodies to have specific recognition for only one antigenic epitope is a crucial aspect of the immune system. In this work, I will present a model antigen-antibody binding event, focusing specifically on the linkages between the two macromolecules. I created an artistic portrayal of the bonding between antibodies and antigenic epitopes, highlighting the flexibility of the antibody molecule itself. This is done both with the bonds between the heavy and light chains and within the hyper variable region of the antibody. Using materials (legos) that bind together with a good amount of strength and materials that allow for the adaptive nature of the binding event to be displayed, the antigen-antibody interaction is given an artistic twist.

Sydney Fasulo
Antigen Presenting Macrophage

Macrophages are produced by white blood cells called monocytes. Macrophages are antigen presenting cells that work to clear the body of infection through phagocytosis. Extracellular pathogens, such as bacteria are engulfed by the macrophage and trapped in a phagosome. The phagosome can fuse with a lysosome which causes the production of an extracellular marker, the major histocompatibility complex class II (MHC Class II), on the cell surface. MHC class II molecules are heterodimers that contain an alpha and beta chain. The size and openness of the two chains allows for larger and more diverse binding. MHC Class II molecules exclusively bind CD4 "helper" T cells which assist in the immune response. In this model, a long and slender *mycobacterium* is engulfed by the long pseudopodia of the macrophage. The *mycobacterium* is enclosed in a vesicle that can fuse with the phagocytic contents of the lysosome. The MHC class II marker is displayed on the cell surface of the macrophage. *Mycobacterium* are known to cause tuberculosis and leprosy and can be resistant to lysosomal degradation.

David Hamilton
Personal Encounters with Autogens

In our day-to-day lives, we walk around feeling relatively safe. Sure, we have fears of people who may want to do us harm, fears that the stock market will collapse, and maybe even have fears of germs and infectious diseases. However, we tend to ignore another, more translucent and omnipresent threat: The autogenic cloud of doom that surrounds us on a day-to-day basis. My collage of photographs taken on our own campus is meant to remove the shroud that covers our eyes, and reveal the extent of autogenic exposure we experience on a day to day basis. This Halloween, it won't be zombies and ghosts you fear, it will be your frying pans and tailpipes.

Victoria Jenkins
Circular Amplification Loop Flipbook

There is a circular amplification loop between macrophages and natural killer (NK) cells spurred on by the release of cytokines from these two cells. Activated macrophages produce interleukin-12 (IL-12), which activates NK cells and causes them to release IFN-γ. Macrophages are activated by IFN-γ, which then causes more IL-12 to be released, and more NK cells to be activated, etc. This loop is represented by a flipbook that starts and ends in the same place with activated macrophages. Through the flip book, the viewer will see the progression from activated macrophage, to IL-12 release, to activated NK cells, to IFN-γ release, and finally, back to activated macrophages.

Chris Lorenc
Lord of the Immune System: The Battle of Measles

In the lands of Mortica, L-Saruman and L-Angmar spread chaos and give rise to a dark force. These evil beings and their creatures are hoping to raise the Dark Lord Sauron of Subacute Sclerosing Panencephalitis. Brave souls step forward to make a stand against these invading monsters. However, will tissue macro-Boromir (26), IL-8-Sam (30), alternative pathway-Frodo (159), DICER-Aragon, complement-Gimli, CD8 cytotoxic-Legalos, and CD4 cytotoxic-Gandalf be enough to rid the land of evil? Or will it be the end of mankind?

Yuqi Mao, Jared Pienkos and Ben Van Arnam
The Cytotoxic Battle Within: What Really Goes on Under the Skin

It's a classic story: virus infiltrates an unsuspecting body, neutrophils converge on the scene, T-cells come to the cytotoxic rescue. See how the evil virus infects a host cell, replicates, and causes cell lysis—spewing new viri throughout the body. A neutrophil phagocytizes the foreign pathogen and presents the specific class II MHC to a CD8 T-lymphocyte. Stalking the body for its target, our hero T-cell locates, recognizes, and neutralizes the intruding virus. This film documents the cellular interactions of the cell-mediated immune response.

Divij Mathew
ROCKY VI Antigen!! Cell No!

This project focuses on the cell differentiation process that B lymphocytes cells undergo to form plasma cells. Before B lymphocyte cells encounter an antigen, they express IgM and IgD antibodies on their surface. However after undergoing cell differentiation, these newly formed plasma cells will have a high affinity for antigens and will be more effective at dealing with pathogens. Also these cells will express significant amounts of only one type of antibody. An important cell that helps in this differentiation process is the CD4 helper T-cell. In this presentation I introduce the science of this process via Rocky/Osmosis Jones movie concept. The setting of the movie is the Hamilton campus in both the real world and dream world. The opening will involve ingestion of food that has fallen on the ground by a "host" providing an access route for antigen into the host's body. Upon falling ill, the host rests, and through dreams the story of the host body fighting the infection is told in true Rocky style. The dream will then come to an end with the host feel significantly better.

Trevor Pedrick
Advanced Electron Micrographs of Various Pathogens

Pathogens come in many different shapes and sizes varying from large parasitic worms to microscopic viruses. Three classes of pathogens include viruses, bacteria, and parasites. All have varying pathogenic qualities that are recognized by the immune system. It is sometimes difficult to conceptualize a virus or bacterium because of their scale. Techniques such as electron microscopy allow for the imaging of microscopic pathogens giving insight to their structure and function. The slide show contains images of a T4 phage, *Shewanella putrefaciens* bacterium, and a sample parasite. These images were produced from both the transmission electron microscope and the scanning electron microscope courtesy of the Hamilton College Biology Department. The T4 phage, Shewanella putrefaciens, and parasite were courtesy of Ken Bart, Mick McCormick, Lindsey Wong ('09), and Ashleigh Smythe.

Benjamin Saccomano
Lego Pixelation of Dendritic Cells

Dendritic cells perform a multitude of functions for the immune system. Some of these functions will be illustrated using legos. The pixel-like nature of Legos will make it necessary to construct models at various levels of magnification. Hopefully, their simplified structure will make it easier to differentiate between the many processes. The bulk of the presentation will be performed in PowerPoint to allow animation of the legos, however, physical models will also be present.

Ryan Seewald
Small Pox Table

Small pox, prior to eradication due to vaccination, was and is one of the most devastating diseases known. The *variola* virus, a member of the poxvirus family, causes small pox, though another member of that family, the *vaccinia* virus, was and is used in modern vaccination. Structural similarities between these two allows makes vaccination with *vaccinia* against *variola* possible. Both contain a 42-kDa glycosylated membrane protein expressed on the extracellular enveloped form of the virus, which allows the virus to exit the cell without lysis. In this projected, I have constructed a table representing the *variola* virus. The surface of a table, constructed from all natural forest products, represents the *variola* virus. The legs represent this 42-kDa glycosylated membrane protein.

Julianne Tylko
Imma Immunoglobin's Life as an Upperclassman at Antibody University

This scrapbook is a collage of memories from Imma's years as an upperclassman at Antibody University. She describes the variety of different individuals, clubs and opportunities present at Antibody University (AU), from the way they look and dress to what they are interested in! Each upperclassman has their own specialized major that will prepare them to achieve their destiny. Imma describes her major, IgM, which is by far the most common major and some of the courses she has to take to complete that major, such as Polymers 101: Ancient History of J-Chain culture. She also describes dating culture at AU. The weekly parties in the student center often consist of upperclassmen girls, who have been set off by a variety of substances, trying to approach the new and exciting freshmen guys. Unfortunately, only a few upperclassmen girls are lucky enough to find the appropriate fit, and some of those that start a relationship are not able to finish school. Imma, because she's very smart, delays the dating scene until she's matured and graduated, and then gets a job in a dating agency in which students can find their "freshmen match!"

Chemical Immunology and Immunopharmacology Presents…

Antigens and Art

Tuesday, November 03, 2009

And

Thursday, November 05, 2009

9:00am to 10:15am

Science Center 3040

Tuesday, November 23 2009

T Cell Receptor – A Users Manual
Matthew Baxter

The T Cell Receptor, or TCR, plays a vital role in antigen recognition. The complexity of the TCR can be challenging for new users, which is why Thymus Industries is here to help through their innovative step-by-step manual. Included are methods for the creation and uses of the TCR, as well as troubleshooting tips. Now with handy quick-reference molecule chart!

A Model Macrophage and the Process of Phagocytosis
Alex Isaacs

Macrophages perform phagocytosis to help rid organisms of microbes. The process of phagocytosis starts with the macrophage recognizing a microbe because of high-affinity receptors expressed on the macrophage. These receptors include pattern recognition receptors and opsonins, which bind to antibodies, proteins and lectins. After the microbe is attached to the macrophage, the pathogen or damaged host cell is engulfed using pseudopodia. Pseudopodia act as arms to begin ingestion of the microbe. Once the microbe is consumed, it is transported in a vacuole called a phagosome. Lysosomes bind to the phagosome and release digestive enzymes like lysozyme and proteases, which result in a phagolysosome. The digestive enzymes chew up the microbe. Next, class II MHC binds to the microbial pieces. Finally, degraded material bound to class II MHC are excytosed out of the cellular membrane and are ready for presentation. This physical model depicts this process of phagocytosis.

Djembe Verse on Lymphocytes
Tom Morrell

This work is a display of my affection for lymphocytes in carefully crafted verse. It is separated into five sections: an introduction, the B lymphocytes, Cytotoxic T lymphocytes, Helper T lymphocytes, and a conclusion. The text reflects the crucial role these cells play in the immune system, and expounds upon their development and function. Each section is accompanied by the percussive beat of a djembe, an ancient West African drum. The entire piece has a rhythmic theme, with each section building on the last to aurally illustrate the interconnections of these cells.

Bridging the Gap: The MHC class I Protein Loading Complex
Nate Schneck

The assembly of class I MHC inside the endoplasmic reticulum is a key component in antigen processing and presentation. Proper protein loading on the class I MHC molecule requires coordinated action from multiple endoplasmic reticulum resident proteins. Tapasin, vlareticulin and ERp57 create a vital link in the assembly complex for class I MHC with the transporter associated with antigen processing (TAP). The simultaneous interaction of these structures stabilizes the peptide-loading complex in manner that facilities peptide binding to the class I molecule. In this project, I have constructed a novel design for a bridge that is modeled after the assembly complex. The bridge binding system spans the TAP Complex and the class I MHC connected binding sites modeled after the actual interactions. The bridge is connected to the TAP complex by a subterranean noncovalent binding complex to the tapasin. The bridge connects to the class I MHC through two unique binding sites with clareticulin and tapasin. The support system for the bridge relies heavily on the unique tapasin and clareticulin interactions with ERp57. The mixed disulfide bond between the ERp57 and tapasin and the b' domain of ERp57 linkage with the clareticulin p-loop produce critical structural supports for the bridge. The bridge design is represented through a small scale model and supplemental blueprint diagrams.

Untitled Childrens Book
Taylor Adams

Open the colorful pages of this unique children's story and step into the world of the immune system! Join a young boy, Nick, who contracts the tuberculosis bacteria when he licks his fingers after having prayed on a rosary sneezed on by his friend, Rick. Then, follow the incredible processes of the immune response as Nick's body finds and destroys the tuberculosis pathogen within his body and leaves him healthy once more. Written in limerick, each page tells the story of one specific component of the immune response. On a background designed with colored pencils, each cell, organ, chemical signal, and receptor is created from construction paper cutouts, each with a unique color and shape. This "cast of characters" is pictorially illustrated in the front of the book for the reader's clarification.

35mm Black and White Photographs of Superfund Sites
Ari Kaphan

Superfund sites are areas designated by the federal government as lands that have been contaminated by toxic or hazardous waste and need to be cleaned because of the hazards to either people or the environment, or both. Some superfund sites have already been cleaned and decontaminated by the government and the Environmental Protection Agency (EPA), while others are still waiting to be cleaned because of a lack of funding. This is a series of images of Superfund sites in our surrounding area, many of which are also on the NPL, or National Priorities List, which lists the most serious uncontrolled or abandoned hazardous waste sites in the country. Below is a website which gives a map of the nearby Superfund sites.

Untitled Childrens Book
Sam Coalillo

The children's book "Yet to be titled" tells the story of an older brother taking his little brother to the doctor for his tetanus shot. When the little brother asks the big brother, a student in Nicole Snyder's phenomenal immunology course, what a vaccine is, the older brother is challenged with explaining the complexity of the underlying humoral immune response to a seven year-old. Through a series of analogies, and a few adventures, the brother explains the point of injections (haha) to his band-aid wearing little brother.

Beauty is Pain…Literally
Valerie Valant

Welcome to ACCEPT Cosmetics!! ACCEPT is a leading brand within the big beautiful world of chemical cosmetology. Our newest project is a line of products that look great and are unbelievably inexpensive!! How did we accomplish the impossible? By using a few "special ingredients" known as paraben and phthalate, we were able to produce a wide range of products, ranging from eye shadows, concealers, blush, eye liner – you name it, we have it! Our "special ingredients" may have a few negative side effects (please refer to the fine print), but like the saying goes: Beauty is Pain, right Ladies? And boys, don't worry we didn't forget about you either. We have a whole line of "male products" specially formulated to help rid you of those tired eyes and graying eyebrows. ACCEPT beauty is an organization that makes products for the consumer – cheap, beautiful cosmetics that make you look (but not always feel) GREAT!

Charlie Brown's Encounter with Ovarian Epithelial Cancer
Sarah Cryer

The ideal world of Charlie Brown is disrupted when the high-risk Human Papillomavirus 16 (HPV), a double-stranded DNA virus, sneaks into Woodstock's nest undetected. His protective friends, Charlie Brown and Snoopy, remain snoozing throughout the attack. The HPV successfully maintains a low profile by remaining in the external periphery of Charlie Brown land, keeping the Snoopy Jr. (a.k.a. the dendritic cell) asleep, and leaving no trail of secreted proteins. Once inside the nest, the HPV easily overpowers Woodstock. After binding and gagging him, the HPV integrates into the mind of Woodstock, the host genome, through a process of hypnosis. Finally in total control of his target, HPV makes a few necessary the host genome and then begins to replicate Woodstock. His goal is to create an army of modified Woodstock clones with which he can take over the ideal world of Charlie Brown. These modified clones are equipped with the oncofetal antigen CEA, the mucin CA-125, and another mucin CA-19-9. Will Charlie Brown and Snoopy wake up before its too late? Or will the army of Woodstock clones take over Charlie land?

Untitled
Tom Nevers

A tremendous number of selection factors are involved in producing functional, non-autoreactive T and B cells. The paucity of easy recognized morphological landmarks such as exist in the study of other body systems, makes the study of the immune system specially challenging. The delicate chemical ballet of T and B cell maturation can be difficult to fully appreciate because of the great variety of chemical factors and cells that make up the complicated process. Using a cohesive narrative of a children's story, the selection mechanisms that underlie T and B cell production are outlined. Of course only a general treatment can be given, but the analogies that come surprisingly organically in the work offer a unique, humanized overview of the processes.

Untitled
David Brown

Immunology is constantly changing. As new discoveries are made our understanding of the prevention of disease continues to increase. This book will recount major advances in the field of immunology and begin to describe where the field may be heading. Immunology has advanced from the description of the first "little animals", to the creation of a novel H1N1 vaccine in less than five months. It is important to look at the achievements of people who came before us so that we can build on their achievements and advance our understanding further.

Varicella-Zoster Protease
Lauren Farver

After observing the extremely uncomfortable and debilitating side-effects of Shingles from a secondary point of view, I became sympathetically interested in the details of the disease. The Varicella-Zoster Virus, more commonly known as Shingles, can appear at multiple stages of one's life. Seen as chicken pox in young children, the virus has the ability to lay dormant in a victim's body for years and then reappear in the middle to late years of life in the form of Shingles. The virus is also commonly known as Herpes. Similar to chicken pox, Shingles produces red scab-like formations on the skin, but whereas the childhood disease produces an incessant itching sensation, the adult form of the virus creates overwhelming pain. Oftentimes triggered by stress or a compromised immune system, the virus produces symptoms that may last for weeks to months. The Varicella-Zoster Protease is an important component in the replication of the virus and its crystal structure has been identified. I used the structure derived by X. Qiu, et. al in the paper *Crystal structure of varicella-zoster virus protease* to create a model of the protease. Reflecting the numerous appearances of the virus throughout life, the paper mache structure is composed of and is decorated with materials used during childhood and adulthood.

Thursday, November 05, 2009

I'm Not Picky… I Just Know What I Want
Sam Cho

The activation of naïve CD4+ T cells is elicited by the presentation of antigenic peptides associated with class II MHC molecules by APCs such as dendritic cells. Of the many class II complexes presented to naïve CD4+ T cells, only a very small number of the complexes are able to initiate a specific T cell response. This project will present the unique specificity that T cells have in their ability to recognize and differentiate between antigenic and self peptides through music and film.

Immunology as Fashion
Andrew Boddorff, Kelley Fitzsimmons, Sara Miller and Laura Radlinski

Antigen receptors play an integral role in the immune response. We will focus on the three major types of receptors – MHC, T-cell receptors, and immunoglobulins – showing their form and functionality through fashionable interpretations of the molecules. Artistic renderings are transformed into wearable pieces of art to be modeled on the runway. They are not only stylish but functional, helping identify antigens presented to our bodies on a daily basis. These subtle differences in functionalities will be illustrated in the outfits we create.

Barry - B: A Musical Bildungsroman
Andrew Branting, Kevin Graepel and Keith Willner

We welcome all to join us in this epic account of the coming of age of one young immature B-cell named Bradley. All are informed of the intricate process of B cell development as Bradley himself tells his tale of maturation during the horrific war against the Clap in the body of Sassy-Pants. When we first come across Bradley he is a fully developed Memory B cell giving his account to an immature B cell Bobby, who reminds Bradley of his younger self. Bradley informs Bobby of the events and cells he will encounter during his own development. He explains such events as opsonization, antigen presentation, and T cell mediated B cell activation in the adaptive immune response to the confused cellular warrior. The audience is left deeply touched by Bradley's account and his compassion towards Bobby, but that is not to say that this story doesn't have its fair share of laughs. Bradley's story is one that every developing cellular being should experience.

C.O.P.S. (Cellular Operatives Preventing Sickness)
Matthew Breen, Peter Garrett and Raul Patrascu

There's a new breed of foreign pathogen in town, and it's up to an elite immunological team to make sure it doesn't get its way! Follow the E-selectin trail and witness the ethically-questionable exploits of T cell and B cell as they struggle to keep order in the face of a malicious viral attack, autoimmunity and immunosuppressant drugs in this gritty new documentary the Journal of Allergy and Clinical Immunology calls "Breathtaking… literally; I think that foreign pathogen spent most of its time in the upper respiratory system." See the inner workings of the system that keeps us all safe at night from illness, and find out how even simple, low affinity individuals can unite to form a menacing, high avidity taskforce with a tendency to harass, poison and devour unwelcome visitors. Whatcha gonna do? Whatcha gonna do when they opsonize you?

Puppet Show
Annie Lee, Shyama Nair and Jack Trieu

Septic shock is the number one cause of death in Intensive Care Units in the US. Mortality rates vary from 40% for uncomplicated sepsis to 80% in those suffering from septic shock and multi-organ dysfunction. In sepsis, mononuclear phagocytes are activated in response to a component of gram-negative bacterial cell wall, known as lipopolysaccharide (LPS). The LPS binds to the CD14 receptor of circulating monocytes and macrophages. In response, these activate mononuclear phagocytes produce TNF-α and IL-1b, which stimulates the production of IL-6, IL-8, and IL-10. High concentrations of IL-6 have been correlated with the severity of the disease, which increases the production of inflammatory mediators. At the same time, the productions of anti-inflammatory mediators are delayed. The delay in anti-inflammatory mediators and increased production of cytokines are related to the high mortality rate. Through a puppet show, we will illustrate the mechanism of septic shock and how it ultimately can lead to multi-system organ failure.

The Rise and Fall of Evil: The Story of Billy Staph, the First Penicillin-resistant *Staphylococcus Aureus*
Phillip Milner

This comic reveals the untold history the development of penicillin resistance in *S. Aureus* during the late 1940s. It opens with the legendary *War of the Ring Finger* in which Lord Saureus, the leader of the 10,000 strong Golden Army of *S. Aureus* bacteria, invade the body of a an unsuspecting human and declare war on his immune system. An epic battle ensues that the pathogens seem likely to win until Penicillin arrives and devastates the evil army. Billy Staph, a young bacterium unwilling to wage war, joins the battle when his father is killed by penicillin, and is found to secrete an enzyme that destroys Penicillin. He raises a clone army and seeks to take over the entirety of his host's body. Only when Methicillin arrives is the Dark Lord's reign finally overthrown and homeostasis returned to the cells of our hero.

Antigens and Allies
Graham Hone and James Langan

Antigens and Allies! The board game that puts your immunological knowledge to the test. You can play as either the Antigen, plotting against the immune system and ultimately, attempting to take over the whole body, or as the Allies, the collective group of macrophages, dendritic cells, T-cells, and B-cells, to defend the attempted takeover of the antigen. Use your currency of IFN-γ or mitosis points to upgrade your armies and fight the good fight. Good luck. And may the best cell win…

The Three Little Cells
Benjamin Weissman

The story is an allegory to the timeless fairytale “The Three Little Pigs”, in which the big bad wolf is represented by a ferocious *Corynebacterium diphtheria* (diphtheria) infection, and the three little pigs are played cells of the pharynx protected by different types of immunity. Instead of huffing, puffing, and blowing down the houses of the three little cells, the diphtheria infection releases a potent exotoxin that blocks adenosine diphosphate (ADP) ribosylation inhibiting protein synthesis resulting in cell death. The first cell is protected only by the innate immune system, which promotes phagocytosis of the bacteria through the alternate pathway of the complement system, but falls prey to the vicious infection. The second cell is protected by the innate immune system and B-cells, but without the help of CD4 T-cells to activate them, the B-cells do little to stem the tide of the infection, so it too falls to diphtheria. The third cell is protected by the full immunes system. With the help of CD4 T-cells to activate the B lymphocytes, its immune system clears the infection and saves the cell.

Antigen Pantigen
Laura Leonard and Rem Myers

In the past, great duos have resounded the halls of arts and sciences: Gilbert and Sullivan, Watson and Crick, Rogers and Hamerstein. Now, a new great duo joins the elite: Leonard and Myers. In a fated meeting, Laura Leonard and Rem Myers joined together to create a musical, none like the world had ever seen: Antigen Pantigen. Our tale follows the journey of a newly mature T-cell who finds himself locked in forbidden love with an outsider: HINI influenza. This show features a myriad of characters including the valiant Natural Killer Cell, the acrobatic Avian Flu, and the ever lovable team of Antibodies. Come experience this once in a lifetime event on Tuesday November 3rd and laugh, cry, and enhance your immunological knowledge with: Antigen Pantigen.

Scholarship—Journal Articles

JOC Article

Stereoselectivity in the Epoxidation of Carbohydrate-Based Oxepines

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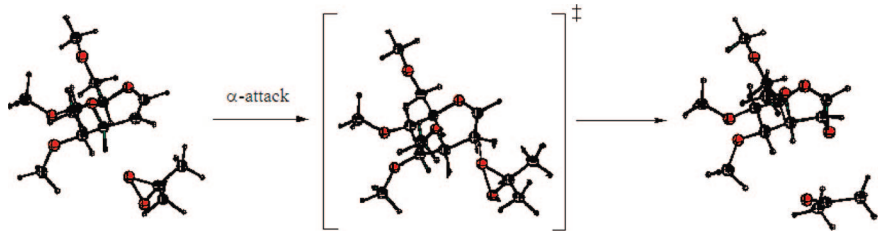

The facial selectivity in the DMDO epoxidation of carbohydrate-based oxepines derived from glucose, galactose, and mannose has been determined by product analysis and density functional theory (DFT, B3LYP/6-31+G**//B3LYP/6-31G*) calculations. Oxepines **3** and **4**, derived from D-galactose and D-mannose, largely favor α- over β-epoxidation. The results reported here, along with selectivities in the DMDO-mediated epoxidation of D-xylose-based oxepine **1** and D-glucose-based oxepines **2** and **5** reported earlier, support a model in which electronic effects, guided by the stereochemistry of the oxygens on the oxepine ring, largely determine the stereoselectivity of epoxidation. Other contributing factors included conformational issues in the oxepine's transition state relative to the reactant, the asynchronicity in bond formation of the epoxide, and the overall steric bulk on the α- and β-faces of the oxepine. Considered together, these factors should generally predict facial selectivity in the DMDO-epoxidation of cyclic enol ethers.

1. Introduction

The formation of glycosidic linkages with use of 1,2-anhydrosugars as donors has enjoyed wide application in the syntheses of oligosaccharides and natural products.[1] Access to these donors from cyclic enol ether (glycals, for example) via epoxidation has been instrumental to their utilization. Because the cyclic enol ethers are often chiral, their reactions with achiral epoxidizing reagents can follow distinct stereochemical pathways which need not be equivalent. In fact, based on the stereochemistry of the cyclic enol ether, high selectivity in the formation of one anhydrosugar in preference to another is common.[2–5]

Our approach to the formation of glycosidic bonds in the septanose series has similarly relied on 1,2-anhydroseptanoses as a donor both directly[4,6,7] or indirectly through the formation of a thiophenyl septanoside.[8] Carbohydrate-based oxepines[9–11] have proven to be useful starting materials for preparing 1,2-anhydroseptanoses, with the oxepines acting as ring-expanded glycals. Selectivity observed in the epoxidation of carbohydrate-

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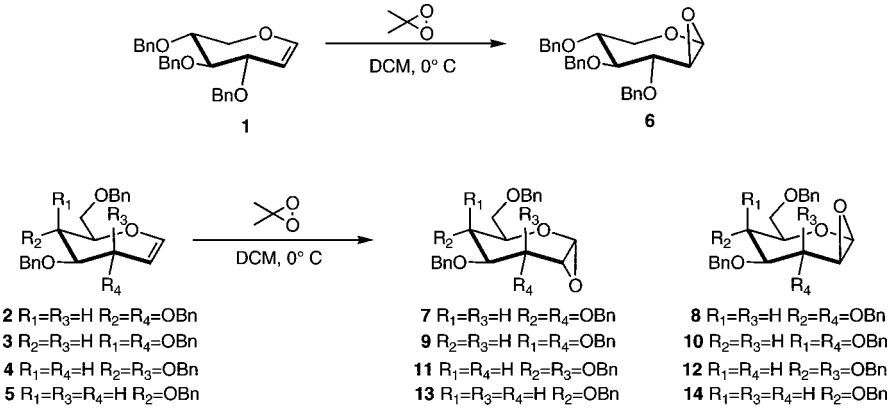
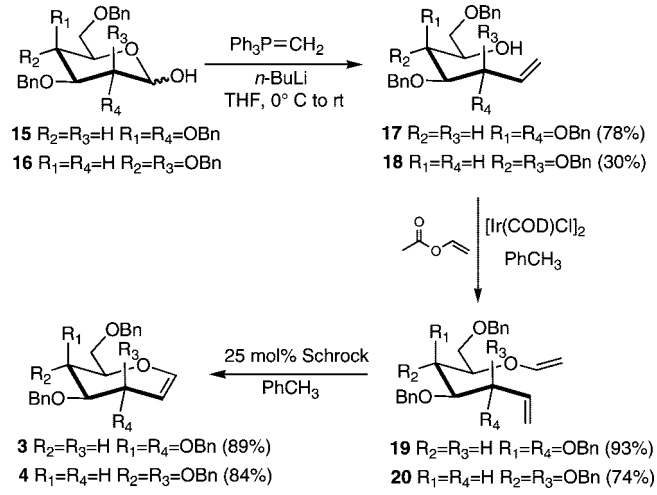
SCHEME 1



based oxepines has been variable and was related to the specific starting material. For example, oxepine **1** was shown to selectively form the β-1,2-anhydroseptanose **6** when using dimethyldioxirane (DMDO) as the oxidant (Scheme 1). Selectivity in this system was determined by ^{1}H and ^{13}C NMR spectroscopy and by analysis of the methyl septanoside product that resulted from methanolysis of the 1,2-anhydroseptanose.[4] DMDO epoxidation of oxepine **2**, on the other hand, proved to be relatively unselective (**7**:**8** = 3:1, Scheme 1). In this case, the ratio of epoxides formed was quantified by characterizing the products of methanolysis.[6,7] Combined, these experiences prompted us to ask the question: What factors determine the selectivity in the epoxidation of carbohydrate-based oxepines? Herein, we analyze the outcomes of the DMDO oxidation of three related oxepines (**2**−**4**) by product analysis and density functional theory (DFT) calculations on the transition states in these reactions. Oxepines **2**, **3**, and **4** are derived from glucose, galactose, and mannose, respectively. The variation of stereochemistry about the ring allowed for an evaluation of the parameters that control the stereochemistry of epoxidation.

Recent reports on the epoxidation stereoselectivity of cyclic enol ethers from the Wei[12] and Rainier[13] laboratories influenced our perspective. Wei described a polarized-π frontier molecular orbital (PPFMO) model that explained selectivity in DMDO epoxidation of a series of 4-deoxypentenosides.[12] In their system, the reactant geometry was similar to that of the transition state, and C−O bond formation was asynchronous in the transition state. Repulsive electronic interactions between the heteroatoms of the ring substituents and the π-bond of the cyclic enol ether polarized the π-bond so that the nucleophilic π-bond had greater electron density on the face with fewer electronegative substituents. This gave rise to the idea of the facial selectivity being due to a "majority rule". The Rainier model similarly emphasized transition state geometry and asynchronous bond formation during the epoxidation of six- and seven-membered-ring cyclic enol ethers. Avoiding unfavorable interactions between DMDO and the allylic C−H bond in the transition state formed the basis for epoxidation selectivity in the systems they evaluated.[14] We were interested in evaluating these models in terms of the DMDO epoxidations of carbohydrate-based oxepines **1**−**5**.

SCHEME 2

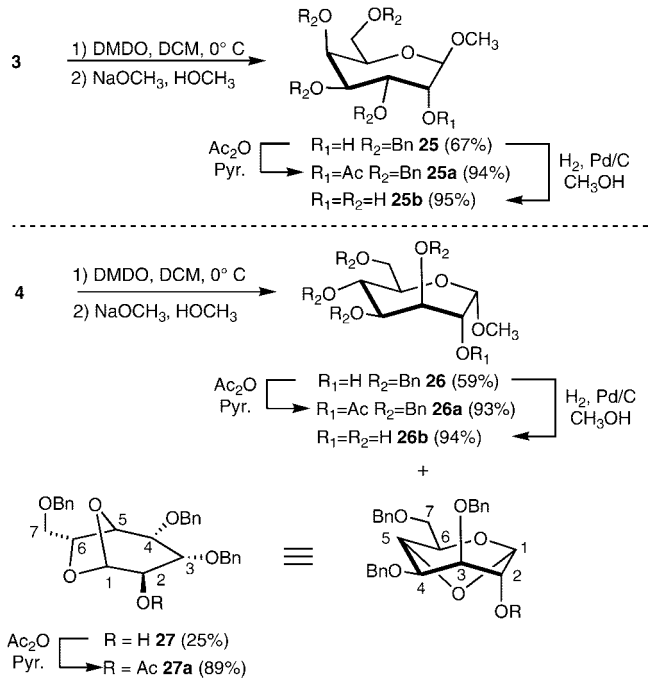


2. Results and Discussion

2.1. DMDO Epoxidations of Carbohydrate-Based Oxepines. Oxepines **1**−**5** served as the starting materials for the DMDO epoxidation reactions. We have previously reported on the details for the synthesis of **1**, **2**, and **5** by a ring-closing methathesis (RCM)-based strategy.[4,6,11] Two new oxepines, **3** and **4**, were prepared by the same route but with a minor modification. Scheme 2 shows the synthesis of **3** and **4** from tetra-*O*-benzyl-D-*galacto*-pyranose **15** and tetra-*O*-benzyl-D-*manno*-pyranose **16**. Wittig methylenation[15] of the lactols gave δ-hydroxy alkenes **17** and **18** in 78% and 30% yields, respectively.[16] The change in synthetic sequence relative to our previous route came in the formation of vinyl ethers **19** and **20**. Vinyl etherification of the hydroxyl group on **17** was carried out with $Pd(OAc)_2$ and ethyl vinyl ether[17] to give diene **19** with

(8) Castro, S.; Fyvie, W. S.; Peczuh, M. W. *Org. Lett.* **2005**, *7*, 4709.
(9) Castro, S.; Peczuh, M. W. *J. Org. Chem.* **2005**, *70*, 3312.
(10) Alcázar, E.; Pletcher, J. M.; McDonald, F. E. *Org. Lett.* **2004**, *6*, 3877.
(11) Peczuh, M. W.; Snyder, N. L. *Tetrahedron Lett.* **2003**, *44*, 4057.
(12) Cheng, G.; Boulineau, F. P.; Liew, S.-T.; Shi, Q.; Wenthold, P. G.; Wei, A. *Org. Lett.* **2006**, *8*, 4545.
(13) Orendt, A. M.; Roberts, S. W.; Rainier, J. D. *J. Org. Chem.* **2006**, *71*, 5565.
(14) Ando, K.; Green, N. S.; Li, Y.; Houk, K. N. *J. Am. Chem. Soc.* **1999**, *121*, 5334.
(15) Martin, O. R.; Saavedra, O. M.; Xie, F.; Liu, L.; Picasso, S.; Vogel, P.; Kizu, H.; Asano, N. *Bioorg. Med. Chem.* **2001**, *9*, 1269.
(16) Attempts to optimize the olefination of tetra-*O*-benzyl-D-*manno*-pyranose were unsuccessful. Elimination of benzyloxy substituents may be a competing reaction. See, for example: Callam, C. S.; Lowary, T. L. *J. Org. Chem.* **2001**, *66*, 8961.
(17) Handerson, S.; Schlaf, M. *Org. Lett.* **2002**, *4*, 407.

SCHEME 3



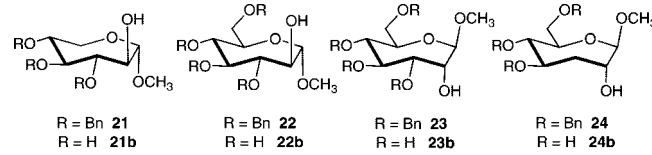
very poor conversion even after refluxing the reaction mixture for several days. To improve the yield in the vinyl etherification reaction, an alternative method with an iridium catalyst as reported by Okimoto et al.[18] was used. Thus, treatment of δ-hydroxy alkene **17** with 10 mol % of $[Ir(COD)Cl]_2$ and vinyl acetate gave diene **19** in 93% yield. The mannose-derived diene **20** was prepared in 74% yield from **18** with use of the same reaction conditions. RCM, using 25 mol % of Schrock catalyst,[19] with **19** and **20** gave oxepines **3** and **4** in 89% and 84% yield, respectively.

As had previously been done for oxepines **1**, **2**, and **5**, the products from basic methanolysis of anhydroseptanoses derived from **3**, **4**, and **5** were analyzed to determine the selectivity of the DMDO epoxidation reaction. For example, methyl α-D-*ido*-septanoside **21** was the product of the epoxidation/methanolysis sequence for **1**, indicating that epoxidation occurred on the β-face of the oxepine. Under the same reaction conditions, oxepine **2** gave rise to methyl α-D-*glycero*-D-*ido*-septanoside **22** and methyl β-D-*glycero*-D-*gulo*-septanoside **23** (1:3 α:β) indicating only a slight preference for α-epoxide formation. C3 deoxygenated oxepine **5** provided β-septanoside **24**[20] through α-1,2-anhydroseptanose **13**, even in the absence of an allylic substituent. Subjecting galactose-derived oxepine **3** to the standard reaction conditions provided only the methyl β-septanoside product **25** in 67% overall yield (Scheme 3). Oxepine **4** showed more complex reactivity, giving methyl α-septanoside **26** and the cyclized product **27** in a combined yield of 84%. On the basis of these product structures, epoxidation of **3** and **4** occurred with high selectivity for the α-face (>25:1) of these oxepines. A complete rationale for the product structures in Scheme 3 is provided below. Results for all of the experimental epoxidation/methanolysis reactions are given in Table 1.

TABLE 1. Experimental Epoxidation Selectivities and Yields for 1−5

oxepine	epoxides (α:β)	products (α:β)	combined yield (%)	C1 ^{13}C δ (ppm)
1	**6** (1:25)	**21**	89[a]	106.2 (**21b**)
2	**7**:**8** (3:1)	**22**:**23** (1:3)	63[b]	106.2 (**22b**); 110.0 (**23b**)
3	**9**:**10** (25:1)	**25**	67	110.1 (**25b**)
4	**11**:**12** (>25:1)	**26**:**27** (7:3)	84	100.7 (**26b**)
5	**13**:**14** (ND)	**24**	61[c]	112.2 (**24b**)

[a] These data are taken from ref 4. [b] These data are taken from ref 6. [c] These data are taken from ref 20.



2.2. Stereochemical Characterization of the Reaction Products. Assignment of accurate structures to the products previously obtained in the epoxidation/methanolysis sequence was critical to understanding the stereoselectivity of the epoxidation reaction. The C2 group consistently reports on this selectivity and is insensitive to any subsequent epimerization/anomerization. The stereochemistry of **21** was assigned by comparison to a known compound from Stevens.[21] Assignment of structures **22** and **23** was concurrent with a conformational analysis of the completely deprotected methyl septanosides corresponding to them.[6] The conformational analysis was in part used to define the stereochemistry in these two structures.

Evidence supporting our structural assignment of the new products has come from X-ray crystallography on methyl 3,4:5,7-di-*O*-isopropylidene β-D-*glycero*-D-*galacto*-septanoside **29** (Figure 1). This molecule was prepared from the di-*O*-isopropylidene protected oxepine **28** via the DMDO/methanolysis sequence in 84% yield (Scheme 4). Deprotection of the acetonide groups in **29** provided methyl β-D-*glycero*-D-*galacto*-septanoside **29b** in 93% yield. Hydrogenolysis of **25** and **26** provided methyl β-D-*glycero*-L-*manno*-septanoside **25b** and methyl α-D-*glycero*-D-*galacto*-septanoside **26b** in 95% and 94% yield (Scheme 3). In addition to the protected septanosides **25**, **26**, and **29**, these deprotected methyl septanosides were utilized in the overall assignment of stereochemistry for the new molecules reported here.

In general, we have observed a partitioning of C1 ^{13}C chemical shifts in methyl septanosides based on the stereochemistry at the anomeric position, especially for completely deprotected methyl septanosides (Table 1).[7,22,23] The C1 ^{13}C chemical shifts for methyl α-septanosides range from 100 to 106 ppm whereas ^{13}C chemical shifts for methyl β-septanosides range from 110 to 112 ppm. On the basis of this trend, **25b** and **29b** fit into the range for β-septanosides with ^{13}C δ for C1 of 110.1 and 110.8 ppm, respectively. The NMR analysis was in accordance with the X-ray structure in the case of **29**/**29b**. Septanoside **26b**, on the other hand, gave a C1 δ of 100.7 ppm, indicating that its anomeric stereochemistry was α. With confidence in the C1 stereochemical assignment, we used an analysis of the ^3J coupling constants to assign the remaining C2 stereochemistry in the product structures.

(18) Okimoto, Y.; Sakaguchi, S.; Ishii, Y. *J. Am. Chem. Soc.* **2002**, *124*, 1590.
(19) Schrock, R. R. *Tetrahedron* **1999**, *55*, 8141.
(20) Castro, S.; Duff, M.; Snyder, N.; Morton, M.; Kumar, C. V.; Peczuh, M. W. *Org. Biomol. Chem* **2005**, *3*, 3869.
(21) Tran, T. Q.; Stevens, J. D. *Aust. J. Chem.* **2002**, *55*, 171.
(22) We have also reported the crystal structure of an α-septanoside. See ref 9.
(23) See the Supporting Information for a complete table of C1 chemical shifts.

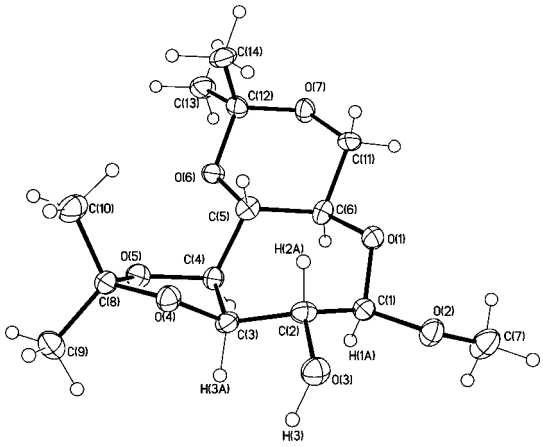
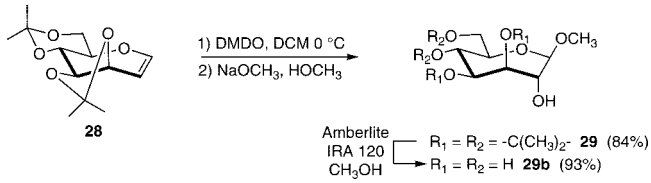


FIGURE 1. ORTEP representation of **29** from X-ray data.

SCHEME 4

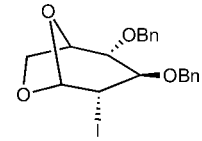


In addition to the X-ray and ^{13}C data, analysis of COSY/NOESY spectra of **25b**, **26b**, and **29b**, along with their AMBER-minimized structures, further supports the stereochemical assignment.[24] Diagnostic NOESY cross-peaks in the spectrum of **25b** are the H1−H4 and H1−H6 cross-peaks. These two transannular cross-peaks are estimated to be 2.4 and 2.1 Å in the calculated structure. For **26b**, the COSY spectrum shows only weak coupling between H1−H2 ($^3J_{H1,H2} = 3$ Hz from ^1H NMR). The NOESY spectrum, however, clearly shows an H1−H2 cross-peak. The calculated structure for **26b** puts the H1−H2 protons approximately 2.3 Å apart and on the same side/face of the ring. The magnitude of the H1−H2 cross-peak is similar to that of the H1-aglycon methyl (−OCH_3) NOE, which is also calculated to be in close proximity (2.3 Å). Other important NOEs observed for **26b** are H2 to H3 and H4 to H6. Calculated distances between these protons were 2.3 and 2.6 Å. Combined, these data strongly suggest the structure of **26b** to the 1,2-*cis* structure as shown. ^1H NMR signals for **29b** were less disperse than those for **25b**, but assignments could still be made. The NOESY spectrum of **29b** showed cross-peaks between H1 and the aglycon methyl (−OCH_3), H1 and H6, and H4 and H6. Interproton distances from the AMBER-minimized structure for **29b** again agree with these observations (2.4−2.6 Å). Overall, the collected crystallographic and spectroscopic data render a consistent picture for the structures of methyl septanosides **25**, **26**, and **29**.

We arrived at the structure for **27** by consideration of the reaction mechanism in combination with NMR experiments. Our previous observation of the intramolecular cyclization of an activated oxepine via its C5 benzyloxy group gave us additional perspective.[25] By considering the potential reaction mechanisms (Scheme 5), there could be five possible reaction paths for anhydroseptanose **11**, three that invoke the anhydrosugar directly and two that proceed via cyclic oxonium ion **33**. The paths are the following: (a) attack of the C3 OBn oxygen to form a C2−C3 epoxide **30**; (b) attack of the C4 OBn oxygen atom to form the bicyclic product **31**; (c) attack of the C7 OBn oxygen atom to form the bicyclic product **32**; (d) attack by methoxide/methanol on the α-face of oxonium ion **33** to form methyl α-septanoside **26**; and (e) attack of the C5 OBn oxygen to the α-face of **33** to form **27**.

Product analysis showed pathway d to be the preferred course of the reaction, giving **26** in 55% yield. Both the ^1H and ^{13}C NMR spectra of the other product, **27** (25%), clearly showed that it was not a methyl septanoside. Notable differences were the lack of an indicative methyl glycoside singlet and also the absence of signals for one of the benzyl groups present in **4** and **11**. Further, the ^1H NMR and ^{13}C NMR did not show signals in the epoxide region, ruling out **30** as the product. HMBC NMR of **27a** showed correlation of H1 to carbons C3, C5, and C6 (all are three-bond couplings), and similarly, C1 correlated to H5 and H3. Also, C4 and C7 both show correlations with benzyl protons in the HMBC while C5 does not. This suggests that the benzyloxy substituent at C5 was the nucleophile, and not the corresponding groups at either C4 or C7. In total, correlations are consistent with **27**, but inconsistent with **31** or **32**.[26]

Structure **27** arises from attack of the C5 OBn oxygen onto the C1 of oxonium ion **33**. We have reported on similar reactivity of a C5 OBn oxygen in the NIS-mediated intramolecular cyclization of oxepine **1**. The bicyclic structure formed in that reaction (**34**) is reminiscent of **27**.[25] The nucleophilicity of the C5 OBn group likely relies on the electronic effects of the group, the stability of the corresponding benzyl/tropylium cation released in the reaction, and the proximity of the reacting groups. Both of the observed products in the epoxidation/methanolysis of **4** (**26** and **27**) arise from a presumed cyclic oxonium ion intermediate **33**. This is in contrast to the other products of methoxide attack on 1,2-anhydroseptanoses, where the products have arisen from S_N2 ring-opening of the epoxide. Although invoking oxonium ion **33** is speculative considering the reaction conditions of the methanolysis ($NaOCH_3/HOCH_3$), it is consistent with both products that have been characterized.[27]



34

2.3. Transition State Calculations for DMDO Epoxidations. The product analyses above established the selectivity in epoxide formation for oxepines **1**−**5** and **28**. We were interested in developing a model for predicting the stereoselectivity of epoxidation in these systems and turned to computational chemistry for insight.

In this computational study, the selectivity of the DMDO-mediated epoxidations of the α- and β-faces of the oxepines was investigated at the B3LYP/6-31+G**//B3LYP/6-31G* level of theory.[28–31] Solvation effects were investigated by using the

(24) Spectra (COSY/NOESY), tabulated interproton distances, and the AMBER minimized structures for **25b**, **26b**, and **29b** are in the Supporting Information.

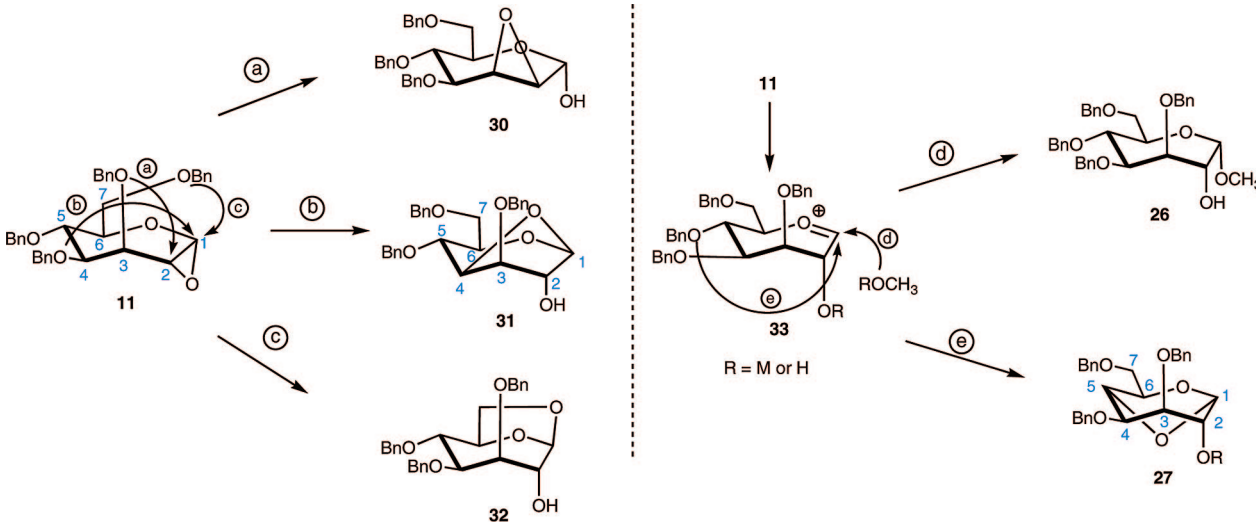
(25) Fyvie, W. S.; Morton, M.; Peczuh, M. W. *Carbohydr. Res.* **2004**, *339*, 2363.

(26) Full NMR spectral characterization (^1H, ^{13}C, COSY, HMQC, HMBC) for **27a** can be found in the Supporting Information.

(27) An investigation aimed at understanding the facility in forming oxonium **33** from **4** is currently underway.

(28) Parr, R. G.; Yang, W. *Density Functional Theory in Atoms and Molecules*; Oxford University Press: New York, 1989.

SCHEME 5



polarizable continuum model (PCM),[32–35] using single-point energy (B3LYP/6-31+G**) calculations with the gas-phase (B3LYP/6-31G*) optimized geometries. (*Note: For the subsequent discussion, we will refer to the B3LYP/6-31+G**//B3LYP/6-31G* level simply as B3LYP, unless otherwise noted.*) Despite changes in the relative ordering of different conformers or transition states, the general trends between the free energies and enthalpies are relatively consistent—all of the energies are provided in the Supporting Information. In the subsequent discussion, the free energies will be presented.

To simplify the computational study, the model systems (**2a**, **3a**, and **4a**—Table 2) with four methoxy substituents were evaluated instead of the experimental systems (**2**, **3**, and **4**) that had benzyloxy substituents at the C3, C4, C5, and C7 positions. By replacing a phenyl group with a hydrogen atom, we anticipate that the **2a**, **3a**, and **4a** models would underestimate the steric effect between the substituents and DMDO; however, this replacement was significantly more computationally tractable for locating and characterizing transition states.

We began the investigation of the facial selectivity by first considering the conformational flexibility of the three model compounds **2a**−**4a**. Using the OPLS-AA force field,[36] we performed a conformational search using a Monte−Carlo protocol available in MacroModel,[37] and for the different compounds, many conformations within ∼3.5 kcal/mol of the global minimum were obtained: 20 conformers for **2a**, 25 conformers for **3a**, and 43 conformers for **4a**. These unique conformations were then fully optimized at the B3LYP/6-31G* level of theory with use of Gaussian03.[38] After this subsequent refinement, there were 4 unique conformers for **2a**, 3 unique conformers for **3a**, and 5 unique conformers for **4a**; all of these had relative free energies within ∼1 kcal/mol of the respective global minimum at the B3LYP level in each case.

The calculated energetic parameters for the low-energy conformers of **2a**, **3a**, and **4a** are shown in Table 2 at the OPLS-AA and the B3LYP level. The B3LYP level predicted a different global minimum from that predicted by the OPLS-AA force field for **2a** and **3a**. For **4a**, even though these two methods predicted the same global minimum, the ordering of the low-energy conformations was different based on these two methods.[39] A similar tendency was observed in our previous study.[7] The geometrical parameters for the OPLS-AA and B3LYP/6-31G* optimized structures are very similar, and obviously structural differences are not critical for the energetic trends by the different computational methods. Our previous study stated that the nature of the difference between molecular mechanics (AMBER*) and electronic structure (HF/6-31G*) methods appeared to be their treatment of important hydrogen-bonding interactions.[7] However, in these systems, hydrogen-bonding interactions are not possible. Therefore, the nature of the difference between the OPLS-AA and the B3LYP methods arises from other interactions.

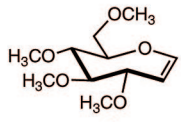
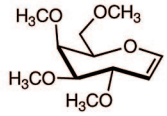
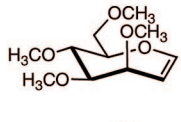
The low-energy conformers of the **2a** and **3a** had very similar geometries with a $^{5}C_{1,2}$ conformation being preferred for the ring (Table 2). The differences between these conformers are related to the orientation of the methoxy substituent on the C3−C7 positions. The low-energy conformers of **4a**, however, possessed unique conformations of the seven-membered ring, including $^{5}C_{1,2}$, $^{1,2}C_{5}$, and $^{5,6}TC_{3,4}$ isomers (Table 2).

To predict the facial selectivity for epoxidation as suggested by Wei,[12] we computed the electrostatic potential (ESP) of the most stable conformer of each oxepine (Table 3), in order to assess a preference for reactivity based on this easily computed diagnostic for the oxepine. The α-face and *β*-face views of the electron density surface, mapping the values of the electrostatic potential (red−blue corresponding to ESP values of −0.01 to +0.01 au), are depicted for the most stable conformer (**2a**-A, **3a**-A, and **4a**-A) in each model system.

As shown in Table 3 for **2a**-A, **3a**-A, and **4a**-A, the α-face had a smaller magnitude of negative charge than the *β*-face for

(29) Labanowski, J. W.; Andzelm, J. *Density Functional Methods in Chemistry*; Springer: New York, 1991.
(30) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(31) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(32) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.
(33) Cammi, R.; Tomasi, J. *J. Comput. Chem.* **1995**, *16*, 1449.
(34) Tomasi, J.; Persico, M. *Chem. Rev.* **1994**, *94*, 2027.
(35) Cramer, C. J.; Truhlar, D. G. *Chem. Rev.* **1999**, *99*, 2161.
(36) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
(37) (a) Osawa, E.; Musso, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 1. (b) Halgren, T. A. *J. Am. Chem. Soc.* **1992**, *114*, 7827. (c) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 616. (d) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(38) Frisch, M. J. et al. Gaussian 03, Revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003.
(39) See the Supporting Information for details.

TABLE 2. Calculated Energetic Parameters for the Low-Energy Conformers of 2a, 3a, and 4a: The Relative Energies (ΔE_{rel}, kcal/mol) with the OPLS-AA Force Field and the Relative Free Energies at 298 K ($\Delta G_{298,rel}$, kcal/mol) at the B3LYP/6-31+G//B3LYP/6-31G* Level of Theory[a]**

Oxepine	Conformers				
	2a-A	**2a**-B	**2a**-C	**2a**-D	
 **2a**					
OPLS-AA E_{rel}	1.4	0.0	1.8	3.3	
B3LYP $G_{298, rel}$	0.0	0.1	0.5	0.7	
	3a-A	**3a**-B	**3a**-C		
 **3a**					
OPLS-AA E_{rel}	0.8	1.2	3.0		
B3LYP $G_{298, rel}$	0.0	0.4	1.0		
	4a-A	**4a**-B	**4a**-C	**4a**-D	**4a**-E
 **4a**					
OPLS-AA E_{rel}	0.0	2.0	0.1	2.2	1.4
B3LYP $G_{298, rel}$	0.0	0.3	0.5	0.6	0.6

[a] All of the energies are relative to the corresponding global minimum for each molecule.

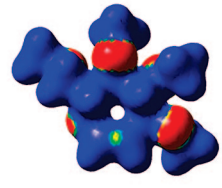
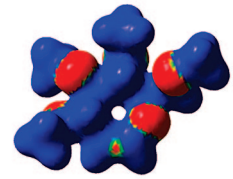
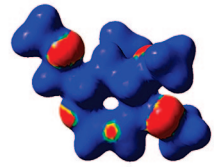
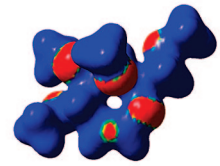
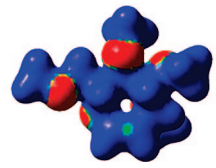
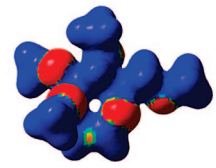
all three oxepines, although the difference was not large. It is well accepted that epoxidation appears to involve electrophilic addition to the alkene.[40,41] The ESP results would suggest a β-face preference for the DMDO epoxidation. This prediction is opposite from the experimental results. While asserting transition state preferences from ground state properties is obviously questionable, it is often synthetically convenient. The disparity between the calculated ESP results and the experimentally observed selectivities indicated that the facial preferences of the transition states were different from the ground state preferences for these carbohydrate-based oxepines. Therefore, to quantitatively predict the facial selectivity, the thermodynamics of the reaction pathways, including comprehensive structural and energetic evaluations of the diastereomeric transition states, were completed. These transition state calculations also considered the different conformations available for each oxepine.

The atomic charges of the transition states and reactant complexes for all of the DMDO epoxidations with the model systems (**2a**, **3a**, and **4a**) were obtained by using a natural population analysis (NPA)[42] at the B3LYP level of theory. For all of the DMDO epoxidations of the various conformers, the NPA results show that negative charge accumulates on the transferring O of DMDO upon progressing from the reactant complexes to the transition states. This indicates that DMDO epoxidations involve electrophilic addition to the double bond of the oxepine, a trend that is consistent with previous studies.[41] On the basis of the NPA results, C2 has a negative charge, in general, and C1, due to the bonded ring oxygen, has a positive charge, and suggests that the C2···O bond would form first for the eventual epoxide in an asynchronous transition state for epoxidation.[39]

The DMDO epoxidations on the α- and β-faces of the most stable conformers of **2a**, **3a**, and **4a** were studied at the gas-phase B3LYP level. The transition state for each pathway was located and then connected to its respective reactant complexes (RC) and products (P). Energetic values for all of the stationary points for α/β facial attack of DMDO with the different

(40) Lynch, B. M.; Pausacker, K. H. *J. Chem. Soc.* **1955**, 1525.
(41) Miaskiewicz, K.; Smith, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 1872.
(42) (a) Reed, A. E.; Weinstock, R. B.; Weinhold, F. *J. Chem. Phys.* **1985**, *83*, 735. (b) Reed, A. E.; Weinhold, F.; Curtiss, J. A. *Chem. Rev.* **1988**, *88*, 899.

TABLE 3. The α-Face and *β*-Face Views of the Electrostatic Potential (Red to Blue Corresponding to Charge Values of −0.01 to +0.01 au) for the Most Stable Conformers of 2a, 3a, and 4a at the B3LYP/6-31G* Level of Theory in the Gas Phase

Oxepine-Conformer	α-face[a]	β-face[a]
2a-A		
3a-A		
4a-A		

[a] The double bond of the oxepine is located at the bottom of each contour plot.

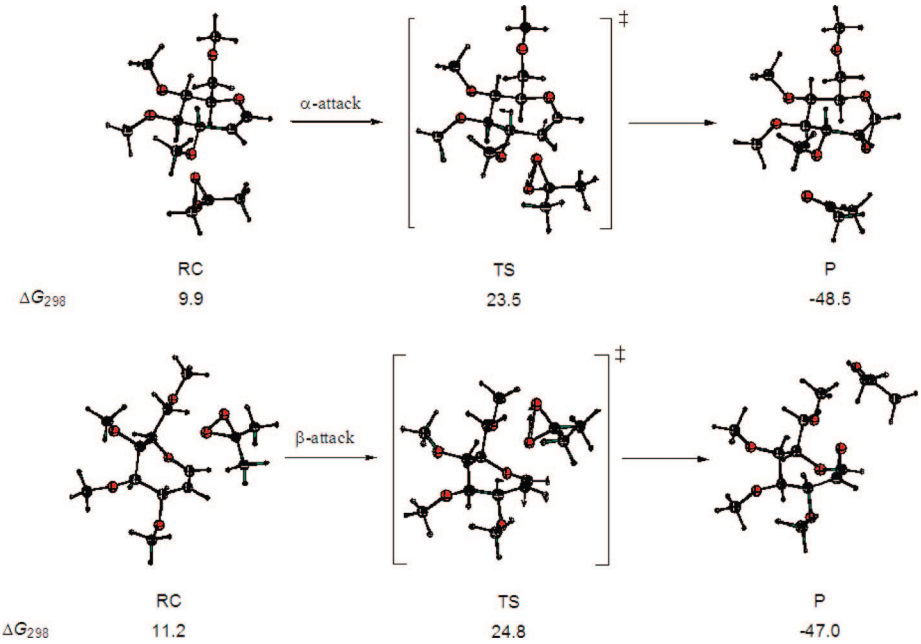


FIGURE 2. Relative free energies at 298 K (ΔG_{298}, kcal/mol) of the most favorable DMDO epoxidations on the α- and *β*-faces of **2a** at the B3LYP/6-31+G**//B3LYP/6-31G* level of theory. All of the energies are relative to the infinitely separated DMDO and the most stable conformer of **2a**.

conformers are listed in the Supporting Information. The energies are presented as relative to the infinitely separated DMDO and the most stable conformer. Considering the effect of implicit solvation with the PCM did not significantly alter the calculated results relative to experiment, therefore, only the gas-phase results are presented here.[43]

(43) All of the PCM results are presented in the Supporting Information.

The calculated results of the most favorable pathways for the DMDO epoxidations on the α- and *β*-faces of **2a** are shown in Figure 2. Among these low-energy conformers (**2a**-A, **2a**-B, **2a**-C, and **2a**-D), **2a**-C underwent the most favorable pathway for the DMDO epoxidation on the α-face of **2a**, while **2a**-B provided that on the *β*-face of **2a**.[39] The DMDO epoxidation on either face was highly exoergic. As shown in Table 4, the free energy of activation for the α-face epoxidation was about

TABLE 4. Difference in the Free Energies of Activation at 298 K ($\delta\Delta G^{\ddagger}$, kcal/mol) and the Ratios of the DMDO Epoxidations on the α- and β-Faces of 2a, 3a,and 4a from Our Computational and Experimental Studies

	theoretical (B3LYP/6-31+G**//B3LYP/6-31G*)[a]				exptl[b]
	α-TS (conf.)	β-TS (conf.)	$\delta\Delta G^{\ddagger}\beta-\alpha$	Facial selectivity (α:β) [c]	Facial selectivity (α:β)
2a	**2a**-C ($^{5}C_{1,2}$)	**2a**-B ($^{5,6}TB_{3,4}$)	1.3	80:20	3:1
3a	**3a**-B ($^{5}C_{1,2}$)	**3a**-B (envelope)	4.6	99.2:0.8	>25:1
4a	**4a**-D ($^{5}C_{1,2}$)	**4a**-A ($^{5}C_{1,2}$)	9.0	100:0	>25:1

[a] Gas-phase calculations at the B3LYP/6-31+G **//B3LYP/6-31G*level of theory (kcal/mol). [b] In this study. [c] Based on calculated $\delta\Delta G^{\ddagger}$ values.

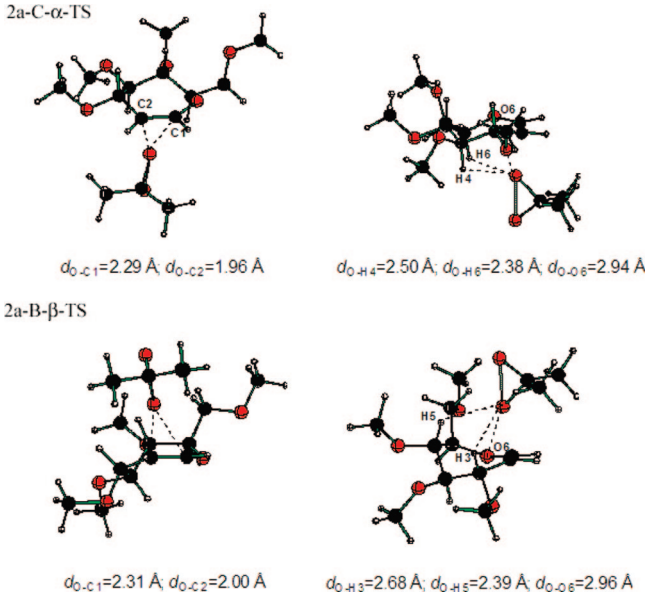


FIGURE 3. Front and side views of the transition state geometries of the most favorable DMDO epoxidations on the α- and β-faces of **2a**, based on the B3LYP/6-31G* calculations.

1.3 kcal/mol lower than that for the β-face. Thus the formation of the α-epoxide is favored kinetically over the β-epoxidation (4:1 α:β). From the calculated $\delta\Delta G^{\ddagger}$ values, the calculated ratio for the α/β facial selectivity by DMDO for **2a** is very similar to our experimental results (3:1 α:β) for the benzyloxy-substituted **2**.

The α-face epoxidation transition states for the four conformers of **2a** have very similar $^{5}C_{1,2}$ ring conformations to the starting oxepine; however, those on the β-face adopt a novel ($^{5,6}TB_{3,4}$) conformation (Figure 3). In all cases, the geometric search for each transition state started with a $^{5}C_{1,2}$ ring structure.[39] No significant differences in the distances between the axial hydrogens and the approaching oxygen could be determined for the epoxidation transition states of the α- and β-faces, and CH···O hydrogen bonding did not contribute to the preference for the α-face epoxidation. As shown in Figure 3, the transition state geometries for the most favorable DMDO epoxidations on the α- and β-faces of **2a** showed a significant difference in torsional strain. The β-face epoxidation of **2a** proceeded through a transition state with a $^{5,6}TB_{3,4}$ ring conformation, which most likely sacrificed some torsional strain to avoid the electrostatic interaction between lone-pair electrons on the incoming oxygen and those on O6 of the oxepine. The more demanding torsional strain for the β-face transition structure probably rationalizes the energetic preference for the α-face epoxidation in **2a**. The epoxidation on either face proceeded through a transition state with an asynchronous spiro geometry for which the C2···O bond forms first (Figure 3). This transition state could minimize the electrostatic interaction between the lone-pair electrons on the incoming DMDO oxygen and those on O6 of the oxepine. We thus postulate that O6 plays an important role in the transition state and also for dictating the α-facial kinetic selectivity.

The calculated results of the most favorable pathway for the DMDO epoxidations on either face of **3a** are shown in Figure 4. Among three low-energy conformers (**3a**-A, **3a**-B, and **3a**-C), the **3a**-B conformer underwent the most favorable pathway for the DMDO epoxidation on either face of **3a**.[39] Similar to **2a**, the epoxidation on either face of **3a** was highly exoergic. The calculated free energy of activation for the epoxidation of the α-face of **3a** was 4.6 kcal/mol lower than that for the β-face; thus, the α-facial selectivity was more significant for **3a** (130:1 α:β) than for **2a** (4:1 α:β), as shown in Table 4. Once again, the calculated ratio for α:β facial selectivity for methyl-substituted **3a** is very consistent with our experimental results (>25:1 α:β) for benzyloxy-substituted **3**.

For the three low-energy conformers of **3a**, the transition states of the α-face epoxidation reactions had very similar conformations as their corresponding conformers with a $^{5}C_{1,2}$ ring structure. During the β-facial attack, the oxepines again adopted novel ring structures, specifically envelope-like conformations.[39] In the α-face transition state (Figure 5), the C1 and C2 positions did not deviate much from the plane, which consists of C3, C4, C6, and O6. Only C5 was displaced from the plane of the other six ring atoms. Similar to **2a**, CH···O hydrogen bonding did not contribute to the preference for the α-face epoxidation. On the other hand, the β-face epoxidation of **3a** proceeded through a transition state with an envelope-like ring conformation, which appears to have sacrificed some significant torsional strain to avoid the electrostatic interaction between the lone-pair electrons on the incoming oxygen of DMDO and those on O6 of the oxepine. However, for this envelope conformation, there are still some electrostatic interactions between the unpaired electrons on O5 and on the incoming O of DMDO, thereby contributing to the α-facial selectively. Similar to **2a**, the epoxidation on either face of **3a** proceeded through a transition state with an asynchronous spirocyclic geometry. For **3a**, O6 appears to play an important role in the formation of the asynchronous transition state, while O5 and O6 seem to control the facial selectivity.

The calculated results of the most favorable pathways for the DMDO epoxidation on the α- and β-faces of **4a** are shown in Figure 6. Among the five low-energy conformers (**4a**-A through **4a**-E), the **4a**-D conformer underwent the most favorable pathway for the α-facial epoxidations, while the **4a**-A conformer provided that preference for the β-face.[44] In a similar manner as for **2a** and **3a**, the epoxidation reactions on either face of **4a** were highly exoergic. The calculated free energy of activation for epoxidation of the α-face of **4a** was 9.0 kcal/mol lower than that for the β-face; thus, the DMDO epoxidation should only occur to the α-face of **4a** (100:0 α:β) as shown in Table 4. The computational results for the model compound **4a** are once again very consistent with our experimental ratio

(44) The transition states of the DMDO epoxidation of **4a**-B and **4a**-E could not be found.

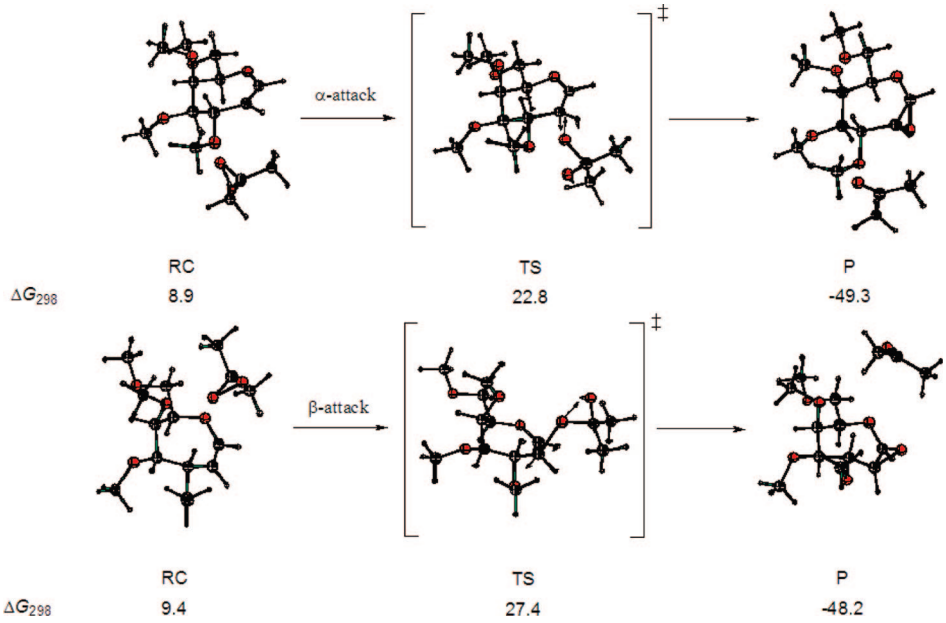


FIGURE 4. The relative free energies at 298 K (ΔG_{298}, kcal/mol) of the most favorable DMDO epoxidations on the α- and *β*-faces of **3a** at the B3LYP/6-31+G**//B3LYP/6-31G* level of theory. All of the energies are relative to the infinite separated DMDO and most stable conformer of **3a**.

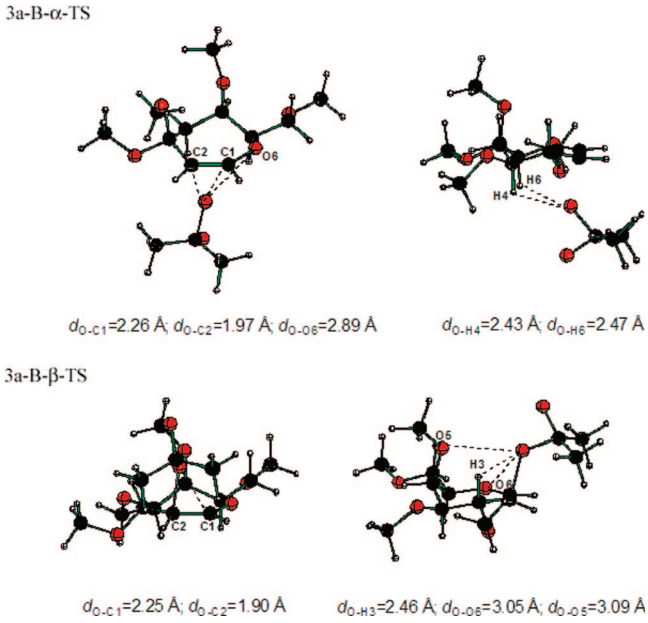


FIGURE 5. Front and side views of the transition state geometries of the most favorable DMDO epoxidations on the α- and *β*-faces of **3a**, based on the B3LYP/6-31G* optimizations.

for the α:*β* facial selectivity (>25:1 α:*β*) for benzyloxy-substituted **4**.

For the five low-energy conformers of **4a**, the epoxidation transition states on either face had very similar conformations as their corresponding starting conformers ($^5C_{1,2}$, $^{1,2}C_5$, and $^{5,6}TC_{3,4}$).[39] This was different from the cases of the other two oxepines, **2a** and **3a**. Similar to **2a** and **3a**, however, CH···O hydrogen bonding did not contribute to the preference for the α-facial epoxidation. Because the transition state geometries for the DMDO epoxidations on the α- and *β*-faces of **4a** did not show any obvious difference in their structures (Figure 3), torsional strain does not appear to be the main contribution to the high preference for the α-facial selectivity. By carefully analyzing the geometries of the α-TS and *β*-TS structures, we believe that the most significant contribution for the α-facial preference was the electrostatic interaction between the lone-pair electrons on the incoming oxygen of DMDO and those on O3 and O6 of the oxepine. To avoid the electrostatic interaction between the unpaired electrons on O3 and the incoming O of DMDO, a $^5C_{1,2}$ ring conformation is adopted in the transition state for *β*-facial epoxidation on **4a**. As shown in Figure 7, the O−O3 distance for **4a**-A-*β*-TS was only 2.79 Å, which was 0.3 Å shorter than the O−O5 distance in the **3a**-B-*β*-TS case. Similar as for **2a** and **3a**, the epoxidation on either face proceeded through a transition state with an asynchronous spiro geometry (Figure 7) in which the forming C1···O and C2···O distances are 2.41 and 1.99 Å, respectively, for the favored α-TS, while they are 2.25 and 1.92 Å, respectively, for the *β*-TS. For **4a**, it appears that O6 plays an important role in the formation of the asynchronous transition state, and O3 controls the facial selectivity.

2.4. Factors that Govern the Stereoselectivity of DMDO Epoxidations. The original impetus for this investigation was to determine the factors that govern selectivity in the epoxidation of carbohydrate-based oxepines. The agreement between the experimentally determined epoxidation selectivities for **2**−**4** and the computational results on **2a**−**4a** provides the basis for enumerating these factors. While there are both steric and electronic contributions to the observed selectivities, the ability of the less sterically demanding oxepines **2a**−**4a** to computationally reproduce the experimental data for the parent compounds **2**−**4** suggested that electronic factors predominate. By incorporating factors of the Wei "majority rule"[12] and the Rainier "asynchronicity rule",[13] a normative model for cyclic enol ether epoxidation develops. A list of key considerations includes the following: (i) the number and orientation of electronegative substituents on the ring relative to the reacting alkene [these factors are a reiteration of the majority rule]; (ii) reorganization of the transition state, driven by repulsive interactions between the ring oxygen of the oxepine (O6) and the O of DMDO, relative to the reactant; (iii) the overall

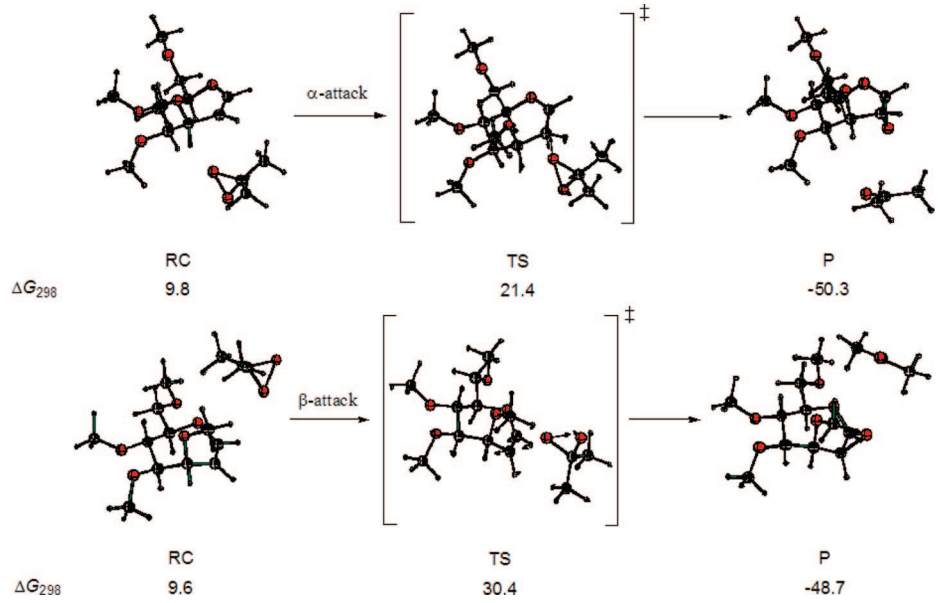


FIGURE 6. The relative free energies at 298 K (ΔG_{298}, kcal/mol) of the most favorable DMDO epoxidations on the α- and *β*-faces of **4a** at the B3LYP/6-31+G**//B3LYP/6-31G* level of theory. All of the energies are relative to the infinite separated DMDO and most stable conformer of **4a**.

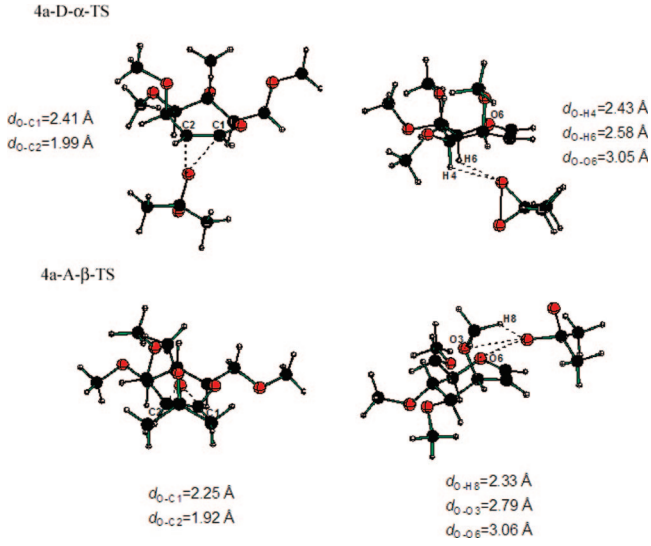


FIGURE 7. Front and side views of the transition state geometries of the most favorable DMDO epoxidations on the α- and *β*-faces of **4a**, based on the B3LYP/6-31G* optimizations.

synchronicity in epoxide bond formation; and (iv) the difference in the overall steric bulk on one side of the ring relative to the other. There is some overlap in these factors, but each is qualified in the proceeding paragraphs.

Application of the Wei majority rule to carbohydrate-based oxepines **2**−**4** allows qualitatively accurate prediction of epoxidation selectivities. This result suggests that the majority rule may be general for substituted cyclic enol ethers. For oxepines **2**−**4**, the orientation of the (protected) C7 hydroxymethyl group was considered among the electronegative substituents that would contribute to the majority rule. The overall electronegative effect may be attenuated due to its remote attachment to the ring, but it should contribute nonetheless. With this in mind, collecting substitutents on the oxepines shows that **2** should give low selectivity, having two "α" substituents at C3 and C5, and two "*β*" substituents at C4 and C6; that is, for **2**, there should be no electronic majority rule. A similar tabulation for **3** and **4** shows that there are more *β* substituents (C4, C5, and C6 for **3** and C3, C4, and C6 for **4**) giving rise to α-selectivity for epoxidation. The C3 deoxygenated oxepine **5** also preferentially formed the α-epoxide as evidenced by the formation of the methyl *β*-3-deoxy-D-*glycero*-D-*gulo*-septanoside **24**. For **5**, there are two *β* substituents (C4 and C6) and one α substituent (C5). The observed α-selective epoxidation for this system is in accordance with the majority rule. Oxepine **1**, which is the only oxepine that gives rise to *β*-facial selectivity, follows this analysis too. It has two α-benzyloxy substituents (C3 and C5) and only one *β*-benzyloxy group at C4. The majority rule is an electronic effect; the associated steric bulk of the benzyl protecting groups in these systems should also make a contribution to the observed selectivities. Wei[12] also used the PPFMO method to predict the facial selectivity for epoxidation of 4-deoxypentenosides; however, the validity of the predictions from PPFMO analysis depends strongly on the similarity between the reactant and transition state structures—a relationship that does not apply to the epoxidation of carbohydrate-based oxepines (**2a** and **3a**). In fact, the observed α-selectivity for epoxidation of **2** may solely reflect the increased steric bulk on the *β*-face of the oxepine due to the size of the protected hydroxymethyl group at C7 plus one benzyloxy group at C4 versus the two α-benzyloxy groups at C3 and C5.

Transition state reorganization and asynchronicity of bond formation in the epoxidation process also play a significant role in determining selectivities. The conformational reorganization observed in the computationally derived transition states for *β*-epoxidation of **2** and **3** contribute to the fact that these are energetically unfavorable routes; that reorganization for *β*-epoxidation of **4** was not seen is somewhat of a surprise. However, as a rationalization, we should note that in the *β*-epoxidation of **4**, the ring adopted a $^{5}C_{1,2}$ ring conformation to avoid repulsive electrostatic interactions between unpaired electrons on O6 and the incoming O of DMDO. In fact, the conformational reorganization in the *β* transition states for **2** and **3** is likely due to the minimization of the repulsive interaction between O6 and the O of DMDO. Anticipation of this repulsive

electronic interation between these oxygens for a given face of a cyclic enol ether allows predictive power in new systems. The asynchronous nature of the bond formation in the reaction plays a significant role for α- over β-epoxidation selectivity of **4**. That is because the pseudoaxial allylic substituent (C3) disfavors epoxidation on the same face based on electronic repulsion. While reorganization and asynchronicity are primarily electronic effects, the steric effects of the benzyl protecting groups also contribute here.

3. Conclusions

We have utilized density functional theory (B3LYP/6-31+G**//B3LYP/6-31G*) to explain the facial selectivity in DMDO-mediated epoxidation of carbohydrate-based oxepines. Through these combined experimental and theoretical treatments, a general model for predicting epoxidation outcomes for highly substituted cyclic enol ethers has been developed. The computational method can quantitatively predict the facial preference of these reactions. It can also provide the transition state structures that can be used to investigate the details of the interactions. In this study, the DMDO epoxidation reactions proceed through a transition state with an asynchronous spiro geometry, and proceed in an electrophilic manner for which the most electron-rich carbon center of the double bond attacks the transferring O of the DMDO oxidant. The lone-pair electrons on O6 prove to play an important role in the formation of the asynchronous transition state. Selectivity is determined by two major electronic factors. First, the collected electronegative substituents on one side or the other of the double bond favors attack on DMDO from the side with greater electron density (the side opposite to the majority of electronegative substituents). Second, the repulsive interaction between the O6 lone-pair electrons and the O of DMDO disfavors one transition over another. This repulsion can even distort the ring conformation of the oxepine in a disfavored transition state. The factors governing facial selectivity enumerated here should be applicable to substituted cyclic enol ethers generally.

4. Experimental Methods

1,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-2-deoxy-D-*galacto*-sept-1-enitol (3). The synthesis was perfomed in a glovebox. To a solution of diene **19** (0.26 g, 0.46 mmol) in toluene (115 mL) was added Schrock catalyst (0.088 g, 0.115 mmol) and the resulting reaction mixture was stirred in the glovebox for 4 h. The reaction vessel was taken out from the glovebox and toluene was removed under reduced pressure to obtain a black residue, which gave **3** (0.22 g, 89%) as a thick liquid after column chromatography (hexanes/ethyl acetate = 9/1). R_f 0.52 (hexanes:EtOAc 7:3); $[\alpha]_D^{22}$ +24.8 (*c* 3.88, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.42 (dd, *J* = 9.0, 7.8 Hz, 1H), 3.56 (dd, *J* = 9.3, 6.4 Hz, 1H), 3.60 (d, *J* = 9.3 Hz, 1H), 3.80 (dd, *J* = 6.6, 6.4 Hz, 1H), 4.04 (s, 1H), 4.37 (AB, J_{AB} = 11.9 Hz, 2H), 4.48 (dd, *J* = 6.5, 2.2 Hz, 1H), 4.66 (d, *J* = 11.8 Hz, 1H), 4.71−4.67 (m, 4H), 4.81 (d, *J* = 11.8 Hz, 1H), 4.91 (d, *J* = 11.8 Hz, 1H), 6.22 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.23−7.38 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.0, 72.9, 73.5, 73.7, 74.2, 74.7, 79.0, 85.4, 107.3, 127.8 (s), 127.9, 128.0, 128.1 (s), 128.5, 128.6 (s), 138.1, 138.6, 138.8, 144. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{35}H_{36}O_5Na^+$ 559.2455, found 559.2445.

1,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-2-deoxy-D-*manno*-sept-1-enitol (4). The synthesis was perfomed in a glovebox. To a solution of diene **20** (0.44 g, 0.78 mmol) in toluene (190 mL) was added Schrock catalyst (0.149 g, 0.195 mmol) in toluene (10 mL) and the resulting solution was stirred in the glovebox for 4 h. Toluene was removed under reduced pressure and the residue was purified by column chromatography (hexanes/ethyl acetate = 9/1) to give **4** (0.35 g, 84%) as a thick liquid. R_f 0.52 (hexanes:EtOAc = 8:2); $[\alpha]_D^{22}$ −17.55 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 (dd, *J* = 10.8, 2.6 Hz, 1H), 3.59 (dd, *J* = 10.8, 5.6 Hz, 1H), 3.66 (dd, *J* = 9.8, 2.2 Hz, 1H), 3.92−4.01 (m, 2H), 4.18 (d, *J* = 11.4 Hz, 1H), 4.30 (d, *J* = 11.4 Hz, 1H), 4.47 (d, *J* = 12.3 Hz, 1H), 4.50 (d, *J* = 12.5 Hz, 1H), 4.56 (d, *J* = 12.3 Hz, 1H), 4.57 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 1.8 Hz, 1H), 4.67 (d, *J* = 12.6 Hz, 1H), 4.73 (dt, *J* = 9.6, 1.8 Hz, 1H), 4.83 (d, *J* = 12.6 Hz, 1H), 6.42 (dd, *J* = 6.9, 2.2 Hz, 1H), 7.04−7.12 (m, 2H), 7.22−7.37 (m, 16H), 7.38−4.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.2, 71.2, 72.1, 72.6, 73.3, 76.0, 77.7, 78.8, 105.4, 127.5, 227.5, 127.6, 127.8, 127.9, 127.9, 128.0, 128.3, 128.3, 128.3, 128.4, 137.5, 138.2, 138.4, 138.8, 146.5. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{35}H_{36}O_5Na^+$ 559.2455, found 559.2441.

1,2-Anhydro-3,4,5,7-tetra-*O*-benzyl-α-D-*glycero*-L-*manno*-septanoside (9). Oxepine **3** (0.22 g, 0.41 mmol) was dried azeotropically with toluene (3 × 10 mL) under reduced pressure and then dissolved in dry DCM (12 mL) and cooled in an ice bath to 0 °C. DMDO (0.55 M) in DCM (2 × 0.88 mL) was added portion-wise and the mixture was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure. ^{1}H NMR of the resulting material showed quantitative conversion (400 MHz, $CDCl_3$) δ 3.12 (d, *J* = 2.3 Hz, 1H), 3.44 (dd, *J* = 9.0, 8.4 Hz, 1H), 3.53 (dd, *J* = 8.7, 5.0 Hz, 1H), 3.75 (dd, *J* = 9.5, 1.3 Hz, 1H), 3.86 (dd, *J* = 8.1, 5.6 Hz, 1H), 3.96 (d, *J* = 0.9 Hz, 1H), 4.36−4.45 (m, 3H), 4.65−4.97 (m, 7H), 7.28−7.43 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 58.7, 69.5, 70.7, 73.4, 74.0, 74.2 (s), 74.7, 76.8, 79.1, 84.1, 127.8, 127.9 (s), 128.0, 128.3, 128.5, 128.6, 128.7, 129.2, 138.2, 138.5, 138.8. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{35}H_{36}O_6Na^+$ 575.2404, found 575.2409.

1,2-Anhydro-3,4,5,7-tetra-*O*-benzyl-α-D-*glycero*-D-*galacto*-septanoside (11). Oxepine **4** (0.20 g, 0.37 mmol) was dried azeotropically with toluene (3 × 10 mL) and then dissolved in DCM (20 mL). DMDO (2.44 mL, 0.22 M) was added at 0 °C and the resulting reaction mixture was stirred for 1 h. The solvent was removed under reduced pressure to give a thick liquid in quantitative yield according to ^{1}H NMR. R_f = 0.35 (hexanes:EtOAc 6:4); $[\alpha]_D^{22}$ +8.07 (*c* 2.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.19 (br s, 1H), 3.65 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.67 (dd, *J* = 8.1, 3.0 Hz, 1H), 3.83 (dd, *J* = 10.5, 5.7 Hz, 1H), 3.92−3.98 (m, 1H), 3.99−4.03 (m, 1H), 4.21 (d, *J* = 2.7 Hz, 1H), 4.30 (d, *J* = 11.4 Hz, 1H), 4.39 (d, *J* = 11.7 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.66 (d, *J* = 12.3 Hz, 1H), 4.74 (d, *J* = 12.3 Hz, 1H), 4.82 (d, *J* = 12.0 Hz, 1H), 4.85 (d, *J* = 12.3 Hz, 1H), 4.96 (d, *J* = 2.7 Hz, 1H), 7.11−7.18 (m, 2H), 7.25−7.50 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 59.2, 69.1, 71.6, 71.7, 71.8, 72.9, 73.1, 75.5, 77.8, 77.9, 80.0, 127.5 (s), 127.55, 127.58, 127.6 (s), 127.7 (s), 127.8, 128.28 (s), 128.3 (s), 128.33 (s), 128.9, 129.6, 137.5, 137.8, 138.0, 138.3.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-D-*galacto*-hept-1-ene (17). To a suspension of H_3CPPh_3Br (0.46 g, 1.29 mmol) in dry THF (5 mL) at 0 °C was added *n*-BuLi (0.76 mL, 1.6 M in hexane) slowly over a period of 10 min and the resulting reaction mixture was stirred for 30 min at 0 °C and 30 min at room temperature. To a solution of *galacto* lactol **15** (0.2 g, 0.37 mmol) in THF (5 mL) was added *n*-BuLi (0.21 mL, 1.6 M in hexane) at 0 °C and the reaction mixture was stirred for 30 min. This solution was transferred to the ylide solution under N_2 at 0 °C and the resulting reaction mixture was stirred at room temperature for 55 h. The reaction mixture was quenched with saturated NH_4Cl (3 mL), THF was removed under reduced pressure, and then extracted with DCM (3 × 10 mL). Purification by column chromatography (hexanes/ethyl acetate = 9.5/0.5) gave a thick liquid (0.155 g, 78%). R_f 0.48 (hexanes:EtOAc 8:2). $[\alpha]_D^{22}$ −4.03 (*c* 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.07 (d, *J* = 8.0 Hz, D_2O exchangeable, 1H), 3.49−3.3.60 (m, 2H), 3.80−3.87 (m, 2H), 4.12 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.16 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.35−4.45 (m, 2H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 11.8

Hz, 1H), 4.71−4.78 (m, 1H), 4.79 (s, 2H), 5.33 (d, J = 8.0 Hz, 1H), 5.38 (d, J = 16.0 Hz, 1H), 5.90 (ddd, J = 16.0, 7.9, 2.5 Hz, 1H), 7.20−7.42 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.7, 70.3, 71.2, 73.08, 73.14, 75.2, 76.6, 80.8, 82.1, 119.1, 127.5 (s), 127.6 (s), 127.7 (s), 128.0 (s), 128.1 (s), 128.3 (s), 135.7, 138.0, 138.1, 138.2. ESI-MS m/z $(M + Na)^+$ calcd for $C_{35}H_{38}O_5Na^+$ 561.2611, found 561.2594.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-D-*manno*-hept-1-ene (18). To a solution of lactol **16** (1.4 g, 2.59 mmol) in THF (15 mL) was added *n*-BuLi (1.7 mL, 1.6 M) at −10 °C and the resulting solution was stirred for 30 min at 0 °C. To a solution of Ph_3PCH_3Br (3.24 g, 9.06 mmol) was added *n*-BuLi (1.6 M, 5.67 mL) at −20 °C and the resulting solution was allowed to warm to 0 °C and stirred for 30 min. The ylide solution was added to the lactol via cannula at −20 °C and stirred for 24 h at rt. Saturated NH_4Cl (5 mL) was added and THF was removed under reduced pressure. The residue was dissolved in DCM (40 mL) and washed with water and brine. The DCM layer was dried and solvent removed under reduced pressure. Purification of the resulting material by column chromatography (hexanes/ethyl acetate = 9/1) gave a thick liquid (0.42 g, 30%). R_f 0.59 (hexanes:EtOAc 7:3). $[\alpha]_D^{22}$ +6.38 (*c* 1.55, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.64 (d, J = 5.8 Hz, 1H, D_2O exchangeable), 3.56 (dd, J = 9.6, 5.4 Hz, 1H), 3.62 (dd, J = 9.6, 3.4 Hz, 1H), 3.83−3.91 (m, 2H), 3.96−4.04 (m, 1H), 4.11 (d, J = 7.4 Hz, 1H), 4.24 (d, J = 11.7 Hz, 1H), 4.47 (s, 2H), 4.48 (d, J = 11.1 Hz, 1H), 4.49 (d, J = 11.2 Hz, 1H), 4.60 (d, J = 11.3 Hz, 1H), 4.62 (d, J = 11.1 Hz, 1H), 4.71 (d, J = 11.2 Hz, 1H), 5.39 (dd, J = 8.8, 1.5 Hz, 1H), 5.42 (d, J = 17.3 Hz, 1H), 5.94 (ddd, J = 17.6, 7.8, 2.4 Hz, 1H), 7.17−7.38 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.9, 70.2, 71.3, 73.3, 73.9, 74.3, 78.6, 80.3, 80.9, 119.7, 127.47, 127.54, 127.66, 127.7, 127.8, 127.84, 128.2, 128.21, 128.3, 128.4, 136.2, 138.0, 138.4, 138.5. ESI-MS m/z $(M + Na)^+$ calcd for $C_{35}H_{38}O_5Na^+$ 561.2611, found 561.2615.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-6-*O*-vinyl-D-*galacto*-hept-1-ene (19). Ethyl vinyl ether (75 mL) and DCM (5 mL) were combined in a flame-dried round-bottomed flask. To this mixture was added 1,10-phenanthroline (0.098 g, 0.54 mmol) followed by $Pd(OAc)_2$ (0.121 g, 0.54 mmol) with stirring for 15 min. To this mixture was added alcohol **17** (1.95 g, 3.62 mmol) in DCM (20 mL) and the resulting reaction mixture was refluxed for 10 d. The solvent was removed under reduced pressure. The residue was directly loaded on a column and purified (hexanes/ethyl acetate = 9.5/0.5) to give a thick liquid **19** (0.27 g, 13%) in 59% yield based on 0.46 g, conversion. R_f 0.64 (hexanes:EtOAc 8:2). Repeated attempts at obtaining HRMS data for **19** provided only the product of vinyl ether hydrolysis (**17**). $[\alpha]_D^{22}$ +6.87 (*c* 5.39, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (dd, J = 9.8, 6.4 Hz, 1H), 3.65 (dd, J = 9.8, 5.9 Hz, 1H), 3.75 (dd, J = 8.0, 3.2 Hz, 1H), 3.99 (dd, J = 6.5, 1.6 Hz, 1H), 4.03 (dd, J = 8.0, 2.7 Hz, 1H), 4.18 (dd, J = 7.8, 3.1 Hz, 1H), 4.32 (d, J = 12.0 Hz, 1H), 4.30−4.34 (m, 1H), 4.37 (dd, J = 14.1, 1.6 Hz, 1H), 4.42 (s, 2H), 4.43 (AB, J_{AB} = 12.0 Hz, 2H), 4.62 (d, J = 12.0 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 5.29 (d, J = 11.3 Hz, 1H), 5.38 (d, J = 17.4 Hz, 1H), 6.00 (ddd, J = 17.8, 7.7, 2.6 Hz, 1H), 6.36 (dd, J = 14.0, 6.5 Hz, 1H), 7.16−7.36 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.6, 69.9, 72.9, 73.8, 74.4, 76.9, 77.2, 79.8, 80.9, 88.3, 118.4, 127.2, 127.23 (s), 127.3 (s), 127.33, 127.4 (s), 127.6 (s), 127.62 (s), 127.68 (s), 127.95 (s), 128.0 (s), 128.1, 128.2, 136.3, 137.7, 138.1, 138.2, 138.23, 151.6.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-6-*O*-vinyl-D-*galacto*-hept-1-ene (19). To a solution of **17** (0.1 g, 0.186 mmol) in toluene (5 mL) was added $[Ir(cod)Cl]_2$ (0.012 g, 0.018 mmol), Na_2CO_3 (0.013 g, 0.113 mmol), and vinyl acetate (0.33 g, 3.72 mmol). The solution was heated to 100 °C and stirred for 12 h. Toluene was removed under reduced pressure and the residue was extracted with DCM (3 × 10 mL). Purification by column chromatography (hexanes/ethyl acetate = 9.5/0.5) gave a thick liquid **19** (0.098 g, 93%). The physical and analytical data were found to be identical as given above.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-6-*O*-vinyl-D-*manno*-hept-1-ene (20). To a solution of alcohol **18** (0.58 g, 1.08 mmol) in toluene (20 mL) was added $[Ir(cod)Cl]_2$ (0.072 g, 0.11 mmol), Na_2CO_3 (0.069 g, 0.65 mmol), and vinyl acetate (0.2 mL, 21.6 mmol). The solution was heated to 100 °C and stirred for 15 h. Toluene was removed under reduced pressure and the residue was extracted with DCM (3 × 10 mL). Column chromatography of the residue (hexanes/ethyl acetate = 9.5/0.5) gave a thick liquid **20** (0.451 g, 74%). R_f 0.57 (hexanes:EtOAc 8:2). $[\alpha]_D^{22}$ −1.56 (*c* 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (dd, J = 10.8, 5.0 Hz, 1H), 3.78 (dd, J = 7.0, 3.5 Hz, 1H), 3.84 (dd, J = 10.8, 2.6 Hz, 1H), 4.02 (dd, J = 6.5, 1.6 Hz, 1H), 4.08 (t, J = 7.7 Hz, 1H), 4.09 (dd, J = 7.0, 3.5 Hz, 1H), 4.16−4.20 (m, 1H), 4.24 (d, J = 11.6 Hz, 1H), 4.34 (dd, J = 14.0, 1.6 Hz, 1H), 4.49 (s, 2H), 4.51 (d, J = 11.3 Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.56 (d, J = 11.5 Hz, 1H), 4.59 (d, J = 12.3 Hz, 1H), 4.70 (d, J = 11.0 Hz, 1H), 5.39 (d, J = 8.6 Hz, 1H), 5.43 (d, J = 15.7 Hz, 1H), 5.96 (ddd, J = 17.6, 7.9, 2.4 Hz, 1H), 6.30 (dd, J = 14.1, 6.5 Hz, 1H), 7.20−7.32 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.9, 69.9, 73.3, 74.4, 74.43, 77.8, 78.7, 80.4, 81.0, 89.2, 119.8, 127.37, 127.42, 127.59, 127.61, 127.7, 128.0, 128.1, 128.2, 128.3, 136.3, 138.2, 138.4, 138.6, 138.7, 151.0. ESI-MS m/z $(M + Na)^+$ calcd for $C_{37}H_{40}O_5Na^+$ 587.2768, found 587.2768.

Methyl-3,4,5,7-tetra-*O*-benzyl-β-D-*glycero*-L-*manno*-septanoside (25). To a solution of epoxide **9** in CH_3OH (5 mL) was added $NaOCH_3$ (20 mg) at 0 °C and the mixture was stirred overnight at rt. The reaction was quenched by adding saturated NH_4Cl (2 mL) and volatiles were removed under reduced pressure. The residue was dissolved in DCM (10 mL) and washed with H_2O. The DCM layer was dried and solvent was removed under reduced pressure. Purification of this material by column chromatography (hexanes/ethyl acetate = 7/3) gave β-methyl septanoside **25** (0.16 g, 67% overall). R_f 0.59 (hexanes:EtOAc 1:1). $[\alpha]_D^{22}$ +41.9 (*c* 3.38, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.84 (d, J = 1.6 Hz, 1H), 3.43 (s, 3H), 3.59−3.73 (m, 3H), 3.83 (dd, J = 8.9, 1.5 Hz, 1H), 3.97−4.02 (m, 2H), 4.23 (d, J = 6.4 Hz, 1H), 4.30 (dd, J = 8.9, 4.4 Hz, 1H), 4.48 (AB, J_{AB} = 11.8 Hz, 2H), 4.58−4.68 (m, 3H), 4.82 (d, J = 12.0 Hz, 1H), 4.84 (d, J = 12.0 Hz, 1H), 4.95 (d, J = 11.6 Hz, 1H), 7.24−7.35 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 69.9, 73.6, 73.7, 74.1, 74.41, 74.44, 75.6, 77.2, 79.1, 79.5, 79.6, 108.1, 127.48 (s), 127.50 (s), 127.7 (s), 127.8 (s), 127.9 (s), 128.1 (s), 128.2 (s), 128.3 (s), 128.4 (s), 138.1, 138.2, 138.6, 138.7. ESI-MS m/z $(M + Na)^+$ calcd for $C_{36}H_{40}O_7Na^+$ 607.2666, found 607.2642.

2-*O*-Acetyl-3,4,5,7-tetra-*O*-benzylmethyl-β-D-*glycero*-L-*manno*-septanoside (25a). To a solution of **25** (0.05 g, 0.086 mmol) in pyridine (0.068 g, 6.5 mmol) was added Ac_2O (0.26 g, 2.56 mmol) and DMAP (2 mg) and the mixture was stirred for 15 h at rt. Volatiles were removed under reduced pressure and the reaction mixture was purified by column chromatography (hexanes/ethyl acetate = 9/1) to give **25a** (0.05 g, 94%). R_f 0.51 (hexanes:EtOAc 7:3); $[\alpha]_D^{22}$ −29.13 (*c* 0.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H), 3.39 (s, 3H), 3.62 (dd, J = 9.8, 5.4 Hz, 1H), 3.68 (dd, J = 9.8, 6.8 Hz, 1H), 3.77−3.82 (m, 1H), 3.84 (dd, J = 8.4, 2.1 Hz, 1H), 3.97 (t, J = 2.4 Hz, 1H), 4.31 (dd, J = 8.5, 3.6 Hz, 1H), 4.35 (d, J = 5.8 Hz, 1H), 4.45 (AB, J_{AB} = 11.8 Hz, 2H), 4.52 (d, J = 11.7 Hz, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 11.7 Hz, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.86 (d, J = 11.7 Hz, 1H), 5.49 (dd, J = 5.8, 3.6 Hz, 1H), 7.18−7.34 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 55.8, 70.0, 73.4, 73.6, 73.9, 74.0, 74.4, 75.2, 78.1, 78.4, 78.9, 105.6, 127.4, 17.6, 127.61, 127.8, 127.9, 128.1, 128.13, 128.2, 128.3, 169.6. ESI-MS m/z $(M + Na)^+$ calcd for $C_{38}H_{42}O_8Na^+$ 649.2772, found 649.2771.

Methyl-3,4,5,7-tetra-*O*-benzyl-α-D-*glycero*-D-*galacto*-septanoside (26) and 1,5-Anhydro-3,4,7-tri-*O*-benzyl-α-D-*glycero*-D-*galacto*-septanose (27). To a solution of oxepine **4** (0.2 g, 0.37 mmol) in DCM (20 mL) was added DMDO (2.44 mL, 0.22 M) at 0 °C and the mixture was stirred for 1 h. Volatiles were removed under

reduced pressure and ^{1}H NMR showed quantitative conversion with formation of a mixture of products. The residue was dissolved in CH_3OH (10 mL) and $NaOCH_3$ (15 mg) was added. The mixture was stirred at rt for 12 h and then quenched by adding saturated NH_4Cl (3 mL). The solvent was removed under reduced pressure and H_2O (15 mL) was added to the mixture. Extraction with DCM (3 × 10 mL), drying, removal of solvent, and purification by column chromatography gave two products. The first eluted with hexanes/ethyl acetate = 9/1 giving **26** (0.12 g, 55%) as a thick liquid. R_f = 0.55 (hexanes:EtOAc 7:3). $[\alpha]_D^{22}$ +55.91 (*c* 2.28, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 (d, J = 4.2 Hz, D_2O exchangeable, 1H), 3.45 (s, 3H), 3.60 (d, J = 4.1 Hz, 2H), 3.78 (dd, J = 9.3, 5.8 Hz, 1H), 3.94 (d, J = 7.7 Hz, 1H), 3.98−4.04 (m, 2H), 4.10 (dt, J = 7.6, 3.8 Hz, 1H), 4.31 (d, J = 11.2 Hz, 1H), 4.44 (d, J = 12.2 Hz, 1H), 4.54 (d, J = 12.2 Hz, 1H), 4.55 (d, J = 11.2 Hz, 1H), 4.64 (d, J = 11.9 Hz, 1H), 4.71 (s, 2H), 4.73 (d, J = 11.9 Hz, 1H), 4.88 (d, J = 3.5 Hz, 1H), 7.08−7.20 (m, 2H), 7.24−7.38 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 70.3, 71.0, 71.4, 73.1, 73.2, 73.4, 73.5, 77.4, 77.9, 79.7, 98.4, 127.4 (s), 127.5 (s), 127.54 (s), 127.6 (s), 127.7 (s), 127.8 (s), 128.0 (s), 128.2 (s), 128.23 (s), 128.3 (s), 138.0, 138.1, 138.3, 138.5. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{36}H_{40}O_7Na^+$ 607.2666, found 206.2671. Further elution with hexanes:EtOAc (8.5:1.5) gave **27** (0.05 g, 29%) as a thick liquid. R_f 0.59 (hexanes:EtOAc 7:3). $[\alpha]_D^{22}$ +67.54 (*c* 2.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.48 (d, J = 4.7 Hz, D_2O exchangeable, 1H), 3.56 (dd, J = 10.8, 4.7 Hz, 1H), 3.62 (dd, J = 10.8, 2.0 Hz, 1H), 3.69 (dd, J = 9.7, 4.3 Hz, 1H), 3.85 (m, 1H), 4.07 (d, J = 2.2 Hz, 1H), 4.25−4.31 (m, 1H), 4.36 (d, J = 4.3 Hz, 1H), 4.40 (d, J = 11.6 Hz, 1H), 4.45 (d, J = 12.3 Hz, 1H), 4.49 (d, J = 11.5 Hz, 1H), 4.51 (d, J = 11.5 Hz, 1H), 4.60 (d, J = 11.4 Hz, 1H), 4.61 (d, J = 12.6 Hz, 1H), 5.27 (d, J = 4.6 Hz, 1H), 7.14−7.35 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.2, 70.3, 71.3, 72.2, 73.4, 73.7, 79.6, 79.7, 82.9, 98.7, 127.6 (s), 127.8 (s), 127.83 (s), 127.9 (s), 128.0, 128.3 (s), 128.4 (s), 128.5 (s), 137.5 (s), 137.6, 137.9. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{28}H_{30}O_6Na^+$ 485.1935, found 485.1929.

2-*O*-Acetyl-3,4,5,7-tetra-*O*-benzylmethyl-α-D-*glycero*-D-*galacto*-septanoside (26a). To a solution of **26** (0.09 g, 0.154 mmol) in pyridine (0.12 g, 1.54 mmol) was added Ac_2O (0.47 g, 4.6 mmol) and DMAP (5 mg). The mixture was stirred for 15 h at rt and volatiles were removed under reduced pressure. The residue was purified by column chromatography (hexanes/ethyl acetate = 9/1) giving **26a** (0.09 g, 93%) as a thick liquid. R_f 0.49 (hexanes:EtOAc 7:3); $[\alpha]_D^{22}$ +34.70 (*c* 1.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01 (s, 3H), 3.39 (s, 3H), 3.57 (dd, J = 10.4, 2.6 Hz, 1H), 3.63 (dd, J = 10.4, 5.1 Hz, 1H), 3.84 (dd, J = 8.9, 6.1 Hz, 1H), 3.85−3.92 (m, 2H), 3.93−4.00 (m, 1H), 4.31 (d, J = 11.2 Hz, 1H), 4.41 (d, J = 12.1 Hz, 1H), 4.52 (d, J = 11.2 Hz, 1H), 4.56 (d, J = 11.2 Hz, 1H), 4.70 (s, 2H), 4.73 (s, 2H), 4.94 (d, J = 3.2 Hz, 1H), 5.34 (dd, J = 7.5, 3.2 Hz, 1H), 7.10−7.34 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0, 55.9, 70.9, 71.0, 72.1, 73.1, 73.3, 73.48, 73.53, 76.7, 76.8, 79.4, 97.2, 127.5, 127.52, 127.54, 127.6, 127.7 (s), 128.1 (s), 128.2 (s), 128.3 (s), 138.0, 138.1, 138.3 (s). ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{38}H_{42}O_8Na^+$ 649.2772, found 649.2775.

1,5-Anhydro-3,4,7-tri-*O*-benzyl-α-D-*glycero*-D-*galacto*-septanose (27a). To a solution of **27** (0.06 g, 0.13 mmol) in pyridine (0.5 mL) was added Ac_2O (2 mL) and DMAP (2 mg) and the mixture was stirred at rt for 15 h. Volatiles were removed under reduced pressure and the material was purified by column chromatography (hexanes:EtOAc 9:1) to give **27a** (0.058 g, 89%) as a thick liquid. R_f 0.47 (hexanes:EtOAc 8:2); $[\alpha]_D^{22}$ +99.85 (*c* 1.06, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.06 (s, 3H), 3.60 (br s, 2H), 3.75 (br d, J = 1.3 Hz, 2H), 4.28 (d, J = 2.2 Hz, 1H), 4.41 (br s, 1H), 4.43 (d, J = 11.4 Hz, 1H), 4.44 (d, J = 11.9 Hz, 1H), 4.51 (s, 2H), 4.57 (d, J = 13.0 Hz, 1H), 4.61 (d, J = 12.2 Hz, 1H), 5.08 (dd, J = 4.4, 2.2 Hz, 1H), 5.58 (d, J = 4.4 Hz, 1H), 7.05−7.23 (m, 2H), 7.25−7.46 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 68.9, 70.1, 71.4, 72.4, 73.3, 73.9, 79.1, 79.9, 80.2, 96.9, 127.8 (s), 127.85 (s), 127.9 (s), 128.1 (s), 128.3 (s), 128.4 (s), 128.5 (s), 137.3, 137.4, 138.1, 169.8. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{30}H_{32}O_7Na^+$ 527.2040, found 527.2042.

Methyl-3,4;5,7-diacetonide-β-D-*glycero*-D-*galacto*-septanoside (29). To a solution of oxepine **28** (0.04 g, 0.156 mmol) in DCM (10 mL) was added DMDO (0.53 mL, 0.36 M) at 0 °C and the mixture was stirred for 1 h. Volatiles removed under reduced pressure and ^{1}H NMR of the residue showed quantitative conversion of **28**. This material was dissolved in CH_3OH (5 mL) and $NaOCH_3$ (7 mg) was added. The reaction was stirred overnight and quenched by adding saturated NH_4Cl (2 mL). Volatiles were removed under reduced pressure and H_2O (15 mL) was added to the residue. Extraction with DCM (3 × 10 mL), drying of the DCM layers, and removal of solvent under reduced pressure was followed by purification via column chromatography (hexanes/ethyl acetate = 4/6) to give **29** (0.04 g, 84% for 2 steps) as a white solid. R_f 0.49 (hexanes:EtOAc 2:8). Mp 96−98 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 6H), 1.41 (s, 3H), 1.48 (s, 3H), 2.42−2.59 (br s, D_2O exchangeable, 1H), 3.47 (m, 1H), 3.50 (s, 3H), 3.63 (d, J = 8.0 Hz, 1H), 4.01−4.10 (m, 4H), 4.21 (d, J = 8.0 Hz, 1H), 4.37 (q, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.4, 26.4, 26.9, 28.1, 56.9, 66.6, 73.2, 73.5, 73.7, 74.2, 78.7, 103.4, 109.4, 110.3. ESI-MS *m*/*z* (M + Na)$^+$ calcd for $C_{14}H_{24}O_7Na^+$ 327.1414, found 327.1410.

Methyl-β-D-*glycero*-L-*manno*-septanoside (25b). To a solution of **25** (0.15 g, 0.26 mmol) in CH_3OH (15 mL) was added $Pd(OH)_2$ (0.17 g, 1.23 mmol) and the solution was stirred under H_2 (1 atm) for 3 h. The catalyst was filtered through a pad of celite and the filtrate was concentrated under reduced pressure to give a thick liquid (0.054 g, 95%). R_f 0.54 (DCM:CH_3OH 9:1). $[\alpha]_D^{22}$ +6.0 (*c* 1.4, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) δ 3.41 (s, 3H, $-OCH_3$), 3.61−3.66 (m, 3H), 3.70 (dd, J = 12.3, 8.2 Hz, 1H), 3.86 (dd, J = 2.8, 2.8 Hz, 1H), 3.91 (d, J = 1.8 Hz, 1H), 3.99 (dd, J = 9.5, 3.7 Hz, 1H), 4.31 (d, J = 4.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 56.8, 63.3, 68.8, 71.7, 72.8, 76.2, 80.3, 110.1. ESI-MS (M + Na$^+$) calcd for $C_8H_{16}O_7Na^+$ 247.0794, found 247.0796.

Methyl-α-D-*glycero*-D-*galacto*-septanoside (26b). To a solution of **26** (0.1 g, 0.17 mmol) in CH_3OH (10 mL) was added $Pd(OH)_2$ (0.115 g, 0.82 mmol) and the solution was stirred under H_2 (1 atm) for 4 h. Catalyst was filtered through a pad of celite and the filtrate was concentrated under reduced pressure to give **26b** as a thick liquid (0.036 g, 94%). R_f 0.52 (DCM:CH_3OH 9:1). $[\alpha]_D^{22}$ +57.31 (*c* 1.5, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) δ 3.43 (s, 3H), 3.60 (dd, J = 8.8, 7.7 Hz, 1H), 3.65 (dd, J = 12.0, 7.0 Hz, 1H), 3.74 (dd, J = 12.0, 3.0 Hz, 1H), 3.74−3.77 (m, 1H), 3.84 (d, J = 1.8 Hz, 1H), 3.86 (d, J = 3.0 Hz, 1H), 3.96 (dd, J = 6.8, 2.2 Hz, 1H), 4.67 (d, J = 3.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 56.4, 64.3, 71.6, 73.2, 73.3, 75.3, 75.6, 100.1. ESI-MS (M + Na$^+$) calcd for $C_8H_{16}O_7Na^+$ 247.0794, found 247.0783.

Methyl-β-D-*glycero*-D-*galacto*-septanoside (29b). To a solution of **29** (0.16 g, 0.526 mmol) in EtOH/H_2O (32 mL, 1:3) was added Amberlite-IR-120 (260 mg) then the solution was refluxed at 110 °C for 3.5 h. The reaction mixture was cooled and filtered through a pad of celite and the filtrate was concentrated under reduced pressure to give **29b** (0.11 g, 93%) as an oil. R_f 0.35 (DCM:CH_3OH 8:2). $[\alpha]_D^{22}$ −13.97 (*c* 1.4, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD) δ 3.34−3.44 (m, 1H), 3.48 (s, 3H), 3.60−3.74 (m, 3H), 3.76−3.88 (m, 3H), 4.22 (d, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 56.7, 64.6, 71.8, 76.2, 76.3, 76.4, 84.5, 110.8. ESI-MS (M + Na$^+$) calcd for $C_8H_{16}O_7Na^+$ 247.0794, found 247.0773.

Computational Methods. A Monte−Carlo conformational search (torsional sampling: 5000 steps; energy window for saving structures: 50 kJ/mol) was used to generate the conformers of **2a**, **3a**, and **4a**. The calculations were performed by employing the OPLS-AA force field[36] as implemented in the MacroModel software (version 8.5)[37] from Schrödinger. This process resulted in 431 unique conformations for **2a**, 655 unique conformations for **3a**, and 750 unique conformations for **4a**.

The 20 most stable conformers of **2a**, 25 most stable conformers of **3a**, and 43 most stable conformers of **4a** from the OPLS-AA conformational searches were further optimized at the B3LYP/6-31G* level of theory.[28–31] All density functional theory (DFT) calculations were performed with Gaussian03[38] at the Ohio Supercomputer Center. The optimized geometries and subsequently the harmonic vibrational frequencies for all of these conformers were obtained, and the relative energies were compared. A scaling factor of 0.9806[45] was used for the zero-point vibrational energy (ZPE) corrections. Enthalpies and free energies were obtained from the calculated thermal and entropic corrections at 298 K, using the unscaled, harmonic vibrational frequencies for the vibrational contribution to the partition function. After this analysis, 4 conformations for **2a**, 3 conformations for **3a**, and 5 conformations for **4a** were obtained, and for which their relative free energies were within ~1 kcal/mol of the global minimum in each case.

The transition states for the epoxidation by DMDO with 10 different conformers of **2a**, **3a**, and **4a** were located at the B3LYP/6-31G* level of theory. Transition state structures were confirmed to have one imaginary vibrational frequency. The desired reactants and products were located by displacement (10%) of the transition state geometries along the normal coordinate of the imaginary vibrational frequency in the positive and negative directions, followed by careful optimization with the analytical second derivatives (opt = calcfc).

Single-point energy and natural population analysis (NPA)[42] calculations were then performed for these stationary points at the B3LYP/6-31+G** level of theory with six Cartesian d functions. The effect of solvation on the gas-phase single-point calculations was investigated by using the polarizable continuum model (PCM)[32–35] for CH_2Cl_2 and CH_3OH as solvents.

(45) National Institute of Standards and Technology, Computational Chemistry Comparison and Benchmark Database Release 12, 2005.

The electrostatic potential for each of the most stable conformers of **2a**, **3a**, and **4a** (**2a**-A, **3a**-A, and **4a**-A, respectively) was generated with Gaussview 3.0[46] at the B3LYP/6-31G* level of theory in the gas phase. The isosurface value was 0.02 au.

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Supporting Information Available: NMR spectra for all new compounds and a summary of enthalpic and free energies, optimized geometries, vibrational frequencies, as well as NPA results for each conformer, reactant complex, transition state and product. This material is available free of charge via the Internet at http://pubs.acs.org. Complete crystallographic data for the structural analysis of methyl septanoside **29** have been deposited in the Cambridge Crystallographic Data Centre (CCDC), No. 655858; copies of this information may be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44−1223−336033; web: www.ccdc.cam.ac.uk/conts/retrieving/html; e-mail: deposit@ccdc.cam.ac.uk).

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Asymmetric Co(II)-Catalyzed Cyclopropanation with Succinimidyl Diazoacetate: General Synthesis of Chiral Cyclopropyl Carboxamides

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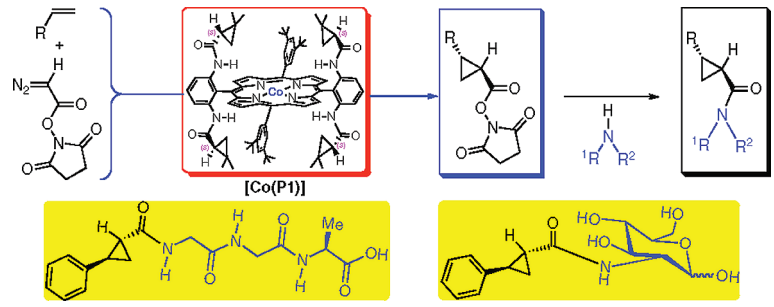
ABSTRACT



[Co(P1)] is an effective catalyst for asymmetric cyclopropanation with succinimidyl diazoacetate. The Co(II)-catalyzed reaction is suitable for various olefins, providing the desired cyclopropane succinimidyl esters in high yields and excellent diastereo- and enantioselectivity. The resulting enantioenriched cyclopropane succinimidyl esters can serve as convenient synthons for the general synthesis of optically active cyclopropyl carboxamides.

The well-documented importance of cyclopropanes in numerous fundamental and practical applications has stimulated vast efforts for the synthesis of these smallest carbocycles.[1] Metal-catalyzed asymmetric cyclopropanation of alkenes with diazo reagents constitutes the most direct and general approach for the stereoselective construction of these unique all-carbon triangular structures.[2] A number of outstanding chiral catalysts have been reported to achieve high diastereo- and enantioselectivity for several classes of cyclopropanation reactions, most of which employed diazoacetates.[1−3] Ongoing endeavors in the field aim at further expanding the substrate scope to include a broader variety of alkenes as

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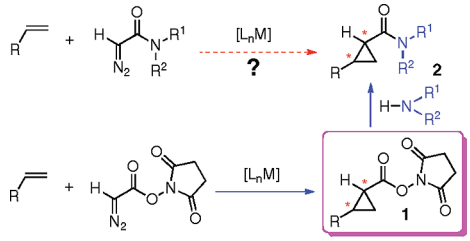
well as to utilize more challenging classes of diazo reagents for use in asymmetric cyclopropanation.

In contrast to the large body of excellent results achieved with diazoacetates,[1−3] diazoacetamides have not been successfully employed for asymmetric intermolecular cyclopropanation (Scheme 1)[4] except for the Rh_2-based

Scheme 1. Routes to Chiral Cyclopropyl Carboxamides



intramolecular reactions by Doyle and co-workers.[5,6] The absence of effective intermolecular asymmetric cyclopropanation with diazoacetamides may be attributed to two major factors: (i) inherent low reactivity of the resulting metal−carbene intermediate due to reduced electrophilicity and increased steric hindrance and (ii) complications resulting from competitive intramolecular C−H insertion.[7] Inspired by their important biomedical applications,[8] we envisioned a postderivatization approach to synthesize chiral cyclopropyl carboxamides **2** in enantioenriched form through reacting preformed cyclopropyl chiral building blocks **1** with various amines (Scheme 1).[9] Herein, we report a cobalt-catalyzed asymmetric cyclopropanation process with succinimidyl diazoacetate (N_2CHCO_2Su),[10] which forms cyclopropanes **1** with excellent diastereo- and enantioselectivities. As a result of the highly reactive hydroxysuccinimide esters present, **1** could serve as convenient synthons for the general preparation of chiral amides **2** through reactions with a range of different amines and without loss of pre-established enantiomeric purity.

Structurally well-defined cobalt(II) complexes of D_2-symmetric chiral porphyrins ([Co(Por*)]) have emerged as a class of effective catalysts for asymmetric cyclopropanation reactions,[11−13] with both electron-sufficient[12a,b] and electron-deficient[12c] olefins using diazoacetates,[12b,c] diazosulfones,[12d] and α-nitro diazoacetates.[12e] Among this family of [Co(Por*)],[12] a group of six derivatives [Co(**P1**)]−[Co(**P6**)] (Figures 1 and S1 (Supporting Information)), possessing

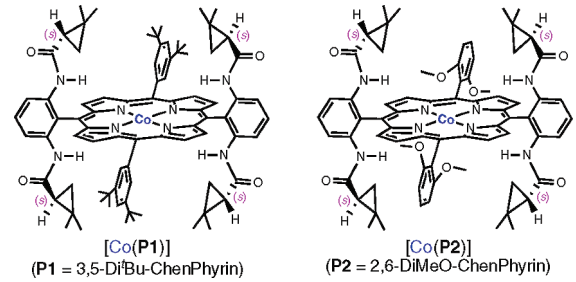


Figure 1. D_2-Symmetric chiral cobalt(II) porphyrins.

diverse electronic, steric, and chiral environments, were evaluated as potential catalysts for the asymmetric cyclopropanation of styrene with the sterically bulky N_2CHCO_2Su (Table 1). As a practical attribute of [Co(Por)]-catalyzed cyclopropanation,[14a] these reactions were carried out *in a one-pot fashion with alkene as limiting reagent and without the occurrence of the common dimerization side reaction*. Upon examination of the results (Table 1), it was evident that the steric bulkiness of the carbene source governed the reactivity difference of these catalysts. For example, no

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(7) For select examples of intramolecular carbene C−H insertion of α-diazoacetamides, see: (a) Brown, D. S.; Elliott, M. C.; Moody, C. J.; Mowlem, T. J.; Marino, J. P.; Padwa, A. P. *J. Org. Chem.* **1994**, *59*, 2447. (b) Gois, P. M. P.; Afonso, C. A. M. *Eur. J. Org. Chem.* **2004**, 3773. (c) Grohmann, M.; Buck, S.; Schaffler, L.; Maas, G. *Adv. Synth. Catal.* **2006**, *348*, 2203.

(8) For select recent examples, see: (a) Garrido, D. M.; Corbett, D. F.; Dwornik, K. A.; Goetz, A. S.; Littleton, T. R.; McKeown, S. C.; Mills, W. Y.; Smalley, T. L.; Briscore, C. P.; Peat, A. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1840. (b) Jiang, T.; Kuhen, K. L.; Wolff, K.; Yin, H.; Bieza, K.; Caldwell, J.; Bursulaya, B.; Wu, T. Y.-H.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2105. (c) Sandanayaka, V. P.; Prashad, A. S.; Yang, Y.; Williamson, T.; Lin, Y. I.; Mansour, T. S. *J. Med. Chem.* **2003**, *46*, 2569. (d) Morain, P.; Lestage, P.; De Nanteuil, G.; Jochemsen, R.; Robin, J.-L.; Guez, D.; Boyer, P.-A. *CNS Drug Rev.* **2002**, *8*, 31. (e) Graham, D. W.; Ashton, W. T.; Barash, L.; Brown, J. E.; Brown, R. D.; Canning, L. F.; Chen, A.; Springer, J. P.; Rogers, E. F. *J. Med. Chem.* **1987**, *30*, 1074.

(9) For an example of ineffective asymmetric cyclopropanation directly with diazoacetamides by current Co(II)-based catalytsts, see Scheme S1, Supporting Information.

(10) The solid N_2CHCO_2Su, which is stable and can be handled safely, has not been previously employed for asymmetric cyclopropanation. For a single report on Ru-catalyzed nonasymmetric cyclopropanation with N_2CHCO_2Su, see: (a) Werle, T.; Maas, G. *Adv. Synth. Catal.* **2001**, *343*, 37. For the use of N_2CHCO_2Su to synthesize diazo derivatives, see: (b) Ouihia, A.; Rene, L.; Guilhem, J.; Pascard, C.; Badet, B. *J. Org. Chem.* **1993**, *58*, 1641. (c) Fuerst, D. E.; Stoltz, B. M.; Wood, J. L. *Org. Lett.* **2000**, *2*, 3521. (d) Clark, J. P.; Middleton, M. D. *Org. Lett.* **2002**, *4*, 765. (e) Grohmann, M.; Buck, S.; Schaffler, L.; Maas, G. *Adv. Synth. Catal.* **2006**, *348*, 2203. (f) Reference 5.

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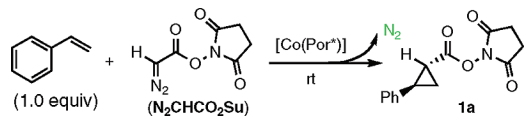
Table 1. Asymmetric Cyclopropanation of Styrene with Succinimidyl Diazoacetate by D_2-Symmetric Chiral Cobalt(II) Porphyrins[a]



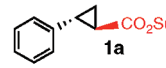
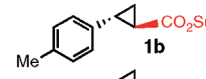
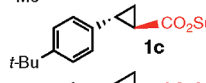
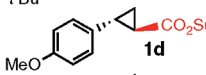
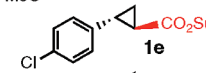
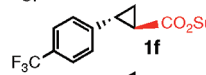
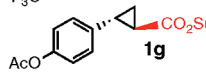
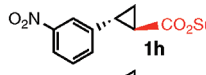
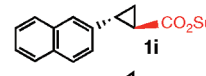
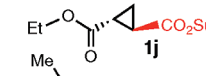
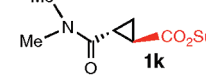
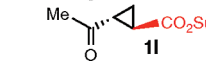
entry	[Co(Por*)][b]	additive	solvent	yield[c,i] (%)	trans:cis[d]	ee[e] (%)
1	[Co(**P1**)]	DMAP	C_6H_5Me	86	>99:1	92
2	[Co(**P2**)]	DMAP	C_6H_5Me	70	>99:1	96
3	[Co(**P3**)]	DMAP	C_6H_5Me	10	>99:1	63
4	[Co(**P4**)]	DMAP	C_6H_5Me	0		
5	[Co(**P5**)]	DMAP	C_6H_5Me	0		
6	[Co(**P6**)]	DMAP	C_6H_5Me	0		
7f	[Co(**P1**)]	DMAP	C_6H_5Me	74	>99:1	91
8[f]	[Co(**P1**)]	NMI	C_6H_5Me	85	>99:1	88
9[f]	[Co(**P1**)]		C_6H_5Me	86	>99:1	88
10[f,g]	[Co(**P1**)]	DMAP	C_6H_5Me	66	>99:1	91
11[f,h]	[Co(**P1**)]	DMAP	C_6H_5Me	64	>99:1	91
12[f]	[Co(**P1**)]	DMAP	C_6H_5Cl	67	>99:1	87

[a] Performed at rt for 48 h using 5 mol % of [Co(Por*)] under N_2 with 1.0 equiv of styrene and 1.5 equiv of N_2CHCO_2Su in the presence of 0.5 equiv of additive; [styrene] = 0.25 M. [b] See Figures 1 and S1 (Supporting Information) for structures. [c] Isolated yields. [d] Determined by HPLC. [e] Trans isomer ee determined by chiral HPLC. [f] 1.2 equiv of N_2CHCO_2Suc. [g] 24 h. [h] 2 mol % of [Co(**P1**)]. [i] Similar olefin conversions with no side reactions.

reactions were observed with the more sterically demanding catalysts [Co(**P4**)], [Co(**P5**)], and [Co(**P6**)] (entries 4−6). Furthermore, the yields of the desired cyclopropane **1a** by the less steric catalysts [Co(**P1**)], [Co(**P2**)], and [Co(**P3**)] were correlated well with the relative hindrance of the ligand environment (entries 1−3). For these reactions, outstanding diastereoselectivities were achieved, with *trans*-**1a** produced as the sole diastereomer. While the best ee was attained by [Co(**P2**)], the use of [Co(**P1**)] afforded the best yield in addition to high enantioselectivity. Reduction of the N_2CHCO_2Su from 1.5 to 1.2 equiv gave similarly high diastereo- and enantioselectivity for the [Co(**P1**)]-catalyzed reaction but resulted in decreased yields (entries 1 and 7). As demonstrated previously,[14b] a more positive *trans* effect of DMAP on enantioselectivity was observed (entries 7−9). Although selectivity was not affected, by lowering catalyst loading or reducing reaction time, decrease in the overall product yield was observed (entries 10 and 11). Finally, toluene seemed to be the solvent of choice as the use of other solvents such as chlorobenzene led to lower yields and decreased enantioselectivities (entry 12).

Under the optimized reaction conditions, different olefin substrates were subject to catalytic cyclopropanation using N_2CHCO_2Su. As shown with select examples (Table 2), both electron-sufficient and electron-deficient olefins could be successfully cyclopropanated by [Co(**P1**)]. For example, asymmetric cyclopropanation of styrene derivatives bearing various substituents, including alkyl and halide groups as well as electron-donating and -withdrawing groups, could be catalyzed by [Co(**P1**)] to form the corresponding cyclopropanes **1a**−**f** in good to high yields with outstanding diastereoselectivities and excellent enantioselectivities (entries 1, 3, 5, 7, 9, and 11). Further

Table 2. [Co(P1)]-Catalyzed Diastereo- and Enantioselective Cyclopropanation of Different Alkenes with N_2CHCO_2Su[a]

entry	cyclopropane	yield (%)[b,h]	trans:cis[c]	ee (%)[d]	[α][e]
1	 **1a**	86	>99:1	92	(−)
2[f]		70	>99:1	96	(−)
3	 **1b**	90	>99:1	95	(−)[g]
4[f]		71	98:2	96	(−)[g]
5	 **1c**	80	>99:1	97	(−)
6[f]		81	>99:1	98	(−)
7	 **1d**	71	>99:1	95	(−)
8[f]		75	99:1	97	(−)
9	 **1e**	66	>99:1	90	(−)
10[f]		48	>99:1	92	(−)
11	 **1f**	77	>99:1	90	(−)
12[f]		30	>99:1	94	(−)
13	 **1g**	71	>99:1	91	(−)
14	 **1h**	50	>99:1	92	(−)
15	 **1i**	33	99:1	91	(−)
16	 **1j**	57	>99:1	89	(−)
17	 **1k**	52	>99:1	96	(−)
18	 **1l**	55	>99:1	91	(−)

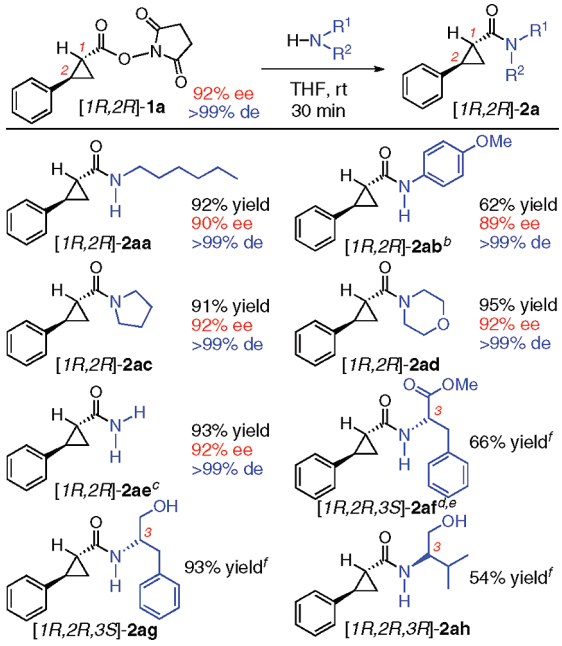
[a] Performed at rt for 48 h using 5 mol % of [Co(**P1**)] under N_2 with 1.0 equiv of styrene and 1.5 equiv of N_2CHCO_2Su in the presence of 0.5 equiv of DMAP; [styrene] = 0.25 M. [b] Isolated yields. [c] Trans:cis ratio determined by NMR or HPLC. [d] Trans isomer ee determined by chiral HPLC. [e] Sign of optical rotation. [f] [Co(**P2**)] as catalyst. [g] [1*R*,2*R*] absolute configuration by X-ray crystal structural analysis and optical rotation. [h] Similar olefin conversions with no side reactions.

improvement in enantioselectivity was achieved uniformly for all these substrates when the relatively bulkier [Co(**P2**)] was employed as the catalyst, albeit in lower yields for most of the cases (entries 2, 4, 6, 8, 10, and 12). In addition, the Co-based catalytic process exhibited functional group tolerance as demonstrated with the reactions of acetoxy- and nitro-substituted styrenes to form **1g**,**h** (entries 13 and 14). Due to the steric bulkiness of N_2CHCO_2Su, the catalytic system was shown to be less efficient for large aromatic olefins as exemplified by the [Co(**P1**)]-catalyzed cyclopropanation reaction of 2-vinylnaphthalene, offering **1i** in 33% yield with 98% de and 91% ee (entry 15). In addition to aromatic olefins, the [Co(**P1**)]/N_2CHCO_2Su-based system could also selectively cyclopropanate challenging electron-deficient olefins such as α,β-unsaturated esters, amides, and ketones (entries 16−18). It is worth noting that the cyclopropanes prepared from these olefins (**1j**,**l**) are highly electrophilic in nature

and have proven to be valuable synthetic intermediates for a variety of applications.[15]

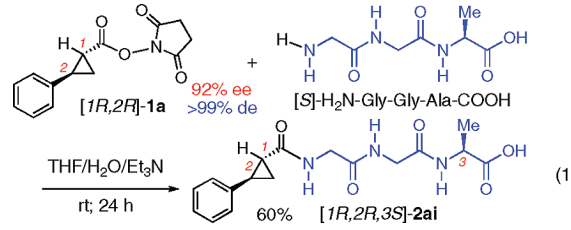
With the established availability of enantioenriched succinimidyl cyclopropyl carboxylate derivatives **1** through the [Co(**P1**)]-catalyzed asymmetric cyclopropanation with N_2CHCO_2Su, their potential application as chiral building blocks for the synthesis of cyclopropyl carboxamides **2** (Scheme 1) was subsequently explored. Using (1*R*,2*R*)-**1a** as a representative synthon, a range of different amines were examined for the postderivatization synthetic approach (Scheme 2). Both aliphatic and aromatic amines

Scheme 2. Post-Derivatization Approach for Synthesis of Chiral Cyclopropyl Carboxamides via Reaction with Different Amines[a]

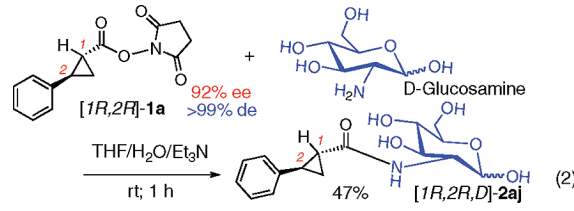


[a] Isolated yields; de determined by NMR or HPLC; ee determined by chiral HPLC [b] 24 h. [c] In dioxane. [d] 1 h. [e] In THF/H_2O with Et_3N. [f] Isolated as single diastereomer.

reacted with **1a** smoothly, affording the desired cyclopropyl carboxamides **2a** with retention of configuration (**2aa** and **2ab**). Cyclic amines, such as pyrrolidine and morpholine, could also be effectively converted to the corresponding amides in high yields with complete preservation of the stereochemistry (**2ac** and **2ad**). *The transformation of* **1a** into the corresponding primary amide using ammonia also occurred in a high yield without loss of diastereo- and enantioselectivity (**2ae**). Owing to the mild and neutral reaction conditions, the postderivatization approach was able to tolerate a number of different functional groups as exemplified by the reactions with chiral α-amino acids such as methyl (*S*)-phenylalaninate as well as chiral β-amino alcohols such as (*S*)-phenylalaninol and (*R*)-valinol (**2af**, **2ag**, and **2ah**). The resulting multifunctional cyclopropyl amides **2af**, **2ag**, and **2ah**, bearing three stereogenic centers, could be isolated as single diastereomers in good to excellent yields.



To further demonstrate the utility of this synthetic approach, (1*R*,2*R*)-**1a** was allowed to react with the unprotected tripeptide (*S*)-H_2N-Gly-Gly-Ala-COOH at room temperature in a mixture of water and THF in the presence of Et_3N (eq 1). The corresponding cyclopropyl tripeptide (1*R*,2*R*,3*S*)-**2ai** was isolated as single diastereomer in 60% yield without affecting the carboxylic acid functionality.



The versatility and functional group tolerance of the synthetic approach was further highlighted with the reaction of (1*R*,2*R*)-**1a** with D-(+)-glucosamine without protecting the hydroxyl groups (eq 2). The reaction proceeded smoothly under mild conditions, forming the desired cyclopropyl carboxamide of the amino sugar (1*R*,2*R*,*D*)-**2aj** in 47% yield.

In summary, a highly diastereo- and enantioselective Co-catalyzed asymmetric cyclopropanation of alkenes with N_2CHCO_2Su has been established for the first time, and the resulting enantioenriched succinimidyl cyclopropylcarboxylates have proven to be valuable synthons for general synthesis of optically active cyclopropyl carboxamide derivatives. The key attributes of the post-derivatization approach include versatility and a high degree of functional group tolerance. Together with the suitability of various olefins for the asymmetric cyclopropanation process, this two-step synthetic scheme should permit straightforward access to a wide range of chiral cyclopropyl carboxamides.[8]

Acknowledgment. We are grateful to USF (Startup Funds), ACS-PRF (AC Grant), NSF (CAREER Award), and NSF (CRIF: MU-0443611, Mass Facility) for financial support.

Supporting Information Available: Experimental procedures and analytical data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Scholarship—Book Chapters

6 Metalloporphyrin-Catalyzed Asymmetric Atom/Group Transfer Reactions

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I. Introduction

Metalloporphyrins have been shown to catalyze many fundamental and practically important chemical transformations in biological systems and in the chemical laboratory. The most notable examples have been accomplished using metalloporphyrin complexes inspired by biology. These complexes have been used to perform atom/group transfer reactions including oxene, nitrene, and carbene transfers, which in turn have provided chemists with direct access to complex and functionally diverse compounds from abundant and inexpensive alkenes and alkanes.

In an effort to improve the catalytic ability and specificity of these 'synthetic enzymes', researchers have focused on the synthesis of 'designed' complexes that are based on well-defined biological/chemical scaffolds. These complexes, which incorporate chiral peripheries into the metalloporphyrin framework, have provided researchers with better control over the reaction system. Three methodologies for synthesizing these derivatives have been developed: (1) porphyrins formed through the classical condensation of chiral aldehydes with pyrrole; (2) porphyrins formed through substitution of amino- and hydroxyl-substituted tetraphenylporphyrins with chiral building blocks; and (3) bridging of the enantiotropic faces of prochiral porphyrins to afford a chiral environment. The porphyrins prepared by these three methods have provided important information regarding the type of chiral environment required to induce enantioselectivity when used as catalysts. The trends indicate that the more rigid the chiral environment, the greater the catalytic stability and the slower the intramolecular decomposition. This in turn provides for a longer catalytic lifetime and higher turnover numbers (TON). On the other hand, more flexible substituents are more vulnerable to degradation, such as oxidation of the porphyrin periphery, and produce products with decreased enantioselectivity.

Another trend observed concerns the proximity of the chiral units to the active center. Chiral units that are far away provide less chiral induction as the approach of the incoming substrate is not substantially affected by the peripheral chiral environment. On the other hand, chiral units that are too close to the metal center often suffer from decomposition as the chiral environment itself becomes the substrate and is then decomposed.

Chiral proximity also plays a role in the enantioselectivity observed with substrates of different sizes and shapes, although the specific requirements are not always as well defined or easy to define. This constraint has led some research groups to incorporate a modular design with steric environments that can be systematically modified to best suit each type of substrate.

The modification and design of porphyrin ligands to create chiral environments has led to a diverse array of catalysts. To categorize these chiral ligands, we

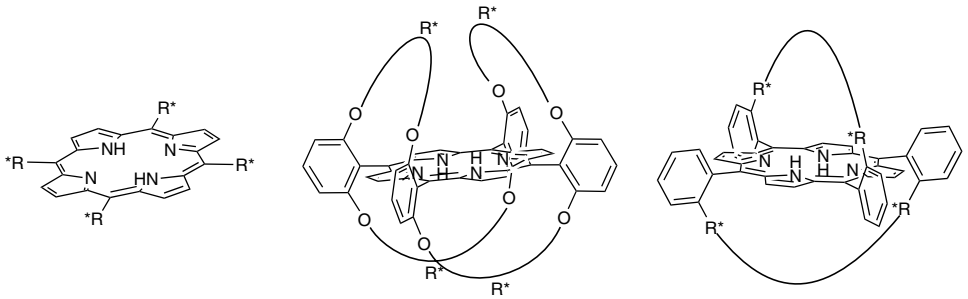


Figure 1. Examples of "picket fence", "strapped", and "basket handle" type porphyrin scaffolds.[1]

will use the classification system outlined by Collman and co-workers in 1993.[1] In this system, chiral porphyrins (Por*) are categorized by class: "picket fence", "strapped", and "basket handle" (Figure 1).

The picket fence class contains porphyrin ligands with chiral units (R*) around the perimeter of the porphyrin ring, but without connections between adjacent or opposite functionalities. Chiral groups can be connected directly to the *meso*- or *beta*-positions or attached to the *meso*-aryl positions in a wide range of orientations.

The strapped and basket handle classes are categorized by the positions of the chiral units bridged around the porphyrin perimeter above and below the porphyrin plane. The major difference between the two classes is the orientation of the chiral bridge. Chiral bridges that connect adjacent positions (e.g., the 5,10-positions) are considered strapped. Chiral bridges connecting opposite positions (e.g., the 10,20-positions) are categorized as basket handle.

This chapter will review the major advancements in asymmetric atom/group transfer reactions utilizing metalloporphyrin catalysts, specifically the asymmetric epoxidation, cyclopropanation, and aziridination of alkenes. Several reports of various forms of C–H functionalization will also be discussed. These works will be organized by ligand class and the literature covers the period through July 2009.

II. Epoxidation

Early work on epoxidation was developed out of studies on cytochrome P-450, a heme-containing monooxygenase enzyme, which catalyzes the incorporation of oxygen from molecular dioxygen into organic substrates.[2] Simple mimics based on iron, manganese, chromium, and ruthenium metalloporphyrin frameworks

have been developed to convert alkenes, alkanes, and aromatic hydrocarbons into valuable commodity chemicals in a controlled and selective fashion.[3] A variety of oxidants have been employed for these systems; including molecular dioxygen, hydroperoxides, peroxyacids, NaOCl, N_2O, and iodosylbenzene (PhIO) and its derivatives.[3] These oxidants have been used to produce high-valent metal intermediates in an effort to replace existing auto-oxidation technologies[4] allowing for stereocontrol of the products.

In 1976, Ullrich and co-workers demonstrated that cytochrome P-450 could catalyze oxygenation reactions through an alternate pathway known as the "peroxide shunt".[5] This pathway allows for the use of exogenous oxygen sources such as PhIO which opened the door to the exploration of alternative oxidants. The mechanistic aspects of these processes will not be covered in this chapter, as they have been extensively addressed by others.[6–8]

Following Ulrich, Groves, and co-workers showed that a number of single-oxygen donors, including hydroperoxides, peroxy acids, and PhIO could be used with metalloporphyrin enzyme mimics to perform oxygen transfer in a manner similar to the fully reconstituted enzyme system.[9,10] For example, the combination of 5,10,15,20-tetraphenylporphinatoiron(III) chloride [Fe(TPP)Cl], and PhIO was used to transfer oxygen to alkenes and alkanes to form epoxides and hydroxides, respectively. Groves and co-workers established that there were dramatic differences in both yield and selectivity between porphyrins with peripheral variations, suggesting that the ligand environment was as intimately involved in the oxygen transfer step as the transition metal ion. Their work provided a foundation for tuning the selectivity and reactivity of metalloporphyrin catalysts through alteration of the porphyrin periphery.[11] Subsequent work by Lindsay-Smith and co-workers illustrated the importance of having a sterically bulky porphyrin periphery to interrupt μ-oxo dimer formation while leaving the metal center open to the approach of the olefin into the active site.[12]

Although the capability of these metalloporphyrins to mimic the oxo-transferase ability of cytochrome P-450 using nonbiological oxidants was impressive, initial catalysts suffered from rapid decomposition of the macrocycle, particularly for porphyrins with open *meso*-positions.[3] Groves and co-workers typically reported TON for epoxidation and hydroxylation of approximately 10 or less, prompting research into the production of catalysts with greater stability.[13] As a result, electron-withdrawing groups were incorporated on to the macrocycle to increase the stability of the porphyrin. Continued efforts by Chang and Ebina[14] and then later by Traylor and co-workers[15] eventually produced metalloporphyrin catalysts that were capable of effectively epoxidizing alkenes in up to 10,000 TON.

Additional insight for improving metalloporphyrin catalysis was provided by studies on metallosalens, close relatives of porphyrins. The salen architecture

offers the advantage of two chiral sp^3-hybridized carbons at the periphery within close proximity to the active metal center.[1] Salens have been shown to be highly effective catalysts for asymmetric epoxidation. In addition to the use of iodosylarenes, the effective use of more practical, inexpensive, oxidants such as sodium hypochlorite (bleach) and molecular dioxygen has made these catalysts especially appealing.[16–27]

Several salens capable of providing greater than 90% enantiomeric excess of *cis*-substituted alkenes have been reported in the literature.[18,28] The major drawback of these complexes is based on the susceptibility of the catalyst to oxidative decomposition. As a consequence, metallosalens typically have low TON (<40). However, the degradation products are generally inactive, thus the products of the reaction remain uncompromised.[1]

The development of a catalytic asymmetric epoxidation system that could match the selectivities of the reported salen systems with the stability and high TON typically reported for metalloporphyrin-catalyzed epoxidation systems would combine the best attributes of both systems to produce the "ideal" epoxidation catalyst.

This section will cover metalloporphyrin-catalyzed epoxidation reactions and, more specifically, investigations into the enantioselectivity of the products generated by these catalysts with a detailed overview of the major contributions to this field.

A. Chiral Picket Fence Porphyrins

Groves and Myers published the first metalloporphyrin-catalyzed asymmetric epoxidation system in 1983.[10] Utilizing a similar scaffold developed by Collman and co-workers for investigating the binding of molecular oxygen to ferrous porphyrinates,[29] Groves and Myers synthesized an $\alpha,\beta,\alpha,\beta$-atropisomer with chiral moieties directed toward the iron metal center (Figure 2). The resulting chiral iron porphyrin contained identical chiral faces, each consisting of two chiral groups. They evaluated the ability of this picket fence porphyrin **1** to epoxidize styrene using PhIO and found that styrene oxide was produced in 65% yield with a moderate enantioselectivity of 31% ee (entry 1, Table 1). The $\alpha,\alpha,\beta,\beta$-isomer **2** and the $\alpha,\alpha,\alpha,\alpha$-$\mu$-oxo-dimer **3** generated racemic products when evaluated under the same conditions (entries 2–3, Table 1).

These results prompted the use of a binaphthyl chiral appendage using the same porphyrin scaffold (Figure 3) in the hopes of generating a relatively large and rigid chiral cavity around the iron center. The resulting chiral porphyrin **4** was evaluated for epoxidation using iodosylmesitylene as the oxidant and as little as 0.625 mol % catalyst loading. The epoxidation of styrene, along with other olefins, proceeded in moderate yields (58–75%) and enantioselectivity (15–51% ee) (Table 2). However,

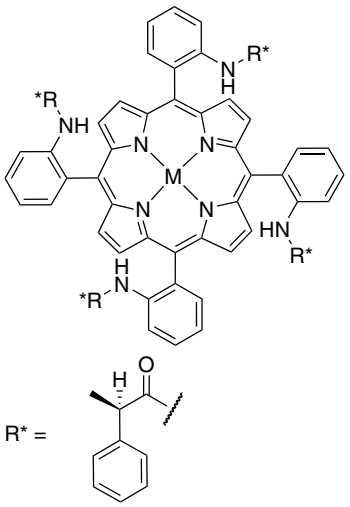
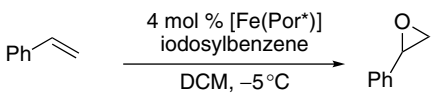


Figure 2. [Fe(HydPP)Cl].[10]

Table 1. Asymmetric epoxidation catalyzed by atropic isomers of [Fe(HydPP)Cl].[10]



Entry[a]	[Fe(Por*)][b]	Yield (%)[c]	ee (%)
1	[Fe(**1**)Cl]	65	31(*R*)
2	[Fe(**2**)Cl]	99	0
3	[Fe(**3**)]$_2$O	93	0

[a] Reactions were performed using 4 mol % of [Fe(Por*)] with 1 equivalent of PhIO as oxidant and 3 equivalents of styrene in DCM. Over a period of 3 hours the oxidant was added at an average temperature of –5°C and was stirred for an additional 45 mins; [b] See Figure 2; [c] Based upon consumed PhIO.

Groves and Myers noted the activity of the metalloporphyrin diminished after 100 catalytic cycles. Presumably, the oxidation of the porphyrin led to unfavorable changes in either the conformation or the electronic properties of the catalyst.

Several years after Groves and Myers seminal work on metalloporphyrin-catalyzed epoxidation, Paolesse and co-workers synthesized chiral porphyrin **5** containing camphanylamido groups on the *ortho*-positions of the *meso*-aryl

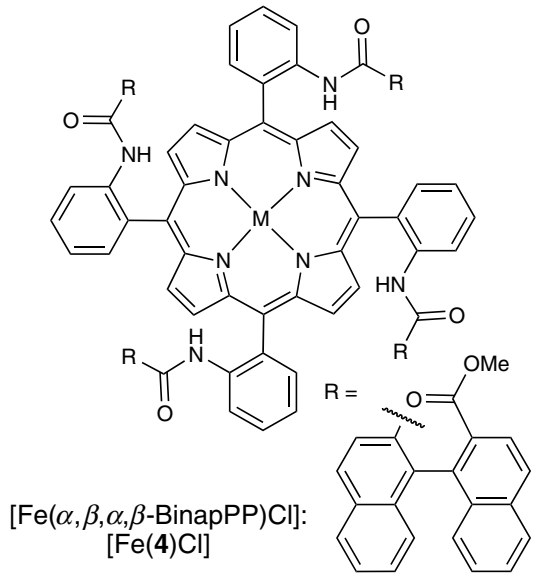
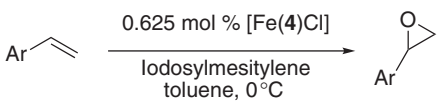


Figure 3. [Fe(**4**)Cl] picket fence catalyst.[10]

Table 2. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**4**)Cl].[10]

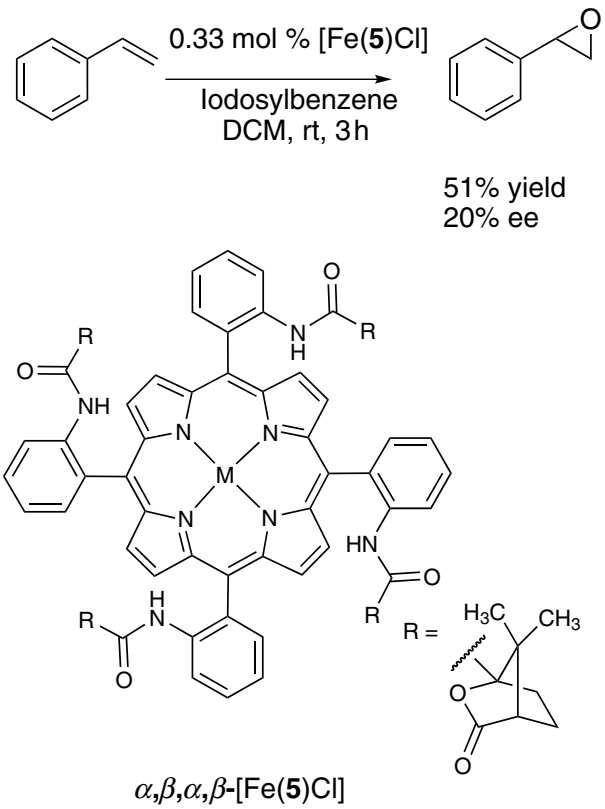


Entry[a]	Substrate	Yield (%)[b]	ee (%)
1	Styrene	67	48 (*R*)(+)
2[c]	4-Chlorostyrene	63	51 (+)
3	4-Methylstyrene	75	43 (+)
4	4-Nitrostyrene	—	36 (+)
5	2-Methylstyrene	58	15 (–)
6	2-Vinylnaphthalene	—	36 (+)

[a] Reactions were performed in a manner similar to that those described in Table 1; [b] Based upon consumed iodosylmesitylene; [c] Performed at –23°C.

groups (Scheme 1).[30] The subsequent catalyst [Fe(**5**)Cl] was used to generate styrene oxide from styrene (5 equivalents) in moderate yields and 20% ee at room temperature in the presence of PhIO.

The binaphthyl group was also used as the chiral moiety by O'Malley and Kodadek in an effort to address catalytic stability.[31] Using the manganese complex of what they termed the "Chiral Wall" (**6**, Figure 4), the epoxidation of styrene



Scheme 1.[30]

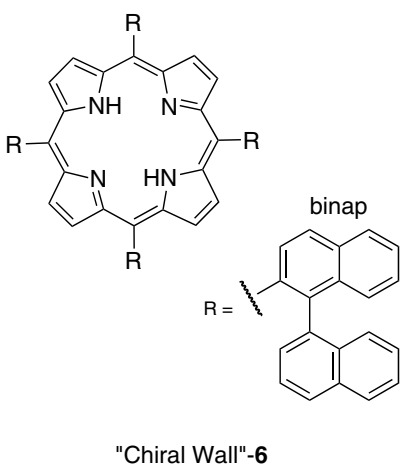
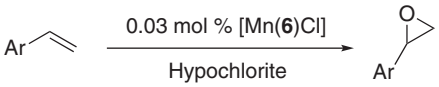


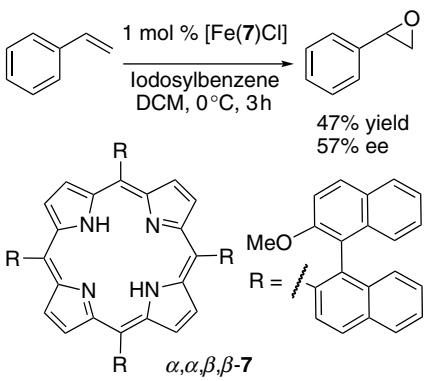
Figure 4. "Chiral wall" porphyrin.[31]

Table 3. Asymmetric epoxidation of aromatic olefins catalyzed by [Mn(**6**)Cl].[31]



Entry[a]	Substrate	Catalytic efficiency[b]	ee (%)
1	Styrene	240	20
2	4-Chlorostyrene	160	20
3	2-Vinylnaphthalene	220	20
4	*trans*-β-Methylstyrene	190	15
5[c]	*cis*-β-Methylstyrene	200	40

[a] Reactions were performed with 0.03 mol % of catalyst with hypochlorite as oxidant; [b] Number of turnovers in 15 min; [c] 7:1 (*cis:trans*).



Scheme 2.[32]

derivatives were accomplished with moderate enantioselectivity (15–40% ee) using hypochlorite and only 0.03 mol % of catalyst to produce the corresponding styrene oxides (Table 3). "Chiral wall" porphyrin **6** was also shown to be quite stable, inducing the same enantioselectivities after several hundred catalytic turnovers (Table 3).

Several years after Kodadek's first report of the "chiral wall" catalyst, Salvadori and co-workers published results based on iron and manganese complexes of a similar ligand **7** (Scheme 2) for epoxidation.[32] The atropisomers of porphyrin **7** were prepared and evaluated for their ability to epoxidize styrene in an asymmetric fashion. Overall, the iron complex of the $\alpha,\alpha,\beta,\beta$-atropisomer [Fe(**7**)Cl] was found to provide the highest asymmetric induction (Scheme 2). It is also worth noting the differences in selectivities produced when different metals were

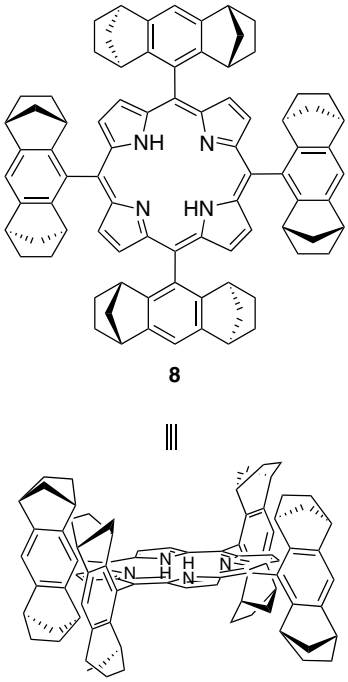


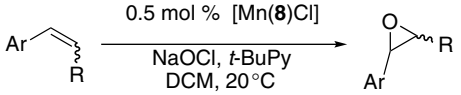
Figure 5. Halterman's D_4-symmetric porphyrin.[33]

used with $\alpha,\beta,\alpha,\beta$-porphyrin **7**: the resulting enantioselectivities of the manganese and iron complexes were 8 and 48% ee, respectively.

In 1991, Halterman and Jan published the synthesis and evaluation of a D_4-symmetric porphyrin **8** (Figure 5).[33] The Halterman porphyrin **8** has become the most investigated porphyrin-based catalyst not only for epoxidation but also for other atom/group transfer reactions that will be discussed later in this chapter. Halterman and co-workers initially reported the use of [Mn(**8**)Cl] with 4-*tert*-butylpyridine as an axial ligand for the epoxidation of styrene derivatives with Clorox® bleach.[34] Under these reaction conditions, high yields and moderate to good enantioselectivities were observed within hours (Table 4). Halterman and co-workers also reported the synthesis and evaluation of several derivatives of **8**. However, the reactivity and selectivity of these systems were either comparable or lower than that of the manganese complex of **8**.[35]

One of the remarkable features of this reaction is the use of excess oxidant without adverse side effects. In most oxidation systems, the oxidant is used as the limiting reagent and a large excess of substrate is used to consume the oxidant quickly to avoid poisoning the catalyst. Using commercially available Clorox® bleach, this catalyst performed well when a 3%-sodium hypochlorite

Table 4. Asymmetric epoxidation of aromatic alkenes catalyzed by [Mn(**8**)Cl].[34]



Entry[a]	Substrate	Time (h)	Yield (%)	ee (%)
1	Styrene	1	90	52 (*S*)
2	1,2-Dihydronaphthalene	1	97	56 (1*R*,2*S*)
3	Indene	1	98	41 (1*R*,2*S*)
4	*cis*-β-Methylstyrene	4	91 (*cis*)	76 (1*R*,2*S*)
			7 (*trans*)	34 (1*S*,2*S*)
5	*trans*-β-Methylstyrene	8	40	4 (1*R*,2*R*)
6	*trans*-β-Phenylstyrene	8	73	10 (1*S*,2*S*)
7	α-Methylstyrene	1	98	6 (*R*)

[a] Reactions were performed with 0.5 mol % of catalyst with 0.5 mol % of *t*-BuPy and 1 equiv. of substrate in a mixture of bleach (2.5 ml) and DCM (2 ml).

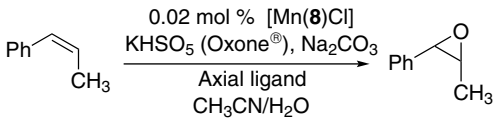
aqueous/DCM medium was employed in the presence of as little as 0.05 mol % of catalyst loading.

The use of the Mn(III) chloride complex of the Halterman porphyrin **8** was further studied by Chang and co-workers using Oxone® ($KHSO_5$) and *cis*-β-methylstyrene.[36] In their work, various axial ligands were employed to evaluate potential *trans* effect on the yield and selectivity of the catalytic epoxidation reaction (Table 5). Although not as effective as Halterman's original catalytic system (entry 4, Table 4), a significant increase in enantiomeric excess was observed when DMAP was employed as the axial ligand (entry 13, Table 5).

Epoxidation employing [Ru(**8**)$(O)_2$] was first investigated by Che and co-workers for the stoichiometric epoxidation of styrene.[37] Moderate yields and enantioselectivities were obtained using styrene as the substrate (Scheme 3). The authors also reported the epoxidation reaction under catalytic conditions with dioxygen as the oxidant (8 atm), generating the desired product with low TON (=10) and moderate enantioselectivity (70% ee) (Scheme 3). The authors commented that higher TON and enantioselectivity should be accessible with the optimization of these reaction conditions.

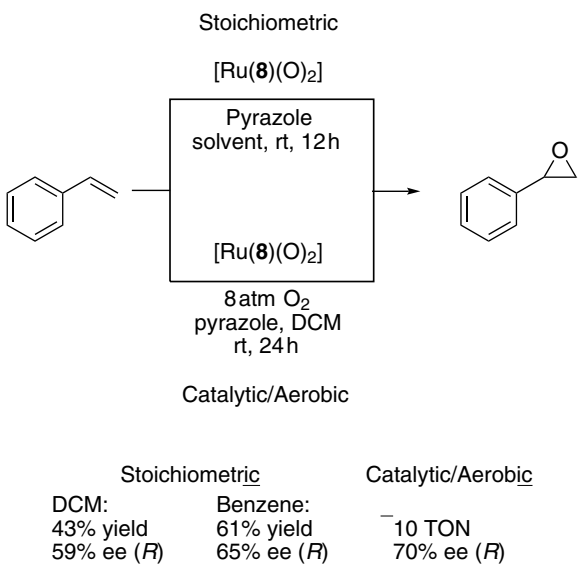
In a separate report, Che and co-workers explored the effects of solvent on the catalytic process with [Ru(**8**)(CO)].[38] The epoxidation of styrene derivatives in either benzene or dichloromethane using [Ru(**8**)(CO)] and PhIO was found to have little effect on the enantioselectivity of the catalytic process (entries 1 and 2, Table 6). Che and co-workers went on to evaluate both

Table 5. Effect of axial ligand on the asymmetric epoxidation of *cis-β*-methylstyrene catalyzed by [Mn(**8**)Cl].[36]



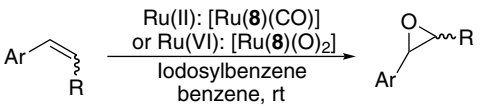
Entry[a]	Axial ligand	Conversion (%)	*cisl trans*	ee (%, cis)
1	—	60	21.3	43
2	Quinoline	24	18.9	25
3	3-Bromopyridine	24	29.6	29
4	4-Chloropyridine	28	10.6	31
5	4-Cyanopyridine	22	17.3	37
6	Pyridine	6	8.0	38
7	4-Phenylpyridine	50	14.0	61
8	4-*tert*-Butylpyridine	18	12.0	65
9	4-Methoxypyridine	30	12.8	69
10	Piperidine	7	25.2	71
11	1,2-Dimethylimidazole	24	10.3	72
12	Pyrrolidine	12	18.2	75
13	DMAP	79	6.4	81

[a] Procedure for oxidation: Oxone® (1 equiv.) and $NaHCO_3$ were dissolved in deionized water, followed by the substrate (1 equiv.), 0.1 mol % of axial ligand, and 0.02 mol % of catalyst in acetonitrile. The mixture was stirred overnight.



Scheme 3.[37]

Table 6. Asymmetric epoxidation of aromatic alkenes catalyzed by [Ru(**8**)(CO)] and [Ru(**8**)$(O)_2$].[38]



Entry	Substrate	Catalyst	Yield (%)	ee (%)
1	Styrene	Ru(II)	57	63 (*R*)
2	Styrene	Ru(II)[a]	71	55 (*R*)
3	Styrene	Ru(VI)[a]	52	51 (*R*)
4	4-Methylstyrene	Ru(II)	35	40
5	4-Methylstyrene	Ru(VI)[a]	41	38
6	4-Chlorostyrene	Ru(II)	66	51
7	3-Nitrostyrene	Ru(II)	40	52
8	*cis*-β-Methylstyrene	Ru(II)	52[b]	52 (1*R*,2*S*)
9	*cis*-β-Methylstyrene	Ru(VI)[a]	53[c]	55 (1*R*,2*S*)
10	1,2-Dihydronaphthalene	Ru(II)	46	62
11	*trans*-β-Methylstyrene	Ru(II)	41	17 *trans*
12	*trans*-β-Methylstyrene	Ru(VI)[a]	31	13 *trans*
13	Cyclohexenylbenzene	Ru(II)[a]	55	8
14	Cyclohexenylbenzene	Ru(VI)[a]	61	7

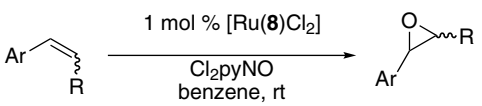
[a] Performed in DCM; [b] *cis:trans* = 6.3; [c] *cis:trans* = 11.

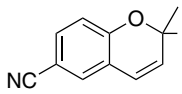
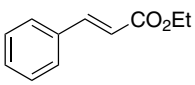
[Ru(**8**)(CO)] and [Ru(**8**)$(O)_2$] as catalysts for the epoxidation of styrene derivatives (Table 6). The [Ru(**8**)(CO)] was found to be slightly more selective than [Ru(**8**)$(O)_2$] (entries 2 and 3, Table 6), although this trend was not observed with all styrene derivatives.

In their continued effort to develop ruthenium complexes of the Halterman porphyrin **8**, Che and co-workers published the synthesis and evaluation of [Ru(**8**)Cl_2] in 2001.[39] For these reactions, 2,6-dichloropyridine-*N*-oxide (Cl_2pyNO) was used as oxidant with 1 mol % catalyst in benzene to generate moderate yields and enantioselectivities with high TON, typically greater than 800 (Table 7). This system was shown to be capable of catalyzing a wide range of substrates, including mono- and di-substituted olefins, having various steric and electronic properties (Table 7). To evaluate the stability of this catalytic system, [Ru(**8**)Cl_2] was reused in four consecutive runs, and it was observed that product conversion diminished with each consecutive run. Through characterization and the analysis of the catalyst after the reaction, it was determined that a new Ru(CO) complex, generated *in situ*, was suspected to be responsible for the decreased efficiency of the catalyst.

Another strategy employed to increase catalytic turnover around the same time was the incorporation of the ruthenium porphyrin complex into sol-gel to

Table 7. Asymmetric epoxidation of olefins catalyzed by [Ru(**8**)Cl_2].[39]



Entry[a]	Substrate	Time (h)	Yield (%)[b]	TON	ee (%)
1	Styrene	1.5	84 (100)	875	69
2	4-Chlorostyrene	4	88 (100)	930	65
3	α-Methylstyrene	5	80 (60)	790	24
4	*cis*-β-Methylstyrene	3	99 (100)	990	68
5	1,2-Dihydronaphthalene	3	78 (100)	890	80
6		2	85 (90)	860	67
7		16	95 (18)	220	16

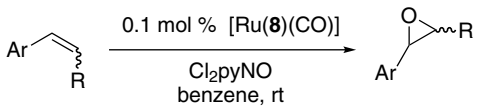
[a] Reactions were performed using 1 mol % of catalyst, 1 equiv. of substrate, and 1.1 equiv of oxidant in benzene at room temperature; [b] Yields are based upon the amount of alkene consumed in the reaction. The value in parenthesis represents conversion of substrate.

produce a heterogeneous matrix. Remarkably, the use of these immobilized catalysts not only increased the TON to more than 10,000 in some cases, but the observed selectivity was comparable to the homogenous catalytic system. This approach was further expanded in a later report by Che and co-workers through the use of mesoporous silica gel, generating TON in excess of 13,000 with comparable enantioselectivities.[40]

The [Ru(**8**)(CO)] complex was evaluated by Berkessel and Frauenkrom for the epoxidation of olefins with 2,6-dichloropyridine *N*-oxide.[41] [Ru(**8**)(CO)] proved to be an effective catalyst under the reported conditions, providing good to moderate yields and moderate enantioselectivities for the epoxidation of dihydronapthalene and styrene derivatives. As shown in Table 8, aliphatic and more hindered olefins were ineffective substrates for catalysis using this system.

Berkessel and co-workers published catalytic studies of ruthenium complexes of a variety of Halterman porphyrin derivatives in 2003.[42] Building on the complexes previously reported by Halterman's group,[43] they modified the *para*-position of the *meso*-aryl group by introducing various groups (Figure 6). The use of electron-withdrawing trifluoromethyl group afforded a very effective catalyst when Cl_2pyNO was employed. This modification led to improved yields and enantioselectivities with a respectable turnover frequency (Table 9). Berkessel speculated that the electron-withdrawing trifluoromethyl group disfavored the oxidative poisoning/destruction of the catalyst by stabilizing the macrocycle.

Table 8. Asymmetric epoxidation of olefins catalyzed by [Ru(**8**)CO].[41]



Entry[a]	Substrate	Conv. (%)	Yield (%)	ee (%)
1	1,2-Dihydronaphthalene	90	85	71
2[b]	1,2-Dihydronaphthalene	90	85	77
3	Styrene	100	79	70
4	Indene	65	55	54
5	1-Hexene	6	5	28
6	*trans*-Stilbene	6	5	0

[a] Reaction were performed using 0.1 mol % of catalyst with 1 equiv. of substrate and 1.1 equiv of oxidant in benzene at room temperature for 48 h; [b] 1 mol % catalyst loading used.

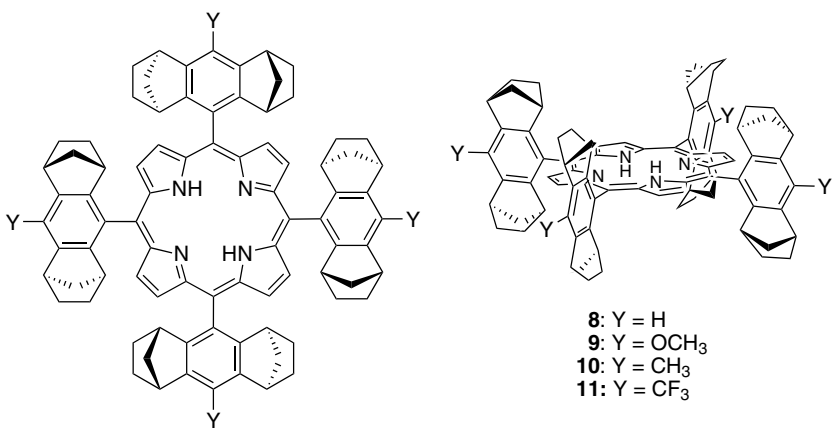
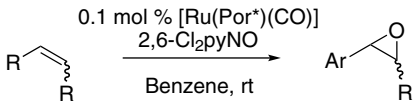


Figure 6. Halterman porphyrin **8** and its derivatives.[42]

Che and co-workers followed up on their previous catalytic work on ruthenium Halterman complexes by developing a molybdenum complex of **8**.[44] [Mo(**8**)(O)(OMe)] was used with *tert*-butylhydrogen peroxide (TBHP) for the epoxidation of aromatic alkenes. Although this molybdenum catalyst was fairly unique, the results as a whole provided low TON (=4) and poor enantioselectivity (16% ee) (Scheme 4).

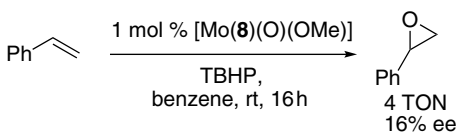
In 1997, Kodadek and co-workers reported the synthesis and catalytic activity of a new derivative of the D_4-symmetric Halterman porphyrin, **12** (Figure 7).[45] The manganese complex of **12** was used for the asymmetric epoxidation of a variety of olefins, including aliphatic olefins (Table 10). Moderate enantioselectivities and high TON were reported.

Table 9. Asymmetric epoxidation of olefins catalyzed by [Ru(**8**–**11**)(CO)].[42]



Entry[a]	Substrate	Por*[b]	Time (h)	Yield (%)	TOF (h^{-1})	ee (%)
1	Styrene	*ent*-**9**	2.5	82	328	76
2	Styrene	*ent*-**10**	2.5	78	312	76
3	Styrene	**11**	2.5	97	388	79
4	Dihydronaphthalene	**8**	48.0	85	—	71
5	Dihydronaphthalene	*ent*-**9**	7.5	66	88	80
6	Dihydronaphthalene	*ent*-**10**	7.5	70	94	78
7	Dihydronaphthalene	**11**	7.5	89	118	83
8	1-Hexene	**8**	45.0	10	2	21
9	1-Hexene	*ent*-**9**	45.0	8	2	36
10	1-Hexene	**10**	45.0	9	2	22
11	1-Hexene	**11**	45.0	16	4	18

[a] Reactions were performed with 0.1 mol % of catalyst, 1 equiv. of substrate, and 1.1 equiv. of oxidant in benzene at room temperature; [b] See Figure 6 for structure of porphyrin ligand.



Scheme 4.[44]

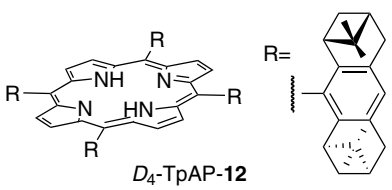
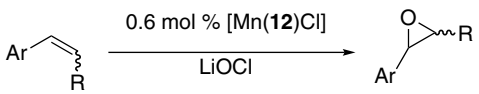


Figure 7. Kodadek's halterman-like D_4-symmetric porphyrin.[45]

Higuchi and co-workers developed a D_4-symmetric chiral porphyrin **13** (Figure 8) related to Halterman porphyrin **8**.[46] The Fe(III) bromide complex of chiral porphyrin **13** was prepared and evaluated for the epoxidation of various aromatic olefins in the presence of PhIO. The results from this investigation are shown in Table 11. While the yields and enantiomeric excess are generally moderate, the selectivity ranged from 8% ee for *trans*-β-methylstyrene to 78% ee for

Table 10. Asymmetric epoxidation of olefins catalyzed by [Mn(**12**)Cl].[45]



Entry[a]	Substrate	Yield (%)	TON	ee (%)
1	Styrene	70	2520	70
2	2-Vinylnaphthalene	46	2918	69
3	Indene	91	2110	24
4	*cis*-β-Methylstyrene	33	957	24
5	2,3,3-Trimethylpent-1-ene	nd	15000	85
6	3,3-Dimethylbut-1-ene	nd	130000	47

[a] Performed at 0°C with 0.6 mol % of catalyst, 1 equiv. of substrate, in a bi-phasic solution of solvent and oxidant.

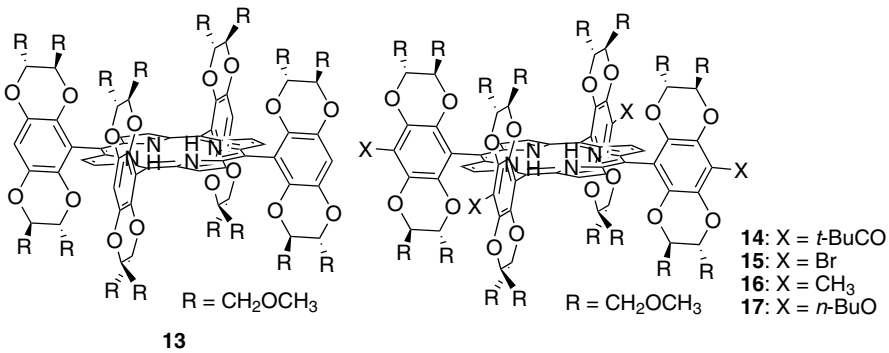
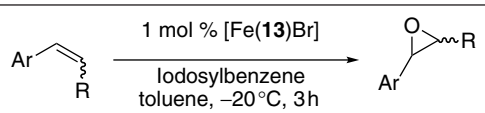


Figure 8. Higuchi's D_4-symmetric chiral porphyrins.[47]

Table 11. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**13**)Br].[46]



Entry[a]	Substrate	Yield (%)	TON	ee (%)
1	Styrene	68	68	47 (*S*)
2[b]	Styrene	64	638	45 (*S*)
3	1,2-Dihydronapthalene	73	73	31 (1*R*,2*S*)
4	2-Vinylnaphthalene	66	66	38 (*S*)
5	*cis*-β-Methylstyrene	70	70	8 (1*S*,2*R*)
6	*trans*-β-Methylstyrene	45	45	42 (1*S*,2*S*)
7	4-Fluorostyrene	59	59	52
8	3-nitrostyrene	62	62	78

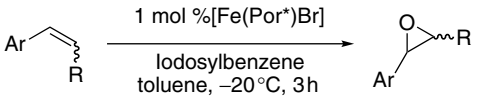
[a] Performed at –20°C for 3 h with 1 mol % of [Fe(**13**)Br], 1 equiv. of oxidant, 10 equiv. of substrate; [b] Performed with 60 equiv. of substrate.

3-nitrostyrene (Table 11). As expected, when the substrate was increased from 10 equivalents to 60 equivalents, it was shown that the TON increased nearly tenfold from 68 to 638 for the epoxidation of styrene. The authors attributed this to decreased poisoning of the catalyst by the oxidant.

The increase in enantioselectivity obtained for the epoxidation of electron-deficient styrene derivatives using the D_4-symmetric iron porphyrin complex [Fe(**13**)Br] led Higuchi and co-workers to further examine the electronic properties of this catalyst.[47] They were able to synthesize derivatives of **13** in which the *para*-position of the *meso*-aryl group was substituted with electron-donating and —withdrawing groups (Figure 8) to determine the effect of substitution on asymmetric induction. As presented in Table 12, no significant differences in enantioselectivity were observed; however, a slight trend of increased enantioselectivity was noted with the incorporation of electron-withdrawing substituents on the porphyrin.

Momenteau and co-workers developed a protocol for the preparation of iron and manganese glycosylated porphyrins (Figure 9) as catalysts for the epoxidation of olefins.[48] The atropisomers of these porphyrins were prepared and used to epoxidize 4-chlorostyrene in the presence of PhIO. Moderate yields and enantioselectivities

Table 12. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**13**–**17**)Br].[47]



Entry[a]	Substrate	[Fe(Por*)][c]	Yield (%)	ee (%)
1	Styrene	**14**	72	42 (*S*)
2	Styrene	**15**	65	44 (*S*)
3	Styrene	**13**	68	47 (*S*)
4	Styrene	**16**	65	49 (*S*)
5	Styrene	**17**	66	52 (*S*)
6	*trans*-β-Methylstyrene[b]	**14**	48	39 (1*S*,2*S*)
7	*trans*-β-Methylstyrene[b]	**15**	43	40 (1*S*,2*S*)
8	*trans*-β-Methylstyrene[b]	**13**	45	42 (1*S*,2*S*)
9	*trans*-β-Methylstyrene[b]	**16**	46	44 (1*S*,2*S*)
10	*trans*-β-Methylstyrene[b]	**17**	49	45 (1*S*,2*S*)
11	3-Nitrostyrene	**14**	68	71
12	3-Nitrostyrene	**15**	70	74
13	3-Nitrostyrene	**13**	61	78
14	3-Nitrostyrene	**16**	70	78
15	3-Nitrostyrene	**17**	63	79

[a] Performed at –20°C for 3 h with 1 mol % of [Fe(Por*)Br], 1 equiv. of oxidant, 10 equiv. of substrate; [b] The *cis*-epoxide was also obtained ion 2–3% yield and 8–10% ee (1*S*,2*R*); [c] See Figure 8 for structures.

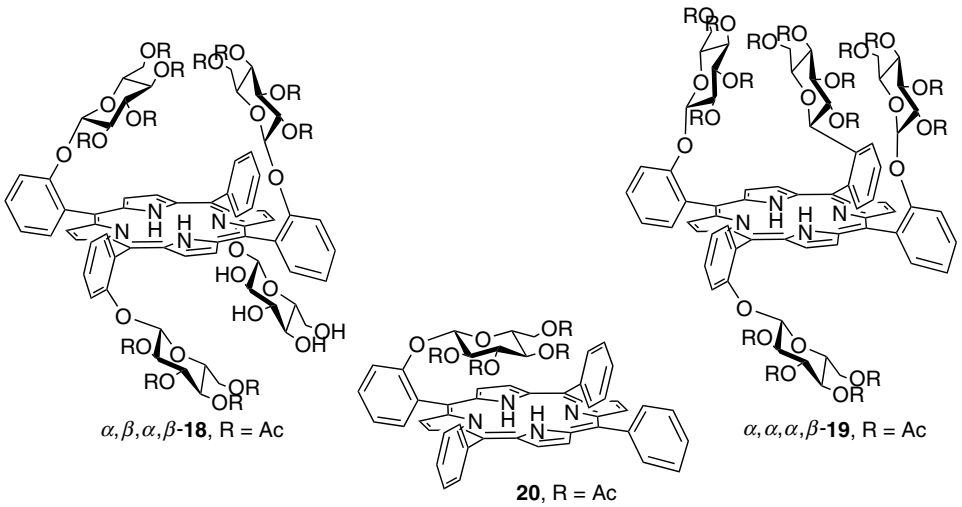
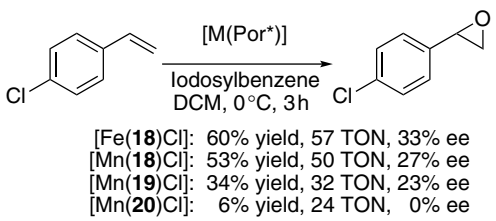


Figure 9. Glycosylated porphyrins.[48]



Scheme 5.[48]

were reported using the Fe(III) chloride complex of the $\alpha,\beta,\alpha,\beta$-atropisomer which was shown to be the most effective catalyst (Scheme 5).

In a later report, Momenteau focused on 2-chloro, 6-glycosylated manganese porphyrins (Figure 10).[49] These glycosylated porphyrins were assessed using 4-chlorostyrene as the olefin substrate as in their previous work. Both PhIO and H_2O_2 were shown to be suitable for catalysis with [Mn(**21**–**23**)Cl] (Table 13). Although the manganese system was not as effective as the [Fe(**18**)Cl]-based system (Scheme 5) with respect to yield or enantioselectivity, the catalyst proved to be relatively more robust, yielding slightly higher TON.

Momenteau and co-workers continued to develop new glycosylated porphyrins *via* the incorporation of larger sugar units onto the *meta*-position of the *meso*-aryl group, as shown in Figure 11.[50] Manganese complexes of these porphyrins, along with those presented earlier, were employed as catalyst to determine the efficacy

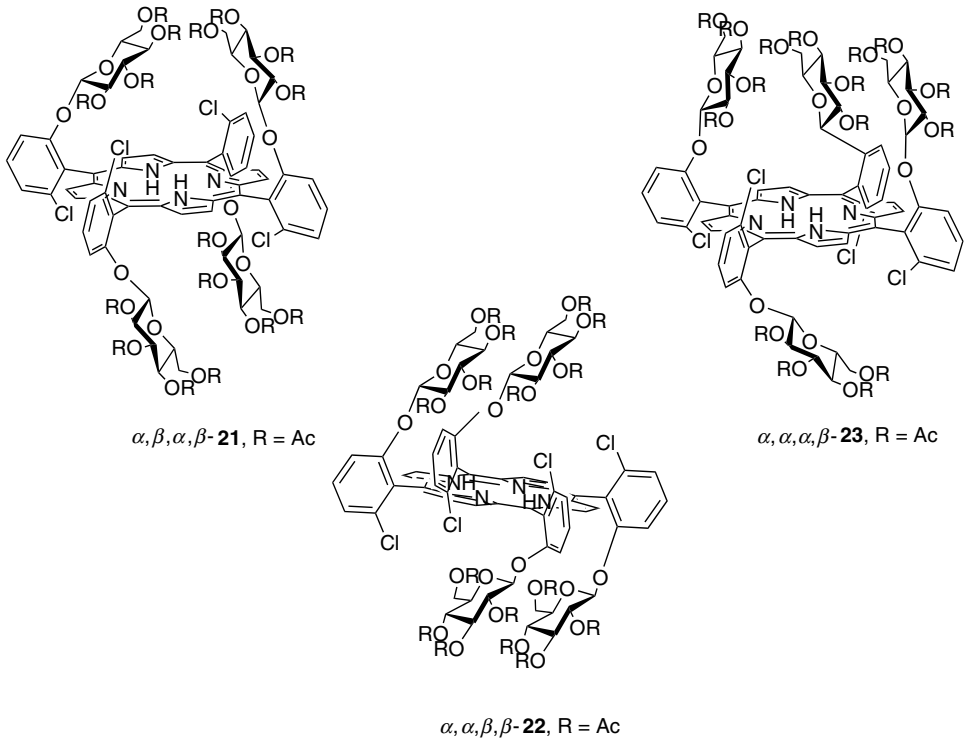
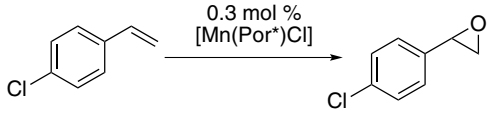


Figure 10. Atropisomers of glycoconjugated porphyrins containing electron-withdrawing groups.[49]

Table 13. Evaluation of glycoconjugated porphyrins **21–23** for the asymmetric epoxidation of 4-chlorostyrene with two different oxidants.[49]



Entry	Oxidant	[Mn(Por*)][c]	Yield (%)	TON	ee (%)
1	PhIO[a]	**21**	25	49	20
2	PhIO[a]	**22**	26	49	19
3	PhIO[a]	**23**	45	57	16
4	H_2O_2[b]	**21**	24	64	23
5	H_2O_2[b]	**22**	31	62	22
6	H_2O_2[b]	**23**	29	58	22

[a] Performed at room temperature for 1 h with 1 equiv. of oxidant, 1.5 equiv. of 4-picoline, and 2.7 equiv. of substrate in DCM; [b] Performed at room temperature for 4 h with 1 equiv. of substrate, 4 equiv. oxidant, 2 equiv. of *t*-Bupy, and 2 equiv. of benzoic acid in DCM; [c] See Figure 10 for structures.

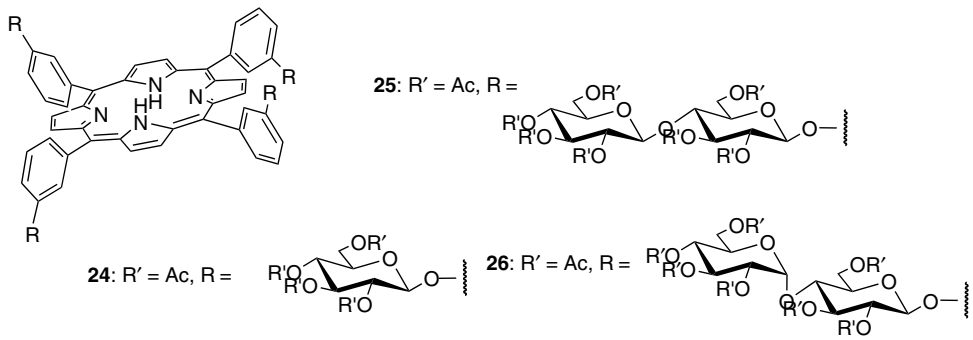
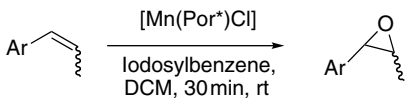


Figure 11. Glycoconjugated porphyrins with chiral substituents at the *meta*-position.[50]

Table 14. Asymmetric epoxidation of aromatic olefins catalyzed by [Mn(**21**–**26**)Cl].[50]



Entry[a]	Substrate	Por*[b]	Yield (%)	ee (%)
1	4-Chlorostyrene	**21**	5	24
2	4-Chlorostyrene	**22**	49	20
3	4-Chlorostyrene	**23**	42	29
4	4-Chlorostyrene	**24**	36	0
5	4-Chlorostyrene	**25**	33	0
6	4-Chlorostyrene	**26**	31	3
7	1,2-Dihydronaphthalene	**25**	62	8

[a] Performed for 30 min at room temperature with ~0.1 mol % catalyst 1.0 equiv. of oxidant, 2.5 equiv. of substrate, and 1.5 equiv. of 4-methylpyridine; [b] See Figures 10 and 11 for structures.

of each complex in catalyzing the epoxidation of 4-chlorostyrene in the presence PhIO. The results, shown in Table 14, demonstrate that the sugar residues need to be in closer proximity to the metal center to induce asymmetry.

"Chiroporphyrins" **27**, developed by Marchon and co-workers, are porphyrins containing chiral cyclopropyl ester and amide moieties attached directly to the *meso*-positions of the porphyrin in an $\alpha,\beta,\alpha,\beta$-arrangement (Figure 12).[51] The manganese complexes of **27** were evaluated for their catalytic ability to epoxidize 1,2-dihydronaphthalene in the presence of the axial ligand pyridine.

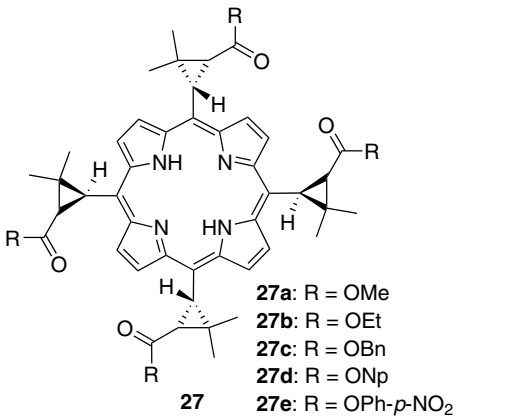


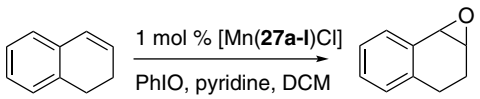
Figure 12. "Chiroporphyrins".[51]

The results revealed that when the *meso*-positions were functionalized with large cyclopropylamide groups, the catalyst afforded the corresponding epoxides with the highest enantioselectivities (Table 15).

With the success of their first generation of "chiroporphyrins", Marchon and co-workers envisioned using electron-withdrawing groups to enhance the activity of the catalytic system.[52] Their efforts led to the preparation of second generation "chiroporphyrins" with pentafluorophenyl groups occupying the 5,15-*meso*-positions of the porphyrin ring, and chiral cyclopropyl groups occupying the remaining 10,20-*meso*-positions (Figure 13). In most cases, these porphyrins were able to completely epoxidize the substrate in an hour or less (Scheme 6). Unfortunately, the enantioselectivities were generally lower in comparison to the first generation "chiroporphyrin" system.

Smith and Reginato developed chiral picket fence porphyrins (A_4-**31**, A_3B-**32**, A_2B_2-**33**, Figure 14) containing chiral ether moieties on the *ortho*-positions of the *meso*-aryl groups of the porphyrins.[53] The Fe(III) chloride complexes of A_4-**31** and A_2B_2-**33** were both evaluated for the epoxidation of styrene with PhIO in dichloromethane. The use of the more bulky [Fe(A_4-**31**)Cl] resulted in lower yields, as expected; the use of the [Fe(A_2B_2-**33**)Cl] provided a quantitative yield with similar enantioselectivity as compared to the A_4-complex **31** (Scheme 7). For the [Fe(A_2B_2-**33**)Cl]-catalyzed reaction, it was shown that lowering the temperature

Table 15. Asymmetric epoxidation of 1,2-dihydronaphthalene catalyzed by [Mn(**27**)Cl].[51]



Entry[a]	R =	Catalyst	ee (%)
1	OMe	[Mn(**27a**)Cl]	60
2	OEt	[Mn(**27b**)Cl]	70
3	OBn	[Mn(**27c**)Cl]	65
4	ONp	[Mn(**27d**)Cl]	73
5	OPh-*p*-NO_2	[Mn(**27e**)Cl]	68
6	OPh-*m*-NO_2	[Mn(**27f**)Cl]	75
7	*O*-(1S)-endo-bornyl	[Mn(**27g**)Cl]	75
8	O^{t}Bu	[Mn(**27h**)Cl]	75
9	N(Me)Ph	[Mn(**27i**)Cl]	80
10	N(Et)Ph	[Mn(**27j**)Cl]	86
11	N(Cy)C(O)NH(Cy)	[Mn(**27k**)Cl]	83
12	NH(Cy)	[Mn(**27l**)Cl]	79

[a] Reactions performed for 1 h with 1 mol % catalyst, 1.0 equiv. of oxidant, 2.5 equiv. of pyridine, and 10 equiv. of olefin.

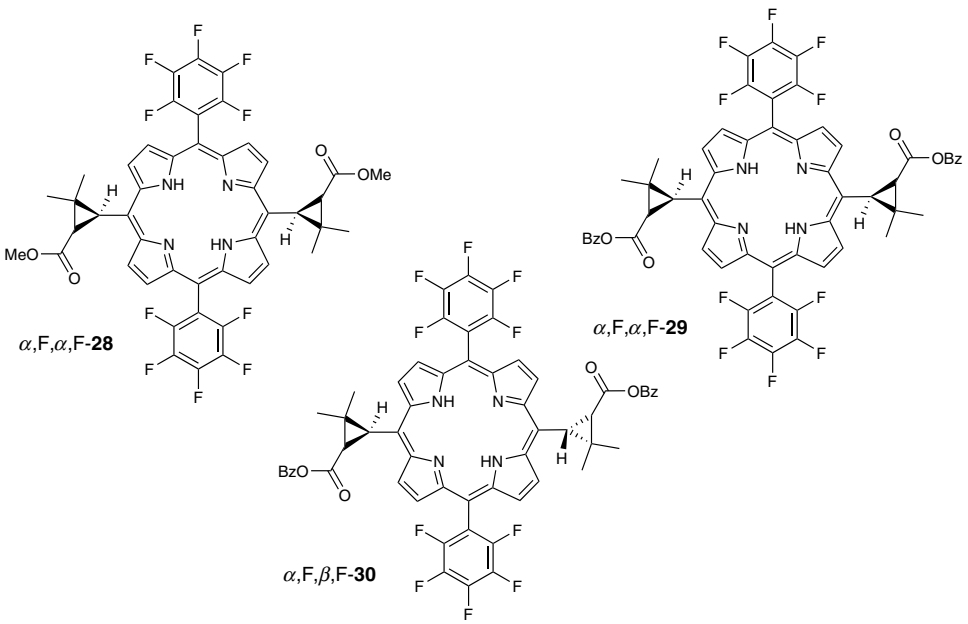
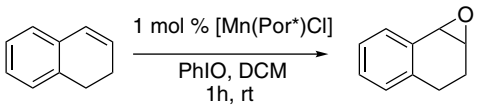


Figure 13. "Chiroporphyrins" with pentafluorophenyl groups.[52]



[Mn(α,F,α,FMeCP)Cl] (**28**): 88% yield, 43% ee
[Mn(α,F,α,FBzCP)Cl] (**29**): 96% yield, 35% ee
[Mn(α,F,β,F)BzCP)Cl] (**30**): 99% yield, 31% ee

Scheme 6.[52]

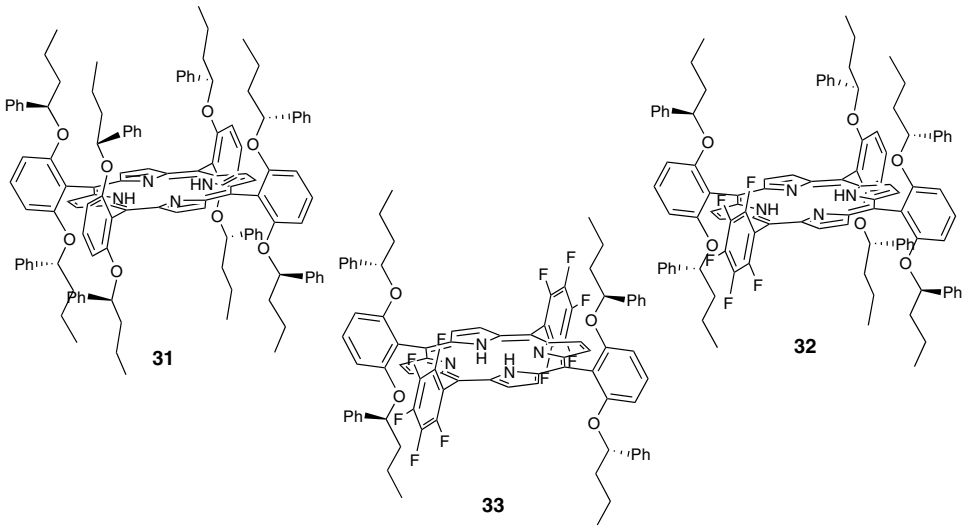
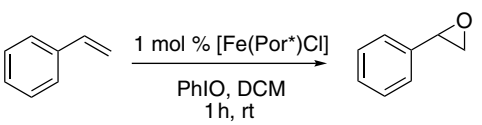


Figure 14. Chiral picket fence porphyrin-bearing chiral ether groups attached to the *ortho*-aryl position.[53]



31: 48% yield, 16% ee
33: 91% yield, 16% ee
33, at 0°C: 99% yield, 23% ee

Scheme 7.[53]

to 0°C increased the enantioselectivity slightly without sacrificing yield. Although the porphyrin catalysts designed for this particular study were only moderately effective in inducing enantioselectivity, the results provided "proof of concept" that rigid and bulky chiral groups could be used to produce more effective catalysts.

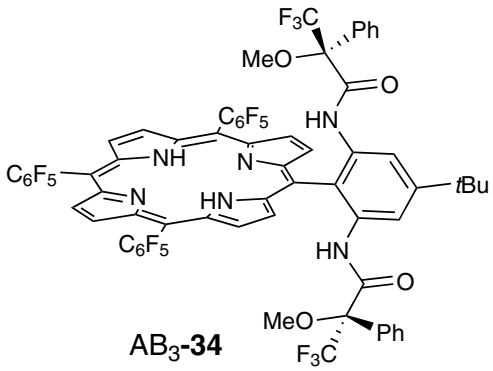
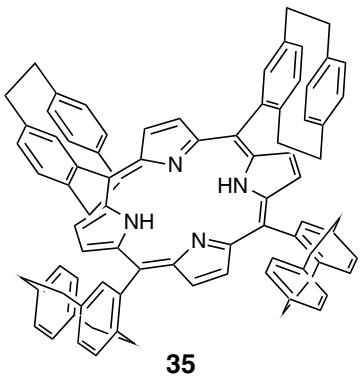


Figure 15. Chiral picket fence porphyrin derived from Mosher's chiral amide.[55]

Prior to Smith and Reginato's work with chiral porphyrin ligands **31–33**, Rose and co-workers prepared a series of chiral porphyrins using Mosher's amide as the chiral moiety on the *ortho*-positions of the *meso*-aryl ring.[54] Among the different complexes examined for catalytic epoxidation, the Fe(III) bromide complex of AB_3-**34**, shown in Figure 15, gave the highest enantiomeric excess, albeit very low (6% ee).[55]

The research groups of Hevesi and Quici also developed porphyrins with chiral moieties on the *meso*-positions.[56,57] Although catalysts **35** and **36** (Figure 16) produced moderate yields and low enantioselectivities for epoxidation of styrene (Scheme 8), [Mn(**36**)Cl] exhibited respectable TON (=195) for the reaction.

In an effort to mimic the chiral environment of the cytochrome P-450 enzyme, Ohkatsu and co-workers attached peptide chains to the *meso*-positions of the porphyrins with a modular approach. This allowed them to tune the steric and electronic properties of the ligand environment to enhance asymmetric induction.[58] Epoxidation reactions of styrene with PhIO were initially performed using porphyrin derivatives containing three peptide chains (Figure 17). It was shown that varying the nature of the amino acid residues resulted in changes in yield and selectivity. For example, when γ-benzyl-L-glutamate (γ-BLG) was used in place of L-phenylalanine (L-Phe), the yield increased 15%; however, enantioselectivity decreased dramatically from 60% ee to 11% ee (Scheme 9). It was noted that lengthening the average peptide chain (n) decreased the yield of the epoxidation reaction as a result of increased hindrance. Several other biomimetic porphyrins with various side chains and compositions were also prepared. However, they provided no significant improvement in yield or enantioselectivity.



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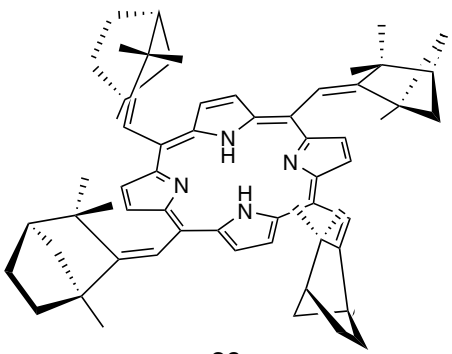
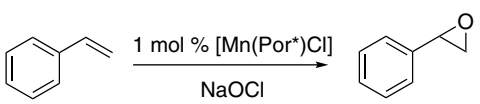


36

Figure 16. Chiral picket fence porphyrins.[56,57]



35: 78% yield, 10% ee
36: 65% yield, 25% ee

Scheme 8.[56,57]

B. Chiral Strapped Porphyrins

Naruta and co-workers developed one of the first examples of chiral strapped porphyrins incorporating binaphthyl groups for use in asymmetric epoxidation (Figure 18).[59,60] The binaphthyl group, which was used to bridge adjacent *meso*-aryl groups through an ether linkage, effectively blocked one face of the catalytic site thus establishing a sterically bulky chiral environment near the metal center. The use of the binaphthyl bridge is similar to the work discussed previously by

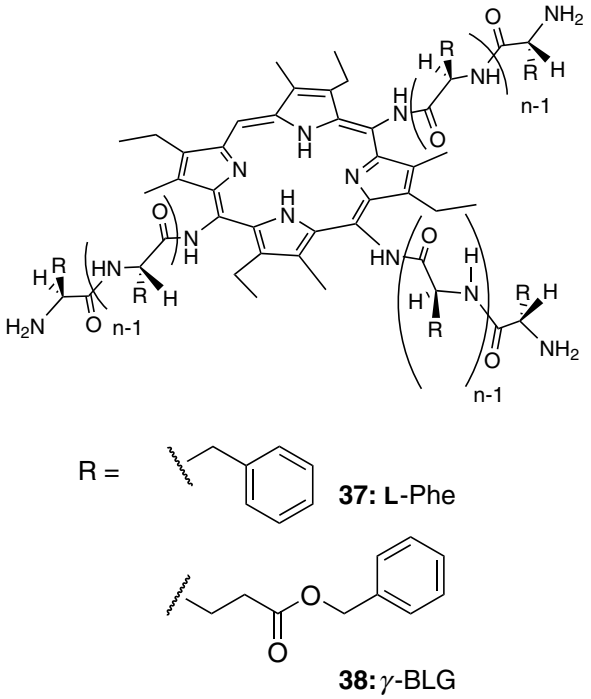
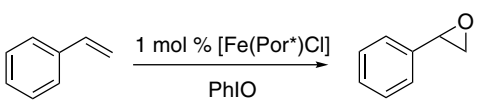


Figure 17. Peptide-bearing chiral picket fence porphyrins.[58]



37: L-Phe-3PCs: n = 4.5, 45% yield, 60% ee
38: γ-BLG-3PCs: n = 4.6, 60% yield, 11% ee

n = average peptide chain length

Scheme 9.[58]

Groves and Myers[10] in 1983. These porphyrins, known as "twin-coronet" porphyrins, are classified by the arrangement of the "straps", which can be in either an eclipsed or staggered orientation, resulting in the generation of two unique ligand environments (Figure 18).

The difference in selectivity resulting from the two different conformations are showcased by the epoxidation of 2-nitrostyrene (Scheme 10). Based on an initial screening, the eclipsed [Fe(**39**)Cl] was further evaluated for the epoxidation of styrenes with PhIO (Table 16). Good enantioselectivities were observed for

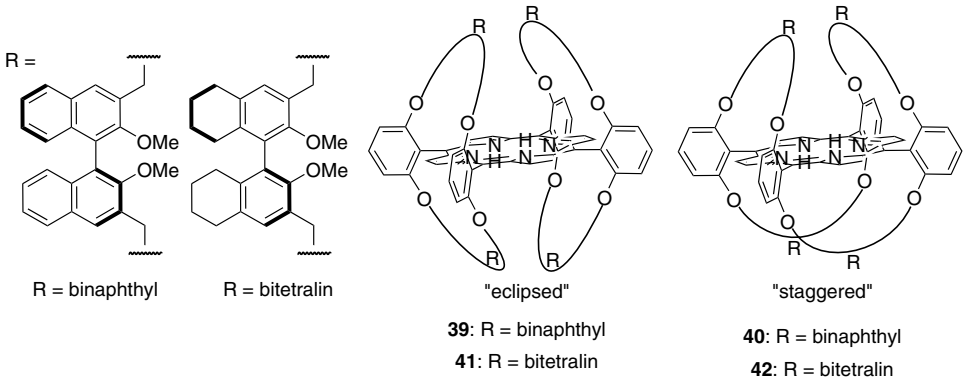
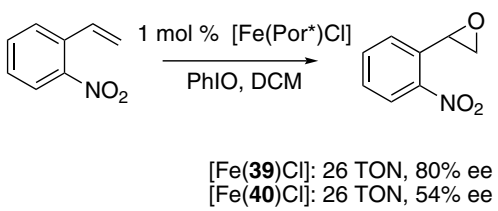
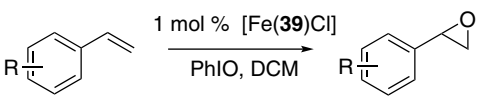


Figure 18. Binaphthyl and bitetralin "twin-coronet" porphyrins.[59–63]



Scheme 10.[59,60]

Table 16. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**39**)Cl].[59,60]



Entry[a]	R =	Time (h)	TON	ee (%)
1	H	3.5	49	20 (*R*)
2	2-NO_2	3	26	80 (*R*)
3	3-NO_2	2	57	60 (*R*)
4	4-NO_2	2	38	54 (*R*)
5	3,5-NO_2	2	20	74 (*R*)
6	F_5	2	36	74 (*R*)
7	4-CF_3	3	42	41 (*R*)
8	4-CH_3	5	48	11 (*R*)
9[b]	4-OCH_3	1.5	0	—

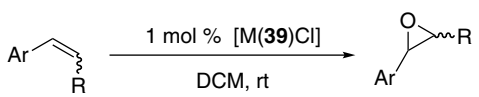
[a]The reactions were performed with 1 mol % of catalyst, 1 equiv. of PhIO, and 5 equiv. of substrate in DCM; [b]Major product was aldehyde. Epoxidation product was not observed.

electron-poor styrene derivatives (entries 2–7, Table 16). Conversely, the more electron-rich substrates displayed lower enantioselectivities (entries 8 and 9, Table 16).

Naruta and co-workers continued their investigations with iron binaphthyl strapped porphyrin [Fe(**39**)Cl] and compared it to [Mn(**39**)Cl] for the epoxidation of styrene and its electron-deficient derivatives.[61] Differences in reactivity and selectivity between the manganese and iron complexes were observed for the epoxidation of *cis*-*β*-methylstyrene (entries 8–9, Table 17). It was shown that enantioselectivities could be marginally improved in select cases by the use of axial ligands and the electron-deficient oxidant iodosyl (pentafluorobenzene), C_6F_5IO.

Naruta and co-workers continued to develop "twin-coronet" porphyrins by producing bitetralin-strapped variation **41** and **42** (Figure 18).[60,62,63] The iron complexes both the eclipsed **41** and staggered **42** forms were evaluated for the epoxidation of styrene with PhIO. The activity of the bitetralin-strapped version of the "twin-coronet" porphyrin correlated well with the binaphthyl-strapped version (Table 18). Additionally, the eclipsed and staggered Fe(III)Cl complexes of **41** and **42** were evaluated for the epoxidation of styrenes with PhIO and again the eclipsed version **41** was shown to be a better catalyst (entries 1 and 2, Table 18). The improved enantioselectivity generated with electron-poor styrene derivatives is highlighted by the 96% ee obtained with the use of 3,5-dinitrostyrene as substrate (entry 6, Table 18).

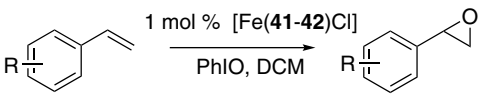
Table 17. Asymmetric epoxidation of aromatic olefins catalyzed by either [Fe(**39**)Cl] or [Mn(**39**)Cl].[61]



Entry[a]	Substrate	[M(39)Cl]	Oxidant	Axial ligand	Time (h)	TON	ee (%)
1	Styrene	[Fe(**39**)Cl]	PhIO	—	3.5	50	22 (*S*)
2	Styrene	[Mn(**39**)Cl]	PhIO	—	9	67	16 (*S*)
3	Styrene	[Mn(**39**)Cl]	C_6F_5IO	—	3	62	0
4[b]	Styrene	[Mn(**39**)Cl]	C_6F_5IO	AcPIm	6	67	23 (*S*)
5	2-Nitrostyrene	[Fe(**39**)Cl]	PhIO	—	3	26	80 (*S*)
6	2-Nitrostyrene	[Mn(**39**)Cl]	PhIO	—	9	22	66 (*S*)
7	*cis*-*β*-Methylstyrene	[Fe(**39**)Cl]	PhIO	—	2	38	19 (1*S*,2*R*)
8	*cis*-*β*-Methylstyrene	[Mn(**39**)Cl]	PhIO	—	4	28	61 (1*S*,2*R*)
9	*cis*-*β*-Methylstyrene	[Mn(**39**)Cl]	C_6F_5IO	—	1	23	47 (1*S*,2*R*)
10[b]	*cis*-*β*-Methylstyrene	[Mn(**39**)Cl]	C_6F_5IO	AcPIm	3	9	70 (1*S*,2*R*)

[a] Reactions were performed with 1 mol % of catalyst, 1 equiv. of oxidant, and 5 equiv. of substrate in DCM; [b] 5 mol % of AcPIm (4-imidazol-1-yl)acetophenone); [c] –20°C.

Table 18. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**41**–**42**)Cl].[62,63]



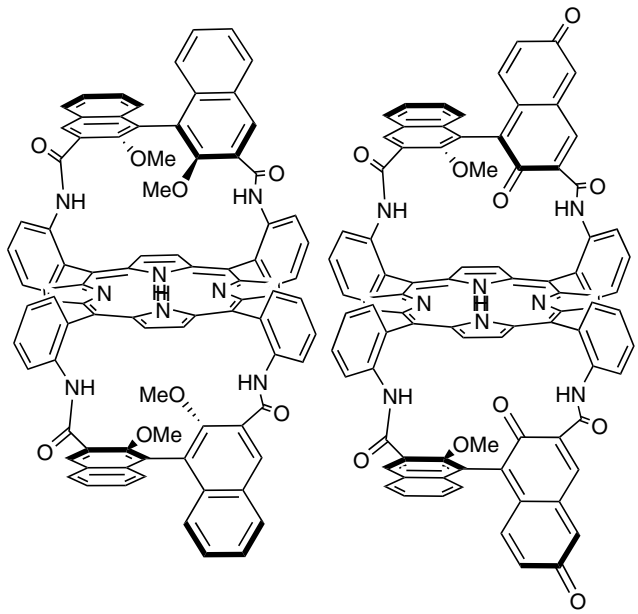
Entry[a]	R =	Catalyst	Time (h)	TON	ee (%)
1	H	**41**	8	84	54 (*S*)
2	H	**42**	7	44	28 (*S*)
3	2-NO_2	**41**	3	17	89 (*S*)
4	3-NO_2	**41**	2	26	74 (*S*)
5	4-NO_2	**41**	3	21	67 (*S*)
6	3,5-NO_2	**41**	4	36	96 (*S*)
7	F_5	**41**	3	10	83 (*S*)
8	4-Br	**41**	3	31	51 (*S*)
9	4-CH_3	**41**	3	30	27 (*S*)
10	3-OCH_3	**41**	2	33	73 (*S*)

[a] The reactions were performed with 1 mol % of catalyst, 1 equiv. of PhIO, and 5 equiv. of substrate in DCM; [b] See Figure 18 for structures.

Collman, Rose, and co-workers developed porphyrins analogous to Naruta's binaphthyl-strapped "twin-coronet" ligands.[64,65] The main difference between the two systems is the chiral bridge. Collman's catalysts are linked to the porphyrin *via* an amide linkage on the *ortho*-positions of the *meso*-aryl-groups, while Naruta's catalysts are linked through an ether linkage. The amide linkage provided for an open, accessible face (Figure 19) yielding higher reaction rates with a variety of olefin substrates, while decreasing oxidative poisoning of the catalyst (Table 19). For example, epoxidation of styrene with Collman's catalyst showed exceptional rate and stability with a TOF = 40/min and up to 5,500 turnovers. In addition, the amide linkage produced a more rigid structure, providing enantiomeric excesses of up to 83% for styrene (entry 1, Table 19).

Collman and co-workers observed an interesting change in enantioselectivity during the course of epoxidation catalyzed by [Fe(**43**)Cl]. They noted that the enantioselectivity increased initially but eventually decreased over the course of the reaction. Investigations into the cause for this observed phenomenon led to the discovery of the iron complex of a new porphyrin **44**, which was generated *in situ* from the oxidation of **43** (Figure 19), as a more selective catalyst. It was rationalized that the initial rise in enantioselectivity was attributed to the generation of **44** and that subsequent decrease in enantioselectivity occurred as a result of the over oxidation of **44** to a less selective catalyst.

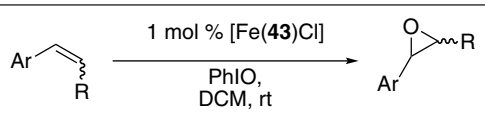
In a separate report, Roschmann and co-workers used Collman's catalyst for the epoxidation of allylic alcohols.[66] This report is especially noteworthy since it was the first example using porphyrins as catalysts for the asymmetric epoxidation



43 **44**

Figure 19. Binaphthyl-derived catalysts by Collman and Rose.[64,65]

Table 19. Asymmetric epoxidation of olefins catalyzed by [Fe(**43**)Cl].[64,65]

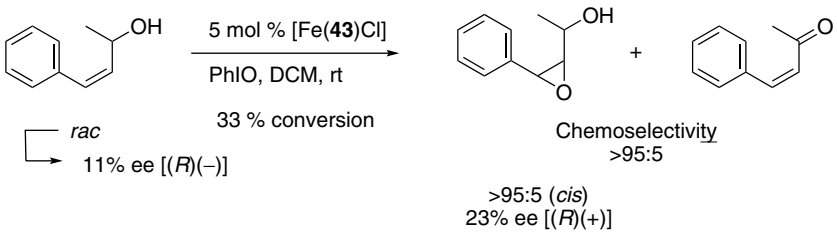


Entry[a]	Substrate	Yield (%)	ee (%)
1	Styrene	95	83 (*S*)
2[b]	Styrene	89	75 (*S*)
3	Pentafluorostyrene	75	88 (*S*)
4	3-Chlorostyrene	90	82 (*S*)
5	3-Nitrosytrene	78	72 (*S*)
6	1,2-Dihydronaphthalene	80	55 (1*S*,2*R*)
7	*cis*-*β*-Methylstyrene	78	49 (1*S*,2*R*)
8	3,3-Dimethyl-1-butene	85	82

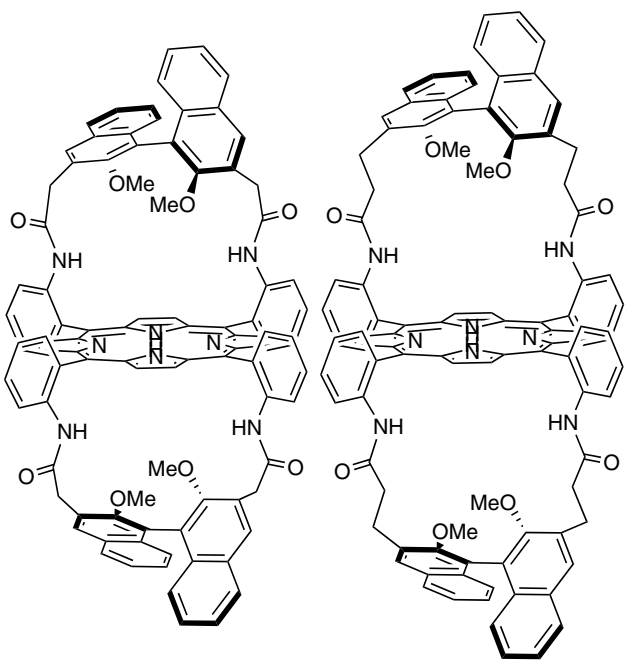
[a] Reactions were performed using 1 mol % of catalyst, 10 equiv. of substrate, and 1.1 equiv. of oxidant in DCM at room temperature; [b] Oxidant is added in 10 portions.

of allylic alcohols. Previous work in this area focused almost exclusively on the use of established catalytic systems, such as the Sharpless system.[67–69]

Using the reaction conditions shown in Scheme 11, racemic allylic alcohols were subjected to catalytic oxidation with PhIO. The resulting epoxides were



Scheme 11.[66]



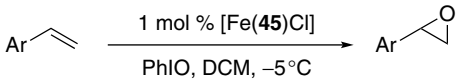
45 **46**

Figure 20. Rose's modified collman catalyst.[70]

formed (33% conversion) in excellent diastereoselectivity and with modest enantioselectivity. The resolution of the allylic alcohol provided an enantiomeric purity of 11% ee. Other allylic olefins were examined using this system and similar results were obtained. The oxidation of allylic alcohols into α,β-unsaturated ketones was the only side reaction reported under the reaction conditions.

Rose and co-workers attempted to induce higher levels of enantioselectivity by extending the linkage on Collman's catalyst **43** to produce catalysts **45** and **46** with a flexible chiral pocket in close proximity to the metal center (Figure 20).[70]

Table 20. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**45**)Cl].[70]



Entry[a]	Substrate	Yield (%)	ee (%)
1	Styrene	96	97 (*R*)
2	Pentafluorostyrene	80	96 (*R*)
3	3-Fluorostyrene	87	93 (*R*)
4	3-Chlorostyrene	90	88 (*R*)
5	3-Nitrostyrene	84	90 (*R*)
6	4-Chlorostyrene	75	84 (*R*)

[a] Reactions were performed with 1 mol % of catalyst, 1 equiv. of oxidant, and 10 equiv. of substrate in DCM at –5°C.

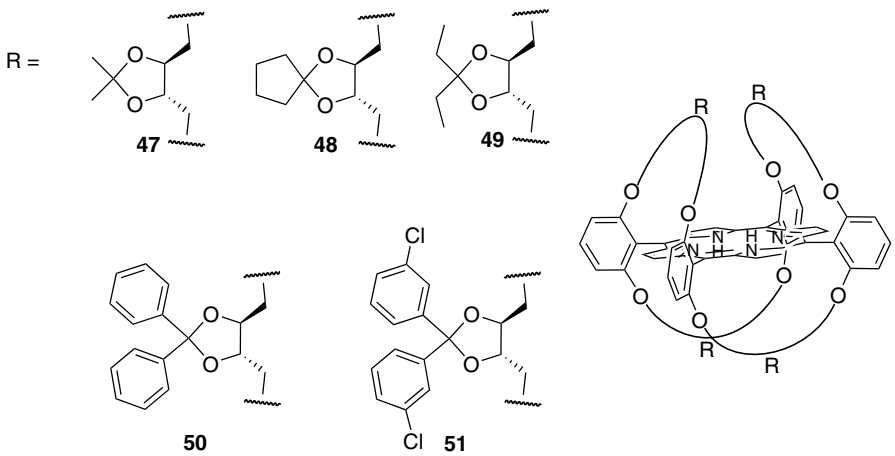
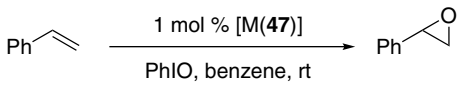


Figure 21. Homochiral threitol-strapped porphyrins.[72–76]

The iron chloride complex of **45** was used with PhIO to epoxidize several styrene derivatives (Table 20). The reported 96% yield and 97% ee for styrene is among the highest reported values for styrene epoxidation; not only for the Collman type catalysts, but also for any metalloporphyrin-based epoxidation system. In 2009, Ren and co-workers also examined the iron complexes of **45** and **46**, generating results similar to those communicated by Collman and Rose.[71]

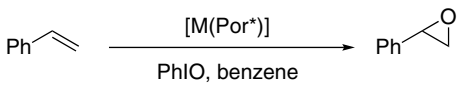
Gross and co-workers also developed a series of porphyrin ligands based on the design of "Twin Coronet" porphyrins.[72,73] Threitol derivatives were appended to adjacent *ortho*-aryl positions into an eclipsed orientation to produce the strapped ligands shown in Figure 21. Manganese, iron, and ruthenium complexes of these porphyrins were evaluated for their ability to serve as asymmetric catalysts in the



[Mn(**47**)Cl]: 77% yield, 10% ee
[Fe(**47**)Cl)]: 65% yield, 44% ee
[Ru(**47**)(CO)]: 47% yield, 42% ee

Scheme 12.[72,73]

Table 21. Asymmetric epoxidation of aromatic olefins catalyzed by iron and ruthenium complexes of **47** and **50**.[74,75]



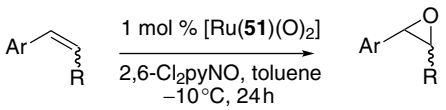
Entry	Substrate	[M(Por*)][e]	Temp (°C)	Yield (%)	ee (%)
1[a]	Styrene	[Fe(**47**)Cl]	25	76	44
2[a]	Styrene	[Fe(**50**)Cl]	23	75	59
3[b]	Styrene	[Fe(**50**)Cl]	–20	59	68
4[c]	Styrene	[Fe(**47**)CO]	25	39	48
5[d]	Styrene	[Ru(**47**)$(O)_2$]	25	29	54
6[a]	4-Fluorostyrene	[Fe(**47**)Cl]	25	68	56
7[a]	4-Fluorostyrene	[Fe(**50**)Cl]	23	70	65
8[b]	4-Fluorostyrene	[Fe(**50**)Cl]	–20	64	73
9[c]	4-Fluorostyrene	[Fe(**47**)CO]	25	39	51
10[d]	4-Fluorostyrene	[Ru(**47**)$(O)_2$]	25	9	53
11[a]	4-Chlorostyrene	[Fe(**47**)Cl]	25	55	53
12[a]	4-Chlorostyrene	[Fe(**50**)Cl]	23	65	63
13[b]	4-Chlorostyrene	[Fe(**50**)Cl]	–20	26	70
14[c]	4-Chlorostyrene	[Fe(**47**)CO]	25	15	45
15[d]	4-Chlorostyrene	[Ru(**47**)$(O)_2$]	25	2	57

[a] Reactions were performed with 0.01 mol % of catalyst, 1 equiv. of PhIO as oxidant, and 10 equiv. of substrate in benzene; [b] Toluene was used as solvent; [c] Reactions were performed with 1 mol % of catalyst, 1 equiv. of PhIO as oxidant, and 10 equiv. of substrate in benzene; [d] Reactions were performed with 0.3 mol % of catalyst, 1 equiv. of 2,6-dichloropyridine *N*-oxide as oxidant, and 1 equiv. of substrate in *m*-xylene for 5 h; [e] See Figure 21 for structures.

epoxidation of styrene (Scheme 12). In general, the Fe(III) chloride complex of **47** generated higher yields and enantiomeric excesses in comparison to the analogous magnesium and ruthenium derivatives.

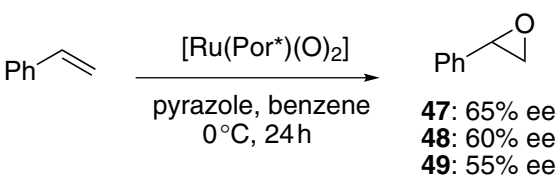
Further exploration and development of the threitol-derived strapped homochiral porphyrin ligand system by Gross and co-workers led to the observation that lower reaction temperatures and higher catalyst loading could provide significant improvements over their previous system (Table 21).[74,75] An additional

Table 22. Asymmetric epoxidation of aromatic olefins catalyzed by [Ru(**51**)$(O)_2$].



Entry[a]	Substrate	TON	ee (%)
1	Styrene	11	75
2[b]	Styrene	55	79
3	3-Chlorostyrene	135	74
4[b]	3-Chlorostyrene	226	81
5	4-Chlorostyrene	24	80
6[b]	4-Chlorostyrene	191	83
7	4-Bromostyrene	19	75
8	4-Fluorostyrene	86	75
9[b,c]	*cis*-β-Methylstyrene	487	69
10[b,d]	*trans*-β-Methylstyrene	242	38

[a] Performed for 1 mol % cat, 1.0 equiv. of oxidant and 1 equiv. of olefin in toluene for 24 h; [b] 48 h; [c] *cis*:*trans* = 15:1; [d] Only *trans*-olefin produced.

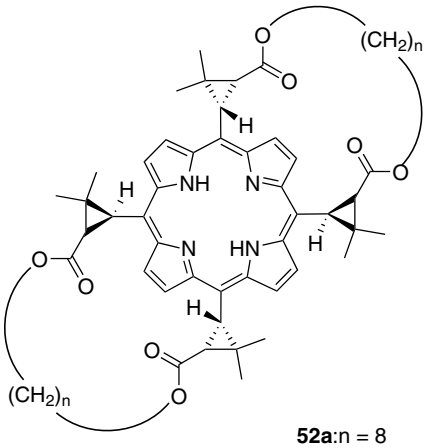


Scheme 13.[76]

development was the use of the ruthenium oxo complex [Ru(**51**)$(O)_2$] (Figure 21), which contains an electron-withdrawing chlorine atom on the aryl group. In many cases, the use of [Ru(**51**)$(O)_2$] resulted in increased enantioselectivities and TON (Table 22).

In addition to their report on catalytic olefin epoxidation by ruthenium complexes of the Halterman porphyrin **8,** Che and co-workers studied catalytic reactions of ruthenium complexes of **48** and **49** (Figure 21), which are derivatives of Gross's homochiral porphyrin **47**.[76] The complexes [Ru(**48**)$(O)_2$] and [Ru(**49**)$(O)_2$] were evaluated for the stoichiometric epoxidation of styrene, generating moderate enantioselectivities (Scheme 13).

Following their successful use of picket fence chiroporphyrin ligands, Marchon and co-workers synthesized a new strapped porphyrin variation consisting of different length cyclopropane moieties (Figure 22).[77] The manganese complexes of these new "bridled chiroporphyrins" were employed for catalytic

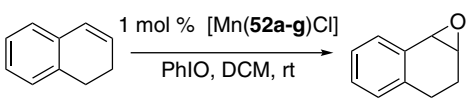


52

52a:n = 8
52b:n = 9
52c:n = 10
52d:n = 11
52e:n = 12
52f:n = 14
52g:n = 16

Figure 22. "Bridled Chiroporphyrin".[77]

Table 23. Asymmetric epoxidation of 1,2-dihydronaphthalene catalyzed by [Mn(**52**)Cl].[77]



Entry[a]	n =	Yield (%)[b]	ee (%)[b]
1	**52a**:8	62	44
2	**52b**:9	63	51
3	**52c**:10	58	63
4	**52d**:11	54	61
5	**52e**:12	60	61
6	**52f**:12	48	53
7	**52g**:12	47	64

[a] The reactions were performed with 1 mol % of catalyst, 1 equiv. of PhIO, and 10 equiv. of substrate in DCM at room temperature; [b] Values reported represent an average from multiple runs.

epoxidation of 1,2-dihydronaphthalene (Table 23). It was shown that both yield and asymmetric induction were affected by the chain length of the catalyst.

Simonneaux and co-workers developed a homochiral porphyrin ligand system containing optically pure cyclohexane auxiliaries which were used to strap

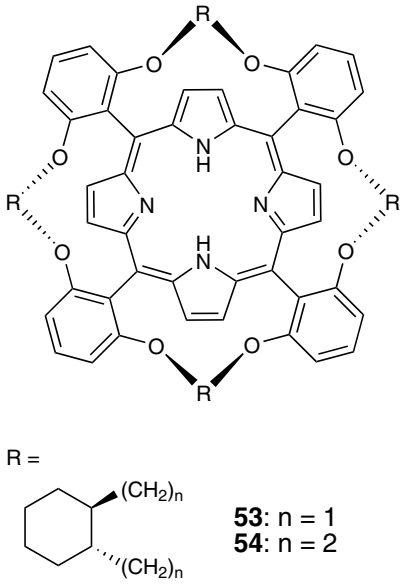
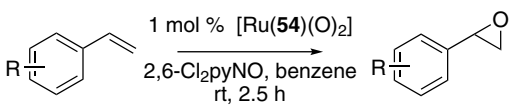


Figure 23. Homochiral-strapped porphyrin with chiral cyclohexane auxiliaries.[78]

Table 24. Asymmetric epoxidation of aromatic olefins catalyzed by [Ru(**54**)$(O)_2$].[78]



Entry[a]	R =	Yield (%)	TON	ee (%)
1	H	84	276	23 (*R*)
2[b]	H	62	204	7 (*R*)
3	2-NO_2	32	107	17 (*R*)
4	3-NO_2	15	50	30 (*R*)
5	2-CF_3	84	276	18 (*R*)
6	3-CF_3	74	244	32 (*R*)
7	4-CF_3	30	97	24 (*R*)
8	4-CF_3	43	144	27 (*R*)
9	4-Br	40	132	21 (*R*)
10	1,2-Dihydronaphtalene	2	33	73 (1*S*,2*R*)

[a] The reactions were performed with 1 mol % of catalyst, 1 equiv. of 2,6-dichloropyridine *N*-oxide, and 1 equiv. of substrate in benzene; [b] [Ru(**53**)$(O)_2$] used as catalyst.

adjacent aryl positions (Figure 23).[78] This ligand system is similar to the threitol-strapped ligand derivatives reported by Gross and co-workers (Figure 21). [Ru(**54**)$(O)_2$] was able to reach an average TON greater than 200 for epoxidation of several of the olefins in this study (Table 24). Unfortunately, the enantioselectivity

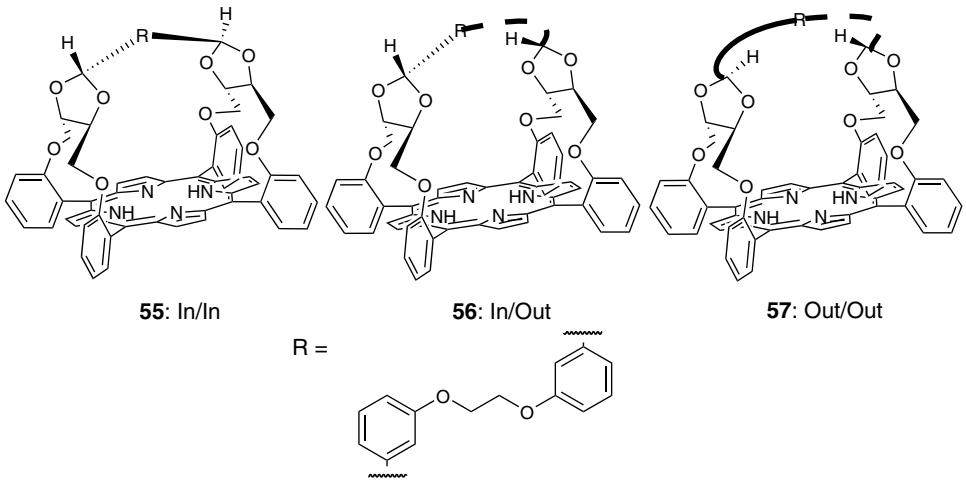


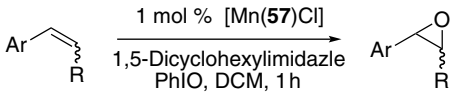
Figure 24. Threitol-strapped porphyrin.[79,80]

induced by these catalysts did not compare favorably with Gross's system (Tables 21 and 24).[72–75]

Collman and co-workers synthesized single-faced threitol-strapped porphyrins containing a bridge across the porphyrin plane connecting the two chiral moieties (**55–57**, Figure 24).[79,80] This system is analogous to basket handle porphyrin ligands, which will be discussed later in this chapter. The open-faced structure of porphyrins **55**–**57** (Figure 24) required an adequate axial ligand to block the achiral face of the catalyst from playing a role in the epoxidation process. The bulky 1,5-dicyclohexylimidazole (DiCyIm) was identified as a suitable axial ligand. The Mn(III) chloride complex of **57**, [Mn(**57**)Cl], was used as a catalyst for the epoxidation of several aromatic olefins in the presence of DiCyIm (Table 25). [Mn(**57**)Cl] was shown to be capable of epoxidation of a range of olefin substrates, including an example of an aliphatic olefin. Good yields and enantioselectivities were generally obtained.

Boitrel and co-workers synthesized and evaluated a series of Fe(III) chloride complexes of porphyrins as catalysts for asymmetric epoxidation. The ligand systems produced, including picket fence, strapped, and basket handle porphyrins, all contained chiral proline units, which served as chiral auxiliaries (Figure 25). The Fe(III) chloride complexes of these ligands were tested for their ability to epoxidize 4-chlorostyrene and 1,2-dihydronaphthalene.[81,82] The enantioselectivity of these reactions is summarized in Table 26. The only catalyst which displayed enhanced enantiomeric performance for both 4-chlorostyrene and 1,2-dihydronaphthalene was $\alpha,\alpha,\beta,\beta$-**61** (entry 7, Table 26).

Table 25. Asymmetric epoxidation of olefins catalyzed by [Mn(**57**)Cl].[79,80]



Entry[a]	Substrate	Temp (°C)	Yield (%)	ee (%)
1	Styrene	25	86	69 (*R*)
2	4-Chlorostyrene	25	83	70 (*R*)
3	2-Nitrostyrene	25	46	74
4	2-Methylstyrene	25	65	79
5	*cis*-β-Methylstyrene	25	89	78 (1*R*,2*S*)
6	*cis*-β-Methylstyrene	0	76	80 (1*R*,2*S*)
7	*trans*-β-Methylstyrene	25	81	21 (1*R*,2*S*)
8	Dihydronaphtalene	25	86	84 (1*R*,2*R*)
9	Dihydronaphtalene	0	67	87 (1*R*,2*R*)
10	Dihydronaphtalene	–10	26	88 (1*R*,2*R*)
11	Indene	25	84	74

[a] The reactions were performed with 1 mol % of catalyst, 1 equiv. of PhIO, and 100 equiv. of substrate in DCM.

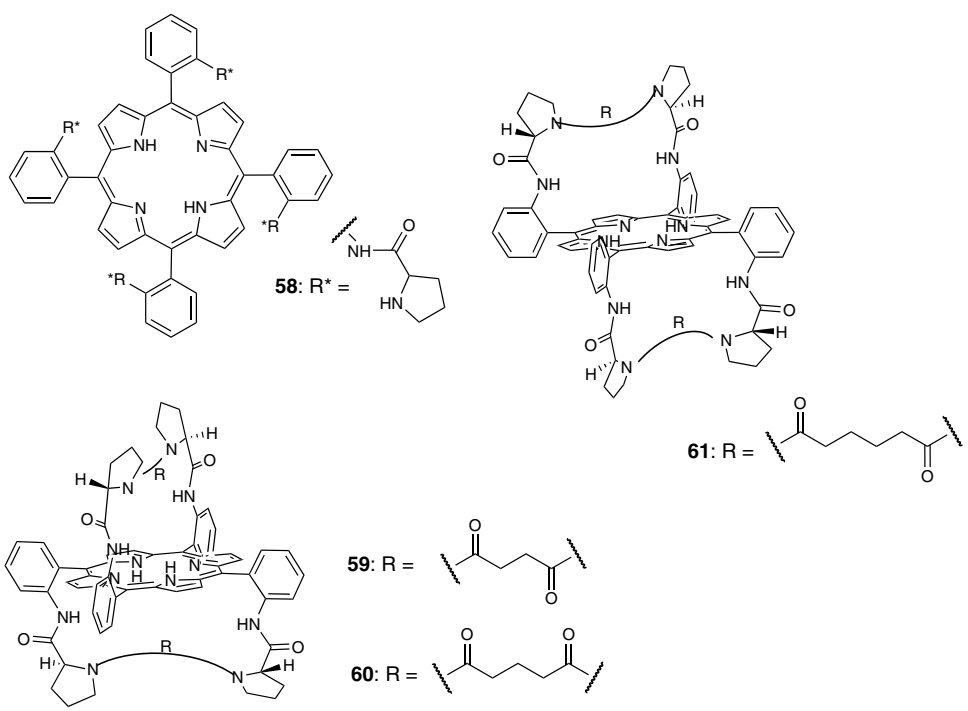
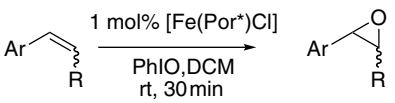


Figure 25. Proline-derived porphyrin ligands.[81,82]

Table 26. Asymmetric epoxidation of olefins by [Fe(**58–61**)Cl].[81,82]



Entry[a]	Por*	4-Chlorostyrene (% ee)	1,2-Dihydronaphthalene (% ee)
1	*αβαβ*-**58**	16	34
2	*ααββ*-**58**	4	9
3	*αααβ*-**58**	10	14
4[b]	*αααα*-**58**	22	10
5	*αβαβ*-**59**	17	0
6	*αβαβ*-**60**	42	4
7	*ααββ*-**61**	31	26

[a] Reactions were performed with 1 mol % of catalyst, 1 equiv. of oxidant, and 10 equiv. of substrate in DCM at room temperature for 30 min; [b] 1-(*tert*-butyl)-5-phenylimidazole used as axial ligand (2.5 equiv.).

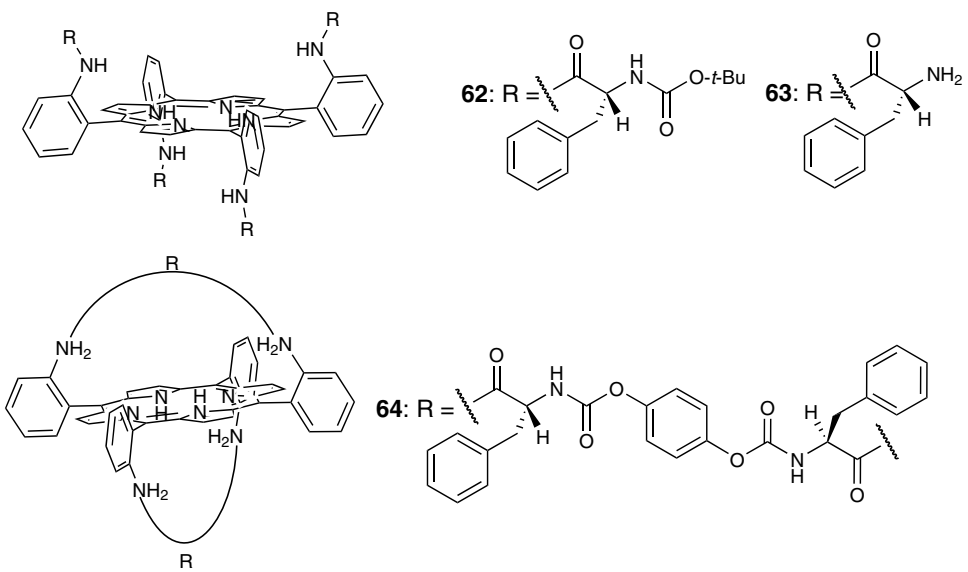
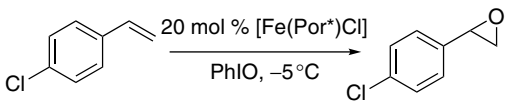


Figure 26. Picket fence and basket handle porphyrins bearing amino acids.[83]

C. Chiral Basket Handle Porphyrins

Mansuy and co-workers reported the first example of a basket handle porphyrin for the epoxidation of 4-chlorostyrene (**64**, Figure 26).[83] The rigid chiral environment was inspired by the desire to mimic the active site of cytochrome P-450. In the course of the development of **64**, precursors **62** and **63** (picket fence type

Table 27. Comparison of picket fence and basket handle porphyrins as catalysts for the asymmetric epoxidation of 4-chlorostyrene.[83]



Entry[a]	Por*	Yield (%)	ee (%)
1	**62**	60	12 (*S*)
2	**63**	45	21 (*S*)
3	**64**	35	50 (*R*)

[a] Reactions were performed with 20 mol % of catalyst, 1 equiv. of oxidant, and 300 equiv. of substrate in DCM at –5°C in a 1:1 ratio of DCM and benzene for 3 h.

ligands) were also evaluated for the epoxidation of 4-chlorostyrene (Table 27). In general, the iron basket handle porphyrin [Fe(**64**)Cl] was shown to be a superior catalyst in terms of selectivity when compared to the picket fence complexes [Fe(**62**)Cl] and [Fe(**63**)Cl]. However, it is noted that this system required high catalyst loadings (20 mol %) and generated only modest yields (35–50%) and enantioselectivities (12–50% ee).

Groves and Viski developed a basket handle derivative of their picket fence porphyrin using a binaphthyl bridge as shown in Figure 27.[84] Iron and manganese complexes of **65** were used as catalysts for the epoxidation of a number of different olefins (Table 28). This system was shown to be suitable for a range of aromatic and aliphatic olefins, generating low to moderate enantioselectivities. The low enantioselectivities reported were believed to result from the oxidative poisoning of the catalyst over the course of the reaction.[85] In the case of bulky substrates such as *cis*-β-methylstyrene, good asymmetric induction was achieved with the iron complex [Fe(**65**)Cl] (entry 6, Table 28).

Collman and co-workers also synthesized a basket handle porphyrin using binaphthyl groups. The iron complex of the "BINAP-capped" porphyrin **66**, shown in Figure 28, was used for the asymmetric epoxidation of mono- and disubstituted aromatic olefins to generate epoxides with moderate yields and enantioselectivities (Table 29).[86]

Inspired by the metalloenzyme cytochrome P-450, Inoue and co-workers synthesized a series of basket handle porphyrin ligands with optical antipodes, as shown in Figure 29. The iron complexes of these ligands were prepared and used as catalysts for the epoxidation of aromatic olefins as shown in Table 30.[87,88] Various axial ligands were used to coordinate to the open face of the porphyrin in

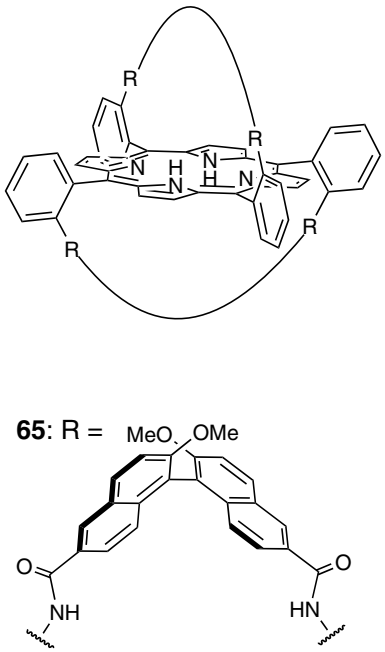
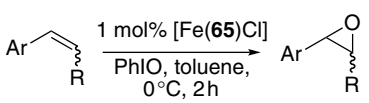


Figure 27. Vaulted binaphthyl porphyrins.[84]

Table 28. Asymmetric epoxidation of olefins catalyzed by [Fe(**65**)Cl].[84,85]



Entry[a]	Substrate	Yield (%)	ee (%)
1	Styrene	23	30 (*R*)
2[b]	Styrene	21	36 (*R*)
3	4-Chlorostyrene	39	38 (*R*)
4	*tert*-Butylethylene	14	40 (*S*)
5	*trans*-β-Methylstyrene	43	~1 (1*S*,2*S*)
6	*cis*-β-Methylstyrene	35	62 (1*S*,2*R*)
7[b]	*cis*-β-Methylstyrene	27	6 (1*S*,2*R*)
8	*trans*-2-Pentene	42	32 (1*S*,2*S*)
9	Indene	70	20 (1*S*,2*S*)
10	1,2-Dihydronaphthalene	61	42 (1*S*,2*S*)

[a] Reactions were performed with 1 mol % of catalyst, 1 equiv. of oxidant, and 10 equiv. of substrate in toluene (iodobenzene for low boiling point products) at 0°C for 2 h; [b] [Mn(**65**)Cl] used as catalyst.

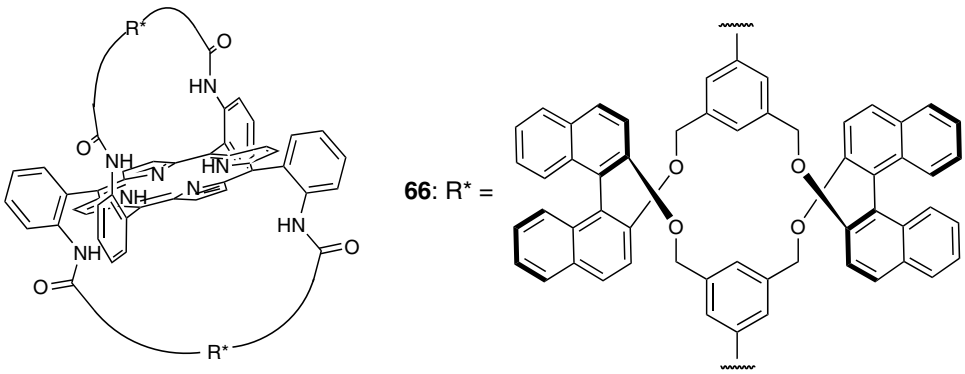
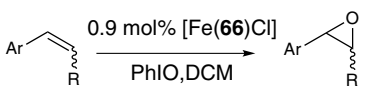


Figure 28. "BINAP-capped" porphyrin.[86]

Table 29. Asymmetric epoxidation of aromatic olefins catalyzed by [Fe(**66**)Cl].[86]



Entry[a]	Substrate	Yield (%)	ee (%)
1	Styrene	62	48 (*S*)
3	4-Chlorostyrene	64	50 (*S*)
4	4-Nitrostyrene	39	56 (*S*)
5	2-Vinylnaphthalene	36	63 (*S*)
6	*cis*-*β*-Methylstyrene	59	29 (1*S*,2*R*)
8	1,2-Dihydronaphthalene	45	21 (1*S*,2*R*)

[a] Reactions were performed with 0.9 mol % of catalyst, 1 equiv. of oxidant, and 27 equiv. of substrate in DCM.

an effort to prevent the generation of racemic products. As anticipated, both enantiomers of catalyst **67** gave similar results with opposite senses of asymmetric induction (entries 1–2, Table 30). The omission of the requisite axial ligand led to an increase in the overall product yield, but with a significant drop in enantiomeric excess as shown in entry 3 (Table 30).

III. Cyclopropanation

Metal-catalyzed cyclopropanation of olefins with diazo reagents has attracted interest due to the fundamental and practical importance of the resulting cyclopropyl units.[89–92] Cyclopropane rings are recurrent motifs in biologically important molecules and can serve as versatile precursors in organic synthesis.[93–96] Currently,

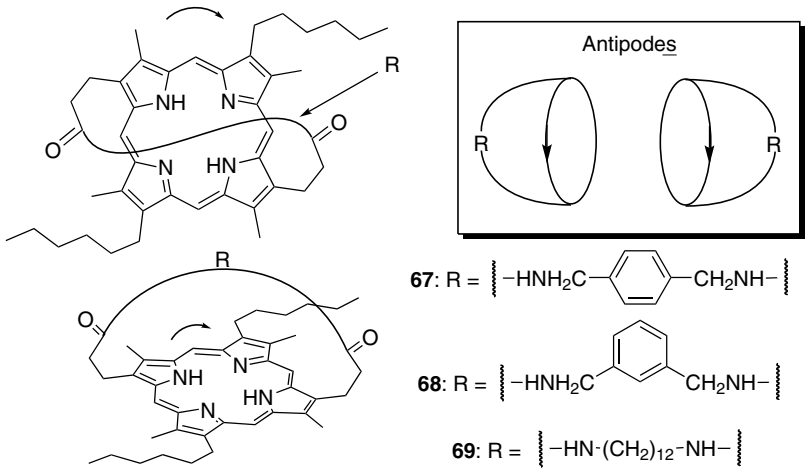
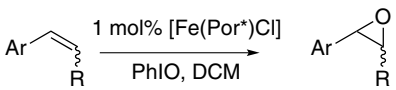


Figure 29. Basket handle type porphyrin antipodes.[87,88]

Table 30. Asymmetric epoxidation of aromatic olefins catalyzed [Fe(**67**–**69**)Cl].[87,88]



Entry[a]	Substrate	Por*[b]	Axial ligand	Yield (%)	ee (%)
1	Styrene	(+)-**67**	Imidazole	43	49 (*R*)
2	Styrene	(–)-**67**	Imidazole	45	48 (*S*)
3	Styrene	(+)-**67**	None	72	18 (*S*)
4	Styrene	(+)-**67**	1-Ethylimidazole	68	50 (*R*)
5	4-Chlorostyrene	(–)-**67**	Imidazole	41	42 (*S*)
6	4-Chlorostyrene	(–)-**67**	2-Methylimidazole	40	42 (*S*)
7	4-Chlorostyrene	(–)-**67**	2-Phenylimidazole	36	8 (*S*)
8	4-Methylstyrene	(+)-**67**	Imidazole	56	47 (*S*)
9	2-Vinylnaphthalene	(+)-**67**	Imidazole	65	42 (*R*)
10	Indene	(+)-**67**	Imidazole	58	58 (1*R*,2*S*)
11	Dihydronaphthalene	(+)-**67**	Imidazole	32	52 (1*R*,2*S*)
12	Styrene	(+)-**68**	1-Ethylimidazole	27	30 (*R*)
13	Styrene	(+)-**68**	None	18	16 (*S*)
14	Styrene	(+)-**69**	1-Ethylimidazole	33	17 (*R*)
15	Styrene	(+)-**69**	None	30	16 (*S*)

[a] Reactions were performed with 1 mol % of catalyst, 10 mol % of axial ligand, 1 equiv. of oxidant, and 5 equiv. of substrate in DCM for 3 h at –10°C; [b] See Figure 29 for structures.

the metal-mediated decomposition of diazo reagents in the presence of olefins constitutes the most direct route to the synthesis of cyclopropanes. This approach provides the advantage of using achiral reagents with the induction of chirality occurring as a direct result of the chiral environment of the catalysts. Prior to the development of catalytic systems capable of decomposing diazo reagents, the Simmons–Smith reaction was the predominant method for cyclopropanation,[91] and chiral starting materials or chiral auxiliaries were required for asymmetric induction.

In the last 20 years, a number of metal-mediated asymmetric cyclopropanation systems incorporating copper, ruthenium, cobalt, rhodium, and various ligands have been developed.[97–104] Copper bisoxazoline and dirhodium complexes are examples of some of the more general and selective catalysts that have been prepared. For example, Doyle's use of dirhodium carboxamides as catalysts for the general and selective asymmetric intramolecular cyclopropanation with diazo reagents has achieved broad use in the chemical literature.[103–110]

Among the wide variety of catalytic systems, metalloporphyrin-based ligand designs are unique, owing to their excellent selectivities and high catalytic TON. Metalloporphyrins were first used as catalyst for cyclopropanation by Callot and co-workers.[111,112] Their initial reports on rhodium-catalyzed cyclopropanation were significantly expanded by Kodadek and co-workers.[113–119] Woo and co-workers showed that osmium porphyrins could also catalyze the cyclopropanation of alkenes, although with less efficiency.[120] Subsequently, Kodadek, Woo, and co-workers jointly reported that iron porphyrins were effective catalysts for the shape-selective and stereoselective cyclopropanation of alkenes.[121] These early works paved a solid foundation for the subsequent development of highly diastereoselective and enantioselective catalysts.

In the following sections, the asymmetric cyclopropanation of alkenes using metalloporphyrins will be discussed. This section will follow the same general outline as the epoxidation section. The robust Halterman porphyrin **8**, examined in various systems for the epoxidation of alkenes, has historically been one of the most widely studied chiral porphyrin ligands for cyclopropanation. More recently, the development of Co(II) complexes of a new family of D_2-symmetric chiral porphyrins capable of high levels of stereocontrol over an expansive substrate scope has broadened the use of metalloporphyrin-based catalysts for asymmetric cyclopropanation.

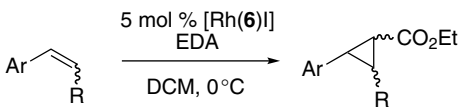
A. Chiral Picket Fence Porphyrins

Inspired by the previous reports of Doyle[105,106,110] and Callot[111,112] using rhodium complexes for the cyclopropanation of olefins, O'Malley and Kodadek employed their "chiral wall" porphyrin (**6**, Figure 4, Sec. II.A) as a ligand for rhodium-catalyzed asymmetric cyclopropanation.[116,117,119] Based on Callot's work, they

believed that the use of the bulky "chiral wall" ligand would favor *cis* cyclopropane isomers, which are normally the minor products in most catalytic cyclopropanations. As anticipated, the *cis*-isomer was generated when the Rh(III) iodide complex of **6** was employed for the asymmetric cyclopropanation of styrene, *cis*-β-methylstyrene, and allyl benzene in the presence of ethyl diazoacetate (EDA). The TON for this catalytic system were outstanding with up to 4,500 TON obtained for the cyclopropanation of *cis*-β-methylstyrene. In all the cases examined, the *cis* isomer was favored over the *trans*, although the *trans* product exhibited significantly higher enantioselectivity (Table 31).

Kodadek and O'Malley designed and synthesized a new chiral porphyrin, termed the "chiral fortress" (**70**, Figure 30), incorporating bulkier aromatic rings

Table 31. Asymmetric cyclopropanation of olefins catalyzed by [Rh(**6**)I].[117,119]



Entry[a]	Substrate	*cis*/*trans*	ee (%) *cis*	ee (%) *trans*	TON
1	Styrene	2.3	10	—	2000
2	*cis*-β-Methylstyrene	7.8	20	50	4500
3	Allylbenzene	4.3	45	60	550

[a] Reactions were performed with 5 mol % of catalyst, 1 equiv. of substrate, and 1 equiv. of EDA in DCM at 0°C. EDA was added in 0.25 equiv. portions over the course of the reaction.

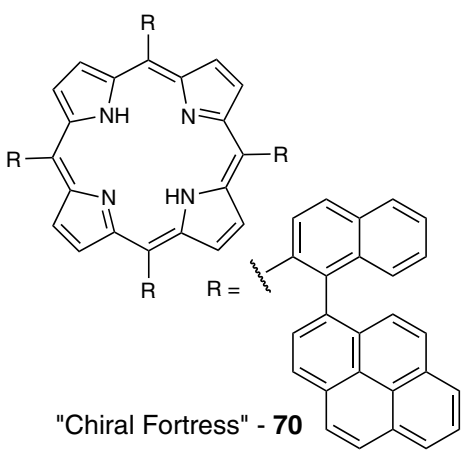
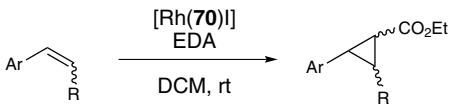


Figure 30. "Chiral fortress" porphyrin.[116]

Table 32. Asymmetric cyclopropanation of olefins catalyzed by [Rh(**70**)I].[116]



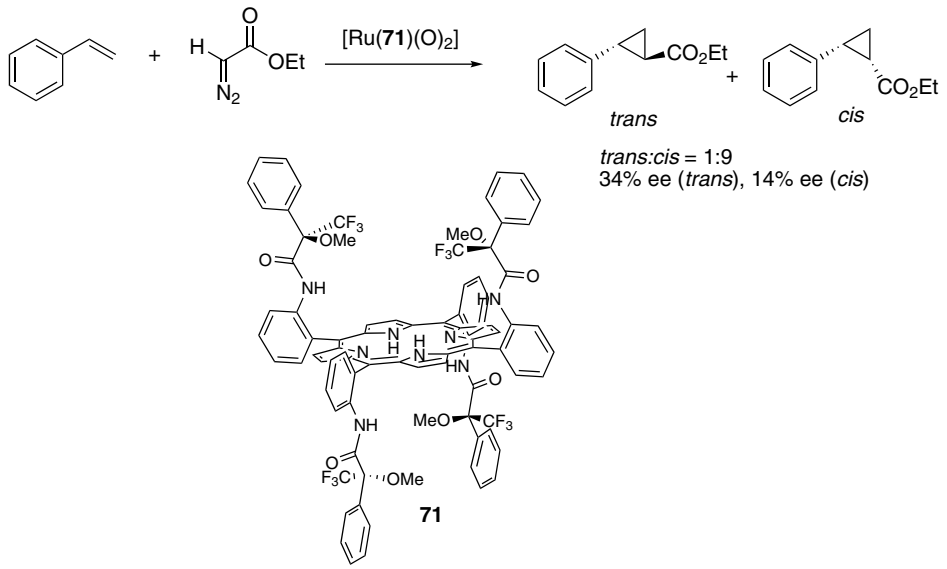
Entry[a]	Substrate	*cis*/*trans*	ee (%) *cis*	ee (%) *trans*	TON
1	Styrene	2.5	15	—	1600
2	*cis*-*β*-Methylstyrene	5.1	25	20	770
3	Allylbenzene	1.0	10	10	420
4	Ethoxyethane	0.83	15	10	130

[a] Reactions were performed with [Rh(**70**)I] as catalyst, 1 equiv. of substrate, and 1 equiv. of EDA in DCM at room temperature. EDA was added in 0.25 equiv. portions over the course of the reaction.

in an effort to enhance the formation of the *cis*-cyclopropanated products.[116] The rhodium complex of **70**, [Rh(**70**)I], was shown to be an active catalyst for the cyclopropanation of olefins. In comparison to the "chiral wall", [Rh(**6**)I], the *cis*:*trans* ratio was only slightly improved for styrene, and a decrease in diastereoselectivity was observed for all other substrates (Table 32). It was concluded that the active site was too sterically encumbered to induce similar levels of selectivity in comparison to the "chiral wall".

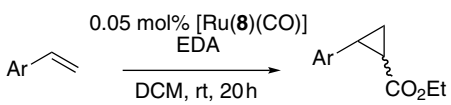
In 1997, Simonneaux and co-workers reported the successful use of ruthenium porphyrins such as [Ru(**71**)$(O)_2$] (Scheme 14) for the asymmetric cyclopropanation of olefins with EDA.[122] Previous studies using this system reported complex mixtures of products, with EDA dimerization identified as the major product of the reaction. The introduction of EDA over several hours minimized the formation of these side products and produced the desired cyclopropane derivatives with good diastereoselectivity albeit low enantioselectivity (Scheme 14). This technique ultimately proved useful for most rhodium, iron, and ruthenium systems.

Che and co-workers expanded their previous work on Halterman complexes by using ruthenium complexes, [Ru(**8**)(CO)] and [Ru(**8**)(CPh_2)] (Figure 5, Sec. II.A), as catalysts for the cyclopropanation of styrene derivatives with diazo reagents (Tables 33 and 34).[123,124] Overall, the [Ru(**8**)(CO)] complex generated higher yields and greater diastereo- and enantioselectivities over a range of aromatic olefins in comparison to [Ru(**8**)(CPh_2)]. In addition, the catalytic efficiency of both of these complexes were shown to be outstanding, providing TON greater than 1,200.



Scheme 14.[122]

Table 33. Asymmetric cyclopropanation of aromatic olefins catalyzed by [Ru(**8**)(CO)].[123,124]

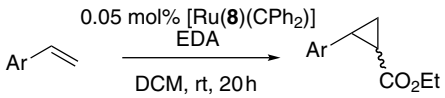


				ee (%)		
Entry[a]	Substrate	Yield (%)	*trans/cis*	*trans*	*cis*	TON
1	Styrene	83	18	87	4	1700
2	α-Methylstyrene	69	3.0	87	35	1400
2	4-Chlorostyrene	66	23	90	4	1300
3	4-Fluorostyrene	83	19	87	3	1700
4	4-Methylstyrene	78	18	81	9	1600
5	4-Methoxystyrene	61	15	85	8	1200

[a] Reactions were performed with 0.05 mol % of catalyst, 1 equiv. of EDA, and 5 equiv. of substrate in DCM at room temperature for 20 h.

The ruthenium carbonyl complex of the Halterman porphyrin, [Ru(**8**)(CO)], was also examined as a potential catalyst for intramolecular cyclopropanation to produce valuable cyclopropyl fused ring products (Table 35). Although the yields and enantioselectivities reported for these cyclopropanes are low to moderate, this represents one of the first reports of asymmetric intramolecular cyclopropanation catalyzed by a ruthenium porphyrin complex.

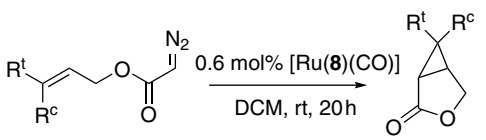
Table 34. Asymmetric cyclopropanation of aromatic olefins catalyzed by [Ru(**8**)(CPh_2)].[123,124]



Entry[a]	Substrate	Yield (%)	*trans/cis*	ee (%) *trans*	ee (%) *cis*	TON
1	Styrene	36	11.0	83	7	720
2	α-Methylstyrene	72	5.6	66	25	1400
2	4-Chlorostyrene	61	5.2	88	40	1200
3	4-Fluorostyrene	71	8.4	85	12	1400
4	4-Methylstyrene	62	4.1	71	—	1200
5	4-Methoxystyrene	75	12	71	9	1500

[a] Reactions were performed with 0.05 mol % of catalyst, 1 equiv. of EDA, and 5 equiv. of substrate in DCM at room temperature for 20 h.

Table 35. Intramolecular asymmetric cyclopropanation catalyzed by [Ru(**8**)(CO)].[123,124]

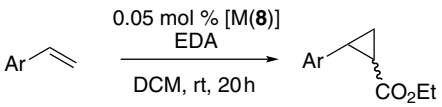


Entry[a]	Rt	Rc	Yield (%)	ee (%)
1	H	H	45	24 (1*R*,5*S*)
2	Me	Me	65	36 (1*R*,5*S*)
3	Me	H	65	28
4	Ph	H	60	85 (1*R*,5*R*)

[a] Reactions were performed with 0.6 mol % of catalyst and 1 equiv. of substrate in DCM at room temperature for 20 h.

Che and co-workers investigated ruthenium and rhodium complexes of the Halterman porphyrin **8** for both inter- and intramolecular asymmetric cyclopropanation.[125] As presented in Tables 36 and 37, for both inter- and intramolecular cyclopropanation systems, [Ru(**8**)(CO)] generated higher diastereo- and enantioselectivities. Despite using catalysts with the same ligand environment, an "enantiomeric switch" was observed for one of the substrates when the metal was exchanged from rhodium to ruthenium (entry 4, Table 37). These results led to the assumption that these two metal complexes cyclopropanate *via* different mechanistic pathways, generating the two different enantiomers.

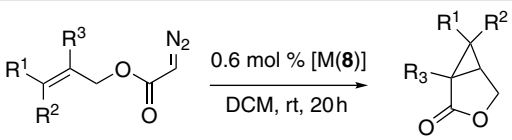
Table 36. Asymmetric cyclopropanation of aromatic olefins catalyzed by rhodium and ruthenium complexes of **8**.[125]



		[Rh(**8**)I]				[Ru(**8**)(CO)]			
				ee (%)				ee (%)	
Entry[a]	Substrate	Yield (%)	*trans/cis*	*trans*	*cis*	Yield (%)	*trans/cis*	*trans*	*cis*
1	Styrene	66	1.5	61	36	83	18	86	4
2	α-Methylstyrene	75	1.0	46	46	69	3.0	87	35
2	4-Chlorostyrene	81	1.2	62	20	66	23	90	4
3	4-Fluorostyrene	72	0.9	62	33	83	19	87	3
4	4-Methylstyrene	71	1.2	49	42	78	18	81	9
5	4-Methoxystyrene	83	1.6	68	44	61	15	85	8

[a] Reactions were performed with 0.05 mol % of catalyst, 1 equiv. of EDA, and 5 equiv. of substrate in DCM at room temperature for 20 h.

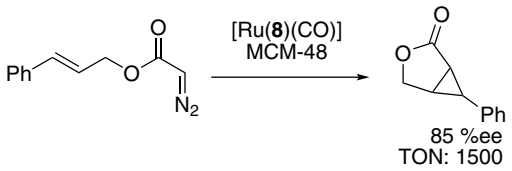
Table 37. Intramolecular asymmetric cyclopropanation catalyzed by rhodium and ruthenium complexes of **8**.[125]



				[Rh(**8**)I]		[Ru(**8**)(CO)]	
Entry[a]	R1	R2	R3	Yield (%)	ee (%)	Yield (%)	ee (%)
1	H	H	H	23	20 (1*R*,5*S*)	48	24 (1*R*,5*S*)
2	Me	Me	H	81	37 (1*R*,5*S*)	65	36 (1*R*,5*S*)
3	Me	H	H	33	24 (1*R*,5*S*)	65	41 (1*R*,5*S*)
4	Ph	H	H	84	20 (1*R*,5*S*)	77	85 (1*S*,5*R*)
5	H	Ph	H	31	31 (1*R*,5*S*)	18	22 (1*R*,5*S*)

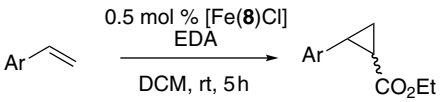
[a] Reactions were performed with 0.6 mol % of catalyst and 1 equiv. of substrate in DCM at room temperature for 20 h.

Che and co-workers also reported the use of ruthenium complex [Ru(**8**)(CO)] encapsulated in mesoporous molecular sieves for intramolecular cyclopropanation.[40] Although only one example was reported (Scheme 15), the catalytic system generated a respectable enantioselectivity (85% ee) and could be reused up to four times. The results from this heterogeneous system were comparable to the corresponding homogenous system (Table 37).



Scheme 15.[40]

Table 38. Asymmetric cyclopropanation of aromatic olefins catalyzed by [Fe(**8**)Cl].[126]



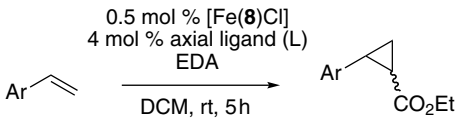
Entry[a]	Substrate	Yield (%)	*trans*:*cis*	trans ee (%)	TON
1	Styrene	71	12:1	80	368
2	4-Chlorostyrene	57	18:1	75	284
3	4-Methylstyrene	56	12:1	79	424
4	4-Methoxystyrene	65	13:1	74	416
5	α-Methylstyrene	68	3:1	81	390
6	1,1-Diphenylethane	72	—	83[b]	410

[a] Reactions were performed with 0.5 mol % of catalyst, 1 equiv. of EDA, and 5 equiv. of substrate in DCM at room temperature for 4 h slow addition is followed by 1 h of stirring in DCM; [b] *Z* isomer.

Iron complexes [Fe(**8**)Cl] was also reported by Che and co-workers for asymmetric cyclopropanation with ethyl diazoacetate. The diastereo- and enantioselectivities reported were similar to those of the aforementioned rhodium and ruthenium complexes (Table 38).[126] However, the iron complex produced the cyclopropanes with lower TON in comparison to the ruthenium complex (Tables 38 and 33). Further investigation of the iron-catalyzed system showed that the use of nitrogen-based axial ligands could improve the diastereo- and enantioselectivity across a range of substrates as shown in Table 39. However, the use of an axial ligand resulted in even lower TON as shown in Table 39.

Berkessel and co-workers evaluated ruthenium complexes of the Halterman porphyrin **8** and its derivatives **9–11** (Figure 6, Sec. II.A)[42] as catalysts for the cyclopropanation of aromatic olefins using the slow addition method for the introduction of EDA (Table 40). Excellent diastereoselectivities and high enantioselectivities were observed with all ligand derivatives when styrene was used as the substrate (entries 1–5, Table 40). Results showed that the derivatization of the *para*-phenyl position of the *meso*-aryl group with electron-donating or -withdrawing groups did not have a pronounced effect on the diastereo- and enantioselectivity in comparison to the metal complexes of the Halterman porphyrin.

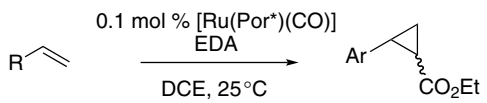
Table 39. Asymmetric cyclopropanation of styrene catalyzed by [Fe(**8**)L].[126]



Entry[a]	Axial ligand (L)	*trans*:*cis*	*trans* ee (%)	TON
1	—	12:1	80	368
2	Pyridine	33:1	82	307
3	DMAP	24:1	81	321
4	1-Methylimidazole	26:1	83	275
5	1-Methylpyrrolidine	27:1	86	293
6	4-Phenylpyridine *N*-oxide	23:1	83	209
7	DMSO	17:1	82	385

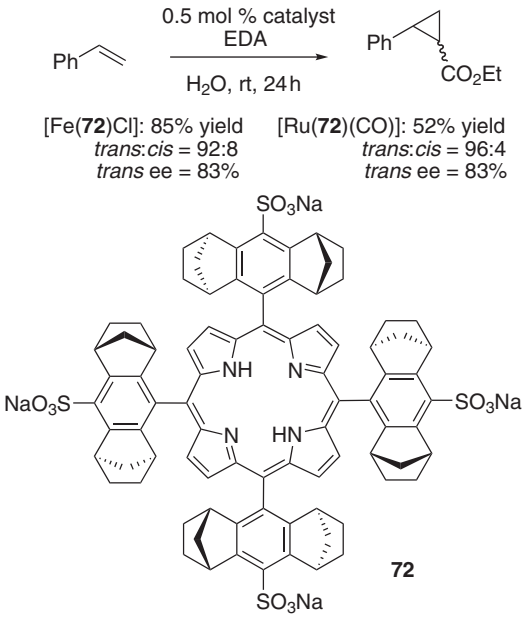
[a] Reactions were performed with 0.5 mol % of catalyst, 4 mol % of axial ligand (L), 1 equiv. of EDA, and 5 equiv. of substrate in DCM at room temperature for 4 h slow addition is followed by 1 h of stirring in DCM.

Table 40. Asymmetric cyclopropanation of olefins catalyzed by [Ru(**8**–**11**)(CO)].[42]



Entry[a]	Substrate	Por*[b]	Conversion (%)	*trans*:*cis*	ee *trans* (%)	ee *cis* (%)
1	Styrene	**8**	80	96:4	87 (1*S*,2*S*)	14 (1*S*,2*R*)
2	Styrene	*ent*-**9**	81	96:4	90 (1*R*,2*R*)	31 (1*R*,2*S*)
3	Styrene	*ent*-**10**	83	96:4	89 (1*R*,2*R*)	11 (1*R*,2*S*)
4	Styrene	**11**	94	97:3	89 (1*S*,2*S*)	<1
5	Styrene	**8**[c]	81	96:4	87 (1*S*,2*S*)	14 (1*S*,2*R*)
6	α-Methylstyrene	**8**	79	66:34	90 (1*S*,2*S*)	38 (1*S*,2*R*)
7	α-Methylstyrene	*ent*-**9**	76	68:32	91 (1*R*,2*R*)	43 (1*R*,2*S*)
8	α-Methylstyrene	*ent*-**10**	81	67:33	91 (1*R*,2*R*)	46 (1*R*,2*S*)
9	α-Methylstyrene	**11**	>98	69:31	91 (1*S*,2*S*)	36 (1*S*,2*R*)
10	α-Methylstyrene	**8**[c]	78	66:34	90 (1*S*,2*S*)	38 (1*S*,2*R*)
11	1-Hexene	**8**	20	86:14	46 (1*S*,2*S*)	9
12	1-Hexene	*ent*-**9**	15	85:15	40 (1*R*,2*R*)	<2
13	1-Hexene	*ent*-**10**	20	82:18	39 (1*R*,2*R*)	<2
14	1-Hexene	**11**	30	85:15	46 (1*S*,2*S*)	4
15	1-Hexene	**8**[c]	42	99.5:0.5	32 (1*S*,2*S*)	6

[a] Reactions were performed with 0.1 mol % of catalyst, 1 equiv. of substrate, and 1 equiv. of EDA in DCE at 0°C. Diazo was added by means of a syringe pump over 2 h; [b] See Figure 6 for structures; [c] [Ru(**8**)PF_3] used as catalyst.



Scheme 16.[127]

Simonneaux and co-workers reported the synthesis of a water-soluble Halterman porphyrin derivative (**72**, Scheme 16) and the use of its iron and ruthenium complexes for the cyclopropanation of styrene with EDA.[127] While both [Fe(**72**)Cl] and [Ru(**72**)(CO)] catalysts displayed very similar diastereo- and enantioselectivities, the iron complex provided superior yields (Scheme 16). It was also noted that the iron complex was recyclable up to four times, with no significant decrease in enantioselectivity while retaining excellent diastereoselectivity. However, the enantioselectivity and yield of the cyclopropanated product generated by the ruthenium complex decreased significantly after the first cycle.

Continued development by Simonneaux and co-workers led to the introduction of heterogeneous catalytic systems for olefin cyclopropanation, and in 2005 they introduced the use of a heterogeneous polymer-supported Halterman porphyrin system as a catalyst for the cyclopropanation of olefins (Figure 31). Iron and ruthenium complexes of the Halterman porphyrin **8** were incorporated into the backbone of the polymer-based system,[128] and the use of an alternative diazo reagent, trifluorodiazoethane (Table 41), was employed for the first time.[129] The diastereoselectivities reported for this polymer-supported system were excellent, however, the yield and enantioselectivity were only moderate.

Following their use of trifluorodiazoethane, Simmonneaux and co-workers explored bulkier diazo reagents for asymmetric cyclopropanation. For example,

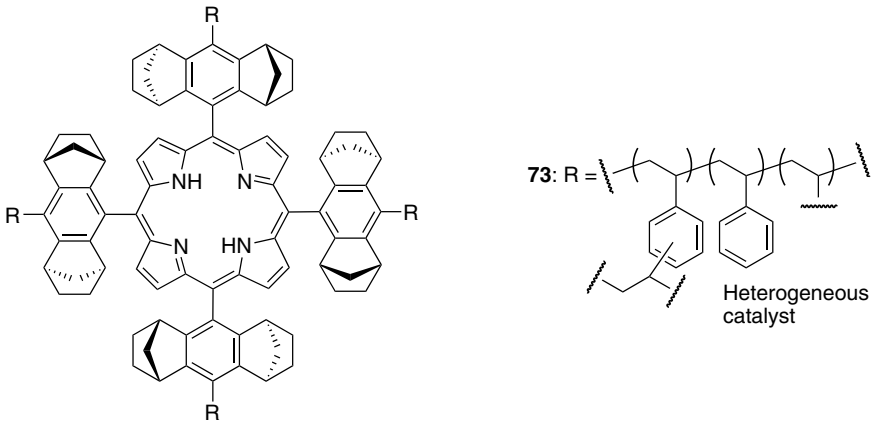
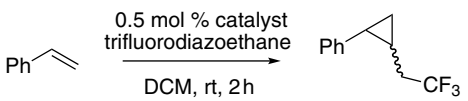


Figure 31. Polymer-supported Halterman ligand.[128]

Table 41. Asymmetric cyclopropanation with trifluorodiazoethane catalyzed by [Fe(**8**)Cl] and [Ru(**73**)(CO)].[128,129]

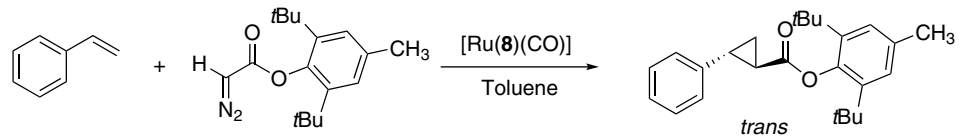


Entry[a]	Catalyst	Yield (%)	*trans:cis*	ee (%, *trans*)
1	[Fe(**8**)Cl]	50	99:1	61
2	[Ru(**8**)(CO)]	32	98:2	58
3	[Fe(**73**)Cl]	52	97:3	56
4	[Ru(**73**)(CO)]	33	99:1	61

[a] Reactions were performed with 0.5 mol % catalyst, 1 equiv. of diazo, and 5 equiv. of substrate in DCM at room temperature for 2 h.

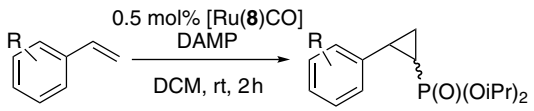
the diazo reagent 2,6-di-*tert*-butyl-4-methylphenyl diazoacetate (Scheme 17) was employed in the cyclopropanation of styrene by [Ru(**8**)(CO)].[130] Although excellent diastereoselectivity was achieved with this system, the yield and enantioselectivity was relatively low. The authors speculated that the low yield and enantioselective outcome was a consequence of the formation of a stable carbene intermediate. Acquisition of a crystal structure of the carbene intermediate supported this claim.

Further studies on the expansion of diazo substrate scope led Simonneaux and co-workers to the use of the ruthenium Halterman complex for the asymmetric cyclopropanation of styrene with diisopropyl diazomethylphosphonate (DAMP).[131] The results, shown in Table 42, demonstrate that excellent diastereo- and



Scheme 17.[130]

Table 42. Asymmetric cyclopropanation of styrene with DAMP catalyzed by [Ru(**8**)(CO)].[131]



				ee (%)	
Entry[a]	R =	Yield (%)	*trans*:*cis*	*trans*	*cis*
1	H	97	96:4	90	34
2	4-Methyl	93	99:1	87	23
3	4-Methoxy	96	97:3	90	23
4	4-Trifluoro	90	95:5	92	5
5	4-Chloros	92	97:3	88	27

[a] Reactions were performed with 0.5 mol % of catalyst, 1 equiv. of DAMP, and 5 equiv. of EDA in DCM at room temperature for 2 h.

enantioselectivities as well as high product yields could be achieved with this diazo reagent.

Simonneaux and co-workers also reported the design and synthesis of a new porphyrin ligand. The incorporation of a mixture of two types of rigid, bulky groups onto the meso positions of the porphyrin ring generated porphyrin ligands **74–76**, as shown in Figure 32. The ruthenium complexes of these Halterman-like porphyrins, bearing 1–4 chiral appendages (with 4 chiral appendages being the Halterman porphyrin **8**) were employed as catalysts for the cyclopropanation of styrene with EDA and DAMP (Table 43). Catalyst [Ru(**74**)(CO)] bearing three chiral groups displayed high levels of asymmetric induction, while ligands [Ru(**75**)(CO)] and [Ru(**76**)(CO)] having only one and two chiral groups, respectively, led to a significant reduction.

In 2006, Cenini and co-workers reported the use of porphyrin **77** for the ruthenium-catalyzed cyclopropanation of α-methylstyrene with EDA (Scheme 18).[132] In comparison to the Halterman porphyrin-based catalysts, this system produced

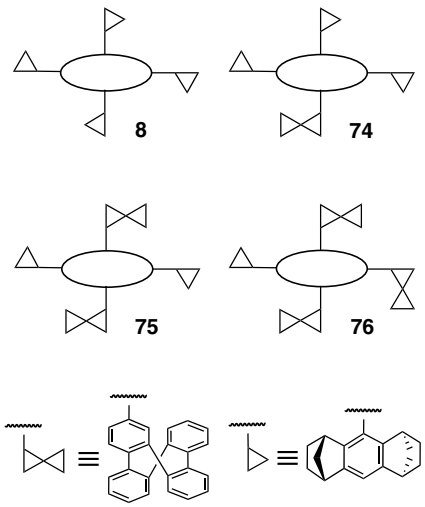
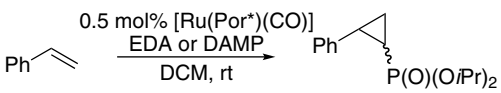


Figure 32. Schematic representation of the chiral porphyrin ligands **74**–**76**.[131]

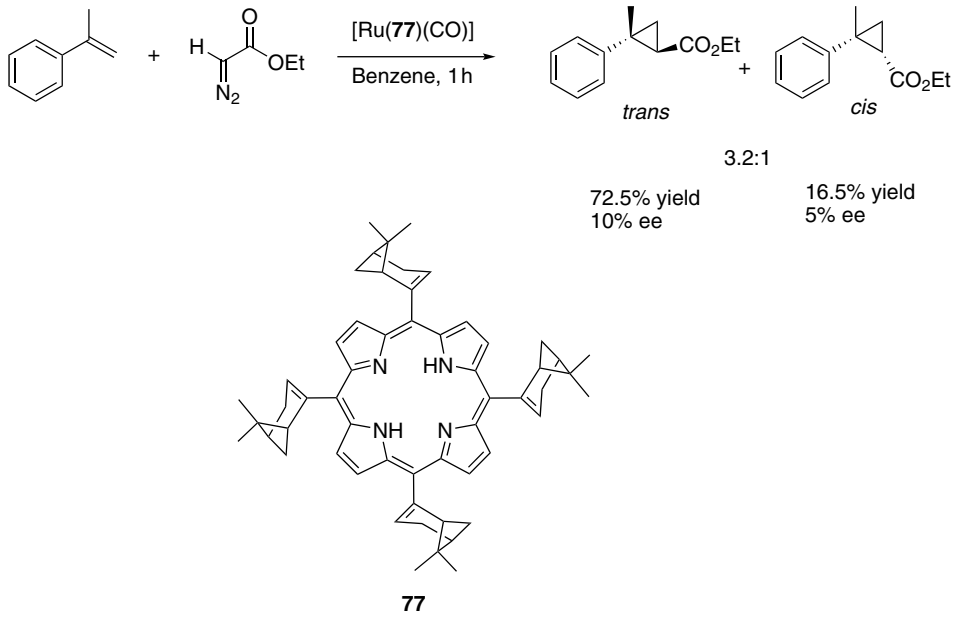
Table 43. Asymmetric cyclopropanation of styrene with DAMP catalyzed by ruthenium complexes of halterman porphyrin **8** and its derivatives **74**–**76**.[131]



Entry[a]	Por*[b]	Diazo	Yield (%)	*trans*:*cis*	*trans* ee (%)
1	**8**	EDA	96	95:5	87
2	**8**	DAMP	97	96:4	90
3	**74**	EDA	95	93:7	65
4	**74**	DAMP	65	96:4	77
5	**75**	EDA	94	90:10	41
6	**75**	DAMP	55	95:5	66
7	**76**	EDA	96	90:10	25
8	**76**	DAMP	13	96:10	33

[a] Reactions were performed with 0.5 mol % of catalyst, 1 equiv. of diazo, and 5 equiv. of substrate in DCM at room temperature for 2 h (EDA) or 48 h (DAMP); [b] See Figure 32 for structures of ligands.

significantly lower selectivities. In the same report, a cobalt complex of **77** was used for the cyclopropanation of styrene with EDA. It was shown that [Co(**77**)] generated slightly greater diastereoselectivities (*trans*:*cis* = 4.4:1) than the ruthenium-catalyzed system. The enantioselectivities of the products generated from this cobalt-catalyzed system were not reported.



Scheme 18.[132]

Independently, Zhang and co-workers also reported some of the first examples of cobalt porphyrin-catalyzed asymmetric cyclopropanation.[133,134] One of the distinct advantages of using cobalt over the existing metal-catalyzed systems was that the slow addition of the diazo reagent was no longer required to inhibit dimer formation.

In the initial report by Zhang and co-workers, cobalt complexes of several chiral porphyrins (**78**–**82**, Figure 33) were prepared and evaluated for the asymmetric cyclopropanation of styrene with EDA (Table 44). Their initial results demonstrated that chiral groups directed toward the metal-center, as with complex **82**, provided the highest selectivities. Continued development of porphyrin ligand systems with chiral groups in close proximity to the metal center led to improved selectivities in Co(II)-catalyzed asymmetric cyclopropanation.

Zhang and co-workers designed and synthesized a series of D_2-symmetric chiral porphyrin for use in cobalt-catalyzed cyclopropanation (Figure 34).[135,136] One notable feature of these systems is the modular design of the porphyrin ligand. This synthetic strategy reported by Zhang and co-workers allows for the direct installation of select chiral amides using a palladium-catalyzed cross-coupling approach between brominated porphyrins and chiral amides. Through selection of the appropriate chiral groups, each of the four diastereomers of the cyclopropanated product could be accessed with excellent enantioselectivity (Scheme 19).

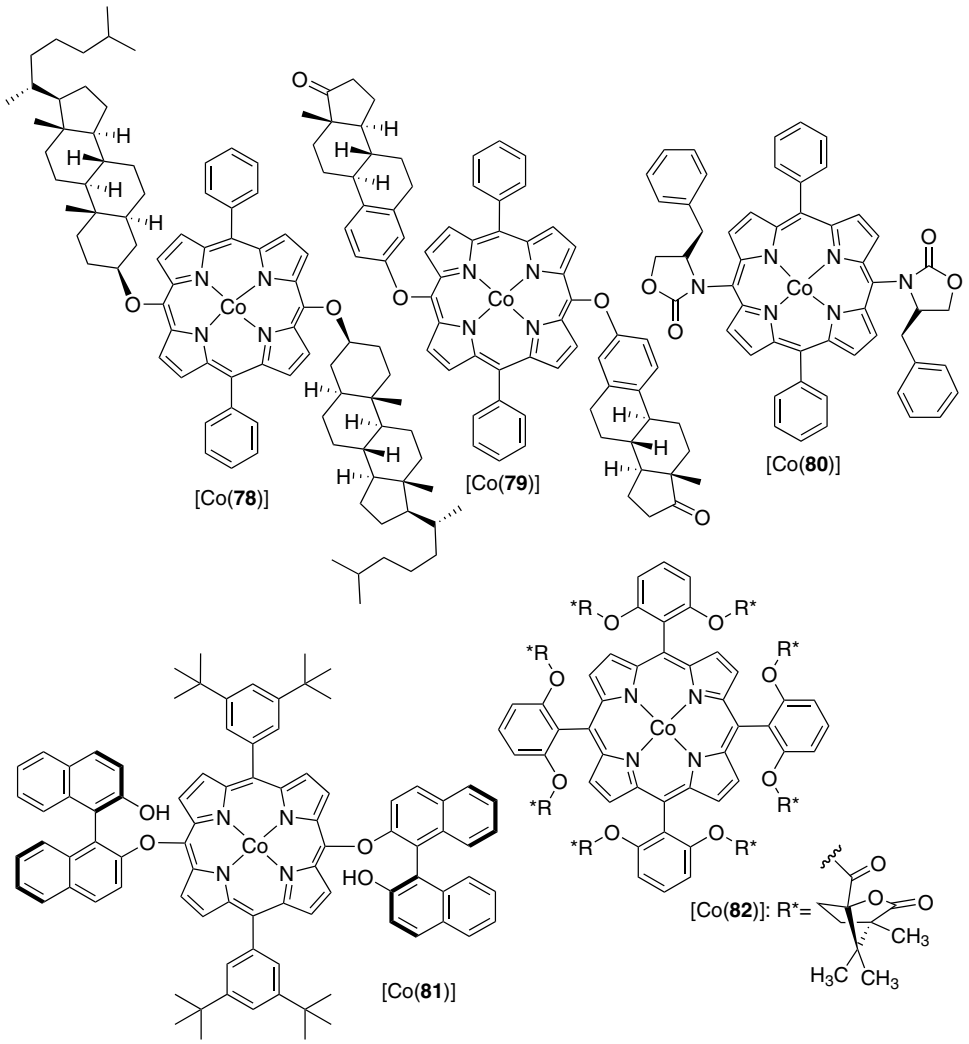
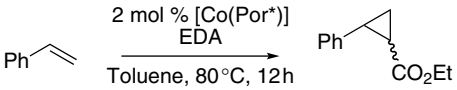


Figure 33. Chiral cobalt porphyrin complexes.[133,134]

The resulting chiral porphyrins (selected examples are shown in Table 45) were evaluated for the cobalt-catalyzed cyclopropanation of styrene with EDA and *tert*-butyl diazoacetate (*t*-BDA). Axial ligands, such as 4-*N*,*N*-dimethylaminopyridine (DMAP), were found to significantly increase diastereo- and enantioselectivity without diminishing the yields (entries 1 and 5, Table 45). Further improvements were achieved by lowering the reaction temperature (entries 8 and 9, Table 45). Given the low cost of cobalt, the "one-pot" process practicality, yield and enantioselectivity generated, chiral cobalt complexes presented themselves as desirable alternatives to other cyclopropanation systems.[137]

Table 44. Asymmetric cyclopropanation of styrene catalyzed by cobalt porphyrin complexes.[133,134]



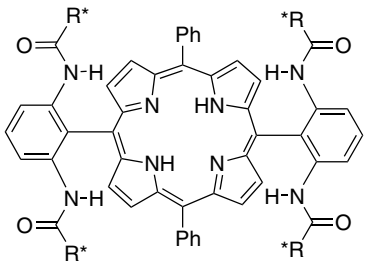
Entry[a]	Por*[b]	Yield (%)	*cis*:*trans*	% ee *cis*	% ee *trans*
1	**82**	73	64:36	77	62
2	**47**	84	48:52	31	10
3	**78**	97	28:72	1	9
4	**79**	99	32:68	1	1
5	**81**	79	36:64	1	1
6	**80**	80	34:66	6	6

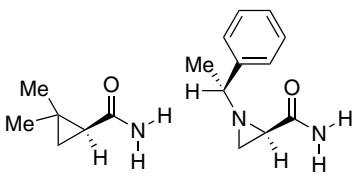
[a] Reactions were performed with 2 mol % catalyst, 1.2 equiv. of diazo, and 1 equiv. of styrene at 80°C for 12 h; [b] See Figures 21 and 33 for structures.

Zhang and co-workers continued to focus their attention on increasing the substrate scope of their cobalt-based system (Table 46).[138] The cobalt D_2-symmetric porphyrin **87** was shown to be a suitable catalyst for the asymmetric cyclopropanation of aromatic olefins (entries 1–5, Table 46). However, the most notable feature of this catalytic system was its ability to cyclopropanate electron-deficient olefins, normally shown to be inactive in other cyclopropanation systems (entries 7–10, Table 46).[138]

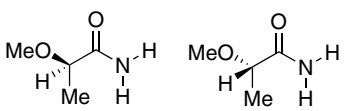
In 2008, Zhang and co-workers reported the use of diazosulfones as diazo reagents for cobalt-catalyzed cyclopropanation.[139] During the course of this study, it was discovered that a new porphyrin ligand would be required to obtain high enantioselectivities (Figure 35). The results, shown in Table 47, demonstrated that the porphyrin complexes examined in the previous cyclopropanation system (**87–88**) did not produce adequate levels of enantioselectivity. Subsequently, ligands **89** and **92** were designed and synthesized in an effort to increase the rigidity and polarity of the chiral environment, thereby enhancing enantioselectivity. The more sterically demanding ligand **92** of the "group of six" (Figure 35) was shown to be the best for supporting cobalt-catalyzed cyclopropanation with diazosulfones (entry 7, Table 47).

With the cobalt complex of ligand **92** identified as the catalyst of choice, the substrate scope was examined (Table 48). Results showed that cobalt-catalyzed cyclopropanation reactions with diazosulfones could be applied to a broad range of olefins, including aromatic and electron-deficient olefins, to generate the desired products with high levels of diastereo- and enantioselectivity.

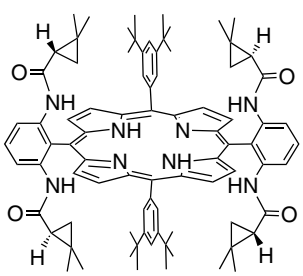




83: R = [*S*]-(+) **84**: R = [*R,R*]-(+)



85: R = [*R*]-(+) **86**: R = [*S*]-(–)

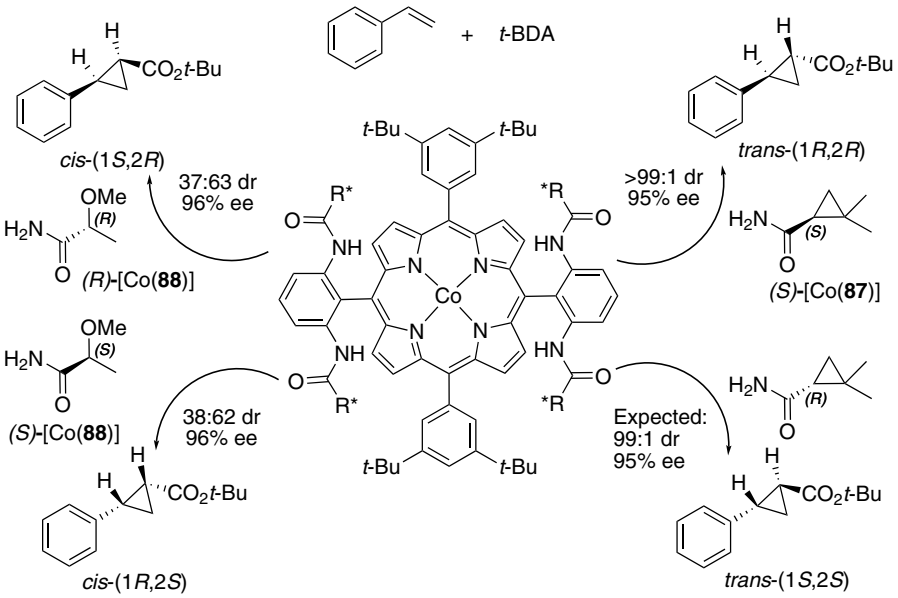


87

Figure 34. D_2-symmetric chiral porphyrins.[135]

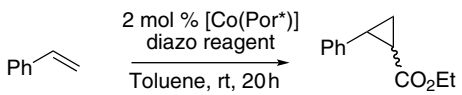
The use of succinimidyl diazoacetate as a carbene source was examined by Zhang and co-workers for the cobalt-catalyzed cyclopropanation of both aromatic and electron-deficient olefins (Table 49).[140] Both ligands **87** and **90** from the "group of six" (Figure 35) provided excellent diastereo- and enantioselectivities. Although the yields associated with the cyclopropanation of electron-deficient olefins were diminished in this system, the stereoselectivities were still very high. The products of these reactions, cyclopropyl succinimidyl esters, are valuable intermediates for the synthesis of cyclopropyl carboxamide derivatives.[140]

Zhang and co-workers also reported on the use of an acceptor/acceptor type diazo reagent, α-nitrodiazoacetate, which was shown to be a suitable diazo source



Scheme 19.[135]

Table 45. Asymmetric cyclopropanation of styrene catalyzed by [Co(**83**–**87**)].[135]

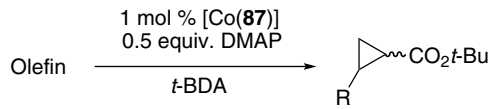


Entry[a]	Por*[c]	Diazo	Additive	Yield (%)	*trans*:*cis*	ee (%)
1	**83**	EDA	—	92	87:13	31
2	**84**	EDA	—	77	66:34	35
3	**85**	EDA	—	92	32:68	48
4	**86**	EDA	—	95	32:68	51
5	**83**	EDA	DMAP	91	96:04	67
6	**87**	EDA	—	89	88:12	43
7	**87**	EDA	DMAP	86	97:03	78
8	**87**	*t*-BDA	DMAP	88	99:01	95
9	**87**	*t*-BDA	DMAP	84	99:01	98

[a] Reactions were performed with 2 mol % catalyst, 1.2 equiv. of diazo, and 1 equiv. of styrene at room temperature for 20 h; [b] –20°C; [c] See Figure 34 for structures.

for use by many cobalt-catalyzed systems.[141,142] The results generated by the "group of six" (Figure 35) in the cyclopropanation of styrene with ethyl α-nitrodiazoacetate (ENDA) is shown in Table 50. Similar to other diazoacetate systems, **87** generated high diastereo- and enantioselectivities (Table 50). Among the solvents examined,

Table 46. Asymmetric cyclopropanation of olefins with *t*-BDA catalyzed by [Co(**87**)].[135–138]



Entry[a]	Olefin	Yield (%)	*trans*:*cis*	ee (%, *trans*)
1	Styrene	84	>99:1	95
2	4-Chlorostyrene	69	98:2	91
3	4-Methoxystyrene	86	98:2	96
4	Pentafluorostyrene	74	97:3	84
5	2-Vinylnaphthalene	84	99:1	98
7[b]	Ethyl acrylate	92	99:1	91
8[b]	Acrylamide	77	99:1	97
9[b]	Pent-1-en-3-one	81	99:1	94
10[b]	Acrylonitrile	83	76:24	93

[a] Reaction conditions: 1 mol % of catalyst, 1.0 equiv. of olefin, 1.2 equiv. of *t*-BDA, and 0.5 equiv. of DMAP in toluene at 25°C under N_2 for 20 h; [b] Chlorobenzene was used as solvent.

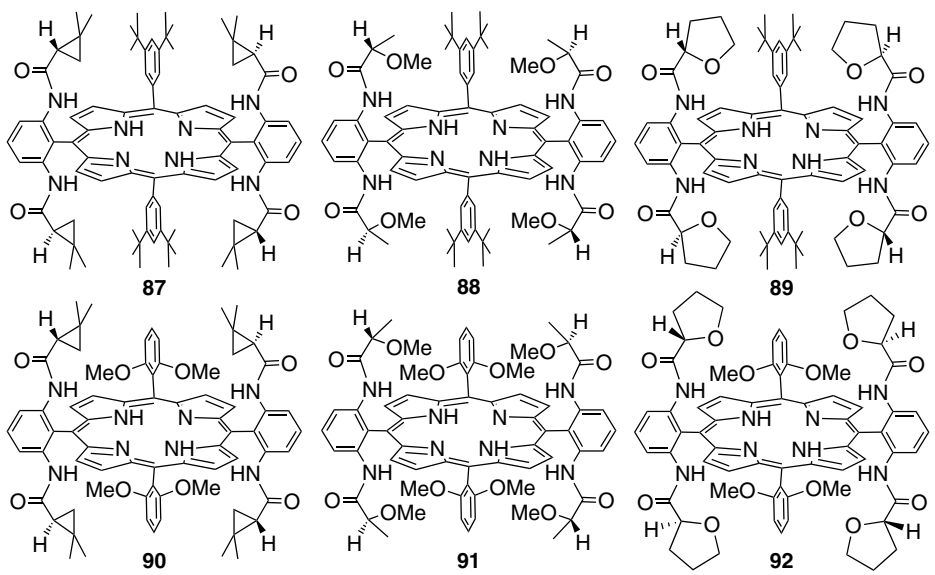
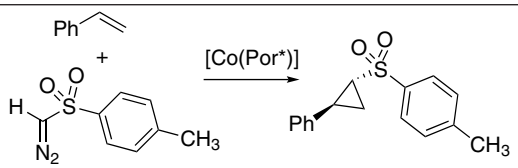


Figure 35. "Group of six" of D_2-symmetric chiral porphyrins.[139]

n-hexane was found to be the preferred solvent, generating the highest enantioselectivity while maintaining high yield and diastereoselectivity (entry 2, Table 50). It was also shown that aromatic and electron-deficient olefins as well as aliphatic olefins were suitable substrates for cyclopropanation with ENDA (Table 51).

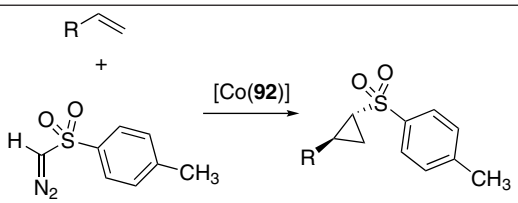
Table 47. Asymmetric cyclopropanation of styrene with diazosulfone catalyzed by Co(II) complexes of the "Group of six" porphyrins.[139]



Entry[a]	[Co(Por*)][b]	Additive	Yield (%)	*trans*:*cis*	ee (%) *trans*
1	[Co(**87**)]	DMAP	–6	>99:01	3
2	[Co(**87**)]	—	86	>99:01	14
3	[Co(**88**)]	—	60	>99:01	23
4	[Co(**89**)]	—	30	>99:01	54
5	[Co(**90**)]	—	78	>99:01	56
6	[Co(**91**)]	—	99	>99:01	61
7	[Co(**92**)]	—	99	>99:01	92

[a] Reaction conditions: Performed at 25°C for 48 h with DCM as solvent using 1 mol % [Co(Por*)] with 1.0 equiv. of styrene and 1.5 equiv. of N_2CHTs; [styrene] = 0.25 M; [b] See Figure 35 for structures.

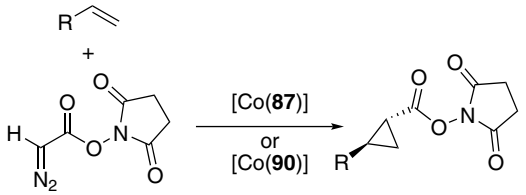
Table 48. Asymmetric cyclopropanation of olefins with diazosulfone catalyzed by [Co(**92**)].[139]



Entry[a]	Substrate	Yield (%)	*trans*:*cis*	ee (%) *trans*
1	Styrene	99	>99:01	92
2	4-*t*-Butylstyrene	57	>99:01	94
3	4-Methoxystyrene	80	>99:01	97
4	4-Trifluoromethylstyrene	72	>99:01	95
5	3-Nitrostyrene	77	>99:01	95
6	2-Vinylnaphthalene	81	>99:01	93
7	Methyl acrylate	96	96:04	89
8	Ethyl acrylate	72	>99:01	90
9	But-3-en-2-one	93	99:01	89
10	Acrylonitrile	81	79:21	61

[a] Reaction conditions: Performed at 25°C for 48 h with DCM as solvent using 1 mol % [Co(**92**)] with 1.0 equiv. of styrene and 1.5 equiv. of N_2CHTs; [styrene] = 0.25 M.

Table 49. Asymmetric cyclopropanation of olefins with succinimidyl diazoacetate catalyzed by [Co(**87**)] and [Co(**90**)].[140]



Entry[a]	Substrate	Yield (%)	*trans*:*cis*	ee (%) *trans*
1	Styrene	86	>99:01	92
2[b]	Styrene	70	>99:01	96
3	4-Methylstyrene	90	>99:01	95
4[b]	4-Methylstyrene	71	98:02	96
5	4-*t*-Butylstyrene	80	>99:01	97
6[b]	4-*t*-Butylstyrene	81	>99:01	98
7	4-Methoxystyrene	71	>99:01	95
8[b]	4-Methoxystyrene	75	99:01	97
9	4-Chlorostyrene	66	>99:01	90
10[b]	4-Chlorostyrene	48	>99:01	92
11	4-Trifluoromethylstyrene	77	>99:01	90
12[b]	4-Trifluoromethylstyrene	30	>99:01	94
13	4-Vinylphenyl acetate	71	>99:01	91
14	3-Nitrostyrene	50	>99:01	92
15	2-Vinylnaphthalene	33	99:01	91
16	Ethyl acrylate	57	>99:01	89
17	*N,N*-dimethylacrylamide	52	>99:01	96
18	But-3-en-2-one	55	>99:01	91

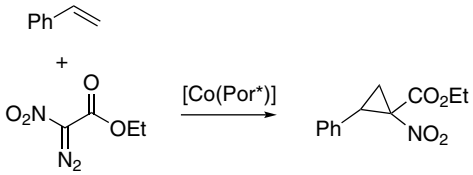
[a] Reaction conditions: Performed at 25°C for 48 h with toluene as solvent using 5 mol % [Co(**87**)] with 1.0 equiv. of styrene and 1.5 equiv. of N_2CHCO_2Suc in the presence of 0.5 equiv. of DMAP; [styrene] = 0.25 M; [b] [Co(**90**)] used as catalyst.

B. Chiral Strapped Porphyrins

In 1998, Simonneaux and co-workers described the use of threitol-strapped ligand **47** (Figure 21), previously reported by Gross and co-workers for epoxidation[72,73] (Sec. II.B), for the ruthenium-catalyzed asymmetric cyclopropanation of aromatic olefins. The ruthenium complex [Ru(**47**)(CO)] was used with ethyl diazoacetate to generate cyclopropane products with excellent yields and moderate diastereo- and enantioselectivities (Table 52).[143]

In 2003, Simonneaux and co-workers reported their work with threitol-strapped ruthenium catalysts for the cyclopropanation of styrene with diisopropyl diazomethylphosphonate (DAMP) as the carbene source.[144] The yield and diastereoselectivities (Scheme 20) produced with [Ru(**47**)(CO)] in the presence of

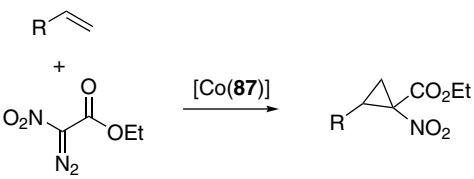
Table 50. Asymmetric cyclopropanation of styrene with ENDA catalyzed by Co(II) complexes of the "Group of six" porphyrins.[141]



Entry[a]	Por*[b]	Solvent	Yield (%)	*Z*:*E*	ee (%) *Z*
1	[Co(**87**)]	CH_2Cl_2	99	91:09	81
2	[Co(**87**)]	*n*-C_6H_{14}	87	92:08	89
3	[Co(**88**)]	CH_2Cl_2	20	67:33	33
4	[Co(**89**)]	CH_2Cl_2	31	66:34	–23
5	[Co(**90**)]	CH_2Cl_2	99	91:09	58
6	[Co(**91**)]	CH_2Cl_2	69	81:19	47
7	[Co(**92**)]	CH_2Cl_2	<5	85:15	nd

[a] Reaction conditions: Performed at 25°C for 48 h with DCM as solvent using 1 mol % [Co(Por*)] with 1.0 equiv. of styrene and 1.2 equiv. of ENDA; [styrene] = 0.25 M; [b] See Figure 35 for structures.

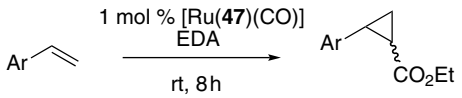
Table 51. Asymmetric cyclopropanation of olefins with α-nitrodiazoacetate catalyzed by [Co(**87**)].[141]



Entry[a]	Substrate	Yield (%)	*Z*:*E*	ee (%) *Z*
1	Styrene	93	92:08	92
2	4-Methylstyrene	86	93:07	90
3	4-Chlorostyrene	82	91:09	90
4	4-Trifluoromethylstyrene	88	92:08	90
5	3-Nitrostyrene	81	93:07	95
6[b]	Methyl acrylate	42	53:47	88
7[b]	Ethyl acrylate	62	56:44	88
8[b]	*N*,*N*-Dimethylacrylamide	92	63:37	75
9[c]	1-Hexene	45	92:08	80

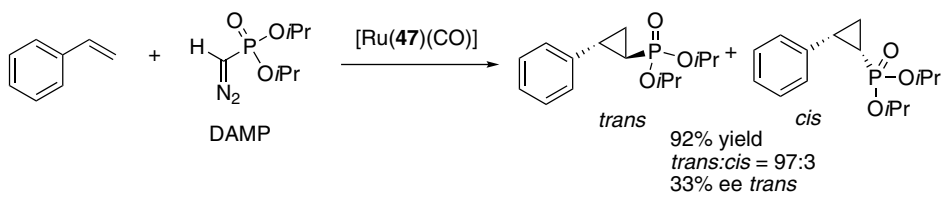
[a] Reaction conditions: Performed at 25°C for 24 h with hexane as solvent using 5 mol % [Co(**87**)] with 1.0 equiv. of styrene and 1.2 equiv. of ENDA; [styrene] = 0.25 M; [b] DCE used as solvent; [c] Reaction performed under neat conditions.

Table 52. Asymmetric cyclopropanation of aromatic olefins catalyzed by [Ru(**47**)(CO)].[143]



Entry[a]	Substrate	Yield (%)	*trans*:*cis*	ee (%) *trans*
1	Styrene	85	4:1	46
2	4-Methoxystyrene	95	6:1:1	47
3	4-Methylstyrene	82	9:9:1	46
4	4-Chlorostyrene	93	8:6:1	52
5	4-Bromostyrene	93	7:1:1	45
6	4-Fluorostyrene	92	11:0:1	50

[a] Reactions were performed with 1 mol % catalyst, 1 equiv. of diazo, and substrate neat at room temperature for 8 h.



Scheme 20.[144]

DAMP were excellent, and comparable to the results generated by the ruthenium complex of the Halterman porphyrin **8** (Sec. III.A). However, unlike the Halterman system, the enantiomeric excess was shown to be significantly lower.

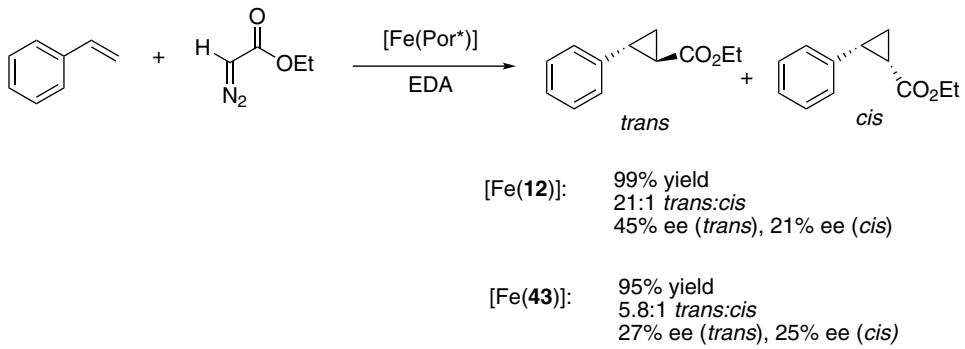
The research groups of Rose and Cenini collaboratively developed an asymmetric cyclopropanation system utilizing cobalt complexes of the binaphthyl-strapped porphyrins **45** and **46** (Figure 20 in Sec. II.B).[145] These porphyrin complexes, first developed by Collman and Rose for asymmetric epoxidation, were evaluated for cobalt-catalyzed cyclopropanation of various styrene derivatives using EDA (Table 53). Although the yields were consistently excellent across a range of substrates, the diastereo- and enantioselectivities reported were not as high as the cobalt-catalyzed cyclopropanation systems developed by Zhang and co-workers.[135]

In 2002, Rose, Woo, and co-workers published a report using the Halterman porphyrin derivative **12** and Collman's bis-binaphthyl strapped ligand **43** for the Fe(II)-catalyzed asymmetric cyclopropanation of styrene with EDA.[146] The Fe(II) complex of the Halterman porphyrin derivative, [Fe(**12**)], generated excellent yields and diastereoselectivities. However, it afforded only a modest 45% ee (Scheme 21). The Fe(III) bis-binaphthyl strapped complex, [Fe(**43**)], showed decreased selectivities.

Table 53. Asymmetric cyclopropanation of aromatic olefins EDA catalyzed by [Co(**45**)] and [Co(**46**)].[145]

		[Co(46)][b]					[Co(45)][b]				
					ee (%)					ee (%)	
Entry[a]	Substrate	Time (h)	Yield (%)	*cis:trans*	*cis*	*trans*	Time (h)	Yield (%)	*cis/trans*	*cis*	*trans*
1	Styrene	36	92	27:33	60	10	48	96	25:75	57	42
2[c]	Styrene	60	89	16:84	50	46	60	93	18:82	66	31
3	α-Methylstyrene	20	95	27:33	76	31	16	94	28:72	39	22
4	4-Chlorostyrene	24	90	29:71	61	8	24	85	17:83	8	21
5	4-Methylstyrene	36	90	27:33	62	10	—	—	—	—	—
6	1,1-Diphenylethylene	72	93	—		56	72	93	—	22	

[a] Reactions were performed with 0.5 mol % of catalyst, 1 equiv. of EDA, and 10 equiv. of substrate in benzene at room temperature; [b] See Figure 20 for structures; [c] *N*-methylimidazole (20 equiv.) was added to reaction mixture.



Scheme 21.[146]

IV. Aziridination

The fundamental and practical significance of aziridine derivatives in chemistry and biology has stimulated intensive research into their synthesis.[147–151] Among the most important routes for the preparation of aziridines, the metal-catalyzed aziridination of alkenes with nitrene sources has received the most attention as this method provides direct access to the desired products.

Despite the success of metalloporphyrins in other atom/group transfer reactions, their use in asymmetric aziridination has been limited.[152–155] Early important efforts in the development of asymmetric aziridination systems by Evans and Jacobsen were based on non-metalloporphyrin systems.[156,157] More recently, several research groups have made significant progress in the development of metalloporphyrin complexes capable of performing asymmetric aziridination. For example, Che and co-workers used manganese and ruthenium Halterman complexes for the asymmetric aziridination of a number of different olefins. In addition, Zhang and co-workers used cobalt complexes of their D_2-symmetric porphyrin catalysts for the aziridination of olefins using azides as nitrene sources. Finally, Marchon and co-workers employed the manganese complex of the "Chiroporphyrin" ligand **27a** developed previously for epoxidation and cyclopropanation for use in asymmetric aziridination.

Traditionally, aziridination systems relied heavily on hypervalent iodine species, such as PhI = NTs and its derivatives.[158–163] Current investigations in the field have focused on the development of alternative nitrene sources, such as the *in-situ* generation of iminoiodane derivatives and azides.[164–172]

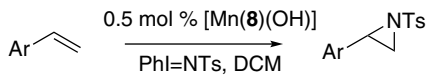
This section will review the current efforts in metalloporphyrin-catalyzed asymmetric aziridination, including recent developments concerning the use of new nitrene sources. As in previous sections, the reaction system will be categorized by the type of porphyrin. The use of alternative nitrene sources will also be highlighted in several studies.

A. Chiral Picket Fence Porphyrins

Che and co-workers investigated the catalytic asymmetric aziridination of olefins by the manganese complex of the Halterman porphyrin **8**, [Mn(**8**)(OH)], using PhI = NTs as nitrene source.[173] Moderate yields and enantioselectivities were achieved with the manganese-catalyzed system, and modest TON were reported (Table 54).

In a later report, Che and co-workers compared the ruthenium and manganese complexes of the Halterman porphyrin **8** (Table 55).[174] In the case of styrene, the manganese complex generated selectivity superior to that of its ruthenium counterpart (entries 1 and 2, Table 55). Comparisons between entries 3 and 4 in Table 55

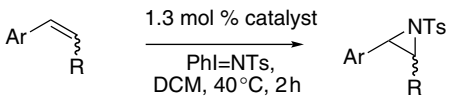
Table 54. Asymmetric aziridination with PhI = NTs catalyzed by [Mn(**8**)(OH)].[173]



Entry[a]	Substrate	Yield (%)	TON	ee (%)
1	Styrene	71	130	49
2[b]	Styrene	73	142	55
3	4-Methylstyrene	66	132	44
4	4-Chlorostyrene	43	86	45
5	3-Chlorostyrene	49	98	49
6	2-Bromostyrene	44	88	62

[a] Reactions were performed with 0.5 mol % of catalyst, 20 equiv. of substrate, and 1 equiv. of PhI = NTs in DCM at 40°C for 2 h; [b] 4-Phenylpyridine *N*-oxide as additive.

Table 55. Asymmetric aziridination of aromatic olefins catalyzed by ruthenium and manganese complexes of **8**.[174]



Entry[a]	Substrate	Catalyst	Yield (%)[b]	ee (%)
1	Styrene	[Mn(**8**)(OH)]	78 (99)	47
2	Styrene	[Ru(**8**)(CO)]	84 (71)	21
3	4-Methylstyrene	[Mn(**8**)(OH)]	76 (99)	44
4	4-Chlorostyrene	[Mn(**8**)(OH)]	93 (99)	50
5	2-Vinylnaphthalene	[Mn(**8**)(OH)]	94 (99)	56
6	Indene	[Ru(**8**)(CO)]	73 (99)	11

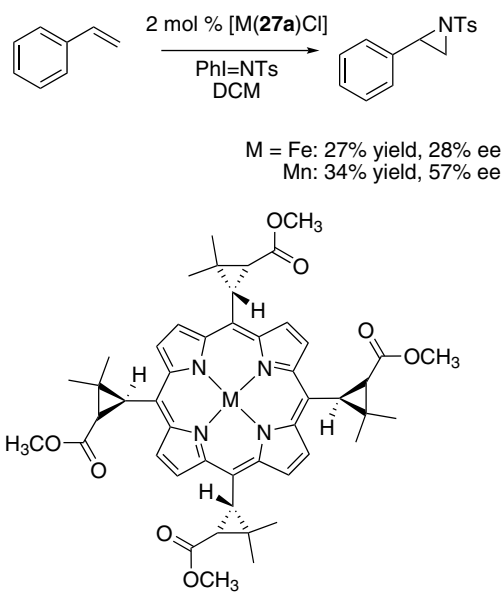
[a] Reactions were performed with 1.3 mol % of catalyst, 1 equiv. of substrate, and 3 equiv. of PhI = NTs in DCM at 40°C for 2 h; [b] Yields are based upon the amount of alkene consumed in the reaction. The number in parenthesis represents conversion of substrate.

and those in Table 54 demonstrate that an improvement in yield could be achieved with higher catalyst loadings without sacrificing enantioselectivity.

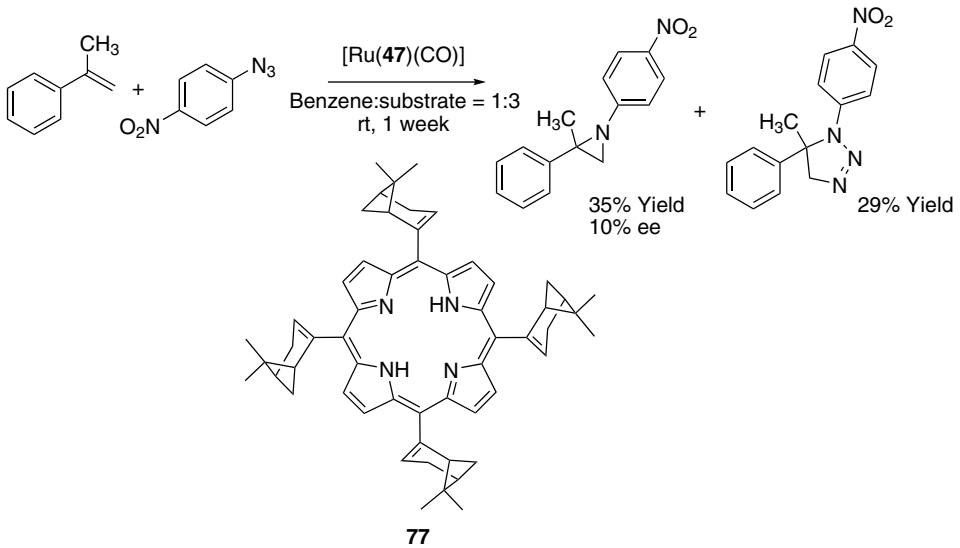
Marchon and co-workers employed iron and manganese complexes of "chiroporphyrin" **27a** for the aziridination of aromatic olefins with PhI = NTs.[175] They reported that [Mn(**27a**)Cl] produced higher yields and greater enantioselectivities than [Fe(**27a**)Cl] when styrene was employed as a substrate (Scheme 22).

In their 2006 report on the use of the ruthenium complex of **77** for cyclopropanation using EDA, Cenini and co-workers also communicated the first report of the ruthenium porphyrin-catalyzed aziridination of olefins using aryl azides (Scheme 23).[132] They observed the formation of the aziridine product in low yield and enantioselectivity with the corresponding triazoline produced as the major side product.

The success of the D_2-symmetric chiral porphyrins developed for asymmetric cyclopropanation encouraged Zhang and co-workers to continue investigating other atom/group transfer reactions, including aziridination.[176] Their research focused not only on using the "group of six" porphyrins (Figure 35, Sec. III.A) as ligands for cobalt-catalyzed aziridination, but also on the use of nitrene sources other than hypervalent iodine reagents. They reported the use of diphenylphosphoryl azide (DPPA) for the asymmetric aziridination of aromatic olefins (Table 56).

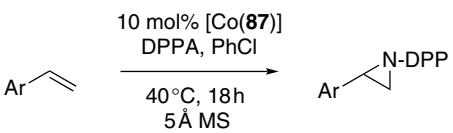


Scheme 22.[175]



Scheme 23.[132]

Table 56. Asymmetric aziridination of aromatic olefins with DPPA catalyzed by [Co(**87**)].[176]



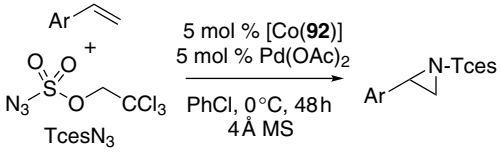
Entry[a]	Substrate	Yield (%)	ee (%)
1	Styrene	88	37
2	4-Methylstyrene	58	37
3	4-*tert*-Butylstyrene	77	53
4	4-Bromostyrene	65	28
5	4-Chlororstyrene	64	6
6	4-Trifluoromethylstyrene	64	44
7	3-Nitrostyrene	58	46

[a] Reactions were performed with 10 mol % of catalyst, 5 equiv. of substrate, and 1 equiv. of DPPA in PhCl at 40°C for 18 h in the presence of 5 Å MS.

While their early attempt at cobalt-catalyzed asymmetric aziridination produced modest yields and enantioselectivities, their report is significant in that it presents one of the first uses of diphenylphosphoryl azide (DPPA) for asymmetric aziridination.

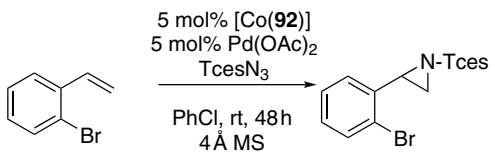
In their continued efforts to develop nitrene sources other than PhI = NTs and its derivatives, Zhang and co-workers synthesized and explored trichloroethoxysulfonyl

Table 57. Asymmetric aziridination of olefins with $TcesN_3$ catalyzed by [Co(**92**)].[177]



Entry[a]	Substrate	Yield (%)	ee (%)
1	Styrene	91	94
2[b]	Styrene	69	94
3	4-Mehtylstyrene	89	90
4	4-Chlorostyrene	93	91
5	4-Trifluoromethylstyrene	88	81
6	3-Nitrostyrene	82	88
7[c]	α-Methylstyrene	48	80
8[d]	Allylbenzene	42	91

[a] Reactions were performed with 5 mol % of catalyst, 5 equiv. of substrate, and 1 equiv. of $TcesN_3$ in PhCl at 0°C for 48 h in the presence of 4 Å MS; [b] Without $Pd(OAc)_2$; [c] Room temperature; [d] 24 h without $Pd(OAc)_2$.



first cycle: 95% yield, 96% ee
second cycle: 89% yield, 94% ee
third cycle: 81% yield, 94% ee

Scheme 24.[177]

azide, $TcesN_3$.[177] The cobalt complex of the D_2-symmetric porphyrin **92** generated excellent yields and enantioselectivities for asymmetric aziridination of both aromatic and aliphatic olefins with $TcesN_3$ (Table 57). The importance of $Pd(OAc)_2$ as a co-catalyst was noted. The addition of sub-stoichiometric amounts of $Pd(OAc)_2$ improved yield without affecting the asymmetric induction (entries 1–2, Table 57). This effect is attributed to the potential activation of the olefin by $Pd(OAc)_2$ as a π-electrophilic Lewis acid.

The [Co(**92**)] catalyst was also demonstrated to be recyclable in the catalytic system. The cobalt catalyst could be precipitated onto molecular sieves with the addition of hexanes. After being filtered and dried, it was reused in subsequent reactions without significant loss of enantioselectivity over at least three cycles (Scheme 24).

V. Carbon–Hydrogen Bond Functionalization

Carbon–hydrogen bond functionalization *via* atom/group transfers represents a direct way to convert hydrocarbons to valuable intermediates.[52,154,178–184] This section will briefly highlight several examples of asymmetric carbon–hydrogen bond functionalization reactions that have been developed using chiral porphyrin catalysts discussed previously in this chapter.

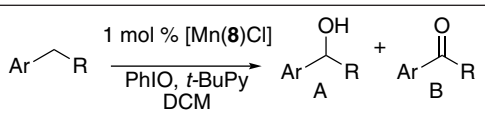
A. Chiral Picket Fence Porphyrins

1. *Hydroxylation*

In 1997, Halterman and co-workers reported use of the manganese complex of porphyrin **8**, [Mn(**8**)Cl], for the asymmetric hydroxylation of hydrocarbons.[34] Although reactions with ethylbenzene (entry 1, Table 58) gave rise to products in low yield and with poor enantioselectivity, reactions with indan and tetrahydronaphthalene resulted in improved yields and higher enantioselectivities (entries 2–3, Table 58). The authors noted that the reactions needed to be carefully monitored, as longer reaction times resulted in over-oxidation and the formation of a ketone side product.

The ruthenium complex of the Halterman porphyrin **8**, [Ru(**8**)(CO)], was also used by Che and co-workers in 1999 for asymmetric hydroxylation.[76,185] The use of the ruthenium carbonyl complex led to significant improvements in enantioselectivity when compared to the [Mn(**8**)Cl] prepared by Halterman. The ruthenium oxide complex [Ru(**8**)$(O)_2$] was also shown to be capable of performing hydroxylation under stoichiometric conditions, although with lower yield and enantioselectivities in comparison to [Ru(**8**)(CO)] (entries 1 and 2, Table 59).

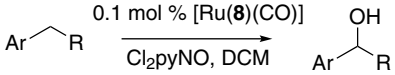
Table 58. Asymmetric benzylic hydroxylation catalyzed by [Mn(**8**)Cl].[34]



Entry[a]	Substrate	Time	Yield (%)	ee (%)	A:B
1	Ethylbenzene	16 h	27	9	1:1
2	Indan	20 m	50	53	5:1
3	Tetrahydronaphthalene	40 m	39	44	3:1
4	2-Ethylnaphthalene	14 h	24	40	2:1

[a] Reactions were performed using 1 mol % of catalyst, 10 equiv. of substrate, and 1 equiv. of PhIO with 20 mol % of *t*-BuPy as additive in DCM.

Table 59. Asymmetric benzylic hydroxylation catalyzed by [Ru(**8**)(CO)].[76,185]



Entry[a]	Substrate	Time	Yield (%)[b]	ee (%)
1	Ethylbenzene	12	62 (13)	72
2[c]	Ethylbenzene	12	30	37
3	4-Ethyltoluene	30	72 (20)	76
4	1-Bromo-4-ethylbenzene	8	63 (14)	74
5	4-Ethylanisole	8	65 (15)	62
6	2-Ethylnaphthalene	20	62 (15)	75
7	Indan	6	65 (54)	12
8	Tetrahydronaphthalene	2	60 (42)	12

[a] Reactions were performed using 0.1 mol % of catalyst, 1 equiv. of substrate, and 1.1 equiv. of Cl_2pyNO as oxidant in benzene; [b] Yields are based upon the amount of alkene consumed in the reaction. The number in parenthesis represents conversion of substrate; [c] [Ru(**8**)$(O)_2$] used under stoichiometric condtitons.

Table 60. Asymmetric sulfoxidation catalyzed by [Mn(**8**)Cl].[34]

Entry[a]	Substrate	Yield A (%)	ee (%, A)	A:B
1	Thioanisole	93	55	44:1
2	Ethyl phenyl sulide	82	42	13:1
3	2-Bromothioanisole	99	68	>95:1
4	4-Bromothioanisole	98	59	34:1
5	4-Methoxythioanisole	99	40	44:1
6	Thiochroman-4-one	99	47	>95:1

[a] Reactions were performed using 0.5 mol % of catalyst, 2 equiv. of substrate, and 1 equiv. of PhIO in DCM.

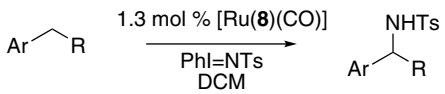
2. *Sulfoxidation*

In conjunction with their previous reports on the epoxidation and hydroxylation using porphyrin **8**, Halterman and co-workers reported sulfoxidation of prochiral aromatic aryl alkyl sulfides.[34] Using the manganese complex [Mn(**8**)Cl], they demonstrated that the sulfoxide products **A** could be generated in high yields with moderate enantioselectivities (Table 60). Similar to the work on hydroxylation, minimal amounts of over-oxidized sulfone side products (**B**) were produced.

3. *Amination*

During the course of their study on the asymmetric aziridination of olefins with metal complexes of **8**, Che and co-workers reported the asymmetric amination of hydrocarbons.[174] The ruthenium complex [Ru(**8**)(CO)] was explored for catalytic amination of various benzylic substrates with PhI = NTs. Moderate to low enantioselectivities were produced (Table 61). Improvements were achieved through the *in-situ* generation of the nitrene source from a sulfonyl amide in the presence of $PhI(OAc)_2$ (Table 62). These systems were able to achieve moderate

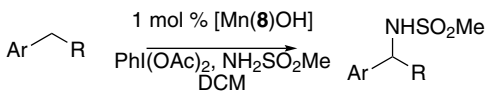
Table 61. Asymmetric amination of benzylic hydrocarbons catalyzed by [Ru(**8**)(CO)].[174]



Entry[a]	Substrate	Yield (%)	ee (%)
1	4-Ethylanisole	82 (29)	22
2	Indan	92 (32)	3
3	Tetrahydronaphthalene	85 (22)	28
4	2-Ethylnaphthalene	84 (23)	47

[a] Reactions were performed using 1.3 mol % of catalyst, 2 equiv. of PhI = NTs, and 1 equiv. of substrate in DCM at 40°C for 2 h; [b] Yields are based upon the amount of alkene consumed in the reaction. The number in parenthesis represents conversion of substrate.

Table 62. Asymmetric amination of benzylic hydrocarbons with *in-situ* generation of nitrene source catalyzed by [Mn(**8**)(OH)].[174]



Entry[a]	Substrate	Yield (%)[b]	ee (%)
1	4-Ethylanisole	84 (26)	50
2	Tetrahydronaphthalene	84 (22)	46
3	2-Ethylnaphthalene	92 (21)	56
4[c]	2-Ethylnaphthalene	90 (34)	40

[a] Reactions were performed using 1 mol % of catalyst, 1 equiv. of $PhI(OAc)_2$, 1.25 equiv. of sulfonyl amide, and 1.5 equiv. of substrate in DCM at 40°C for 2 h; [b] Yields are based upon the amount of alkene consumed in the reaction. The number in parenthesis represents conversion of substrate; [c] Catalyzed by [Ru(**8**)(CO)].

enantioselectivities across a variety of benzylic substrates. It was also shown that [Mn(**8**)OH] was able to induce a higher level of asymmetry than the ruthenium complex [Ru(**8**)(CO)] (entries 3 and 4, Table 62).

B. Chiral-Strapped Porphyrins

1. *Hydroxylation*

In 1999, Gross and co-workers reported the use of the threitol-strapped porphyrin **51** (Figure 21) for the ruthenium-catalyzed hydroxylation of a racemic tertiary alkane.[75] The desired hydroxylation products were generated in 38% enantiomeric excess (Scheme 25). The result is remarkable since the asymmetric induction relies on the differentiation of the similar methyl and ethyl groups on the substrate by the active site of the catalyst (Scheme 25).

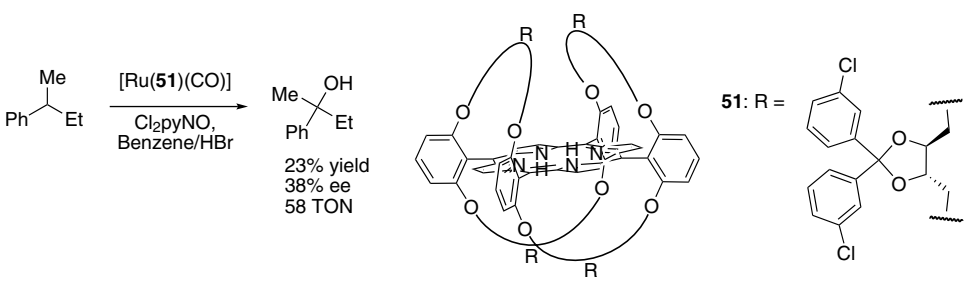
2. *Carbene Insertion*

In addition to using porphyrin ligand **47** for the ruthenium-catalyzed asymmetric cyclopropanation of olefins with EDA, Simonneaux and co-workers reported the ability of the catalyst to perform carbene insertion with thiols.[143] Although high yields were generated with this system, the resulting asymmetric induction was low (Scheme 26).

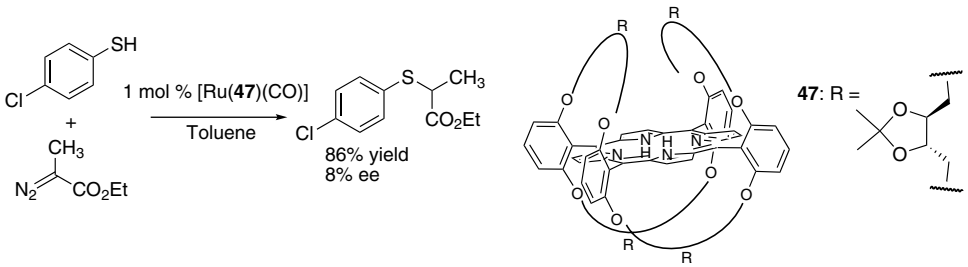
C. Chiral Basket Handle Porphyrins

1. *Hydroxylation*

Groves and co-workers employed both iron and manganese complexes of "vaulted" binaphthylporphyrin **65** (Figure 27) for studying catalytic hydroxylation of hydrocarbons.[84] Several substrates were screened for hydroxylation with

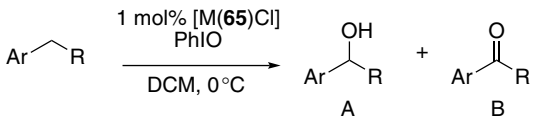


Scheme 25.[75]



Scheme 26.[143]

Table 63. Asymmetric sulfoxidation catalyzed by iron and manganese complexes of ligand **65**.[84]



		[Fe(**65**)Cl]			[Mn(**65**)(Cl)]		
Entry[a]	Substrate	Yield (%)	ee (%)	A:B	Yield (%)	ee (%)	A:B
1	Ethylbenzene	40	40	2.3:1	77	26	1:3:1
2	1-Phenylpropane	32	44	5.0:1	98	16	1:4:1
3	Indan	72	54	7.2:1	82	18	4:5:1
4	Tetrahydronaphthalene	47	72	20.2:1	46	12	1:3:1

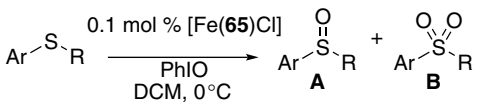
[a] Reactions were performed with 1 mol % of catalyst, 1 equiv. of PhIO, and 10 equiv. of substrate in DCM at 0°C.

[Fe(**65**)Cl] and [Mn(**65**)Cl] as catalysts (Table 63). Although the yields generated by the manganese complex were typically higher, the iron-catalyzed system gave higher enantioselectivities. In addition to the desired hydroxylation products, ketones were observed as the side products, even in the presence of a tenfold excess of substrate.

2. *Sulfoxidation*

The iron complex of "vaulted" binaphthyl porphyrin **65** was also used by Groves and co-workers for asymmetric sulfoxidation of aryl sulfides.[84] Similar to the results by Halterman with [Mn(**8**)Cl] (Table 60), it was shown that the [Fe(**65**)Cl]-based sulfoxidation process could successfully generate the desired sulfoxide products **A** in good yields but low enantioselectivities with sulfones **B** as the over-oxidation side products (Table 64).

Table 64. Asymmetric sulfoxidation catalyzed by [Fe(**65**)Cl].[84]



Entry[a]	Substrate	Yield (%)	ee (%)	A:B
1	Thioanisole	84	24	8.2:1
2	Ethyl phenyl sulide	73	42	7.9:1
3	2-Bromothioanisole	74	48	8.4:1
4	4-Bromothioanisole	88	20	7.5:1
5	4-Methoxythioanisole	70	14	6.2:1
6	Thiochroman-4-one	67	28	8.9:1

[a] Reactions were performed using 0.5 mol % of catalyst, 1 equiv. of substrate, and 10 equiv. of PhIO in DCM at 0°C.

VI. Conclusions

The use of chiral porphyrin ligands for asymmetric atom/group transfer reactions has significantly evolved since the first report by Groves and Myers in 1983. Various metal complexes of chiral porphyrins have been used for catalytic asymmetric epoxidation, cyclopropanation, aziridination, and in several reports of carbon–hydrogen bond functionalizations. The greatest efforts have been focused on the development of metalloporphyrin catalysts for asymmetric epoxidation. Much of the research in this area has been devoted to the synthesis and evaluation of metal complexes of Halterman porphyrin **8**. Catalytic systems based on this robust porphyrin have been shown to be capable of achieving several thousand TON for some catalytic processes. Complexes of Collman's BINAP-strapped porphyrins **43**–**44**, including its derivatives **45**–**46** prepared by Rose, have been demonstrated with the highest enantioselectivities for metalloporphyrin-catalyzed asymmetric epoxidation reactions.

The successes of both Halterman and Collman's BINAP-strapped porphyrins emphasize a balanced need for open access and steric hindrance of catalyst's active site where the approach of substrates and subsequent reactions take place. The desired balance of the two factors in these successful metalloporphyrin-catalyzed epoxidation processes allowed for facilitation of reaction rates and reduction of catalyst degradation while reaching high selectivities.

Research activities on metalloporphyrin-catalyzed asymmetric cyclopropanation were also dominated by the use of metal complexes of Halterman porphyrin **8**. Similar to the development of metalloporphyrin-based asymmetric epoxidation, various metal complexes of Halterman porphyrin have been employed as catalysts for asymmetric cyclopropanation. High diastereoselectivity and enantioselectivity

along with high TON have been achieved for some asymmetric cyclopropanation processes. The recent development of Co(II) complexes of the D_2-symmetic chiral porphyrins **87–92** by Zhang and co-workers has provided some of the most selective and practical cyclopropanation catalytic systems. These Co(II)-catalyzed cyclopropanation systems have been demonstrated to be effective for a wide range of alkene substrates and with different types of diazo reagents, providing the desired cyclopropane products in high yields with excellent diastereoselectivities and enantioselectivities.

The development of metalloporphyrin-catalyzed asymmetric aziridination has lagged behind in comparison to epoxidation and cyclopropanation reactions. Many of the same catalytic systems employed for asymmetric cyclopropanation have been extended to asymmetric aziridination. Although this direction of efforts have been further advanced by the recent publications from the research groups of Che, Zhang, and others, many challenging issues remain to be solved in the development of catalytic systems for asymmetric aziridination.

The demonstrated examples of asymmetric carbon–hydrogen bond functionalization processes, developed in conjunction with other atom/group transfer reactions, are another indication of the catalytic versatility of metalloporphyrin-based catalysts. Further development for these more challenging catalytic transformations is one of the most important on-going objectives in asymmetric catalysis by metalloporphyrins.

Finally, the diverse porphyrin ligand systems already developed and the great amount of progress achieved in epoxidation and cyclopropanation reactions ensure that the continued development of metalloporphyrin-based asymmetric atom/group transfer processes will remain an active goal for researcher around the world in the years to come.

VII. Acknowledgments

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VIII. References

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11 Porphyrin Functionalizaton *via* Palladium-Catalyzed Carbon–Heteroatom Cross-Coupling Reactions

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I. Introduction

Porphyrins and related tetrapyrrolic macrocycles play essential roles in biological systems in such processes as electron transfer, light harvesting, dioxygen transport, and substrate transformations.[1] The significance of these processes has stimulated intense interest in synthesizing novel porphyrin complexes that mimic these systems. These porphyrins have important applications in a number of fields, including catalysis,[2–13] polymer synthesis,[14] materials,[15,16] and photodynamic therapy.[17–26]

Chiral porphyrins have found a range of useful applications in asymmetric catalysis, chiral recognition/sensing, and enzymatic mimicry.[10,27–31] Several approaches have been described for the syntheses of chiral porphyrins.[3,32,33] Historically, the preparation of substituted porphyrins has been limited by the acidic and oxidative reaction conditions which restrict substituent incorporation on the porphyrin macrocycle. These methods are typically associated with low-yields and tedious purifications.[34] As a result, the development and application of new synthetic strategies to overcome these difficulties has been the focus of porphyrin research during the last several decades.

In recent years, considerable progress has been made in the development of palladium-catalyzed cross-coupling reactions for the synthesis of chiral porphyrins. Smith and co-workers were the first to apply C–C cross-coupling methodologies to haloporphyrins.[35] Since their seminal report in 1989, reactions developed by Suzuki, Stille, Heck, Sonogashira, and others have been used to create new carbon–carbon bonds using palladium-mediated catalysis.[36–64] With these systems, a single porphyrin precursor can be converted into a large number of functionalized derivatives.[65] The generation of suitable precursors has been the key to the success of these reactions, and research has shown that bromo- and iodo-porphyrins have played the most important roles.[36,37,66,67]

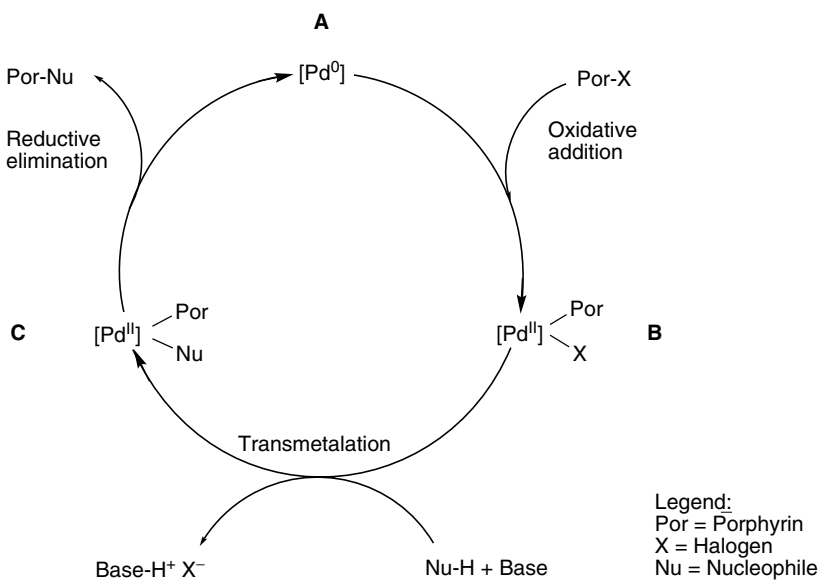
In light of the results obtained from halogenated porphyrins in transition metal-mediated carbon–carbon bond formation reactions, the application of palladium-catalyzed carbon–heteroatom bond cross-couplings was a natural extension. Therien and co-workers' 1998 report on C–B bond formation provided the first examples of palladium-catalyzed carbon–heteroatom cross-coupling reactions using porphyrins.[66] Since then, a variety of boronic esters, amines, amides, alcohols, thiols, selenols, diphenylphosphines, diphenylphosphine oxides, and

phosphanes have been coupled to many different porphyrin scaffolds, typically in high yields.

This chapter provides a detailed overview of important, recent advancements in palladium-catalyzed porphyrin carbon–heteroatom bond formation through July of 2009. The reactions presented in this chapter will be categorized by the type of carbon–heteroatom bond formed, and subcategorized by the position functionalized on the porphyrin, including the *meso*- and β-positions on the porphyrin macrocycle and the aryl groups post ring cyclization.

A. General Mechanistic Overview

The general palladium-catalyzed cross-coupling reaction with porphyrin halides consists of a palladium source, ligand(s), an aryl halide, a soft nucleophile, a base, and the solvent. As shown in Scheme 1, palladium(0) ligand complex **A** undergoes oxidative addition with the porphyrin halide, increasing both the oxidation state and coordination number of the palladium by two to form **B**. Complex **B** then undergoes transmetalation with a base-activated nucleophile to form complex **C**. Complex **C** then undergoes reductive elimination to release the coupled-porphyrin product, decreasing both the oxidation state and coordination number by two to regenerate the initial palladium(0) species **A**.



Scheme 1.

The typical catalytic cycle begins with a palladium(0) complex undergoing oxidative addition. This step, which is typically the rate-determining step, is affected by several factors. First, bulky, unsaturated palladium species containing four or less phosphine ligands typically react rapidly to initiate the catalytic cycle. $Pd(PPh_3)_4$ and $Pd_2(dba)_3$, are two of the most common palladium sources, followed by the air-stable Pd(II) precursors $Pd(PPh_3)_2Cl_2$, $Pd(dppf)Cl_2$, and $Pd(OAc)_2$, which are readily reduced to active palladium(0) complexes through the addition of other phosphine ligands. Two additional factors also affect the reaction rate. First, the relative reactivity of the aryl halide or pseudo-halide plays an important role. Reactivity follows the expected trend of I > OTf > Br » Cl.[68] Second, the reaction is typically faster when electron-withdrawing groups are employed and the substrate is less sterically hindered.

The mechanism of the transmetalation step is highly dependent on the reaction conditions and organometallic substrates involved. Several palladium carbon–heteroatom cross-coupling mechanisms containing intricate and complex catalytic cycles have been reported and reviewed elsewhere.[69–73]

Once reductive elimination occurs, the product is released to regenerate the initial Pd(0) complex. The elimination of organic substrates occurs from a *cis*-orientation and the order of elimination for C–C bond formation is diaryl > aryl/alkyl > dipropyl > diethyl > dimethylpalladium(II). This suggests that π-orbital overlap aids in the resulting bond formation, and an increase in steric bulk assists in the dissociation of the product.

The choice of potential reaction solvents is extremely broad, and includes nonpolar, noncoordinating solvents such as toluene and benzene; polar, coordinating solvents such as tetrahydrofuran (THF), dimethoxyethane (DME), *N*,*N*-dimethylformamide (DMF), and dimethylsulfoxide (DMSO); and polar, protic solvents such as methanol, ethanol, and even water. Solvent mixtures are often used to optimize the solubility of the substrates.

The base may be used either in solution or as a suspension. Common base choices include alkali carbonates, alkoxides, hydroxides, and phosphates.

B. Oxidative Addition of Palladium with Porphyrin Halides

Haloporphyrins are large electron-rich substrates capable of undergoing palladium-catalyzed cross-coupling reactions. The electron-rich nature of the porphyrin ring decreases the reactivity of the oxidative addition step, slowing the rate of the reaction. In addition, the steric environment of many porphyrin substrates results in a more hindered approach of the nucleophile during the transmetalation step. These challenges account for much of the delay in applying palladium-catalyzed cross-coupling reactions to porphyrin substrates.

The recent isolation and characterization of several *meso*-η^1-palladio intermediates by Arnold and co-workers has provided important detail into the mechanistic aspects of the oxidative addition step of palladium-catalyzed cross-coupling reactions with porphyrin substrates.[74–77] This work, outlined below, provided an important framework for the successful preparation of mono-, di-, tri-, and tetra-coupled products, as well as the stepwise formation of dimers, trimers, and complex arrays.

5,15-Diphenylporphyrin H_2(DPP) **1**, pioneered by Therien for Stille and Negishi type couplings,[37] was brominated at either one or both of the sterically unencumbered *meso*-positions to form *meso*-haloporphyrins **2** and **3** (Figure 1). These *meso*-haloporphyrins were then subjected to oxidative addition to form the corresponding *meso*-η^1-palladio-species **B**. Arnold and co-workers were the first to isolate and characterize the first peripherally metalated porphyrins, *meso*-η^1-palladioporphyrin **4** and **5** (Figure 2).[74]

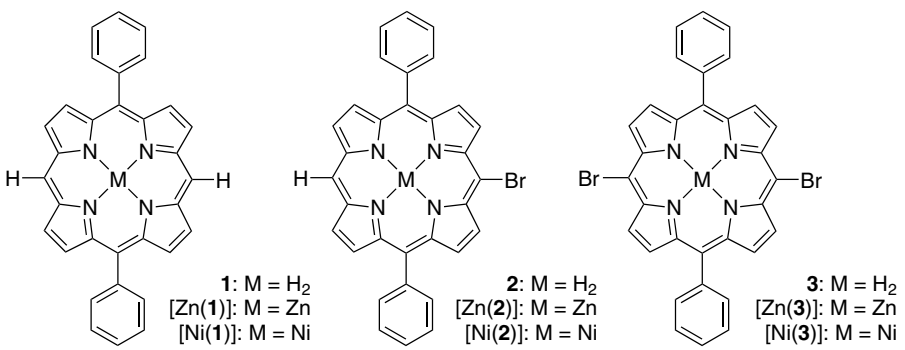


Figure 1. Structure of **1** diphenylporphyrin (DPP); **2** *meso*-bromodiphenylporphyrin (mono-BrDPP); and **3** *meso*-dibromodiphenylporphyrin (di-BrDPP).

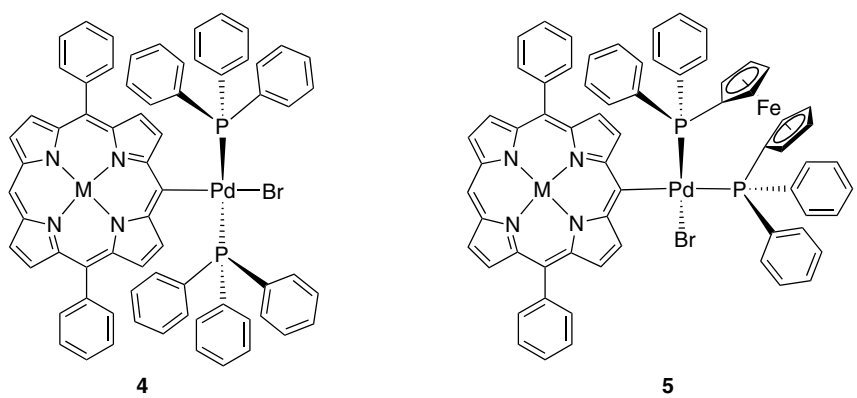


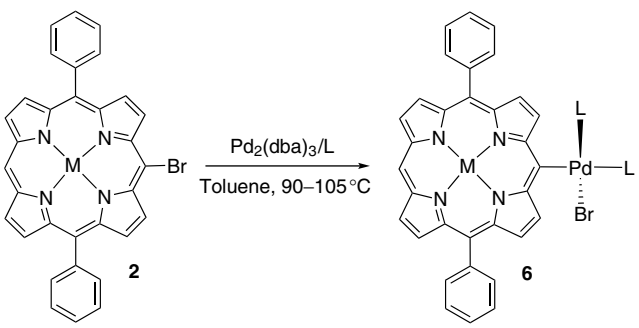
Figure 2. Geometry of σ-bound palladium *trans*-bis(triphenylphosphine) derivatives and the dppf organopalladioporphyrin.[74]

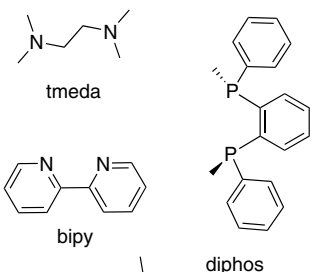
Stoichiometric amounts of both the H_2(DPP) and Ni(DPP) *mono*-bromoporphyrins (**2** and [Ni(**2**)], Figure 1) when reacted with $Pd(PPh_3)_4$ in near-boiling toluene, led to the rapid (less than 40 minutes), quantitative formation of the corresponding *meso*-η^1-palladioporphyrins (Figure 2) in up to 80% yield. Bidentate diphosphines, such as 1,2-bis-(diphenylphosphino)ethane (dppe), 1,2-bis-(diphenylphosphino) propane (dppp), and 1,2-bis-(diphenylphosphino)ferrocene (dppf), were also evaluated, and the reactions proceeded in a similar fashion to the monophosphines. The more crowded and rigid alkenyl *cis*-1,2-bis-(diphenylphosphino)ethylene provided only the reduced (dehalogenated) H_2(DPP) product **1**.

The concurrent double oxidative addition of the dibromodiphenylporphyrin required much longer reaction times and higher palladium/ligand loading. UV-vis spectroscopy was used to show that the addition of one electron-donating $Pd(PPh_3)_2Br$ led to a more basic (electron-rich) porphyrin ring, as evidenced by a red shift of UV-vis absorption. It was concluded that the enriched electronic character of the porphyrin ring significantly decreased the rate of the second oxidative addition step.

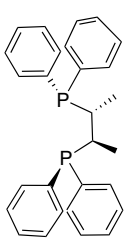
Arnold and co-workers explored palladium complexes of bidentate nitrogen donor ligands and chiral bisphosphine ligands for the oxidative addition of mono-brominated Ni(DPP) ([Ni(**2**)], Table 1).[75,77] Tetramethylethylenediamine

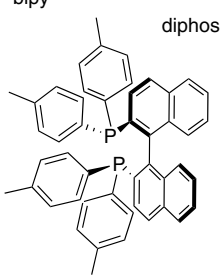
Table 1. *meso*-η^1-Palladioporphyrin complexes of bidentate nitrogen donor ligands and chiral bisphosphine Ligands.[75,77]





Entry	Por	Ligand (L)	Yield (%)
1	**2**	tmeda	93
2	**2**	bipy	91
3	[Ni(**2**)]	(*R,R*)-diphos	57
4	[Ni(**2**)]	(*R,R*)-CHIRAPHOS	76
5	[Ni(**2**)]	(*R*)-tol-BINAP	80


CHIRAPHOS


tol-BINAP

(tmeda) and 2,2′-bipyridine (bipy) were evaluated for their ability to force a *cis*-configuration of the metal center for use in self-assembled supramolecular systems with success.

Arnold and co-workers also used diphos, which is chiral at both phosphorus atoms; CHIRAPHOS, which has a chiral carbon backbone; and tol-BINAP, which is chiral as a result of atropisomerism about the binaphthyl bond. The reactivity decreased in the order diphos > CHIRAPHOS > tol-BINAP. It initially appeared that there was a substantial steric component; however, evaluation of dppf, a nonchiral bulky bisphosphine ligand, was shown to proceed rapidly in direct contrast to the trends established with diphos, CHIRAPHOS, and tol-BINAP.

In an effort to address these observations, ^{31}P-NMR was used to explore the influence of decreasing electron density on the palladium-coordinated phosphorus atom using ^{31}P-NMR. The resulting chemical shifts indicated that decreasing electron density led to an increase in activity: CHIRAPHOS > diphos > dppf > tol-BINAP. Although the requisite balance of both of these factors toward efficient oxidative addition is indisputable, the relative influences of increased steric bulk versus the electron density remain unclear.

II. Palladium-Catalyzed C–B Coupling

The palladium-catalyzed syntheses of arylboronic acids have received much attention in recent years. These compounds are generally thermally stable and inert to atmospheric conditions, making them particularly useful synthetically. For this reason, they have become key reagents for transition metal-catalyzed Suzuki–Miyaura cross-couplings, providing a powerful and general methodology for accessing a broad range of carbon–carbon bond formations.[78–80]

In 1995, Miyaura and co-workers reported the palladium-catalyzed reaction of the pinacol ester of diboronic acid with haloarenes to give direct access to arylboronic esters.[68] Later, Masuda and co-workers described a series of reactions using pinacolborane, a hydroborane, as a boron source for palladium-catalyzed cross-couplings.[81] The methodologies developed for preparing these organoboron compounds from aryl halides have played an important role in the synthesis of borylated porphyrins. The desired borylated porphyrins are typically functionalized further *via* Suzuki–Miyaura couplings to produce the corresponding porphyrin analogs.

Pinacolborane and the pinacol ester of diboronic acid are the most common boron sources and have been applied to porphyrin borylation to produce high yields of porphyrinyl *meso*-, β-, and aryl boronates, including those with base-sensitive functional groups. Stronger bases, such as K_3PO_4 and K_2CO_3, promote the continued palladium-catalyzed reaction of the organoborate with unreacted

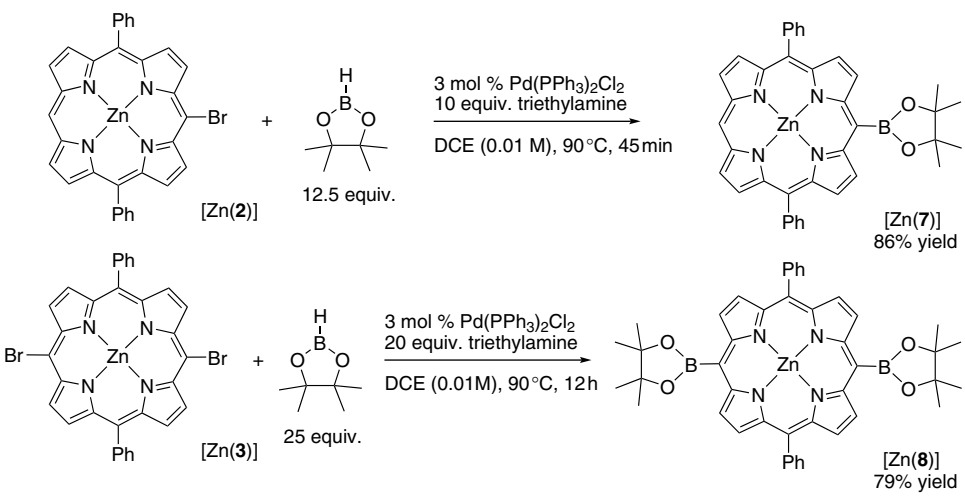
halogenated porphyrin precursors to give the undesired Suzuki–Miyaura dimer product.[68] The use of weaker bases, such as triethylamine or acetates, minimizes this alternate reaction pathway.

It is worth noting that porphyrins containing borylated aryl groups are generally synthesized from borylated aldehydes, rather than through palladium-catalyzed borylation after the cyclization of the porphyrin macrocycle. While this approach has contributed significantly to porphyrin functionalization, it will not be discussed in this chapter.

A. *meso*-Borylation

Pinacolborane was used as the transmetalating agent in the palladium-catalyzed cross-coupling reaction reported by Masuda and co-workers in 1997 to produce arylboronates from a wide range of aryl halide precursors.[81] This report inspired Therien and co-workers to use pinacolborane to prepare the first examples of *meso*-borylated porphyrinyl species where the boronic esters were directly appended to the Zn(II) porphyrinato framework (Scheme 2).[66]

The formation of the Zn(II) mono-substituted porphyrin [Zn(**7**)] was complete in 45 minutes as monitored by thin layer chromatography; however, the Zn(II) di-substituted porphyrin [Zn(**8**)] required 12 hours for complete conversion. Porphyrins [Zn(**7**)] and [Zn(**8**)] were then used as transmetalating reagents in Suzuki–Miyaura couplings.[66] Therien and co-workers continued efforts in this area focused on synthesizing more elaborate donor–bridge–acceptor, porphyrin–spacer–quinone complexes for use as electron transfer assemblies.[83]



Scheme 2.[66]

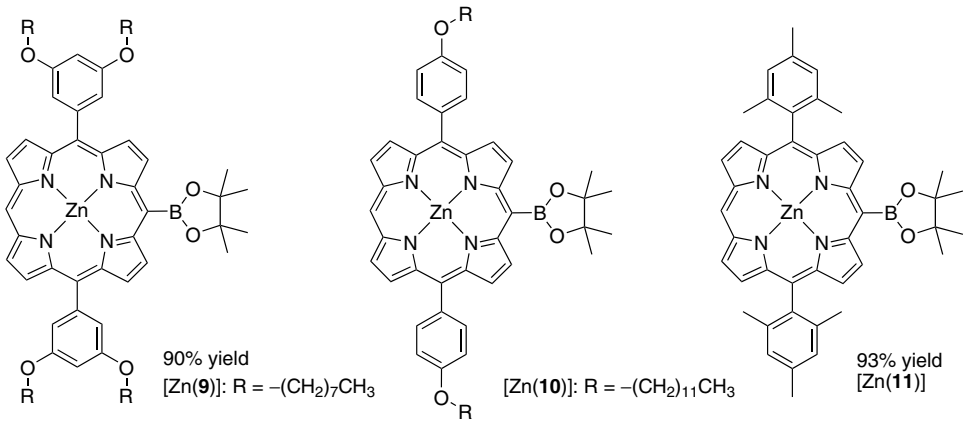


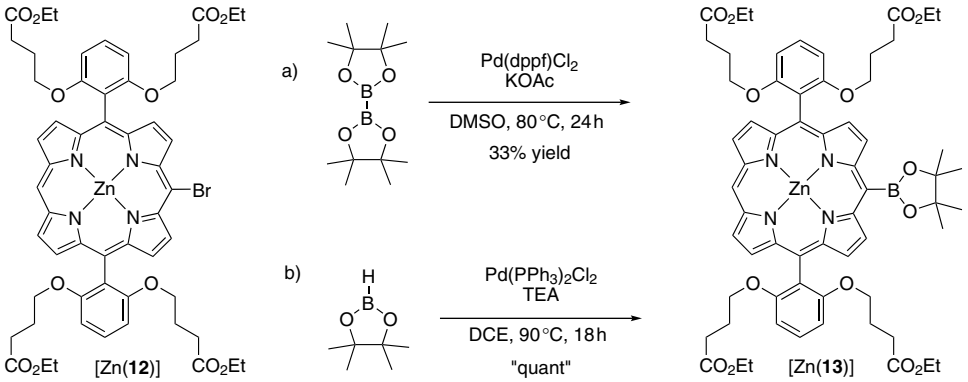
Figure 3. Synthesis of Zn(II) *meso*-boronyl-diphenylporphyrin derivatives.[49,82,83]

In a separate report, Osuka and co-workers used the methodology employed for the synthesis of porphyrin [Zn(**7**)] to produce mono-boronyl complexes of bis(3,5-dioctyloxyphenyl)porphyrinatozinc(II) [Zn(**9**)] and *p*-dodecyloxyphenyl derivative [Zn(**10**)] (Figure 3). The porphyrylboronate products obtained were subsequently used in the synthesis of *meso–meso* linked hetero-metalated multi-porphyrin arrays and 1,4-phenylene-bridged diporphyrin arrays.[49,82]

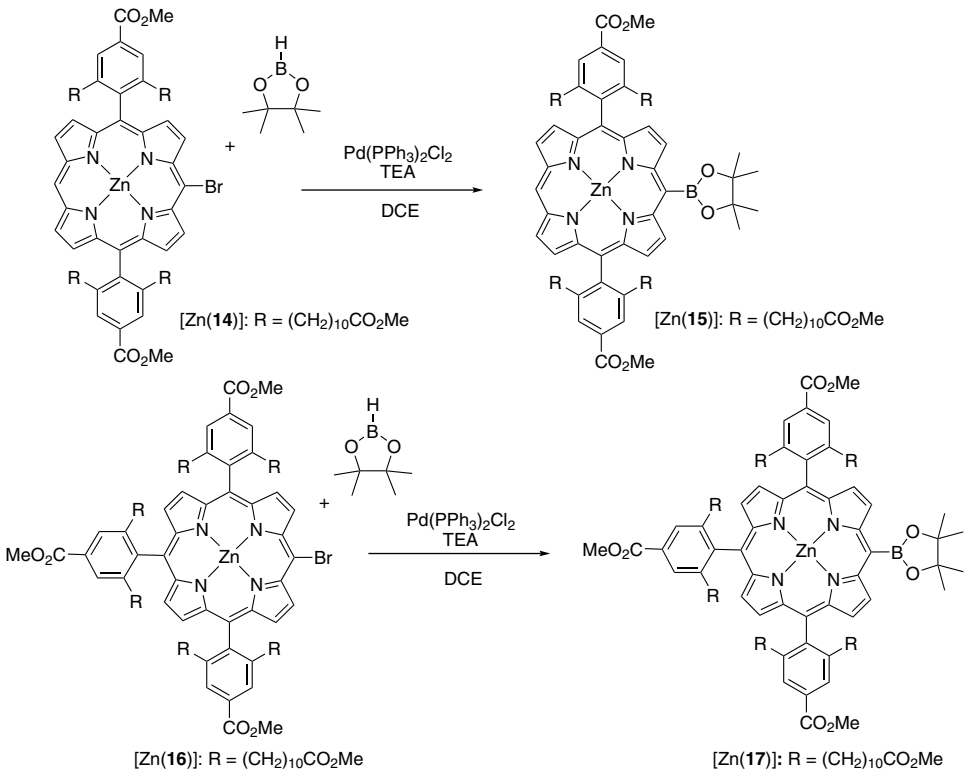
Lindsey and co-workers also used this methodology to synthesize the Zn(II) *meso*-boronyl dimesitylporphyrin ([Zn(**11**)], Figure 3), which was then *meso*-coupled with 5-(4-iodophenyl)-dipyrrin to form a dipyrrinato-porphyrin.[84] The excited-state energy transfer of triads composed of two porphyrins bridged with a bis(dipyrrinato)metal complex were studied, and it was concluded that the bis(dipyrrinato)zinc complexes underwent highly efficient energy transfer.

Diederich and co-workers designed and synthesized dendritic metalloporphyrins with hydrogen-bonding capability as mimics of hemoglobin (Hb). Low yields were reported when *meso*-brominated porphyrins were used as the electrophile in Suzuki cross-coupling reactions. As a result, they developed an alternative synthesis using porphyrinyl boronates as transmetalating agents, and compared two different carbon–boron bond forming methodologies. Their results showed that Therien and co-worker's pinacolborane method (Scheme 3b) generated greater yields for this porphyrin substrate when compared to Miyaura's diboronic acid method (Scheme 3a).[85,86]

Mitzutani and co-workers developed two versions of water-soluble gable-type porphyrin DNA intercalators. Their synthesis consisted of two borylated intermediates: *meso*-boronyl-diphenylporphyrin [Zn(**15**)], and *meso*-boronyl-triphenylporphyrin [Zn(**17**)] (Scheme 4).[87] The methodology developed by Therien and co-workers



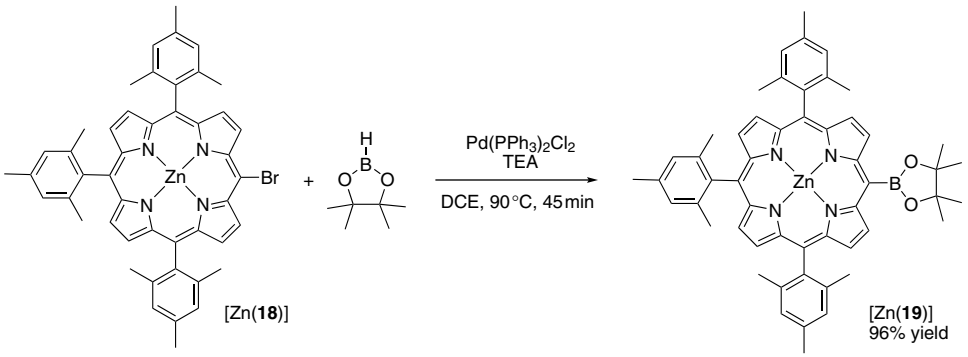
Scheme 3.[85,86]



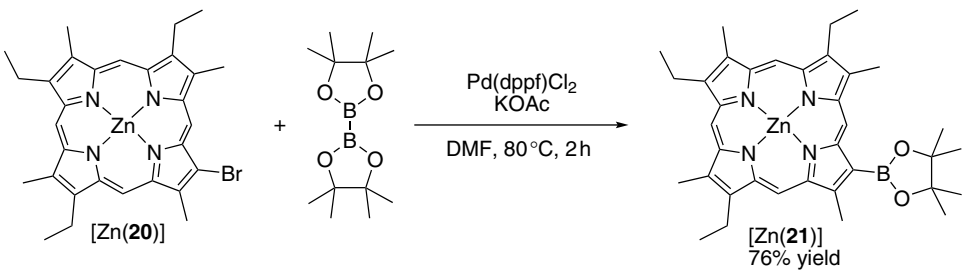
Scheme 4.[87]

was employed for the borylation step in both syntheses. The gable-type porphyrins were produced in overall yields of 44 and 51%, respectively.

Meso-Boronyl-trimesitylporphyrin [Zn(**19**)] was later synthesized in 96% yield by Nocera and co-workers using the Therien method (Scheme 5).[88,89]



Scheme 5.[88,89]



Scheme 6.[47]

Dihalogenated xanthene and dibenzofuran linkers were used to synthesize cofacial bisporphyrins containing [Zn(**19**)] to give the Suzuki–Miyaura coupling product. The corresponding "Pacman" porphyrins were metalated with manganese and successfully applied to the catalytic disproportionation of hydrogen peroxide. Later work by Nocera and co-workers used [Zn(**19**)] as the structural base for Fe(III) "Hangman" porphyrins which also showed the ability for catalytic O–O activation.[88]

B. β-Borylation

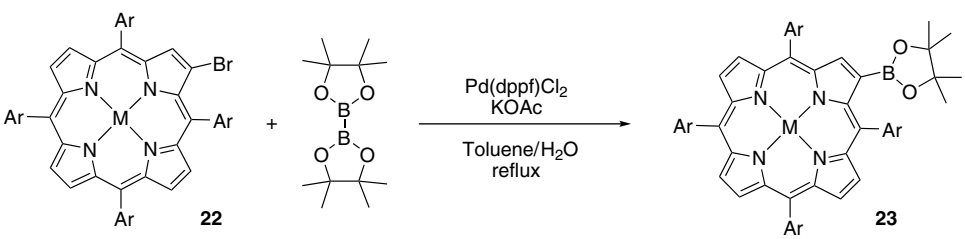
Modification of the β-positions usually requires lengthy syntheses, lower yields, and difficult separations. However, β-functionalized porphyrins more strongly resemble the photosynthetic reaction centers of biological photocatalysts, and as such are targeted for use as biomimetic light-harvesting complexes.

In one of the first examples of β-functionalization *via* palladium-catalyzed cross-coupling, Nocera and co-workers reacted 2-bromo-7,12,17-triethyl-3,8,13,18-tetramethylporphyrinatozinc(II) [Zn(**20**)] with bis(pinacolato)diboron to give the desired borylated product [Zn(**21**)] in 76% yield (Scheme 6).[47] The

β-borylated porphyrin was then subjected to Suzuki–Miyaura coupling conditions to produce the corresponding β,β′-bisporphyrin.

In 1974, Callot reported on the efficient synthesis of β-brominated tetraphenylporphyrin **22a** using *N*-bromosuccinimide (NBS).[90] The yield and scale of β-bromotetraphenylporphyrin (β-BrTpp) **22a** was later improved by Zhang and co-workers.[91] These two developments provided a foundation for the synthesis of β-functionalized tetraarylporphyrin derivatives.

In 2005, Zhang and Suslick reported the synthesis of β-boronyl-tetraphenylporphyrinato Zn(II) [Zn(**23**)] (Scheme 7).[92] Bringmann and co-workers expanded on this work in 2008 using electron-rich and electron-deficient free-base and metallo-tetraphenylporphyrin derivatives to produce the corresponding β-boronyl-tetraarylporphyrins (Table 2).[93] They also showed the synthesis of these porphyrins could be accelerated through the addition of 10 mol% 18-crown-6 or by microwave irradiation. The study by Bringmann and co-workers also confirmed the solvent dependence of this reaction as originally reported by Chen and co-workers in 2006.[94]



Scheme 7.[92,93]

Table 2. Synthesis of β-boronyl-tetraarylporphyrins.[93]

Entry[a]	Ar	Br-por	Time (h)	Yield (%)
1	phenyl	**22a**	3	70
2	4-tolyl	**22b**	3	65
3	4-ClPh	**22c**	2	56
4	4-MeOPh	**22d**	3	64
5	phenyl	[Zn(**22a**)]	3	62
6	4-tolyl	[Zn(**22b**)]	3	67
7	phenyl	[Ni(**22a**)]	3	58
8	4-tolyl	[Ni(**22b**)]	3	60
9	phenyl	[Cu(**22a**)]	4	53
10	4-tolyl	[Cu(**22b**)]	4	50
11	phenyl	[Pd(**22a**)]	4	61

[a]Reactions were performed with 20 mol % $Pd(dppf)Cl_2$ with 2.5 equiv. of bis(pinacolato)diboron and 10 equiv. of KOAc in 3.35 mM toluene/H_2O (83 % v/v) at 110°C.

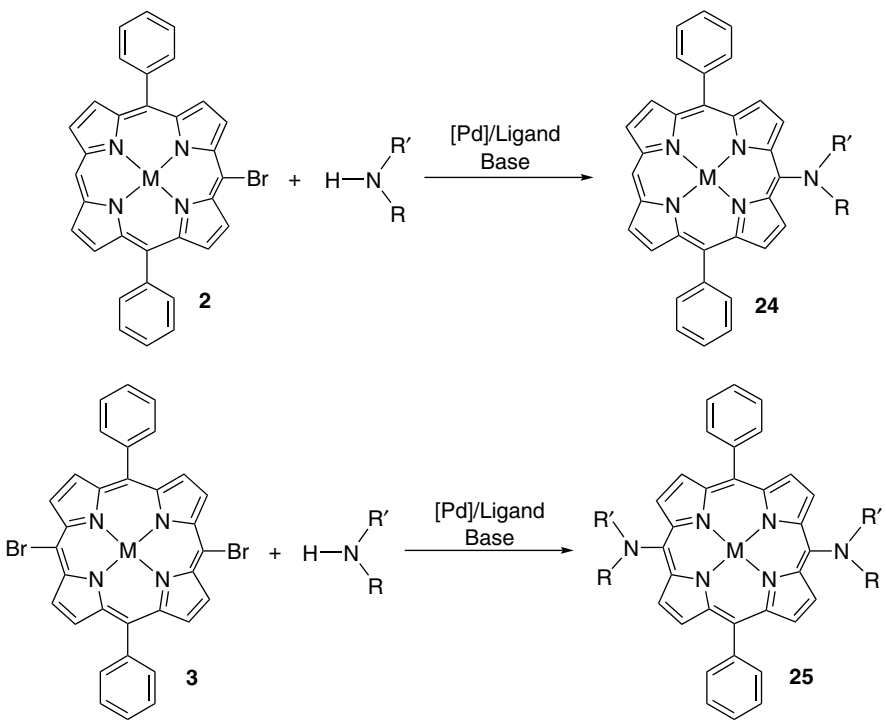
Bringman and co-workers went on to report the synthesis of chiral, β-β'-linked, bis(tetraarylporphyrins) *via* an optimized Suzuki–Miyaura coupling procedure.[93] The resulting chiral bis-porphyrins bearing different *meso*-aryl residues or different metal centers could be employed as asymmetric mono- or bi-metallic catalysts or as chiral reporter groups.

III. Palladium-Catalyzed C–N Coupling

A. Amination

1. *meso-Amination*

The alkyl and aryl-amino couplings to *meso*-halogenated porphyrins were reported in 2003 by Chen and Zhang.[95] In this study, both the free-base and zinc complex of 10-mono-bromo-5,15-diphenylporphyrin **2** and 10,20-di-bromo-5,15-diphenylporphyrin **3** were coupled with a variety of amines (Scheme 8). Combinations of the palladium source, phosphine ligand, and base were screened in THF or toluene to identify optimal reaction conditions.



Scheme 8.[95,97–99]

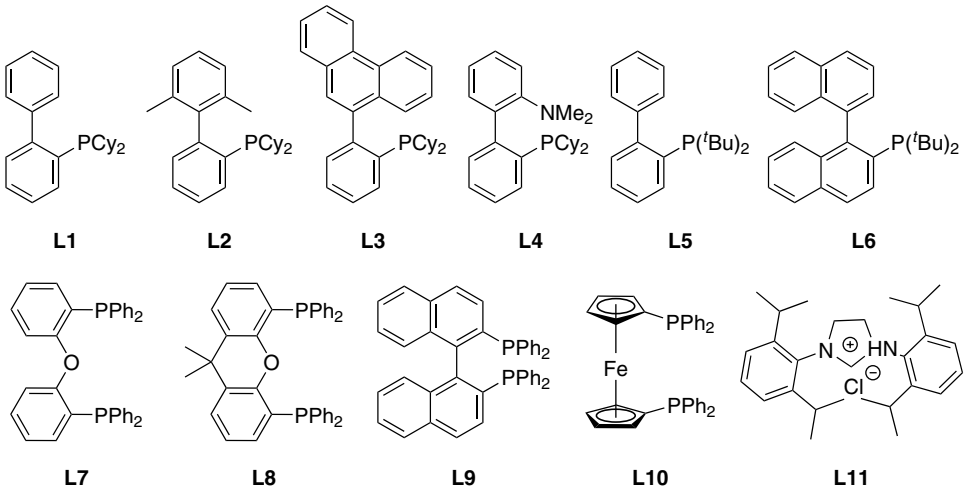


Figure 4. Common ligands: mono-phosphines, bis-phosphines, and *N*-heterocyclic carbene.

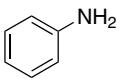
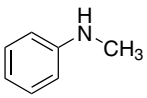
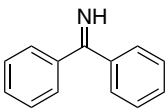
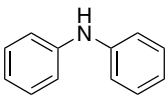
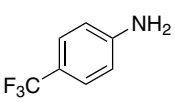
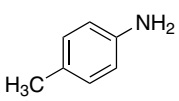
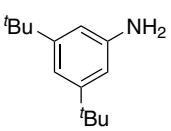
Both Pd(II) acetate and tris(dibenzylideneacetone)dipalladium(0) were evaluated in combination with a variety of electron-rich, bulky biphenyl mono-phosphine (**L1**, **L4**, and **L5**, Figure 4) and bis-phosphine ligands (**L7** and **L9**, Figure 4) as catalysts for the amination reactions. Results showed that there was no significant difference between the two palladium sources; however, the DPEphos ligand **L7** was selected for continued study because of its high catalytic activity and low cost.

Although the yields generated in THF and toluene were comparable, THF was chosen as the solvent since it provided greater homogeneity. Finally, screenings showed that K_2CO_3 was catalytically ineffective and the strong base NaO^{t}Bu led to side-products; therefore, the weak base Cs_2CO_3 was chosen as the preferred base. Increased reaction temperatures were used as the reaction was shown to be sluggish at room temperature.

Based on the initial screenings, optimized reaction conditions were established and a variety of amines were evaluated as substrates for palladium-catalyzed cross-coupling with bromoporphyrins. In general, both electron-rich and electron-poor substrates were suitable, and typically high yields were produced in less than 24 hours (entries 1–12, Table 3). However, sterically demanding diarylamines and aliphatic amines (entries 13–16) were sluggish, requiring much longer reactions times.

The use of 18-crown-6 for the palladium-catalyzed amination of aryl iodides with secondary cyclic amines was first reported by Wolfe and Buchwald in 1997.[96] The rate acceleration observed in this reaction was shown

Table 3. Amination of *meso*-bromoporphyrins with amines.[95]

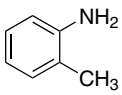
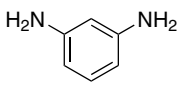
Entry[a]	Amine	Br-por[b]	Time (h)[c]	Yield (%)[d]
1		**2**	19	98
2		[Zn(**2**)]	13	95
3		**3**	20	65
4		[Zn(**3**)]	13	82
5		**2**	19	94
6		[Zn(**2**)]	13	99
7		**3**	15	71
8		[Zn(**3**)]	17	82
9		**2**	24	84
10		[Zn(**2**)]	22	94
11		**3**	15	95
12		[Zn(**3**)]	16	84
13		**2**	40	66
14[e]		[Zn(**2**)]	25	61
15		[Zn(**3**)]	50	30
16	*n*-HexNH$_2$	[Zn(**2**)]	50	80
17		[Zn(**3**)]	17	90
18		[Zn(**3**)]	16	94
19		[Zn(**3**)]	62	95

[a]Reactions were carried out at 68°C under N_2 using 1 equiv. of Br-pro. 3.6 (M = Zn) or 4.8 (M = Zn) equiv. of amine, 5 mol % $Pd(OAc)_2$ with 7.5 mol % DPEphos (Pd: **L7** = 1:1.5) in the presence of 1.4 equiv. of base per Br. Concentration: 0.01 M Br-por in THF; [b]Structures of Br-por are shown in Figure 1; [c]Reaction times have not been optimized; [d]Represents isolated yields of >95% purity as determined by ^{1}H-NMR; [e]The reaction was conducted using 10 mol % $Pd(OAc)_2$ and 15 mol % DPEphos in the presence of 2.8 equiv. of NaO-*t*-Bu.

to be a result of the activation of NaO*t*-Bu by 18-crown-6 through solvation of Na^+.

Inspired by Wolfe and Buchwald's report, Suda and co-workers used crown ether additives to establish a methodology that would be suitable for the palladium-catalyzed cross-coupling of aliphatic secondary amines with bromoporphyrin

Table 4. Palladium-catalyzed amination of *meso*-bromo-substituted porphyrin **2** with Amines.[97]

Entry[a]	Amine	Br-por[b]	Time (h)	Yield (%)[c]
1[d]		**2**	0.5	ND
2[e]	*n*-hexylNH$_2$	[Zn(**2**)]	0.5	55
3		[Ni(**2**)]	0.5	85
4	*i*-PrNH$_2$	[Ni(**2**)]	4	80
5		[Ni(**2**)]	2.5	81
6		[Ni(**2**)]	3	quant.
7	Ph(Me)NH	[Ni(**2**)]	3	quant.
8		[Ni(**2**)]	5	96

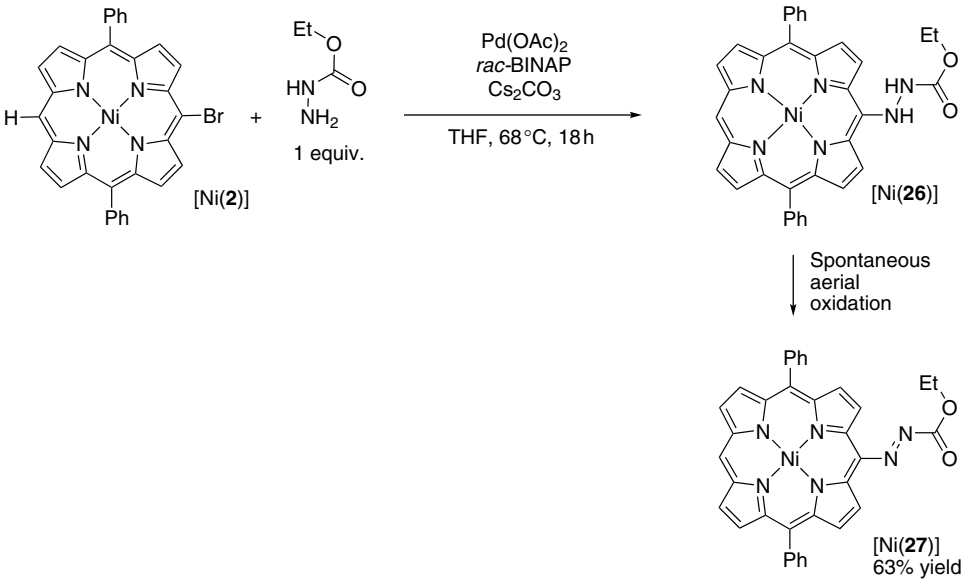
[a]Reactions were carried out at 68°C under N_2 in THF with 2 equiv. of amide, 6.7 mol % $Pd(OAc)_2$; and 19.4 mol % *rac*-BINAP in the presence of 23 equiv. of NaO^{t}Bu and 4.5 mol % 18-crown-6. Concentration: 0.01 M Br-por in THF; [b]See Figure 1 for Structures; [c]Isolated yields; [d]Recovered 70% of **2**; [e]Recovered 12% of [Zn(**2**)].

synthons.[97] In their initial report in 2003, they employed *meso*-bromo-5,15-diphenylporphyrin and its zinc and nickel complexes for the preparation of *meso*-amino substituted porphyrins.

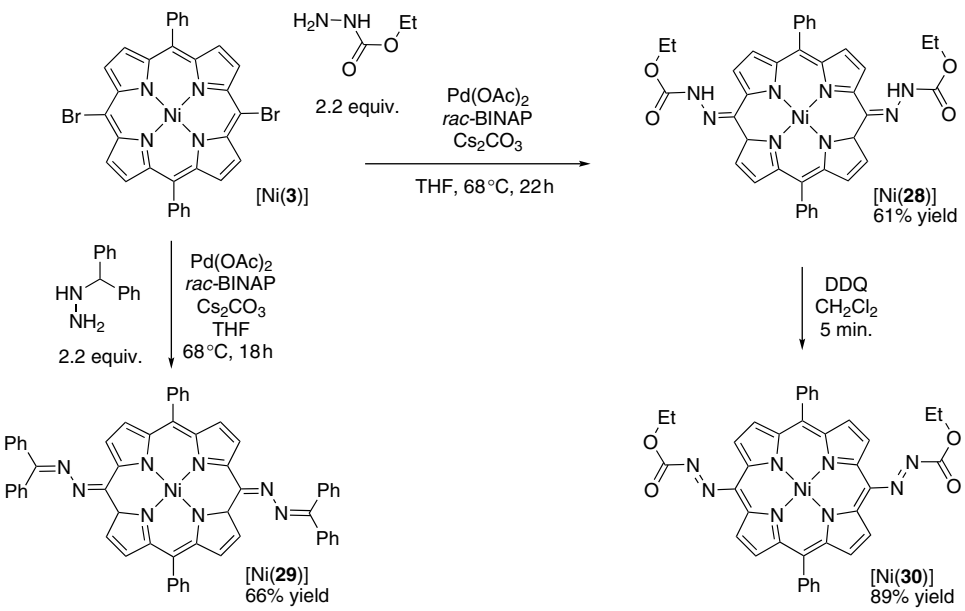
As illustrated in Table 4, the reported use of crown ether additives in the palladium-catalyzed cross-couplings provided Suda and co-workers with access to a wide variety of aminoporphyrin analogs, including alkyl amines (entries 1–4), cyclic amines (entry 5), and primary and secondary aromatic amines (entries 6–7).

In general, the reactions proceeded efficiently and the desired products were produced in moderate to high yields. Of particular interest was the reaction of *m*-phenylenediamine (entry 8, Table 4) which produced the corresponding *meso*-amino substituted Ni(II) porphyrin in 96% yield. This reaction proceeded with minimal production of the dicoupled product, providing a free amino group as a synthetic handle for further functionalization.

Carbazates and azoesters are known to be biologically active.[100] Their combination with porphyrins that are well known for their photosensitizing and tumor-localizing abilities inspired Arnold and co-workers to extend the scope of carbon–nitrogen bond couplings to protected hydrazines. In 2005, they reported the first synthesis of *meso*-substituted azoporphyrins *via* palladium-catalyzed cross-coupling (Schemes 9 and 10).[98]



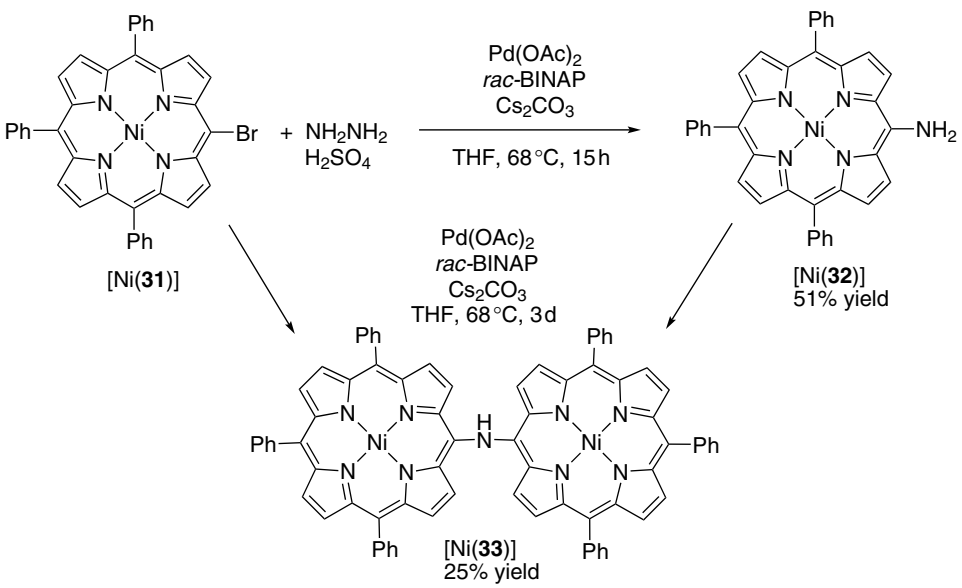
Scheme 9.[98]



Scheme 10.[98]

The palladium-catalyzed cross-coupling of ethyl and *tert*-butylcarbazates with *meso*-bromoporphyrins resulted in the expected mono-*meso*–azo-product which spontaneously oxidized in air to produce [Ni(**27**)]. In addition, despite beginning with only one bromo substituent, a significant amount of the bis-aminated product [Ni(**30**)] (Scheme 10) was observed. Bis-amination presumably resulted from the activation of the opposite *meso*-C–H bond by addition of the first azo (or hydrazo). Arnold and co-workers also evaluated the scope of the hydrazination/oxidation reaction using benzophenone hydrazone to produce [Ni(**29**)] in comparable yield.

In 2006, Arnold and co-workers used a palladium-catalyzed cross-coupling approach to synthesize an aminoporphyrin using the sulfate salt of hydrazine (Scheme 11).[99] This was the first reported example using an unprotected hydrazine sulfate as an ammonia surrogate. When 2 equivalents of *meso*-bromotriphenylporphyrin [Ni(**31**)] were reacted in the presence of 1 equivalent of hydrazine, the *meso*-aminotriphenylporphyrin [Ni(**32**)] was produced in 51% yield. However, if equivalent molar ratios of the porphyrin and hydrazine were reacted for an extended period, the bis(diporphyrinylamine) [Ni(**33**)] was produced in 25% yield. The authors noted that the delocalized electronic structure of the bis(diporphyrinylamine) and its analogs may have relevance for use in molecular electronic devices.

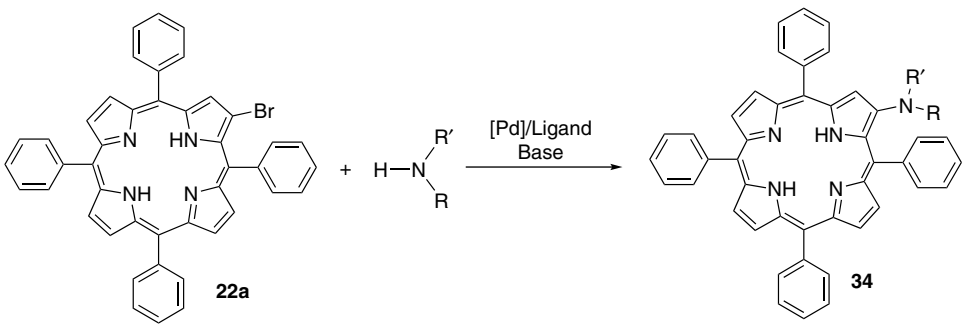


Scheme 11.[99]

2. *β-Amination*

In 2001, van Lier and co-workers reported palladium-catalyzed carbon–heteroatom intermolecular cross-coupling using porphyrin substrates.[101] The focus of their report was the convenient and efficient synthesis of a host of new photodynamic therapy agents from a single porphyrin starting material. The report was vague regarding the conditions employed and the yields obtained. However, this work is important because it highlighted the first successful application of Buchwald–Hartwig conditions to the amination of mono- and di-*β*-bromoporphyrins using a variety of amines (Scheme 12 and Figure 5).

In 2007, Zhang and co-workers investigated the direct employment of free-base *β*-bromoporphyrins **2** and **3** in palladium-catalyzed amination reactions (Scheme 12 and Table 5).[91] Their report began with an improvement on the synthesis of *β*-BrTPP from tetraphenylporphyrin, first developed by Callot.[90] The



Scheme 12.[91,101–103]

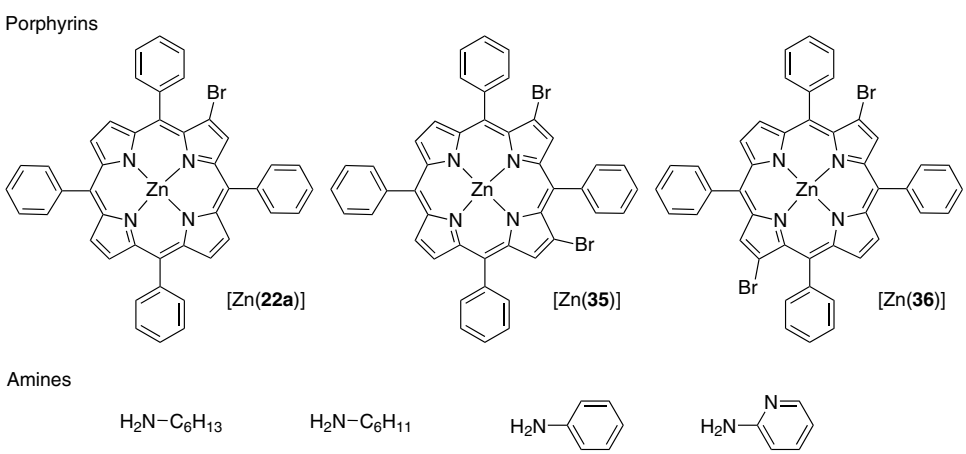
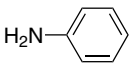
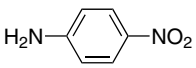
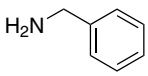
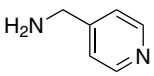


Figure 5. Van Lier's Buchwald-Hartwig cross-coupling partners.[101]

Table 5. Synthesis of β-aminotetraphenylporphyrins *via* palladium-catalyzed C–N bond formations of β-BrTPP with amines.[91]

Entry[a]	Amine	Br-por[b]	Time (h)	Yield (%)
1		**22a**	24	48
2[b]		**22a**	24	75
3[b]		**22a**	5	46
4[b]		**22a**	24	71

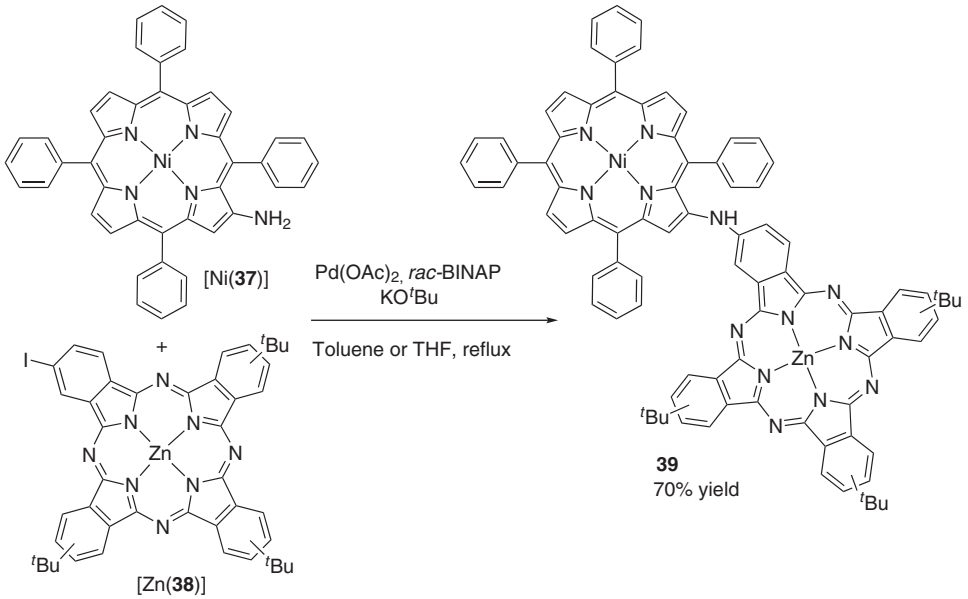
[a]Reactions were carried out at 100°C under N_2 in THF with 1 equiv. of **22a**; 4 equiv. of amine, 10 mol % $Pd(OAc)_2$; and 20 mol % BINAP in the presence of 2 equiv. of Cs_2CO_3. Concentration: 0.01 M **22a** in THF. [b]4 equiv. of Cs_2CO_3.

authors noted that when the bromination reaction was carried out by slow addition of 3 equivalents of pre-dissolved NBS to a 0.4 mM solution of the desired tetraphenylporphyrin in pyridine and chloroform at 60°C, a 64% yield of the mono-brominated product **22a** was obtained. In addition, they noted that the new reaction conditions were amenable to multigram scales.

Zhang and co-workers used free-base β-BrTPP **22a** to demonstrate the efficient synthesis of a variety of β-substituted alkyl and aryl amines, amides, alcohols, and thiols.[91] The palladium sources $Pd(OAc)_2$ and $Pd_2(dba)_3$ were screened in combination with the bis-phosphine ligands DPEphos **L7**, Xantphos **L8**, and BINAP **L9** as potential catalytic systems. The coupling of alkyl and aryl amines to the β-position generally favored product formation using a combination of $Pd(OAc)_2$ and BINAP **L9**. The resulting porphyrins were used to further examine the correlation between porphyrin structure and catalytic reactivity for Co(II) porphyrin-based asymmetric cyclopropanation.

In 2007, Torres, Guldi, and co-workers used a nitrogen-based linker to attach porphyrins to phthalocyanines.[102] The resulting products provided an extended π-conjugated system capable of promoting energy transfer. The authors envisioned the pH and redox-sensitive nitrogen linker could serve as an "active" spacer or switch for use in the field of molecular materials.

Two approaches were used by Torres, Guldi, and co-workers to prepare porphyrin-phthalocyanine dyads from β-aminoporphyrins *via* palladium-catalyzed amination (Schemes 13 and 14).[102] In the first method, [Ni(**37**)] and [Zn(**38**)] were cross-coupled to produce the corresponding porphyrinylaminophthalocyanine **39**



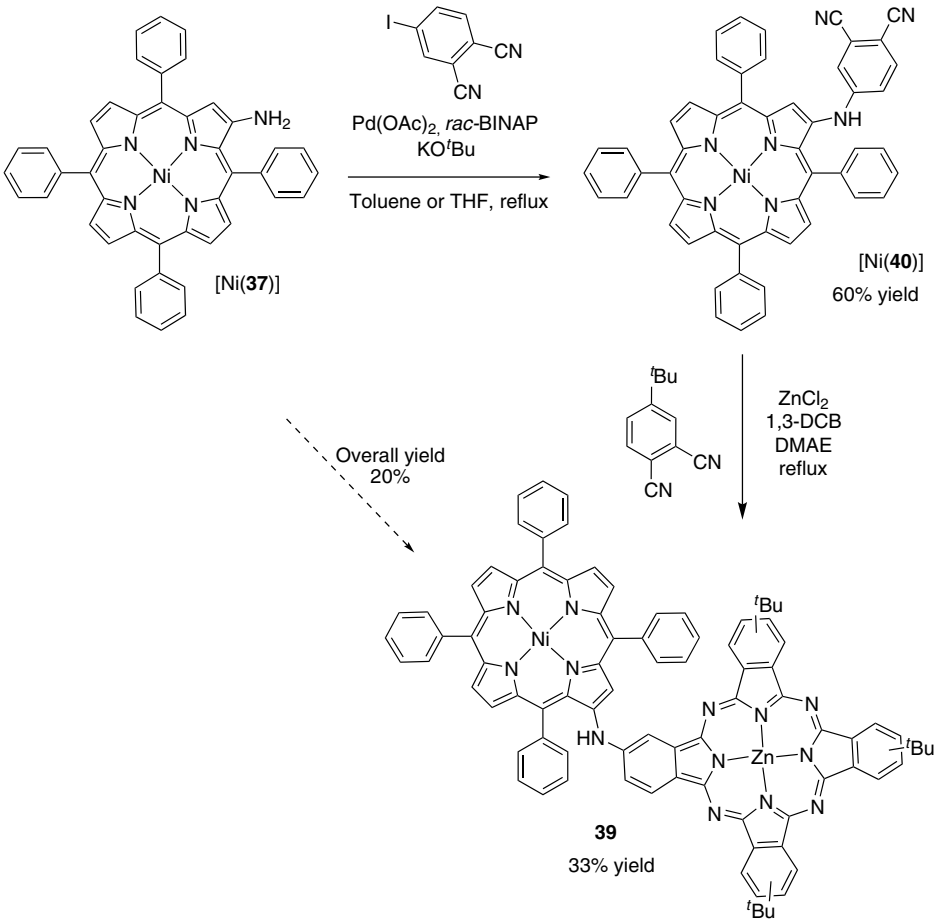
Scheme 13.[102]

in 70% yield (Scheme 13). In the second method, 4-iodophthalonitrile was cross-coupled with [Ni(**37**)] under identical conditions to form nickel complex of porphyrinylaminophthalonitrile [Ni(**40**)] in 60% yield (Scheme 14).

Although the synthetic yields indicate that the two cross-coupling approaches employed are comparable, Torres, Guldi, and co-workers made note of some important considerations that should be taken into account when preparing dyad **39**. Specifically, hexabutoxyiodo Zn(II)phthalocyanine [Zn(**38**)] (Scheme 13), which was formed as a mixture from reaction of two different phthalonitriles in a 1:3 ratio in the presence of $Zn(OAc)_2 \cdot 2H_2O$ under refluxing conditions using *N*,*N*-dimethylaminoethanol (DMAE) as the solvent, displayed strong tendencies toward aggregation. As a result of aggregation, the desired mono-iodophthalocyanine [Zn(**38**)] was only isolated in a low 19% yield.

In the second method, the phthalocyanine portion of the dyad **39** was then prepared through condensation of the nickel complex of porphyrinylaminophthalonitrile [Ni(**40**)] with an excess of 4-*tert*-butylphthalonitrile in a refluxing solution of *o*-dichlorobenzene and DMAE. Zinc chloride was added during macrocycle formation (template procedure) to provide the porphyrin-phthalocyanine dyad **39** in 33% yield. The overall yield for the two-step synthesis is 20% (Scheme 14).

In 2008, Cavaleiro and co-workers compared a classical condensation approach with a palladium-catalyzed cross-coupling approach for the preparation

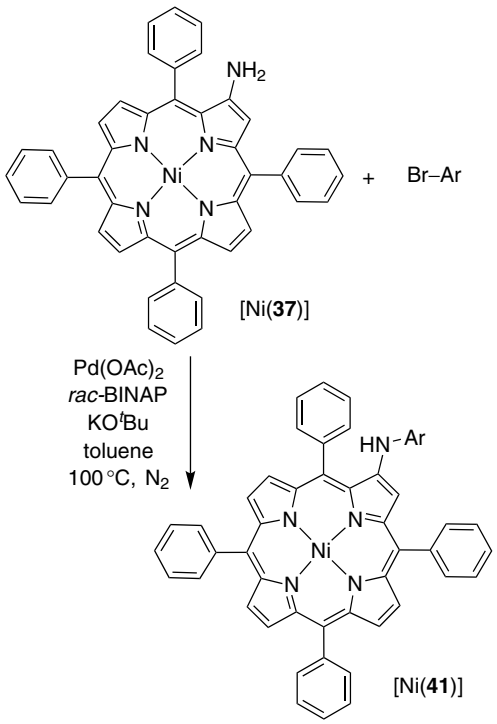


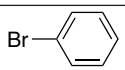
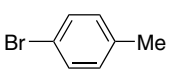
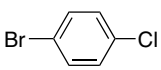
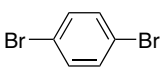
Scheme 14.[102]

of *N*-arylaminoporphyrins [Ni(**41**)] (Table 6).[103] In the first case, *β*-nitrotetraphenylporphyrin was refluxed with aniline or *p*-toluidine for 20 hours under a nitrogen atmosphere to give [Ni(**41**)] in 53 and 32% yield, respectively. When this methodology was applied to electron-withdrawing *p*-bromoaniline and *p*-chloroaniline, however, it failed to produce the desired products. The reasons for this are still unknown.

In an effort to improve this system, aryl bromides were coupled to *β*-aminotetraphenylporphyrinatonickel(II) [Ni(**37**)] using a palladium-catalyzed cross-coupling approach to provide the corresponding aminated products [Ni(**41**)] in 45–78% yields (entries 1–4, Table 5).[103,104] These products could be used to prepare porphyrins bearing fused rings through the oxidative cyclization of *N*-arylaminoporphyrins to give **42** (Figure 6).[103,104]

Table 6. Palladium-catalyzed cross-coupling of Ni(II) aminoporphyrins with aryl halides.[103,104]

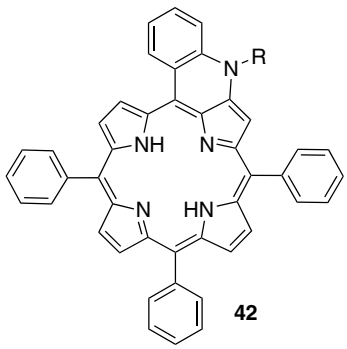


Entry[a]	Br–Ar	Time (h)	Yield (%)
1		24	69
2		2	78
3		2	68
4		24	45

[a]Reactions were carried out at 100°C under N_2 using 1 equiv. of aminoporphyrin [Ni(**37**)], 2.8 eqiuv. of aryl bromide, 29 mol % $Pd(OAc)_2$ with 25 mol % rac-BINAP (Pd: L = 1.16:1) in the presence of 2.13 equiv. of KO^{t}Bu. Concentration: 6.4 mM aminoporphyrin in toluene.

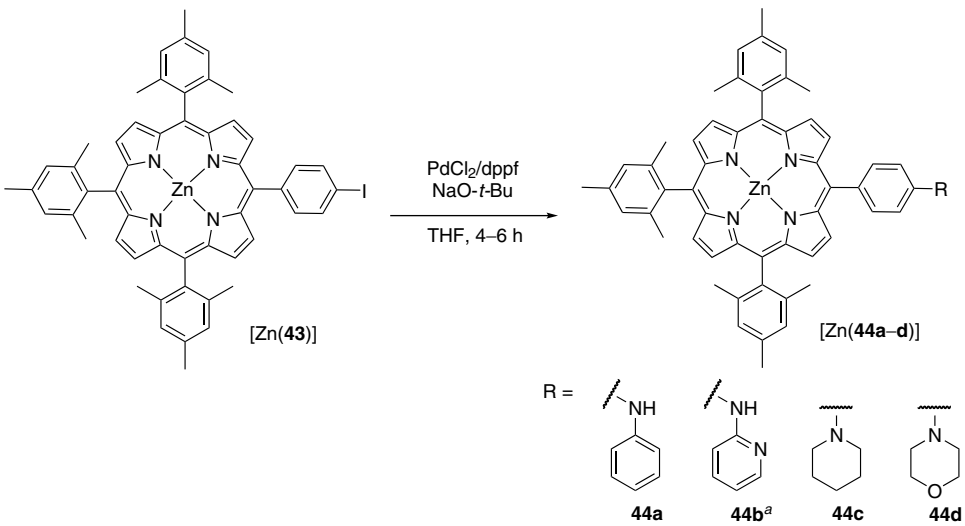
3. *aryl–Amination*

In 2001, van Lier and co-workers reported the first palladium-catalyzed carbon–heteroatom intermolecular cross-coupling reactions using porphyrin substrates.[101]



42

Figure 6. *N*-Aryl aminoquinolinoporphyrin.[103]

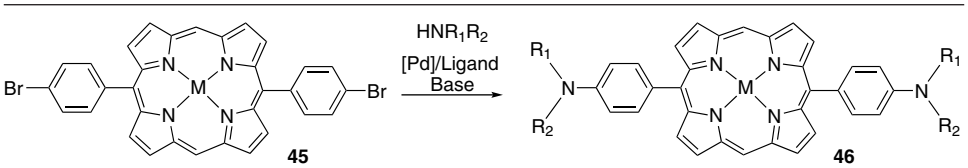


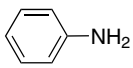
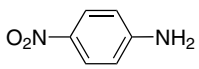
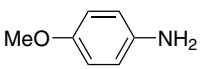
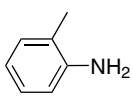
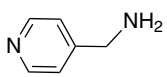
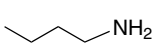
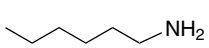
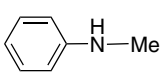
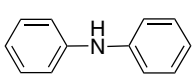
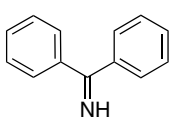
Scheme 15.[101]

In their communication, they successfully coupled 5-(4′-iodophenyl)-10,15,20-trimesityl-porphyrinatozinc(II) [Zn(**43**)] with aniline, pyridin-2-amine, piperidine, and morpholine using conditions previously employed by Hartwig and co-workers (Scheme 15).[69–72] No yields were reported in this study.

In 2003, Zhang and co-workers used *para*-bromo-substituted diphenyl and tetraphenylporphyrins to synthesize a series of alkyl and arylaminoporphyrins *via* palladium-catalyzed amination (Tables 7 and 8).[105] In their report, a practical "one-pot" protocol was used to perform double and quadruple couplings in good to excellent yields. As shown in Table 7, electron-rich and electron-deficient

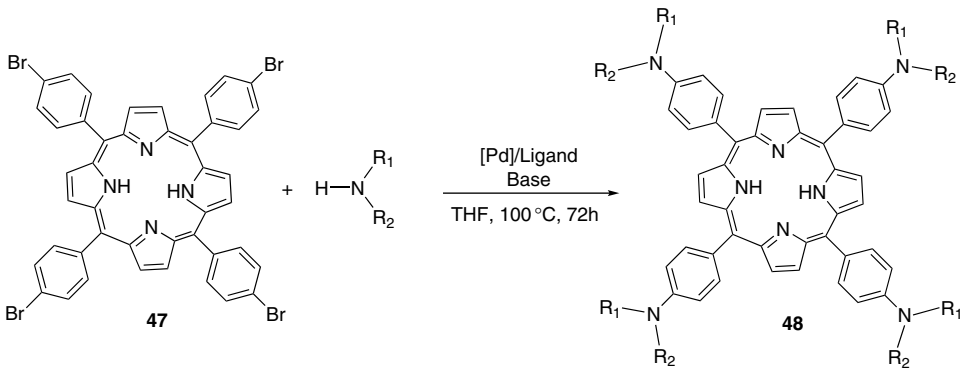
Table 7. Palladium-catalyzed double amination reactions of bromoporphyrins with various amines.[105]

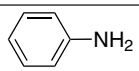
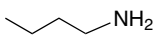
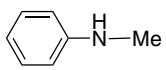
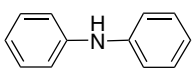


Entry[a]	Amine	Br-por	Ligand	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1		**45**	**L9**	THF	100	24	95
2[c]		[Zn(**45**)]	**L9**	Toluene	100	48	66
3		**45**	**L9**	Toluene	100	48	76
4		**45**	**L2**	THF	100	48	93
5		[Zn(**45**)]	**L6**	THF	100	48	68
6		**45**	**L2**	THF	100	48	87
7		[Zn(**45**)]	**L6**	THF	100	48	73
8		**45**	**L9**	THF	100	48	88
9		**45**	**L1**	THF	100	48	96
10		**45**	**L2**	THF	68	48	96
11		[Zn(**45**)]	**L6**	THF	100	48	93
12		**45**	**L2**	THF	100	48	90
13		[Zn(**45**)]	**L8**	THF	100	48	53
14		**45**	**L2**	THF	100	48	88
15		[Zn(**45**)]	**L2**	THF	100	48	73
16		**45**	**L2**	THF	100	48	81
17		[Zn(**45**)]	**L6**	THF	100	48	57
18[d]		**45**	**L5**	THF	100	48	52

[a]Reactions were carried out under N_2 with 1 equiv. of Br-por, 4 equiv. of amine, 2.5 mol % Pd(OAc)2, and 5 mol % ligand in the presence of 4 equiv. of NaO^{t}Bu per Br. Concentration: 0.01 M Br-por/solvent; [b]Represents isolated yields of >95% purity as determined by ^{1}H-NMR; [c]Cs_2CO_3 used instead of NaO^{t}Bu; [d]$Pd_2(dba)_3$ used instead of $Pd(OAc)_2$.

Table 8. Palladium-catalyzed quadruple amination reactions of bromoporphyrins with various amines.[105]



Entry[a]	Amine	Br-por	Ligand	Yield (%)[b]
1		**47**	**L9**	91
2		**47**	**L6**	86
3		**47**	**L9**	82
4		**47**	**L2**	81

[a]Reactions were carried out at 100°C under N_2 with 1 equiv. of Br-por, 4 equiv. of amine, 2.5 mol % $Pd(OAc)_2$, and 5 mol % ligand in the presence of 4 equiv. of NaO^{t}Bu per Br in THF for 72 h. Concentration: 0.01 M Br-por in THF; [b]Represents isolated yields of >95% purity as determined by ^{1}H-NMR.

arylamines (entries 3–5), sterically hindered amines (entries 6–7), aliphatic amines (entries 8–13), secondary aliphatic-aromatic amines (entries 14–15), and aromatic-aromatic amines (entries 16–17), as well as imines (entry 18) were very suitable substrates. The quadruple amination of tetra(*para*-bromophenyl)porphyrin proceeded with similar success (Table 8). In both cases, the zinc porphyrin complexes were generally produced in lower yields in comparison to the free-base porphyrins.

In their report, Zhang and co-workers observed that a strong ligand dependency existed for palladium-catalyzed amination (Tables 7 and 8). For example, simple aromatic amines were successfully coupled using the sterically hindered mono- and bis-phosphines, **L6** and BINAP **L9**, respectively. Electron-withdrawing and sterically hindered aromatic amines were also successfully coupled with the

bulky monophosphine, **L2**. Similarly, alkyl amines as well as both types of secondary amines preferred the bulky monophosphines, **L2** and **L6**. However, the sterically demanding imine performed best with the hindered monophosphine, **L5**.

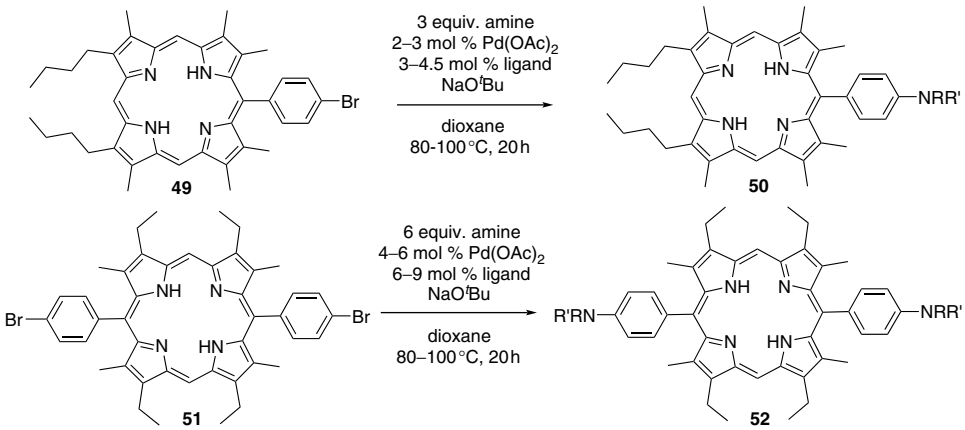
In 2007, Beletskaya and co-workers reported on the successful palladium-catalyzed cross-coupling between hydroxypiperidenes and *meso*-bromophenyl polyalkylated porphyrins (Table 9).[106] Their work, inspired by the reported anti-HIV activity of *trans*-3,4-dihydroxypiperidines, specifically focused on the preparation of different combinations of hydroxypiperidines and porphyrin pharmacophores.

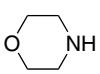
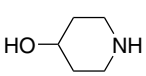
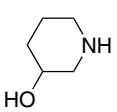
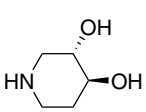
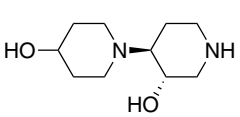
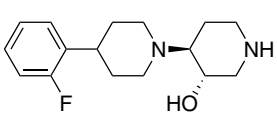
The authors showed that the correct choice of ligand was very important in the successful formation of the desired products. For example, simple secondary amines, such as piperidine (entries 1–3, Table 9) and morpholine (entry 4, Table 9), could be coupled with either 5-(4′-bromophenyl)-2,3,7,8,12,18-hexamethyl-13,17-dibutylporphyrin **49** or 5,15-bis(4′-bromophenyl)-3,7,13,17-tetramethyl-2,8,12,18-tetraethylporphyrin **51** in 77–83% yields without complications using ligands **L2**, **L3**, and **L4** (Table 9).[106] However, the aminations of hydroxypiperidines and their derivatives were complicated by two factors: the competitive cross-coupling of the hydroxyl group and reduction of the aryl halide, which is a known problem in the arylation of aliphatic amines.[107–109]

The couplings of monohydroxypiperidines (entries 5, 8, and 14, Table 9) with mono-brominated porphyrin **49** using ligand **L3** led to the desired products in 50–70% yields. However, when the dibrominated porphyrin substrate **51** was used with ligands **L3** or *t*-Bu_3P-HBF_4 (entries 6–7 and 9–10, Table 9), three products were produced: the diaminated product; the partially aminated/partially reduced product; and the fully reduced product. Hydroxypiperidines containing secondary alcohols were shown to function as powerful reducing agents in the coupling reactions. The palladium-mediated coupling reaction of mono-brominated porphyrin **49** with dihydroxypiperidines using *t*-Bu_3P-HBF_4 as a supporting ligand gave the desired product in less than 20% yields (entry 12, Table 9). As a result of reduction, the reaction of dibrominated porphyrin **51** with dihydroxypiperidines produced only the mono-aminated products (entries 11 and 13, Table 9).

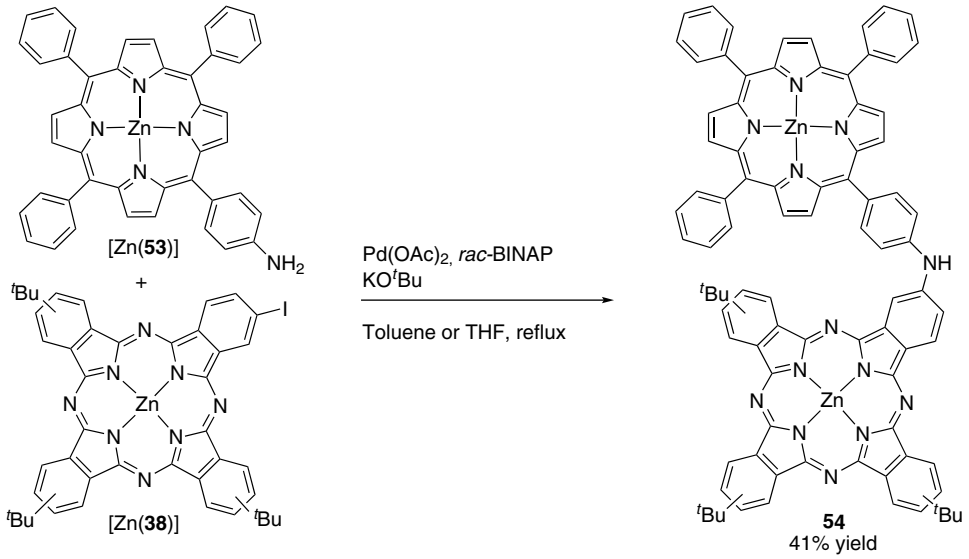
Torres, Guldi, and co-workers constructed porphyrin-phthalocyanine dyads using palladium-catalyzed amination of iodophthalocyanine [Zn(**38**)] with arylaminoporphyrins.[102] Using the same Buchwald–Hartwig conditions described in their β-aminated examples (Schemes 13 and 14), 5-(4′-aminophenyl)-10,15,20-triphenylporphyrin [Zn(**53**)] and Zn(II)-2(3), 9(10), 16(17)-tri-*tert*-butyl-23-iodophthalocyanine [Zn(**38**)] were cross-coupled to give the porphyrinylaminophthalocyanine **54** in 41% yield (Scheme 16).

Table 9. Amination of 5-(4′-Bromophenyl)-2,3,7,8,12,18-hexamethyl-13,17-dibutylporphyrin **49** and 5,15-bis(4′-bromophenyl)-3,7,13,17-tetramethyl-2,8,12,18-tetraethylporphyrin **51**.[106]



Entry	Amine	Br-por	Ligand	Temp (°C)	NaO*t*-Bu (equiv.)	Isolated yields (%) di	mono	Reduced
1[a,c]		**49**	L4	100	4	—	83	—
2[b,c]		**51**	L2	100	4	82	—	—
3[b,c,d]		**51**	L3	100	4	82	—	—
4[b,e]		**51**	L2	100	4	82	—	—
5[a]		**49**	L3	100	8	—	70	71
6[b]		**51**	L3	100	16	44	30	trace
7[b]		**51**	*t*-Bu$_3$P-HBF$_4$	80	16	7	21	26
8[a]		**49**	L3	80	8	—	50	45
9[b]		**51**	L3	100	20	19	23	19
10[b]		**51**	*t*-Bu$_3$P-HBF$_4$	80	16	11	28	28
11[b]		**51**	*t*-Bu$_3$P-HBF$_4$	80	18	trace	11	47
12[a]		**49**	*t*-Bu$_3$P-HBF$_4$	80	16	—	18	48
13[b]		**51**	*t*-Bu$_3$P-HBF$_4$	80	28	—	25	34
14[a]		**49**	L3	100	16	—	56	34

[a]Reaction conditions amine (3 equiv.) $Pd(OAc)_2$ (2–3 mol %), ligand (3–4 5 mol %) in dioxane for 20 h; [b]Reaction conditions amine (6 equiv.) $Pd(OAc)_2$ (4–6 mol %), ligand (6–9 5 mol %) in dioxane for 20 h; [c]20 equiv. of amine; [d]Cs_2CO_3 used as base; [e]5 equiv. of amine.



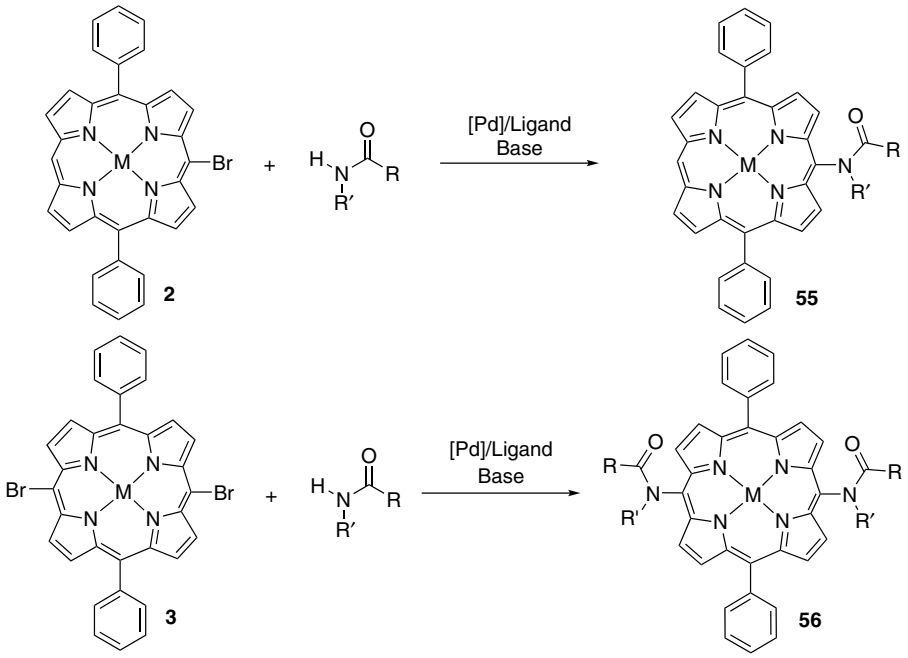
Scheme 16.[102]

B. Amidation

1. *meso-Amidation*

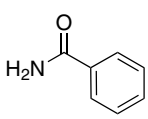
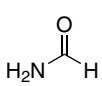
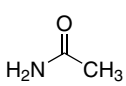
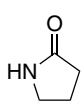
Suda and co-workers reported a useful methodology for the palladium-catalyzed cross-coupling of primary and secondary amides (Scheme 17).[97] They explored the use of *meso*-bromo-5,15-diphenylporphyrin **2** and its zinc and nickel complexes for the preparation of *meso*-amido-substituted porphyrins. The effect of the central metal ion on the reaction with benzamide showed a remarkable acceleration when the nickel complex [Ni(**2**)] was used. In addition to the nearly quantitative production of amidoporphyrin using benzamide (entry 3, Table 10), the amidation system was shown to be effective with formamide (entry 4, Table 10), acetamide (entry 5, Table 10), and pyrrolidinone (entries 6, Table 10).

Zhang and co-workers independently developed a method for the palladium-catalyzed cross-coupling of free-base and zinc bromoporphyrins with primary and secondary amides (Scheme 17).[110] In addition to benzamide (entries 1–3, Table 11), the amidation system reported by Zhang and co-workers could be applied to acetamide and other alkyl amides (entries 4–11, Table 11), as well as carbamates and *N*-phenylacetamides (entries 12–19, Table 11). The amidation system was also shown to catalyze reactions with cyclic amides, such as pyrrolidinone and 4-benzyloxazolidin-2-one in good yields (entries 20–25, Table 11). The efficient use of free-base porphyrins as synthons for palladium-catalyzed cross-coupling highlights the importance of this methodology as it eliminates the need for extra steps



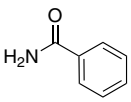
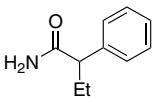
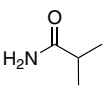
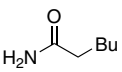
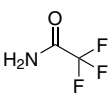
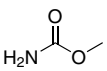
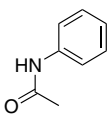
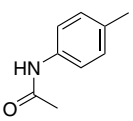
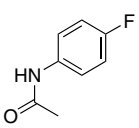
Scheme 17.[97,110]

Table 10. Amidation of Ni(II) *meso*-bromoporphyrin [Ni(**2**)].[97]

Entry[a]	Amide	Br-por[b]	Time (h)	Yield (%)
1[c]		**2**	24	ND
2[c]		[Zn(**2**)]	24	32
3		[Ni(**2**)]	5	>99
4		[Ni(**2**)]	2	85
5		[Ni(**2**)]	1	83
6		[Ni(**2**)]	1	80

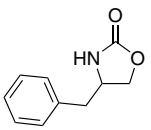
[a]Reactions were carried out at 100°C under N_2 in dioxane with 2 equiv. of amide, 6.7 mol % $Pd(OAc)_2$; and 19.4 mol % BINAP in the presence of 2.4 equiv. of Cs_2CO_3. Concentration: 0.01 M Br-por in dioxane; [b]See Figure 1 for structures; [c]Reactions conditions same as in a, except they were done at 68°C in THF in the presence of 12 equiv. of Cs_2CO_3. Concentration: 0.01 M Br-por in THF.

Table 11. Palladium-catalyzed cross-coupling of *meso*-bromoporphyrins with amides.[110]

Entry[a]	Amide	Br-por	Temp (°C)	Time (h)	Yield (%)
1		**2**	68	19	88
2[c,f]		[Zn(**2**)]	80	17	68
3		**3**	68	22	60
4[b]		**2**	68	21	71[k]
5[h]		**2**	100	22	69[k]
6[i]		**2**	80	10	70[k]
7		**2**	80	18	83[k]
8		[Zn(**2**)]	80	16	60[k]
9		**2**	80	18	89
10[e,g,i]		[Zn(**2**)]	80	19	75
11		**3**	100	2	73
12[f]		**2**	100	8	65
13		**2**	80	17	71
14		**2**	100	24	92
15		[Zn(**2**)]	80	17	24
16		**3**	80	19	66[k]
17		**2**	100	19	83
18		[Zn(**2**)]	100	17	21
19		**3**	100	30	57[k]
20[b]		**2**	68	21	83
21[d,g]		[Zn(**2**)]	80	17	62
22		**3**	100	30	59

(*Continued*)

Table 11. (*Continued*)

Entry[a]	Amide	Br-por	Temp (°C)	Time (h)	Yield (%)
23[c]		**2**	80	19	60
24[c,f]		[Zn(**2**)]	80	17	46
25		**3**	68	22	62[k]

[a]Reactions were carried out under N_2 in THF with 1 equiv. of Br-por; 4 equiv. of amide, 10 mol % $Pd(OAc)_2$; and 20 mol % Xantphos in the presence of 2 equiv. of Cs_2CO_3 Concentration: 0.01 M Br-por in THF; Br-por **3**:**8** equiv. of amide and 4 equiv. of base; [b]2.5 mol % $Pd_2(dba)_3$; [c]5 mol % $Pd_2(dba)_3$; [d]10 mol % $Pd_2(dba)_3$; [e]20 mol % $Pd(OAc)_3$; [f]20 mol % Xantphos; [g]40 mol % Xantphos; [h]2 equiv. of amide and 1.5 equiv. of base; [i]NaO*t*-Bu; [j]8 equiv. of amide; [k]Exists as a mixture of stereoeoisomers.

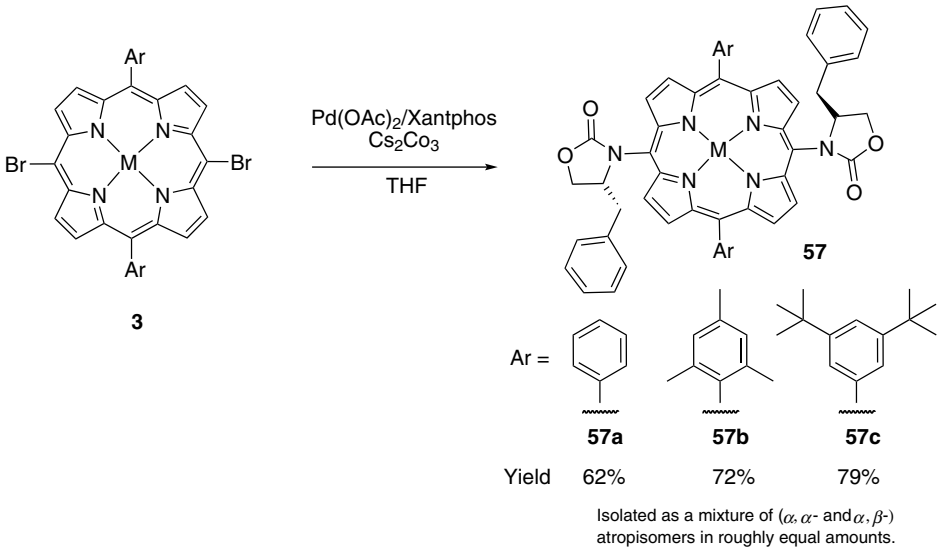
of metalation and demetalation which would result in reduced yields of the desired cross-coupled products.

Chiral porphyrins have found a range of useful applications in several areas such as asymmetric catalysis, chiral recognition/sensing, and enzymatic mimicry.[27–31] As a part of their program to develop metalloporphyrins as practical catalysts for atom/group transfer reactions,[111–130] Zhang and co-workers systematically probed the relationship between the structure of the porphyrin and reaction selectivity in asymmetric cyclopropanation reactions.

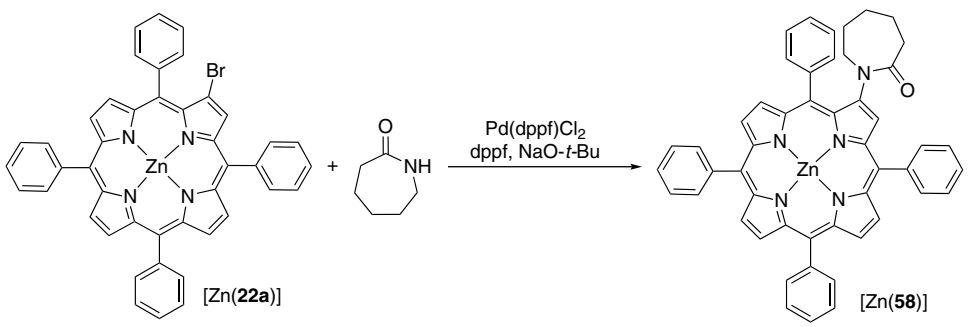
meso-Dibromoporphyrins **3** bearing three different *meso*-aryl groups (Scheme 18) were readily prepared in gram scale *via* selective bromination of the corresponding 5,15-diarylporphyrins.[38,48,53] These three synthons were then coupled with (*R*)-(+)-4-benzyl-2-oxazolidinone, a commercially available chiral amide, under the palladium-catalyzed amidation conditions previously established. The corresponding chiral *meso*-amidoporphyrins **57a–c** were produced as a mixture of two atropisomers (α,α- and α,β-isomers) in approximately equal amounts. The cobalt complexes of these products, [Co(**57**)], were subsequently evaluated for their ability to perform cyclopropanation reactions with styrene and ethyl diazoacetate. Although cyclopropane products were generated in high yields, the diastereoselectivity was moderate (*cis*:*trans* = 26:74) and enantioselectivity was low (≤12 % ee).[131]

2. *β-Amidation*

As discussed in the section on β-amination, van Lier and co-workers also reported in the same 2001 paper on palladium-catalyzed carbon–heteroatom intermolecular cross-coupling between β-substituted porphyrins and amides.[101] They noted that a moderate yield was obtained for the amidation of β-BrTPP [Zn(**22a**)] with the cyclic amide, ε-caprolactam (Scheme 19).

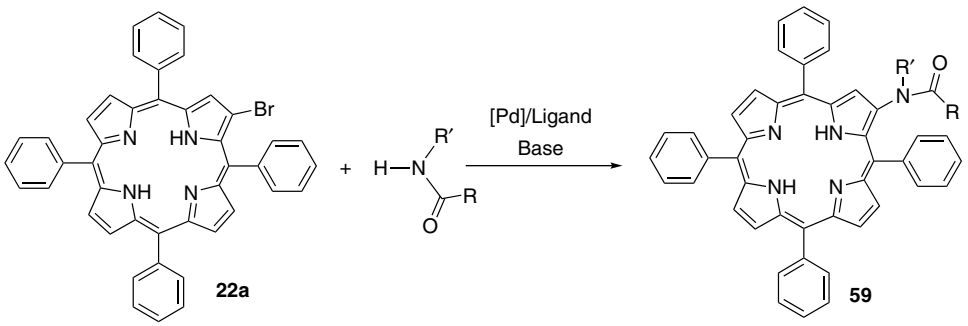


Scheme 18.[131]



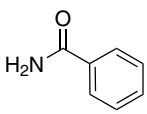
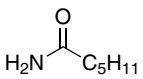
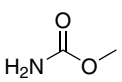
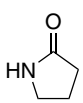
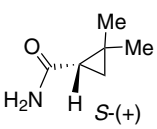
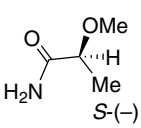
Scheme 19.[101]

In 2007, Zhang and co-workers reported the efficient synthesis of a variety of *β*-substituted porphyrins by coupling *β*-BrTPP **22a** with alkyl and aryl amines, amides, alcohols, and thiols (Scheme 20).[91] The palladium sources $Pd(OAc)_2$ and $Pd_2(dba)_3$ were screened in combination with various bidentate phosphine ligands such as DPEphos **L7**, Xantphos **L8**, and BINAP **L9**. Using a Xantphos **L8**/$Pd_2(dba)_3$-based catalytic system, amidation reactions of *β*-BrTPP **22a** with both aromatic and aliphatic amides were successfully catalyzed to produce desired *β*-amidoporphyrins in 65–76% yields (entries 1–2, Table 12). The amidation system was also productively applied to methyl carbamate and pyrrolidinone (entries 3–4, Table 12).[91]



Scheme 20.[91]

Table 12. Synthesis of β-amidotetraphenylporphyrins.[91]

Entry[a]	Amide	Br-por	Time (h)	Yield (%)
1		[Zn(**22a**)]	24	65
2		**22a**	28	76
3[b]		**22a**	5	45
4		**22a**	24	71
5		**22a**	24	86
6		**22a**	24	46

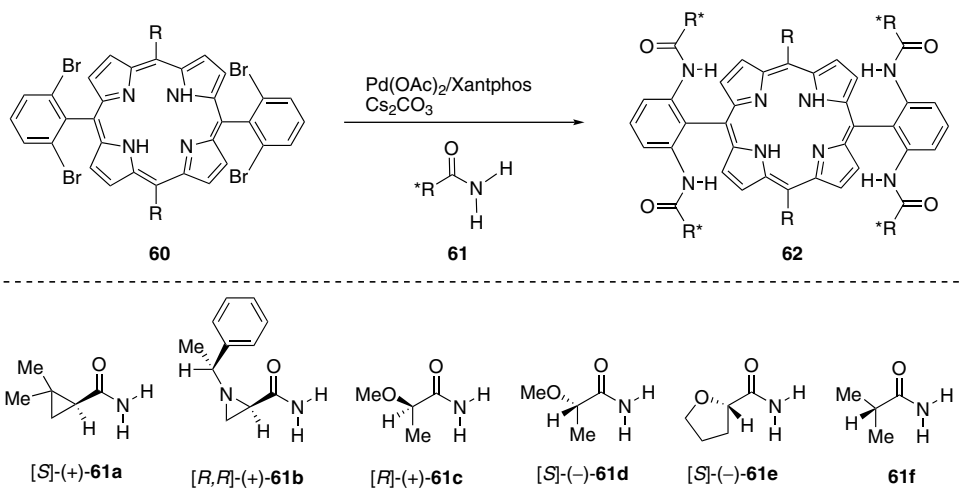
[a]Reactions were carried out at 100°C under N_2 in THF with 1 equiv. of **22a**; 4 equiv. of amide, 10 mol % $Pd(OAc)_2$; and 20 mol % Xantphos in the presence of 2 equiv. of Cs_2CO_3. Concentration: 0.01 M **22a** in THF; [b]4 equiv. of Cs_2Co_3.

One of the practical aspects of the palladium-catalyzed cross-coupling amidation method developed by Zhang and co-workers is its mild reaction conditions under which potential racemization of chiral centers is prevented. This feature was demonstrated by reactions of **22a** with commercially available chiral amides (*S*)-(+)-(2,2-dimethylcyclopropanecarboxamide and (*S*)-(−)-2-methoxypropionamide (entries 5–6, Table 12). It was shown the corresponding chiral β-amidoporphyrins could be formed in 86 and 46% yields, respectively, without racemization.

3. *aryl-Amidation*

In 2004, Zhang and co-workers reported that 5,10-bis(2′,6′-dibromophenyl)porphyrins **60a–k** were versatile synthons for the modular construction of chiral porphyrins *via* palladium-catalyzed amidation reactions with chiral amides.[132] The quadruple carbon–nitrogen bond formation reactions could be accomplished in high yields under mild conditions and with different chiral amide building blocks, forming a series of D_2-symmetric chiral porphyrins (Scheme 21). The Co(II) complexes of these chiral porphyrins were shown to be excellent catalysts for the enantioselective and diastereoselective cyclopropanation of a variety of alkene substrates with diazoacetates.

Zhang and co-workers continued to develop D_2-symmetric chiral porphyrins to further explore the catalytic properties of these systems. The new designs they



Scheme 21.[132]

Table 13. Amidation of D_2-symmetric chiral porphyrins.[122,123,132]

Entry[a]	R	60	61	62	Yield (%)[b]
1	ph	**60a**	**61a**	**62a**	78
2	ph	**60a**	**61b**	**62b**	64
3	ph	**60a**	**61c**	**62c**	75
4	ph	**60a**	**61d**	**62d**	71
5	4-*t*-BuPh	**60b**	**61a**	**62e**	86
6	4-CF_3Ph	**60c**	**61a**	**62f**	77
7	pentaFPh	**60d**	**61a**	**62g**	46
8	4-AcetylPh	**60e**	**61a**	**62h**	66
9	2,4,6-triMePh	**60f**	**61a**	**62i**	84
10	2,6-diMeOPh	**60g**	**61a**	**62j**	59
11	2,6-diMeOPh	**60g**	**61d**	**62k**	63
12	2,6-diMeOPh	**60g**	**61e**	**62l**	60
13	2,6-diMeOPh	**60g**	**61f**	**62m**	59
14	3,5-diMeOPh	**60h**	**61a**	**62n**	88
15	3,5-di-*t*-BuPh	**60i**	**61a**	**62o**	85
16	3,5-di-*t*-BuPh	**60i**	**61c**	**62p**	79
17	3,5-di-*t*-BuPh	**60i**	**61d**	**62q**	72
18	3,5-di-*t*-BuPh	**60i**	**61e**	**62r**	63
19	3,5-di-*t*-BuPh	**60i**	**61f**	**62s**	65
20	4-*n*-heptyl	**60j**	**61a**	**62t**	74
21	H	**60k**	**61a**	**62u**	79

[a]Reactions were carried out under N_2 in THF with 1 equiv. of Br-por; 16 equiv. of amide, 10 mol % $Pd(OAc)_2$; and 20 mol % Xantphos in the presence of 8 equiv. of Cs_2CO_3. Concentration: 0.01 M Br-por in THF; [b]Isolated yields.

reported were based on the hypothesis that it is possible to control diastereoselectivity and enantioselectivity through the combined use of the chiral R* and *meso*-R groups.[123] This is illustrated by catalysts derived from chiral porphyrins containing the bulkier 2,6-methoxyphenyl groups at the *meso* positions (**60g**, Table 13). Palladium-catalyzed amidation reactions between tetrabromoporphyrin **60g** and chiral acyclic amide **61c** or **61d**, both of which possess intramolecular O–H–N hydrogen bonding interactions, resulted in chiral porphyrins, Co(II) complexes of which were shown to be effective catalysts with improved enantioselectivity for cyclopropanation and aziridination of a range of alkene substrates.

In their continuing efforts to create more effective catalysts, Zhang and coworkers synthesized chiral porphyrins containing rigid and cyclic structures as well as functional groups capable of participating in intramolecular O–H–N hydrogen bonding. Their work ultimately led to the development of chiral porphyrins **62l** and **62r** using (*S*)-(−)-2-tetrahydro furancarboxamide (entries 12 and 18, Table 13).[123] The Co(II) complexes of these chiral porphyrins were shown to impart a substantial increase in enantioselectivity for catalytic cyclopropanation

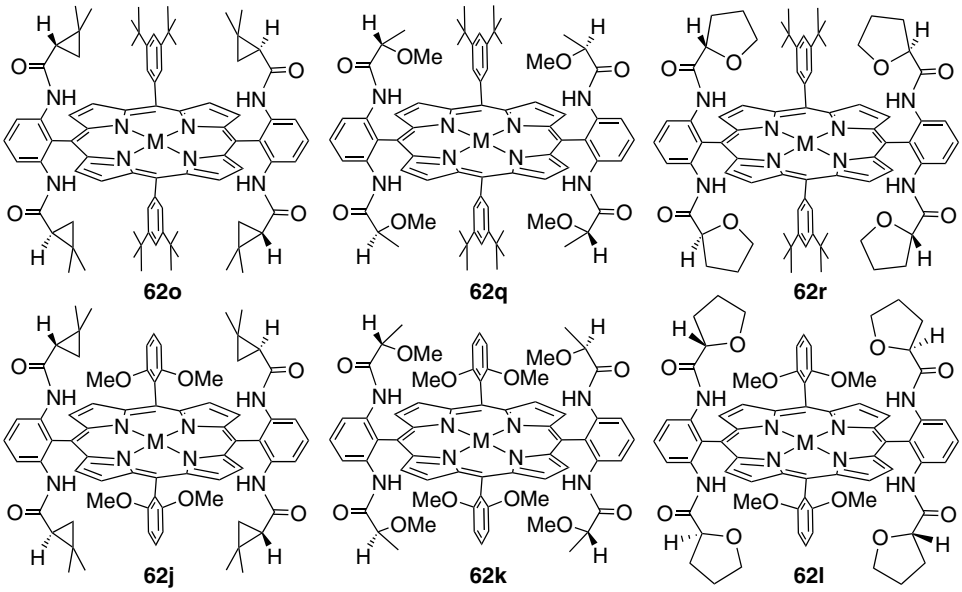


Figure 7. The "group of six" chiral D_2-symmetric porphyrins.

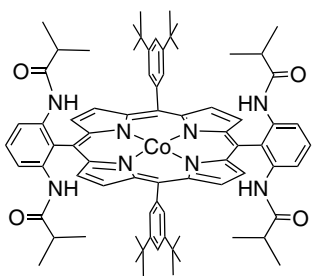


Figure 8. Co(II) complex of 3,5-di*t*butyl-*i*-BuPhyrin.[122]

with diazosulfones over previously reported Co(II) porphyrin catalysts. Through these investigations, a "Group of Six" D_2-symmetric chiral porphyrins (Figure 7) have emerged and their Co(II) complexes were demonstrated to have catalytic capabilities for a range of carbene and nitrene transfer reactions.

Zhang and co-workers investigated the use of Co(II) porphyrins for the catalytic aziridination of styrene and its various derivatives with sulfonyl azides.[122] 2,6-Diamidophenyl-substituted porphyrin **62s** (entry 19, Table 13) was designed and synthesized based on potential hydrogen bonding interaction in the presumed metal–nitrene intermediate of the catalyst. The cobalt complex [Co(**62s**)] (Figure 8)

was indeed shown to be a highly effective catalyst for the aziridination of a variety of aromatic olefins with various arylsulfonyl azides.

IV. Palladium-Catalyzed C–O Coupling

Porphyrins and metalloporphyrins that possess aryloxy and alkoxy groups have been shown to serve as catalysts, liquid crystal complexes, and photoinduced electron transfer agents.[134–152] However, in comparison to the large numbers of synthetic porphyrins with carbon-based peripheral substituents, only a limited number of porphyrins with aryloxy- and alkoxy-substituents have been reported.

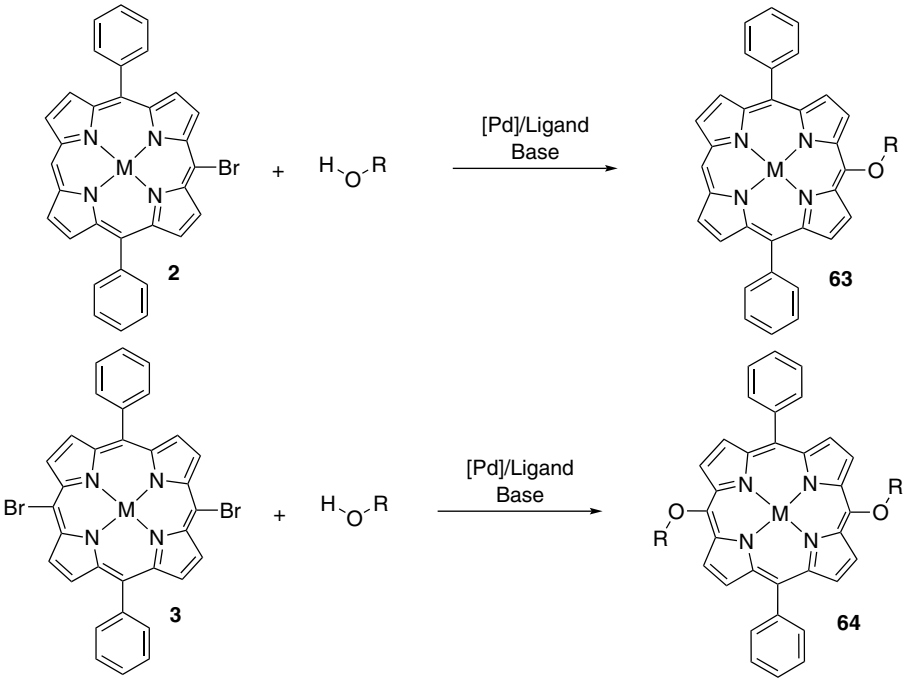
Aryloxy- and alkoxyl-substituted porphyrins have been traditionally prepared *via* nucleophilic substitution of pyrroles.[141–152] Considering the multiple steps and electronic requirements associated with alkoxypyrrole synthesis,[141–143] the development of alternative methods to synthesize this class of porphyrin conjugates was necessary to exploit these compounds.[141–152] The following section highlights recent advancements made in C–O bond formations *via* palladium-catalyzed cross-coupling.

A. *meso*-Etheration and Hydroxylation

Zhang and co-workers explored the palladium-catalyzed etheration[153–160] of meso-bromoporphyrins **2** and **3** for the versatile synthesis of *meso*-aryloxy- and alkoxyl-substituted porphyrins (Scheme 22).[131,133] The reactions were performed under mild conditions with a host of alcohols, producing a family of novel porphyrins in moderate to high yields (Tables 14 and 15). In general, simple bidentate phosphine ligands, such as DPEphos (**L7**) and Xantphos (**L9**), worked best for these transformations.

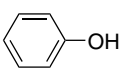
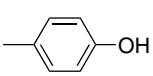
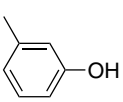
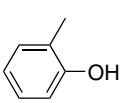
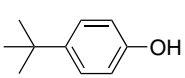
Under the general reaction conditions, free-base and zinc complexes of both *meso*-monobrominated and *meso*-dibrominated 10,20-diphenylporphyrins **2**, [Zn(**2**)], and **3** were effectively coupled with a variety of different alcohols. As summarized in Table 14, phenol and cresol isomers (entries 1–9, Table 14) were successfully employed to give the desired *meso*-monoaryloxyporphyrins in high yields. Sterically hindered 2-isopropylphenol gave the desired porphyrin in good yields (entries 12–13).

Electron-rich and electron-deficient phenol derivatives such as 4-methoxyphenol (entries 17–19) and 4-fluorophenol (entries 21–22) were also efficiently coupled under the reported reaction conditions. In addition, electron-deficient 3- and 4-nitrophenols, which failed to react with **3**, could be successfully coupled with 5,15-dibromo-10,20-bis-(3,4,5-trimethoxyphenyl)porphyrin, a diaryl derivative of **3**. The etheration methodology was also extended to a variety of aliphatic alcohols, including electron-rich and electron-deficient benzyl alcohol derivatives (entries 1–5,



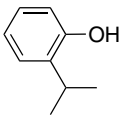
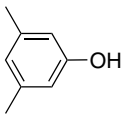
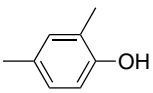
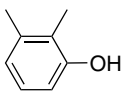
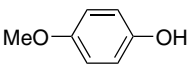
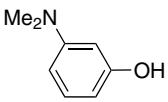
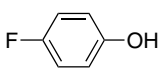
Scheme 22.[131,133]

Table 14. Synthesis of *meso*-aryloxytetraphenylporphyrins *via* palladium-catalyzed C–O bond formations of *meso*-bromotetraphenylporphyrins with aromatic alcohols.[133]

Entry[a]	Br-por[b]	Alcohol	Temp (°C)	Time (h)[c]	Yield (%)[d]
1[e]	[Zn(**2**)]		100	23	80
2[e]	**3**		80	4	66
3	[Zn(**3**)]		80	5	44
4[e]	[Zn(**2**)]		100	24	65
5	**3**		100	24	69
6	[Zn(**2**)]		100	16	78
7[f]	**3**		100	23	61
8	[Zn(**2**)]		100	16	89
9[g]	**3**		100	48	59
10[e]	[Zn(**2**)]		100	18	73
11	**3**		100	48	45

(*Continued*)

Table 14. (*Continued*)

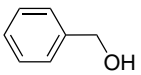
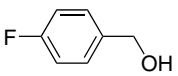
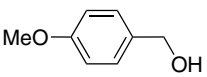
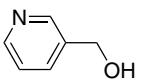
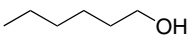
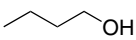
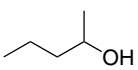
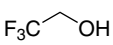
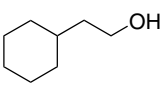
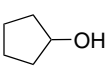
Entry[a]	Br-por[b]	Alcohol	Temp (°C)	Time (h)[c]	Yield (%)[d]
12[e]	[Zn(**2**)]		100	17	72
13	**3**		100	25	50
14	**3**		100	21	58
15	**3**		100	21	62
16	**3**		100	21	54
17[e]	[Zn(**2**)]		100	17	93
18[f]	**3**		80	16	78
19	[Zn(**3**)]		100	18	68
20[f]	**3**		80	18	44
21[e]	[Zn(**2**)]		100	17	78
22[e]	**3**		80	4	55

[a]Reactions were carried out in bluene under N_2 with 1 equiv. of Br-por, 2–4 equiv. of alcohol, 5 mol % $Pd_2(dba)_3$, and 10 mol % DPEphos in the presence of 2 equiv. of Cs_2CO_3 per Br. Concentration: 0.01 M Br-por in toluene; [b]Structures of bromoporphyrins are shown in Figure 1; [c]Reaction times have not been optimized; [d]Represents isolated yields of >95% purity as determined by ^{1}H-NMR; [e]$Pd(OAc)_2$ was used instead; [f]Xantphos was used instead; [g]K_3PO_4 was used instead.

Table 15), linear primary and secondary alcohols (entries 6–14), cyclic primary alcohols (entries 15–16) and cyclic secondary alcohols (entries 17–18).

In a follow-up report, Zhang and co-workers applied this methodology to di-*meso*-bromo-diarylporphyrins using chiral alcohols (Table 16).[131] A combination of $Pd_2(dba)_3$ and DPEphos (**L7**) could be used to perform the desired double C–O coupling reactions. Secondary cyclic alcohol, (+)-dihydrocholesterol, formed the corresponding *meso*-chiral porphyrins in 45–82% yields (entries 1–4, Table 16). The aromatic alcohol (+)-estrone was used to produce the corresponding *meso*-chiral porphyrin in 98% yield (entry 5, Table 16). Only one set of resonances was

Table 15. Synthesis of *meso*-slkoxytetraphenylporphyrins *via* palladium-catalyzed C–O bond formations of *meso*-bromotetraphenylporphyrins with aliphatic alcohols.[133]

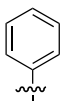
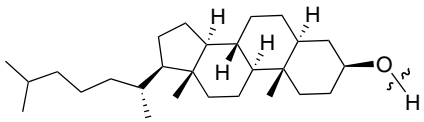
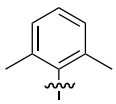
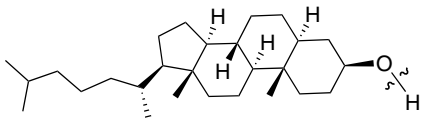
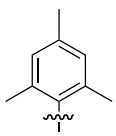
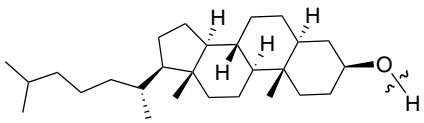
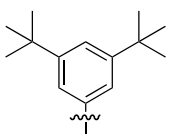
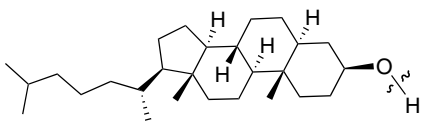
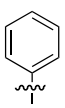
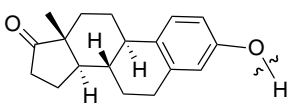
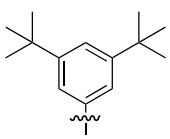
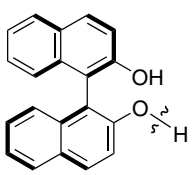
Entry[a]	Br-por[b]	Alcohol	Temp (°C)	Time (h)[c]	Yield (%)[d]
1	**3**		80	18	40
2[e]	**3**		80	20	48
3[e]	**3**		80	4	41
4[e]	[Zn(**3**)]		80	5	44
5[e]	**3**		80	17	62
6[e]	**3**		80	17	51
7	[Zn(**3**)]		100	25	54
8[e]	**3**		100	39	66
9	**3**		100	47	50
10[e]	**3**		100	39	30
11	**3**		100	48	33
12[e]	**3**		80	17	63
13[e]	[Zn(**3**)]		100	23	51
14	**3**		80	48	62
15	[Zn(**3**)]		100	48	50

[a]Reactions were carried out in toluene under N_2 with 1 equiv. of Br-por, 2–4 equiv. of alcohol, 5 mol % $Pd_2(dba)_3$, and 10 mol % DPEphos in the presence of 2 equiv. of Cs_2CO_3 per Br. Concentration: 0.01 M Br-por in toluene; [b]Structures of bromoporphyrins are shown in Figure 1; [c]Reaction times have not been optimized; [d]Represents isolated yields of >95% purity as determined by ^{1}H-NMR; [e]Xantphos was used instead.

observed in both ^{1}H and ^{13}C-NMR spectra of these compounds, suggesting free rotation around the C–O bond at ambient temperature.

When *R*-(+)-BINOL was used in excess, the double etheration reaction could be controlled to provide a *meso*-chiral porphyrin where only one of the two hydroxyl groups reacted (entry 6, Table 16). The observation of multiple ^{1}H-NMR resonances suggested the product exists as a mixture of two atropisomers (α,α- and

Table 16. Synthesis of *meso*-chiral porphyrins.[131]

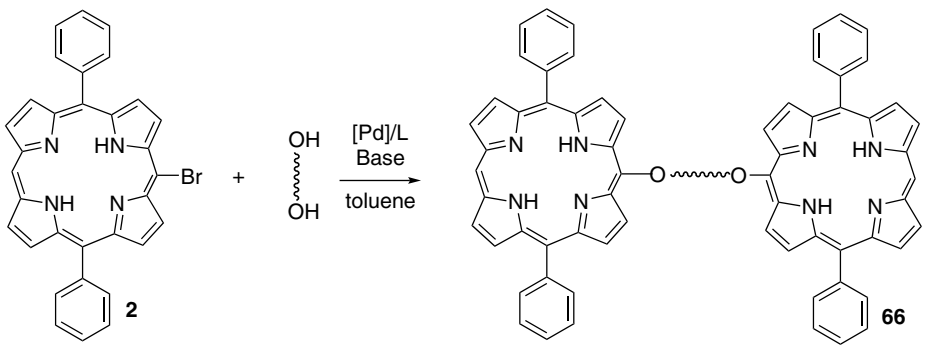
Entry[a]	Ar group of **2**	*ROH	Yield (%)[b]
1			45
2			82
3			80
4			79
5			98
6			35

[a]Reaction were performed in tolune at 100°C for 17–40 h in the presence of $Pd_2(dba)_3$ DPEphos, and Cs_2Co_3; [b]Represents isolated yields of >95% purity as determined by ^{1}H-NMR.

α,β-isomers), presumably due to an increased rotation barrier around the C–O bond. Attempts to separate the atropisomers were unsuccessful.

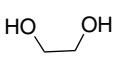
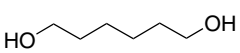
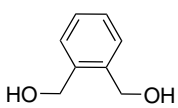
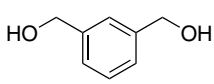
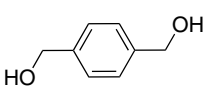
The *meso*-chiral diphenylporphyrins in Table 16 were then chelated with cobalt and used as catalysts for the cyclopropanation of styrenes with ethyldiazoacetate. The desired cyclopropanes were produced in high to excellent yields with moderate *trans*-selectivities, but low enantioselectivities. The authors speculated that the orientation and flexibility of the chiral appendages are likely responsible for the low enantioselectivities observed. For the *R*-(+)-BINOL-coupled Co(II) porphyrin catalyst, the existence of atropisomers created additional problems.

In 2008, Zhang and co-workers extended their methodology to demonstrate that diporphyrins could be selectively synthesized from bromoporphyrin precursors *via* palladium-catalyzed cross-coupling.[61] Using the diol as limiting reagent, a series of homo-diporphyrins containing different types of spacers were formed in high to excellent yields (Scheme 23, Table 17). The methodology developed was shown to be general for a number of diols and porphyrin compounds.

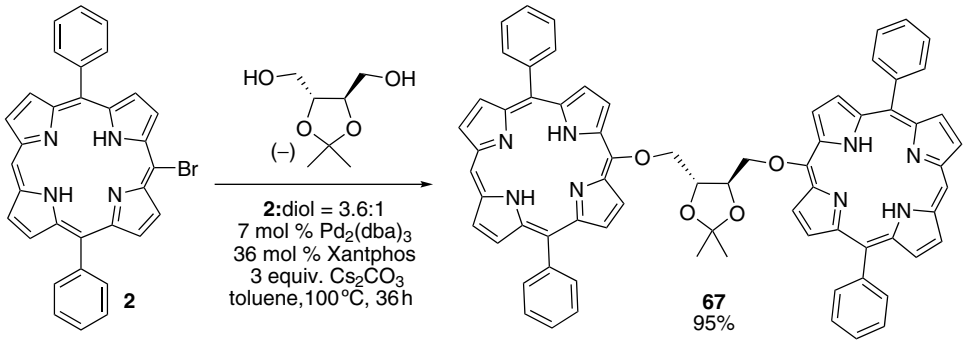


Scheme 23.[161]

Table 17. Synthesis of homo-diporphyrins.[161]

Entry[a]	Diol	Yield (%)
1		53
2		96
3[b]		73
4[b]		70
5[b]		91

[a]General reaction conditions 1 equiv. of 2 in toluene at 100°C under a N_2 atmosphere with 2.5–4.5 equiv. of alcohol, 10–12 mol % $Pd_2(dba)_3$ 30–50 mol % DPEphos **L7** in the presence of 4–6 equiv. of Cs_2CO_3 per Br. Concentration: 0.01 M in toluene for 24–28 h; [b]In toluene at 80°C.



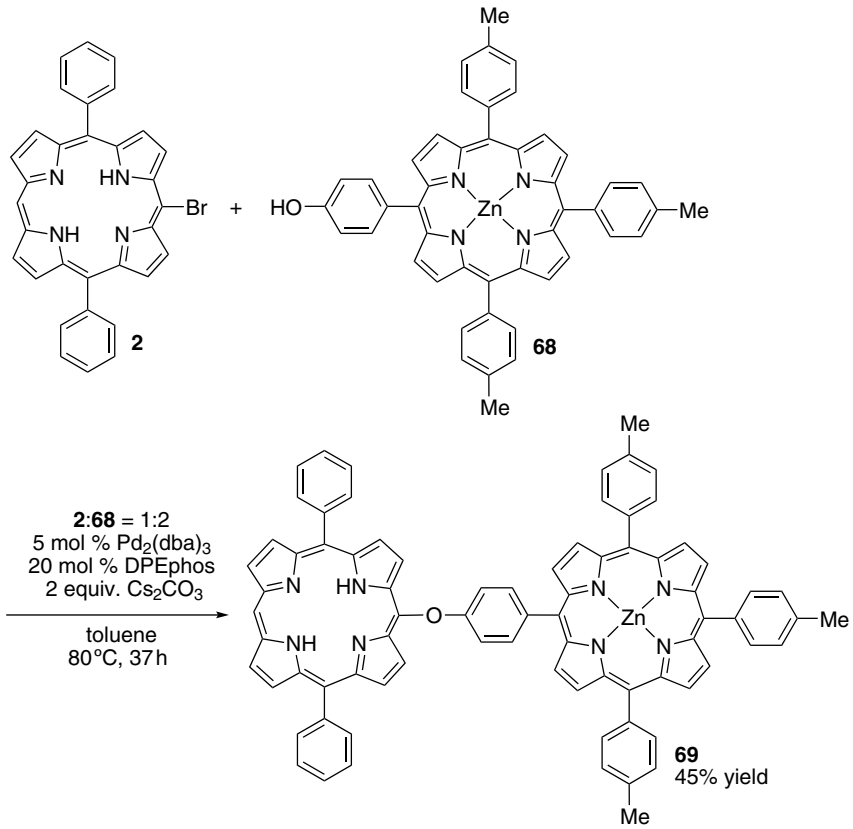
Scheme 24.[161]

Using the same approach, chiral diporphyrins could be readily constructed through the use of optically active diols. As illustrated in Scheme 24, the *meso*-chiral diporphyrin **67** could be effectively prepared in 95% yield *via* palladium-catalyzed C–O cross-coupling between *meso*-bromodiphenylporphyrin **2** and chiral diol (–)-2,3-*O*-isopropylidene-D-threitol.[161]

The same strategy was used to provide access to hetero-diporphyrins and triporphyrins, including free-base and metalloporphyrin hetero-dimers. This methodology was also successfully employed for construction of hetero-diporphyrins with direct C–O–C linkages between two porphyrin units. For example, hetero-dimer **69** was prepared from reaction of hydroxyphenyl porphyrin **68** and *meso*-bromoporphyrin **2** in 45% yield (Scheme 25). To reach the optimized yield, bromoporphyrin **2** was used as the limiting reagent under the reaction conditions.

A stepwise approach was applied to synthesize hetero-diporphyrins with different linkages *via* palladium-catalyzed etheration.[161] For example, the etheration reaction of bromoporphyrin **2** with excess 1,6-hexanediol could be catalyzed to selectively form the mono-coupled product **70** in 64% yield (Scheme 26). Porphyrin **70**, which bears a pendant hydroxyl group, was then effectively coupled with bromoporphyrin **71**, forming hetero-diporphyrin **72** in 72% yield (Scheme 26). This stepwise approach also provided a straightforward route for the synthesis of homo- and hetero-triporphyrins with free-base or metalloporphyrin units. For example, coupling of dibromoporphyrin **74** with excess amount of hydroxyporphyrin **73** could furnish the hetero-triporphyrin **75** in 52% yield (Scheme 27).

Palladium-catalyzed hydroxylation of bromoporphyrin was reported with nickel porphyrin [Ni(**31**)] by Arnold and co-workers in 2006 (Scheme 28).[99] It was concluded that hydrated cesium carbonate was the potential nucleophile responsible for the hydroxylation, despite the fact that the experiments were conducted with base that was rigorously dried. The resulting hydroxyporphyrin complex



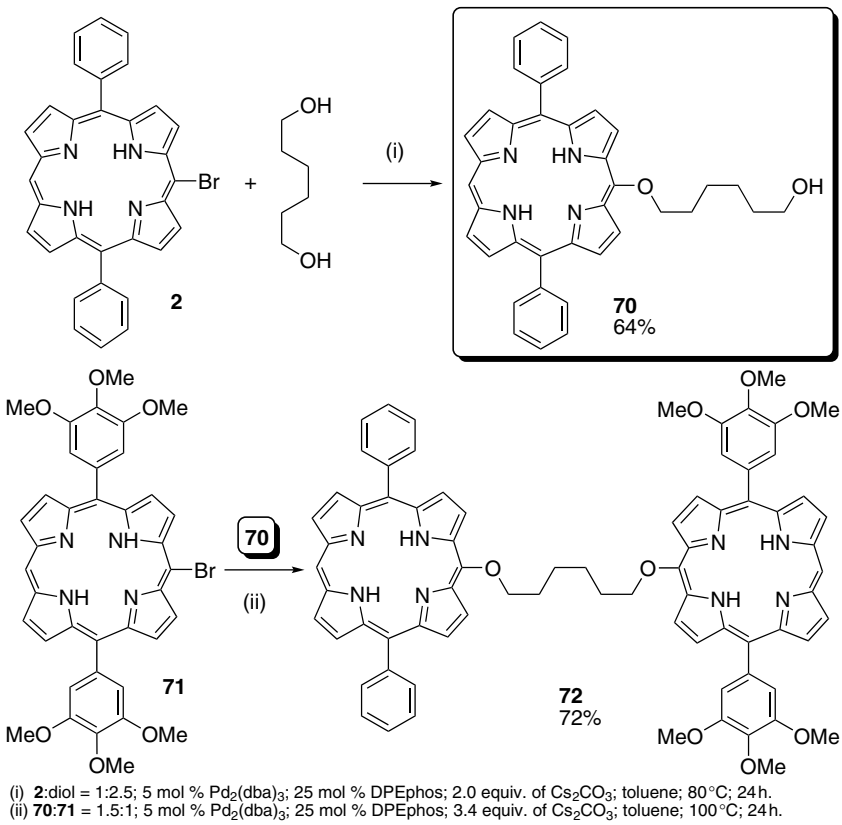
Scheme 25.[161]

[Ni(**65**)] was shown to be readily oxidized and deprotonated to give oxygen-centered radicals. However, [Ni(**65**)] could be "trapped" as the corresponding acetate upon treatment with acetic anhydride and pyridine.

B. β-Etheration

In 2007, Zhang and co-workers reported the efficient synthesis of a variety of β-substituted porphyrins by reacting β-BrTPP **22a** with alkyl and aryl alcohols (Scheme 29).[91] Palladium sources $Pd(OAc)_2$ and $Pd_2(dba)_3$ were screened in combination with the bidentate phosphine ligands DPEphos **L7**, Xantphos **L8**, and BINAP **L9** as potential catalysts.

The C–O couplings of alkyl and aryl alcohols with **22a**, could be successfully catalyzed using $Pd_2(dba)_3$ and DPEphos **L7**, forming the desired β-alkoxy/aryloxy-substituted porphyrins in good yields (Table 18). Both aromatic and



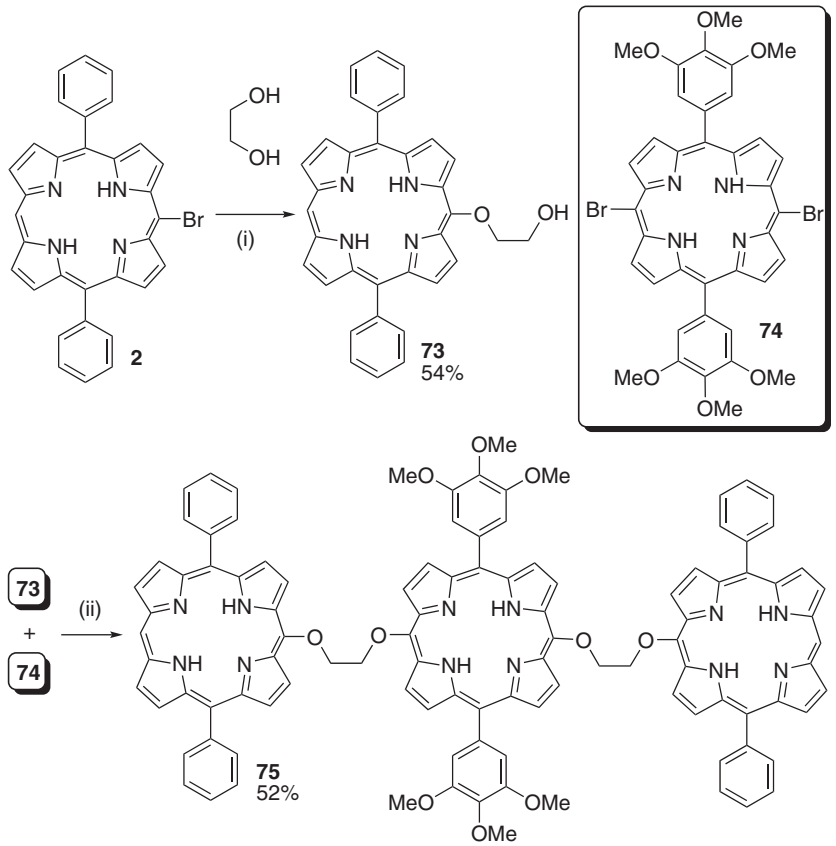
Scheme 26.[161]

aliphatic alcohols were shown to be suitable substrates for the coupling although aliphatic alcohols required additional reaction time (Table 18).

V. Palladium-Catalyzed C–S and C–Se Coupling

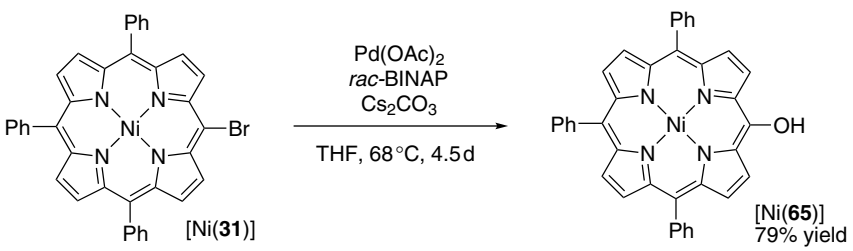
A. *meso*-Sulfanylation and Selenation

In their 2004 paper, Zhang and co-workers extended their synthetic strategy to palladium-catalyzed C–S bond formation[162–167] for porphyrin synthesis. They reported a general method for the synthesis of *meso*-arylsulfanylporphyrins from reaction of bromoporphyrins with thiols using the combination of $Pd_2(dba)_3$ and DPEphos **L7** as catalyst (Scheme 30 and Table 19).[168] For example, 2-naphthalenethiol, as well as 2-methyl-, 4-chloro-, and 4-methoxythiophenol were shown to be suitable coupling partners with *meso*-bromoporphyrin **2** and its zinc complex (entries 1–10, Table 19). Although a low yield was obtained with benzyl thiol (entry 11, Table 19),



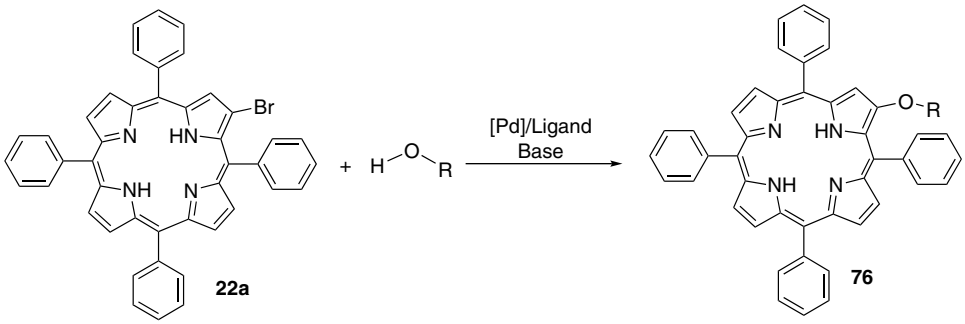
(i) **2**:diol = 1:10; 5 mol % $Pd_2(dba)_3$; 25 mol % DPEphos; 4 equiv. of Cs_2CO_3; toluene; 100°C; 24h.
(ii) **73**:**74** = 2:1; 10 mol % $Pd_2(dba)_3$; 40 mol % DPEphos; 4 equiv. of Cs_2CO_3; toluene; 80°C; 42h.

Scheme 27.[161]



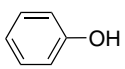
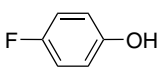
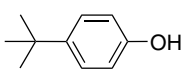
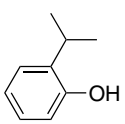
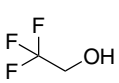
Scheme 28.[99]

alkylsulfanyl-substituted porphyrins were produced in moderate to high yields from the reactions with corresponding acyclic primary and cyclic secondary aliphatic thiols (entries 12–17, Table 19). When *N*-(4-mercaptophenyl)-acetamide was used, the catalytic reaction proceeded selectively with the thiol group



Scheme 29.[91]

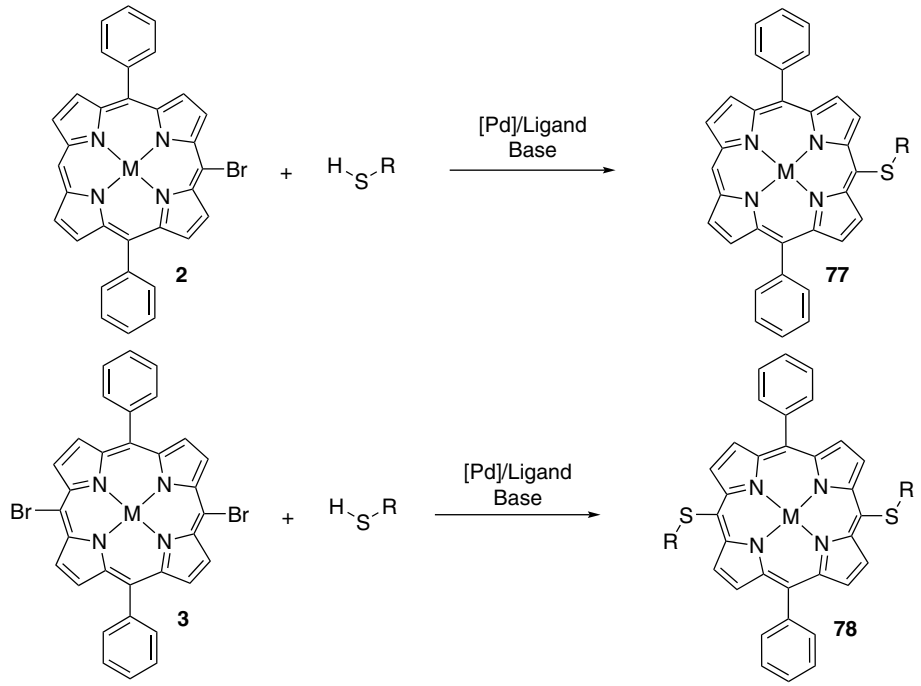
Table 18. Synthesis of β-aryloxy/alkoxy-tetraphenylporphyrins *via* palladium-catalyzed C–O bond formations of β-BrTPP with alcohols.[91]

Entry[a]	Alcohol	Br-por	Time (h)	Yield (%)
1		**22a**	24	66
2[b]		**22a**	26	65
3		**22a**	24	77
4		**22a**	26	79
5		**22a**	40	71

[a]Reactions were carried out at 100°C under N_2 in toluene with 1 equiv. of **22a**, 4.0 equiv. of alcohol, 10 mol % $Pd_2(dba)_3$, and 20 mol % DPEphos in the presence of 2 equiv. of Cs_2CO_3. Concentration: 0.01 M **22a** in toluene; [b]4 equiv. of Cs_2CO_3.

without affecting the amide functionality (entries 18–19, Table 19). In addition, heterocyclic thiols such as benzothiazole-2-thiol were converted to the desired *meso*-monosulfanyl-substituted porphyrin in high yield (entries 20–21, Table 19).

In the same report, it was noted that when an excess amount of propane-1,3-dithiol was used, the coupling reaction could be controlled to take place with only one of the thiol groups, affording a porphyrin with an appended free thiol functionality



Scheme 30.[168]

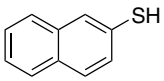
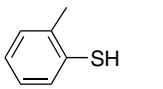
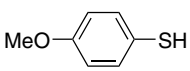
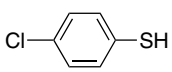
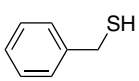
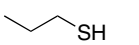
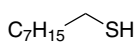
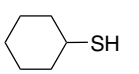
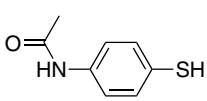
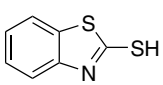
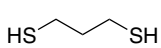
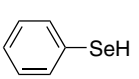
in good yield (entry 22, Table 19). Considering the results obtained from the synthesis of *meso*-diporphyrins *via* C–O bond formation,[161] this novel functional thioporphyrin might be useful for the construction of unsymmetric diporphyrins or triporphyrins upon further reaction with brominated porphyrins.[169–170] In view of the commercial availability of dithiols with varied chain lengths, this methodology should provide convenient access to thiol-derivatized porphyrins, which have found interesting applications for sensors, optics, and information storage.[171–179]

Under similar conditions, a *meso*-seleno-substituted porphyrin was also prepared using a selenol *via* palladium-mediated C–Se bond formation (entry 23, Table 19).[168] This represents the first synthesis of a *meso*-seleno-substituted porphyrin.

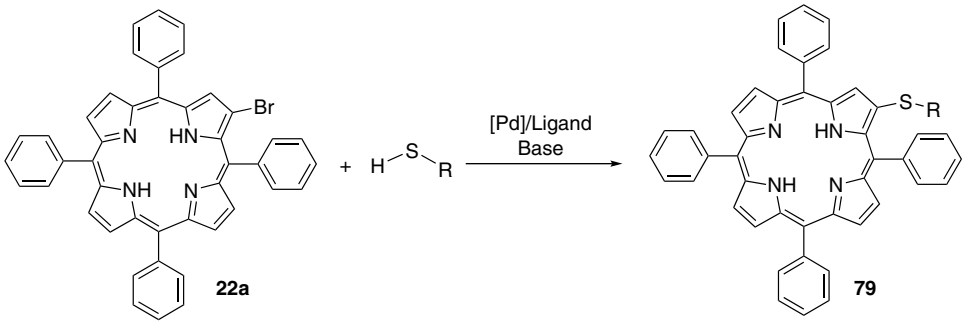
B. β-Sulfanylation

In 2007, Zhang and co-workers reported the efficient synthesis of a variety of β-mercaptoporphyrins from reactions of β-BrTPP **22a**, with alkyl and aryl thiols *via* palladium-catalyzed sulfanylation (Scheme 31).[91] Palladium sources $Pd(OAc)_2$ and $Pd_2(dba)_3$ were screened in combination with bidentate phosphine ligands

Table 19. Synthesis of *meso*-mercaptotetraphenylporphyrins *via* palladium-catalyzed C–S bond formations of *meso*-bromotetraphenylporphyrin with thiols.[168]

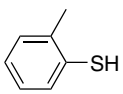
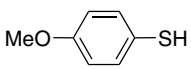
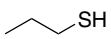
Entry[a]	Thiols	Br-por	Ligand	Temp (°C)	Time (h)	Yield (5)[b]
1		**2**	**L7**	100	20	64
2		**2**	**L7**	100	20	68
3		[Zn(**2**)]	**L7**	100	24	79
4[c]		**3**	**L7**	100	27	71
5		**2**	**L5**	100	21	94
6		[Zn(**2**)]	**L7**	100	24	75
7[c]		**3**	**L7**	100	27	87
8		**2**	**L7**	100	20	71
9		[Zn(**2**)]	**L9**	100	24	75
10		**3**	**L7**	100	25	72
11		**2**	**L7**	80	24	38
12		[Zn(**2**)]	**L9**	100	24	77
13		**3**	**L7**	100	24	75
14		**2**	**L7**	80	48	52
15[d]		**2**	**L9**	100	23	66
16		[Zn(**2**)]	**L9**	100	24	81
17		**3**	**L7**	100	24	74
18[d,e]		**2**	**L8**	100	24	61
19[f]		[Zn(**2**)]	**L9**	100	32	64
20		**2**	**L7**	100	20	77
21		[Zn(**2**)]	**L7**	100	32	54
22[g]		**2**	**L5**	100	25	70
23		**2**	**L7**	60	12	49

[a]Reaction conditions Br-por **2**: reactions were carried out in toluene under N_2 with 1 equiv. of Br-por, 3 equiv. of thiol, 5 mol % $Pd_2(dba)_3$ and 20 mol % of ligand in the presence of 4 equiv. of Cs_2CO_3. Reaction conditions Br-por [Zn(**2**)]: same as **2** except for 4 equiv. of thiol and used 2 equiv. of Cs_2CO_3. Reaction conditions Br-por **3**: same as **2** except for 8 equiv. of thiol and 10 mol % $Pd_2(dba)_3$. Concentration: 0.01 M Br-por in toluene; [b]Isolated yields; [c]40 mol % of ligand; [d]4.0 equiv. of thiol and 2.0 equiv. Cs_2CO_3; [e]5 mol % $Pd(OAc)_2$; [f]10 mol % of ligand; [g]8.0 equiv. of thiol and 2.5 mol % of $Pd(dba)_3$.



Scheme 31.[91]

Table 20. Synthesis of β-mercaptotetraphenylporphyrins *via* palladium-catalyzed C–S bond formations of β-BrTPP with thiols.[91]

Entry[a]	Thiol	Br-por	Time (h)	Yield (%)
1[b]		**22a**	40	35
2		**22a**	28	24
3		**22a**	28	32

[a]Reactions were carried out at 100°C under N_2 in toluene with 1 equiv. of **22a**; 4 equiv. of thiol, 10 mol % $Pd_2(dba)_3$; and 20 mol % BINAP in the presence of 2 equiv. of Cs_2CO_3. Concentration: 0.01 M **22a** in toluene; [b]4 equiv. of Cs_2CO_3.

DPEphos **L7**, Xantphos **L8**, and BINAP **L9** for these reactions. The coupling of alkyl and aryl thiols to form β-arylsulfanyl- and alkylsulfanyl-substituted porphyrins proceeded smoothly when $Pd_2(dba)_3$ and **L9** were used. To explore the scope and limitations of this catalytic system, different thiol substrates, including sterically hindered aryl thiol, electron-rich aryl thiol, and short-chain aliphatic thiol, were selected for the C–S coupling reaction (Table 20). While all the three kinds of thiols could be coupled with **22a** to form the corresponding products in low to moderate yields, the sterically hindered aromatic thiol required longer reaction time.

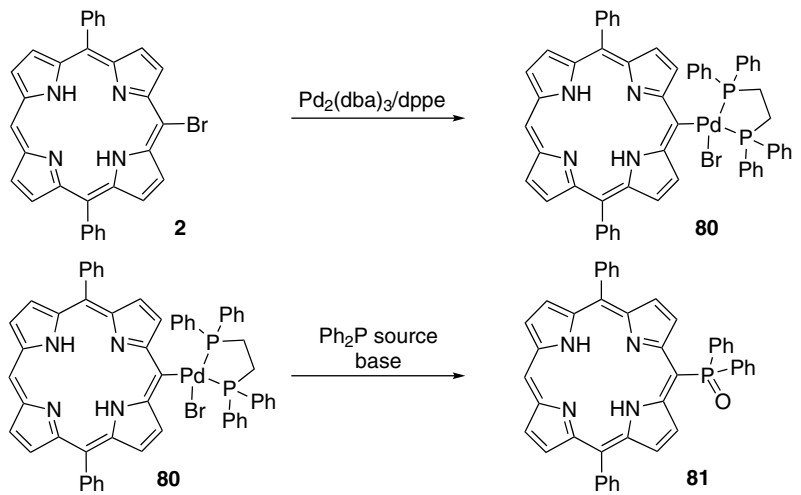
VI. Palladium-Catalyzed C–P Coupling

Hirao and co-workers reported the first palladium-catalyzed C–P bond formation in 1980.[180] Since then, this methodology has been expanded to generate various

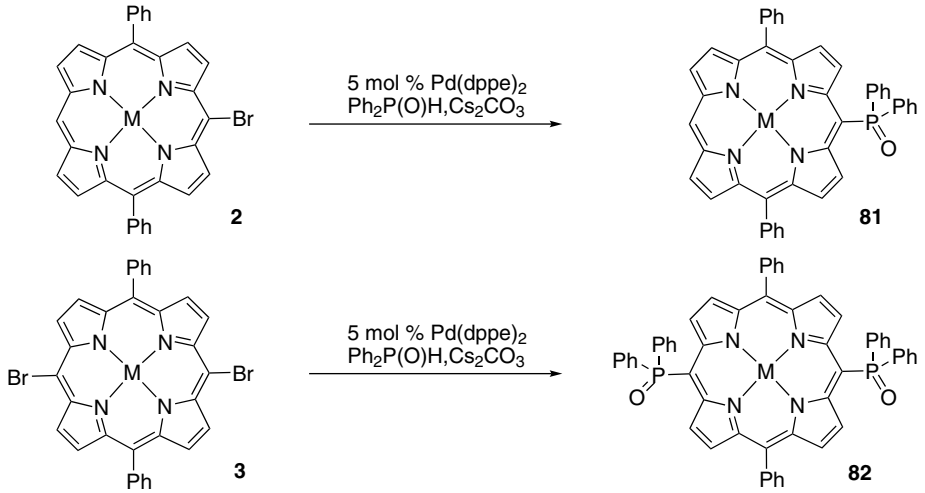
phosphonates, phosphites, phosphine oxides, and phosphines[65,181] and has been applied to the functionalization of porphyrins.

A. *meso*-Phosphoration

In 2006, Arnold and co-workers turned their attention from isolating organopalladium porphyrins with chelating diphosphine ligands, as discussed in Sec. II.B, to palladium-catalyzed C–P cross-coupling of bromoporphyrin synthons to form porphyrinylphosphine oxides.[76] In their initial report, Arnold and co-workers were only able to obtain the phosphine oxide in low yield using conditions developed by Stille[182] due to the formation of the reduced, dehalogenated porphyrin as the major product. In an effort to circumvent dehalogenation, Arnold and co-workers were able to demonstrate C–P bond formation from the direct use of *meso*-η^1-palladioporphyrin **80** as starting material (Scheme 32).[76] It was speculated that the dppe-palladioporphyrin moiety served to orient the bromide into a *cis*-conformation with the porphyrin unit where the desired C–P bond formation *via* reductive elimination is facilitated. While the initial experiment used a stoichiometric amount of palladioporphyrin **80** to react with diphenylphosphinic acid (DPA), subsequent reactions under catalytic conditions were shown to give similar results (Scheme 33).[76] For example, the C–P coupling reactions of monobromoporphyrin **2** and dibromoporphyrin **3** with DPA could be catalyzed with 5 mol % $Pd(dppe)_2$, leading to the *meso*-porphyrinylphosphine oxides in high yields (Table 21). The authors also reported a straightforward



Scheme 32.[76]



Scheme 33.[76]

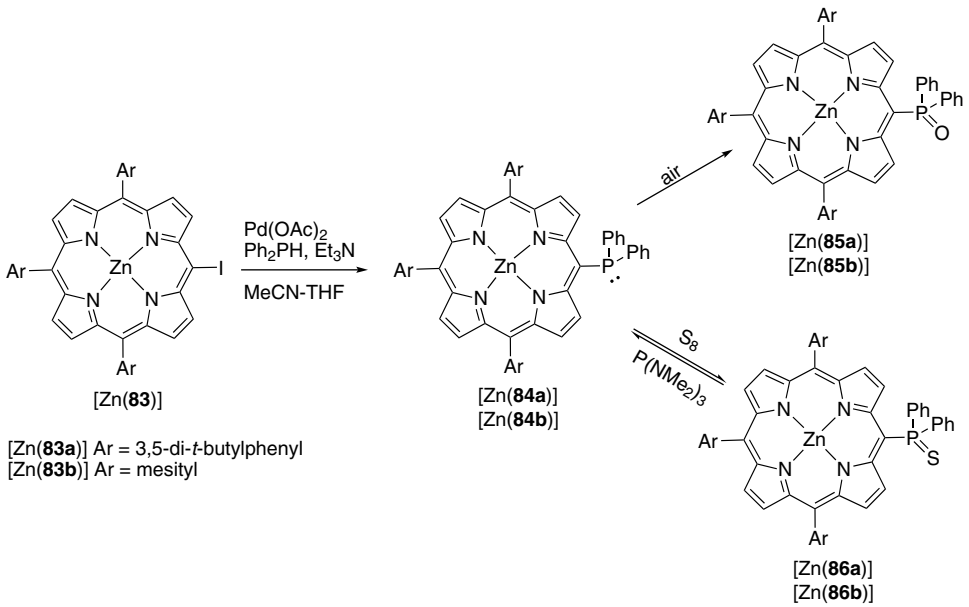
Table 21. Synthesis of *meso*-porphyrinylphosphine oxides *via* palladium-catalyzed C–P bond formation.[76]

Entry[a]	Br-por	Ratio Br-por:DPA	Time (h)	Yield (%)
1[a]	**2**	1:1:1	24	>95
2[b]	**3**	1:2:2	40	>95
3[a]	[Ni(**2**)]	1:1:1	46	>95
4[b]	[Ni(**3**)]	1:2:1	24	>95
5[a]	[Zn(**2**)]	1:1:4	24	61[d]
6[c]	[Ni(**3**)]	1:2:2	24	80[e]

[a]Reactions were carried out at 84°C under N_2 in toluene with 1.1 equiv. of $Ph_2P(O)H$; 5 mol % $Pd(dppe)_2$ in the presence of 1 equiv. of Cs_2CO_3. Concentration: 0.01 M Br-por in toluene; [b]Same as (a), but with 2.2. equiv. of $Ph_2P(O)H$; 10 mol % $Pd(dppe)_2$ in the presence of 2 equiv. of Cs_2CO_3. Concentration: 0.005 M Br-por in toluene; [c]Same as (a), but with 2.2 equiv. of Cs_2CO_3; [d]Yield by ^{1}H-NMR higher, purification problematic; [e]^{1}H-NMR of reaction mixture shows only one porphyrin, purification problematic.

protocol for the high-yielding isolation of the porphyrinylphosphine oxides as either free-bases or nickel complexes (entries 1–4, Table 21). It was noted, however, that the zinc complexes proved difficult to isolate as they were insoluble in most organic solvents.

Subsequently, Imahori and co-workers explored the synthesis of *meso*-phosphanylporphyrins.[183] The isolation of the porphyrinylphosphines were complicated by the autooxidation of the phosphine units.[184–186] However, a strategy



[Zn(**83**)]

[Zn(**83a**)] Ar = 3,5-di-*t*-butylphenyl
[Zn(**83b**)] Ar = mesityl

[Zn(**84a**)]
[Zn(**84b**)]

[Zn(**85a**)]
[Zn(**85b**)]

[Zn(**86a**)]
[Zn(**86b**)]

Scheme 34.[183]

based on the use of phosphine oxides and phosphine sulfides was shown to be an effective entry point to the isolation of the corresponding phosphines.

Palladium-catalyzed C–P cross-coupling reactions of *meso*-iodoporphyrins [Zn(**83a**)] and [Zn(**83b**)] (Scheme 34) with diphenylphosphane produced the corresponding *meso*-phosphanylporphyrins [Zn(**84a**)] and [Zn(**84b**)],[183] which were shown to be rapidly oxidized to the corresponding *meso*-porphyrinylphosphine oxides [Zn(**85a**)] and [Zn(**85b**)]. To circumvent the difficult isolation of the air-sensitive [Zn(**84a**)] and [Zn(**84b**)], elemental sulfur was added to convert them to the corresponding air-stable *meso*-thiophosphorylporphyrins [Zn(**86a**)] and [Zn(**86b**)] in 87–92% yields. After isolation, [Zn(**86a**)] and [Zn(**86b**)] were cleanly converted back to [Zn(**84a**)] and [Zn(**84b**)] in 90–95% yields after refluxed with $P(NMe_2)_3$ in toluene.

It was further shown by Imahori and co-workers that treatment of [Zn(**84a**)] and [Zn(**84b**)] with certain palladium (or platinum) salts could result in the formation of metal-linked co-planar porphyrin dimers **87** and **88**, which contain interesting phosphanemetallacycle linkages (Figure 9).[183] By altering the ratio of *meso*-phosphanylporphyrin and palladium salt, it was demonstrated that each of the two different dimers could be formed preferentially through regioselective β-C–H activation by the Pd(II) salt. These peripherally fused phosphanemetallacycles that have p_π–d_π orbital interactions through the peripheral β-carbon–metal

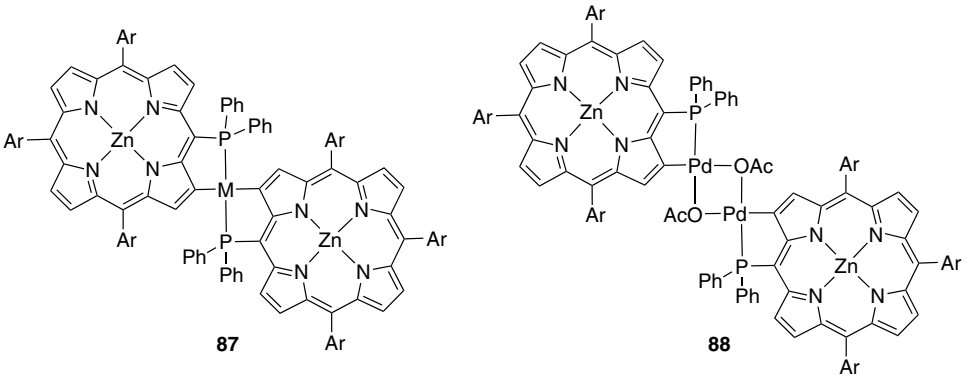


Figure 9. Structures of β-η^1-palladioporphyrins.[183]

bond could potentially be used to tune the optical and electrochemical properties of these co-planar porphyrin systems.

VII. Conclusions

Over the last 20 years, palladium-catalyzed carbon–heteroatom cross-coupling reactions have developed into practical, reliable synthetic methods for the functionalization of porphyrins. The scope of catalytic reactions has evolved significantly to include borylation, amination, amidation, etheration, sulfanylation, selenation, and phosphoration of halogenated porphyrins and metalloporphyrins.

Early efforts using Suzuki–Miyaura cross-coupling reactions for the preparation of functionalized porphyrins led to the production of borylated porphyrins, which could be subsequently employed as coupling partners for further Suzuki–Miyaura coupling reactions to form C–C bonds.

The majority of the efforts in applying palladium-catalyzed carbon–heteroatom bond formation reaction for porphyrin synthesis have been focused on amination and amidation reactions. Developments in palladium-catalyzed C–N cross-couplings have led to the successful synthesis of a diverse array of amino- and amido-substituted functional porphyrins *via* mono, double, triple, and even quadruple amination/amidation reactions. The practicality and utility of these palladium-catalyzed reactions are highlighted by the stepwise formations of more complex porphyrin systems, such as dimers, trimers, and even higher arrays.

Palladium-catalyzed etheration has proven to be a powerful method for the one-pot production of aryloxy- and alkoxy-substituted porphyrins, which were previously accessed only through often difficult and low-yielding multi-step preparations. It has been demonstrated that a broad range of alcohol substrates can

be successfully coupled with a variety of halogenated porphyrins to produce the desired porphyrins in moderate to high yields.

The reliability and diversity of palladium-catalyzed carbon–heteroatom cross-coupling reactions will continue find practical applications in porphyrin synthesis as highlighted by recent developments in functionalization of porphyrins *via* C–S, C–Se, and C–P bond formations. Further advancements in this field will continue to provide valuable synthetic tools for the construction of a diverse array of porphyrins for applications in catalysis, energy transfer, and medicine, among other areas.

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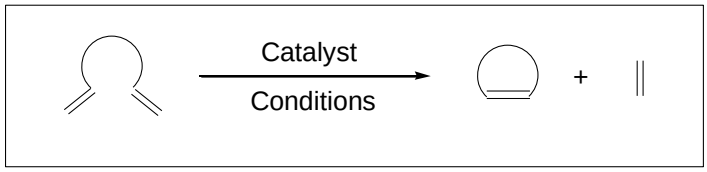
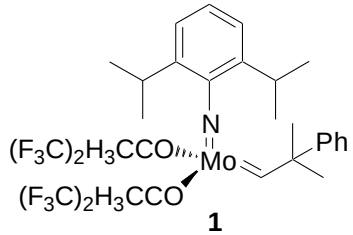
5.3 Ring-closing Metathesis

Nicole L. Snyder and Kevin W. Graepel

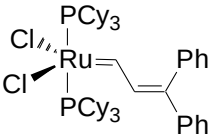
5.3.1 Description



Catalysts:



1



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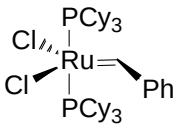

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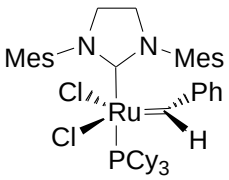

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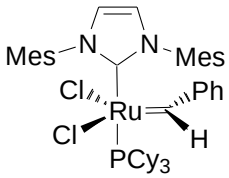

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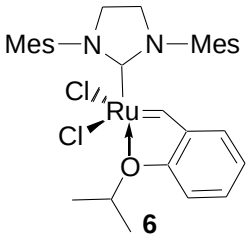


6

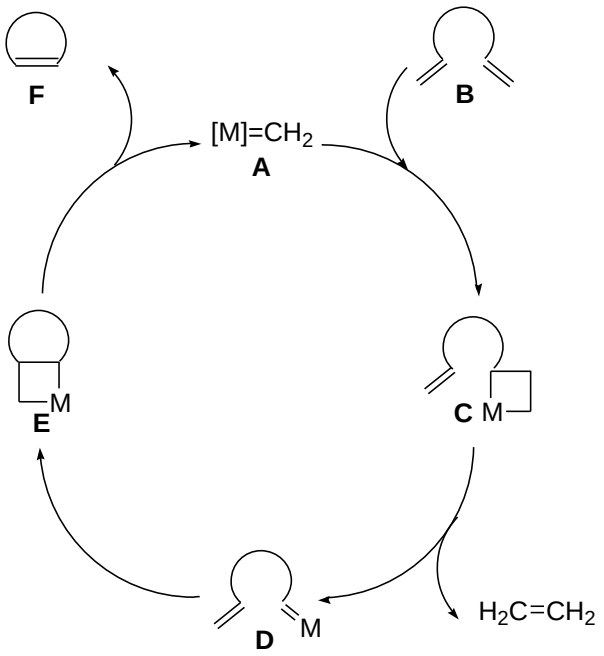
Ring-closing or olefin metathesis is the intramolecular redistribution of alkylidene moieties between two alkenes in the presence of a catalytic amount of a metal carbene to provide for a new product olefin and a byproduct olefin that is usually volatile in nature.

5.3.2 Historical Perspective

Ring-closing metathesis reactions were first used in 1980 by Tsuji[1] and Villemin.[2] However, it was not until the early 1990's that well-defined, single-component catalytic systems were developed independently in the research groups of Richard R. Schrock[3] and Robert H. Grubbs.[4] In 2005, Schrock and Grubbs shared the Nobel Prize in chemistry with Yves Chauvin for their work in this area. Schrock's molybdenum catalyst (**1**), Grubbs first and second generation catalysts (**2**–**5**) and the Grubbs–Hoyveda catalysts (**6**) are the most common catalysts used in ring-closing metathesis today.

Over the past twenty years ring-closing metathesis has become a powerful tool for the synthesis of a wide range of carbocyclic compounds. Carbocycles containing as small as five and as large as eighteen carbon atoms have been prepared. Traditionally, five, six, and seven membered ring carbocycles have been the most common targets for ring-closing metathesis. Recent developments in catalyst design and a better understanding of the substrate requirements and reaction conditions required for ring-closing metathesis have led the increased use of this reaction in the synthesis of large carbocycles and macrocycles.

5.3.3 Mechanism



The mechanism for ring-closing metathesis using ruthenium complexes **3** and **4** has been well established both experimentally[5] and theoretically.[6] Entry into the catalytic cycle begins with the initial dissociation of the phosphine ligand to form the active 14-electron metalacarbene complex (**A**). This complex then coordinates with the an α,ω-diene (**B**) to form a 16-electron system. Migratory insertion, presumably via a [2 + 2] cycloaddition gives rise to the corresponding metallacyclobutane intermediate (**C**), which has been characterized by NMR spectroscopy.[7] Metallacyclobutane (**C**) then undergoes a retro [2 + 2] cycloaddtion to form a new 16-electron carbene intermediate (**D**) with the concomitant release of an alkene, usually ethylene. Intermediate **D** then undergoes an intramolecular [2 + 2] cycloaddition

reaction to form a new metallocyclobutane **E**, which subsequently undergoes a retro [2 + 2] cycloaddition to produce the desired cyclic olefin (**F**), while regenerating the active 14-electron metalacarbene complex **A**. The reaction is entropically driven and there is still some debate as to rate limiting step of the reaction.

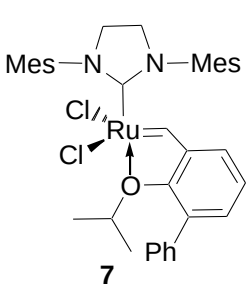
Schrock's catalyst **1**, a molybdenum imido complex, has been shown to be highly active; however its sensitivity to oxygen, moisture, and some polar functional groups has limited the utility of this catalyst in the synthesis of complex and highly functionalized compounds. Grubbs' first and second generation catalysts **2**–**5**, and the Grubbs–Hoyveda catalyst **6**, which consist of ruthenium carbene, are less sensitive to oxygen and moisture. In addition, the functional group tolerance exhibited by **2**–**6** have made them the catalysts of choice in the synthesis of many complex synthetic targets.[8] On the other hand, Schrock's catalyst has been shown to be more effective in forming rings with high steric and electronic demands.

In general, ring closing is fastest for smaller rings (5–7 membered ring) for which enthalpic and entropic factors are favorable.[9] The formation of eight, nine, and ten membered rings are particularly problematic. Conformationally directed ring-closing metathesis using cyclic precursor has been widely used to overcome many of the problems associated with the synthesis of larger rings.[10] Gem–dialkyl[11] and Thorpe–Ingold effects[12] have also been exploited in an effort to produce larger rings via ring-closing metathesis. Research by Hoye and co-workers has also suggested that substrates containing allylic alcohols are activated towards ring-closing metathesis.[13]

The reaction conditions for ring-closing metathesis have also been studied.[14] In general, low concentrations (0.1M–0.1mM) of catalyst favor ring formation, while higher catalyst concentrations favor cross metathesis and polymerization reactions. Ring-closing metathesis reactions are also strongly dependent on the solvent and temperature of the reaction. Experimental evidence has shown that more polar solvents lead to higher initiation rates, as they are able to better stabilize the active 14-electron metalacarbene complex.[15] As a result of these studies, dichloromethane is one of the most frequently used solvents for ring-closing metathesis. A recent study by Adjiman, Taylor and co-workers has shown that cyclohexane and acetic acid may, in fact, be the best solvents for ring-closing metathesis.[16] Benzene and toluene are also commonly employed, as catalyst degradation is often slower in these solvents. Finally, the use of higher temperatures over long reaction periods, has been shown to lead to catalyst degradation.

5.3.4 Variations and Improvements

The most notable improvements for ring-closing metathesis have been in the development of new catalysts.[17] In particular, the "phosphine mimic's" **4**–**6** as well as several variants of these complexes have been shown to be more reactive with electronically deactivated or sterically hindered alkenes that have normally been troublesome in ring-closing metathesis. For example, the Blechert catalyst **7**[18] and Grela catalyst **8**[19] have found application in the synthesis of the substrates that showed little or no reactivity when catalyzed with **1**–**6**. Grubbs–Hoyveda derivative **9** has been employed by Grubbs and co-workers in the synthesis of tetra substituted olefins.[20]



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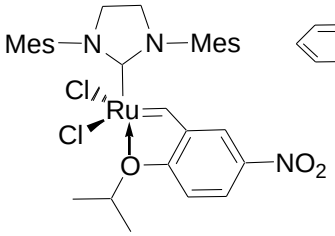

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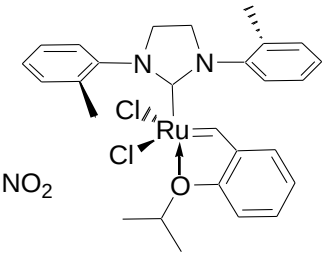
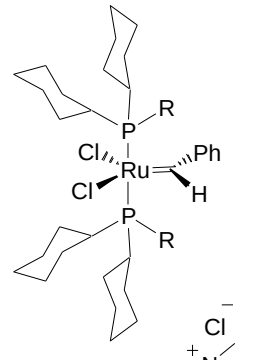


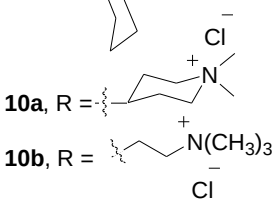
9

In addition to sterics, a major limitation with the ruthenium-based catalysts has been the formation of stable Fischer carbene species with alkenes, such as vinyl ethers, that tend to be electronically deactivated towards metathesis. Previously, most groups were able to circumvent this issue by building sterics into olefins prone to Fischer carbene formation, thus driving the reaction forward by forcing the catalyst to coordinate with the less reactive alkene. The advent of new catalysts, such as the commercially available second generation catalysts **4**–**5** and the Grubbs–Hoyveda catalyst **6**, as well as catalysts **7**–**9** has opened new opportunities in this area. For systems that remain problematic, relay ring-closing metathesis has proved useful in directing catalyst coordination. This strategy, pioneered by Hoye and co-workers[21] has been largely successful for previously unreactive substrates. This technique has also found utility in the ring-closing metathesis of sterically hindered substrates.

The development of several water soluble catalysts, including several derivatives of Grubbs' first generation catalyst (**10a,b**[22]–**11**[23]), second generation catalyst (**12**[24]), and the Grubbs–Hoyveda catalyst (**13a,**[25]**b**,[26]**c**[27]) has expanded the scope of these catalysts to substrates that require more polar reactions conditions. These catalysts have also found wide applications in green chemistry processes[28] and for olefin metathesis in chemical

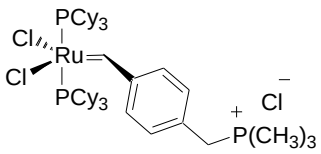
biology.[29] The recent development of ruthenium olefin metathesis catalyst bearing carbohydrate ligands (**14**–**15**) is also of promise in this area.[30]





10a, R =

10b, R =



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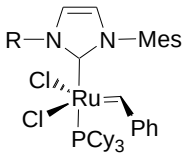
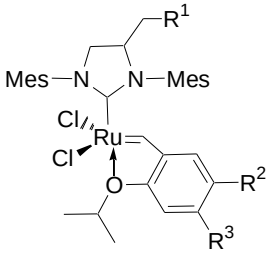


R = $CONH(CH_2)_2OPEG\text{-}CH_3$

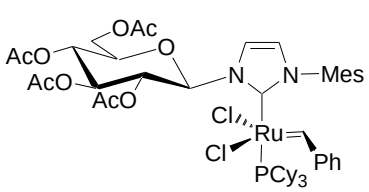
12



13a, $R^1 = CH_2OPEG\text{-}CH_3$
$R^2, R^3 = H$

13b, $R^1 = CH_2NH_3^+Cl^-$
$R^2 = CH_2N(CH_3)_3^+Cl^-$
$R^3 = H$

13c, $R^1, R^2 = H$
$R^3 = NEt_2CH_3^+I^-$



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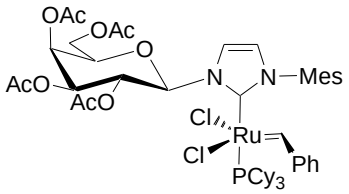


15

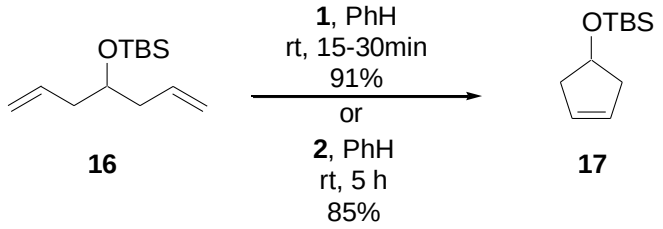
Finally, two important limitations with ruthenium-based catalysis are catalyst-lifetime and the efficient removal (and subsequent recovery) of residual catalyst and decomposition products from the desired reaction products.[31] Currently, only the Grubbs–Hoyveda catalyst **6** can be readily recovered by flash chromatography. Numerous special work-up and purification methods have been developed to solve the later problem.[32] However, recent immobilization of ring-closing metathesis catalysts through *N*-heterocyclic carbenes to a solid support is gaining increasing attention as solution to both of these problems, in most cases providing for greater efficiency and easy recovery.[33] A recent report by Grubbs and co-workers on

a well-defined silica-supported olefin metathesis catalyst for catalysis offers new promise in this area.[34]

5.3.5 Synthetic Utility

5.3.5.1 General Utility

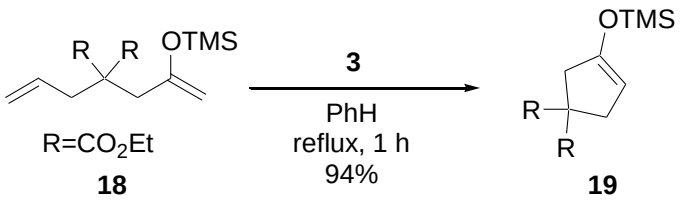
In 1993, Grubbs and co-workers independently reported the first examples of ring-closing metathesis using **1**[35] and **2**[36] to prepare carbocyclic compounds. Reaction of 16 with 2–4 mol% either **1** or **2** in benzene at room temperature furnished the corresponding five membered ring carbocycle in 91% (using **1**) and 85% (using **2**).



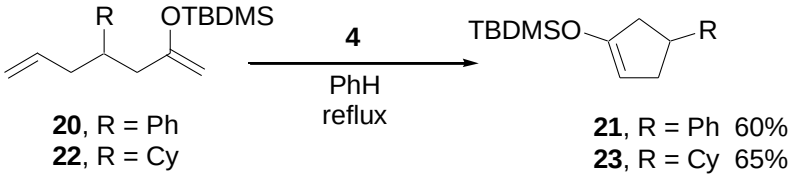
Since their initial report, this methodology has been extended to the formation of six, seven, eight and larger ring carbocyclic systems. The sections below highlight significant application of the ring-closing metathesis strategy in the synthesis of these carbocyclic systems.

5.3.5.2 Five Membered Rings

Shibasaki and co-workers used a ring-closing metathesis approach to prepare a number of five, six and seven membered rings from electron-deficient olefins.[37] Acyclic enol ether **18** was subject to ring-closing metathesis using 7 mol% of **3** in refluxing benzene to provide the corresponding cyclic enol ether **19** in 90% yield, respectively. Deprotection of the silyl ether **19** resulted in the corresponding cyclic ketone, a valuable synthetic intermediate. The authors reported additional examples of the synthesis of five membered ring carbocycles as part of this study.



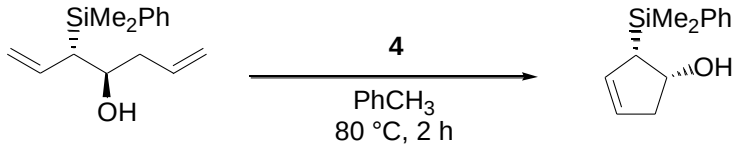
Independently, Aggarawa and co-workers prepared five, six and seven membered carbocyclic methyl and silyl enol ethers bearing phenyl and cyclohexyl substituents.[38] Dienes **20** and **22** required upwards of 20 mol% of **4** in refluxing benzene to achieve modest yields of the desired products **21** and **23**, respectively. The authors attributed the reduced yields to a lack of *gem*-diester substituents, a pattern which has been known to dramatically increase the rates of cyclization.[39]



20, R = Ph
22, R = Cy

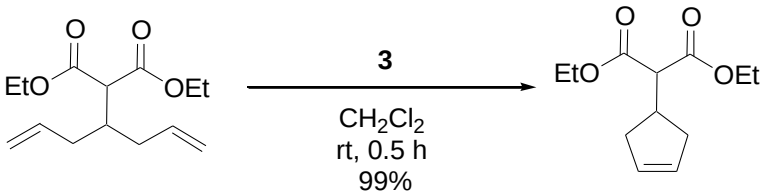
21, R = Ph 60%
23, R = Cy 65%

Roush and co-workers used ring-closing metathesis as a key step in the enantio- and diastereoselective preparation of several cyclic β-hydroxy allylsilanes.[40] Reaction of β-hydroxy allylsilane **24** with 10 mol% of **4** in toluene gave the corresponding carbocycle **25** in 73% yield. The authors also applied this strategy to the formation of six membered ring carbocycles bearing cyclic β-hydroxy allylsilanes.



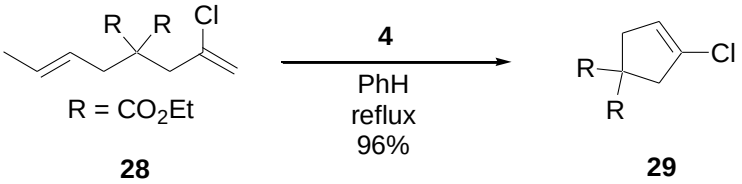
24 **25**

6,6-Bicyclic malonamides are important intermediates in the synthesis of natural products and have recently gained attention as potential donor atoms for ligand-metal complexes. Hutchinson and co-workers employed ring-closing metathesis as a key step in the synthesis of malonamides.[41] Treatment of diethyl malonate diene **26** with a catalytic amount of **3** in dichloromethane gave quantitative conversion to the corresponding carbocycle **27** in under a half an hour.



26 **27**

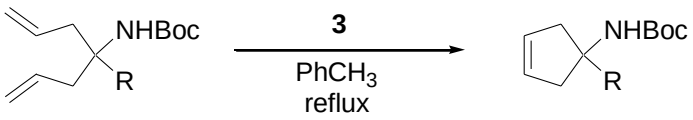
Weinreb and co-workers reported one of the first examples of a ring-closing metathesis strategy employing vinyl chlorides.[42] Vinyl chloride **28**, when reacted in the presence of 10 mol% of **4** in refluxing benzene, gave the corresponding carbocyclic derivative **29** in 96% yield. Reactions with analogous vinyl bromides using similar reaction conditions gave none of the desired carbocylic products. The authors speculated that this was due to the formation of a stable, unreactive Fischer-type carbene.



28 **29**

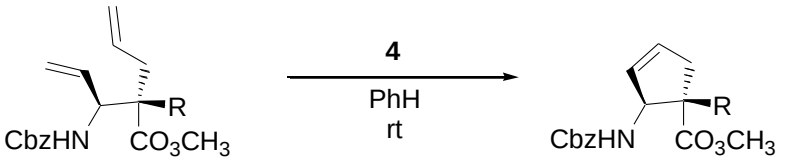
A number of research groups have used ring-closing metathesis to prepare conformationally constrained α- and β-amino acids. The corresponding peptides that incorporate these unusual amino acid residues often exhibit interesting biological properties. Several examples of constrained amino acid residues incorporating five membered ring carbocycles are illustrated below.

Kotha and co-workers used a ring-closing metathesis strategy in their synthesis of α,α-dialkylated amino acids for the preparation of novel conformationally constrained peptide therapeutics.[43] Treatment of diene precursors bearing different amino acid substitutions, when with 10 mol% of **3** in refluxing toluene, provided the corresponding α,α-amino acid derivatives in yields ranging from 49-90%. Undheim and co-workers employed a similar ring-closing metathesis strategy in their synthesis of α,α-dialkylated constrained amino acids.[44] Ple and co-workers also used an olefin metathesis strategy to prepare several constrained examples of α-alkoxy and α-amino esters.[45]



30, R=CONH(L)PheOCH$_3$
31, R=CONH(L)PheOCH$_3$ 75%
32, R=CONH(L)ValOCH$_3$
33, R=CONH(L)ValOCH$_3$ 90%
34, R=CONH(L)Ala(L)LeuOCH$_3$
35, R=CONH(L)Ala(L)LeuOCH$_3$ 50%
36, R=CONH(L)Leu(L)AlaOCH$_3$
37, R=CONH(L)Leu(L)AlaOCH$_3$ 49%
38, R=CONH(D)Val(L)ValOCH$_3$
39, R=CONH(D)Val(L)ValOCH$_3$ 53%
40, R=CONH(D)Val(L)LeuOCH$_3$
41, R=CONH(D)Val(L)LeuOCH$_3$ 75%

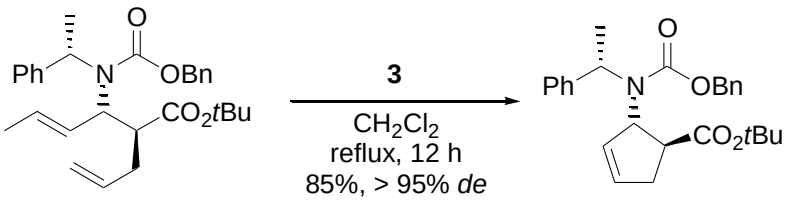
Abell and co-workers employed ring-closing metathesis in the synthesis of a number of five, six, and seven membered ring β-amino acids for incorporation into β-peptide mimetics.[46] β-peptide mimetics have been shown to selectively disrupt bacterial cell membranes over mammalian cell membranes, and as such are key targets for the synthesis of new antibiotics. Treatment of diene **42** or **44** with a 5 mol% of **4** in benzene furnished the corresponding β-amino acid derivatives **43** and **45** in 92 and 93% yield, respectively. Attempts to increase yields by refluxing in toluene led to a reduction in the amount of product produced.



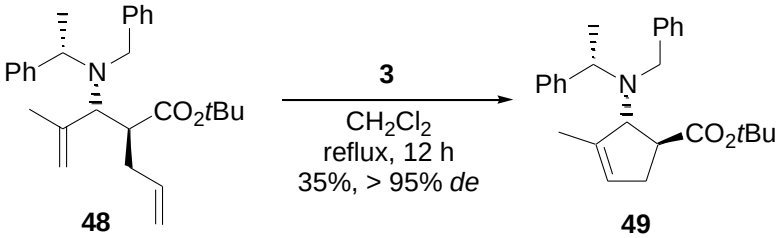
42, R = H
44, R = CH_3

43, R = H 92%
45, R = CH_3 93%

Davies and co-workers also employed a ring-closing metathesis strategy in the preparation of constrained β-amino acid derivatives.[47] However, in their work, they employed a chiral auxiliary to achieve the desired products in a stereoselective fashion. Treatment of diene precursors **46** and **48** with 4 mol% of **3** in refluxing dichloromethane gave the corresponding carbocyclic β-amino acid derivatives **47** and **49** in 85 and 35% yields with greater than 95% diastereoselectivity in both cases. The diminished yield in the case of **49** is presumably due to sterics effects.



46 **47**

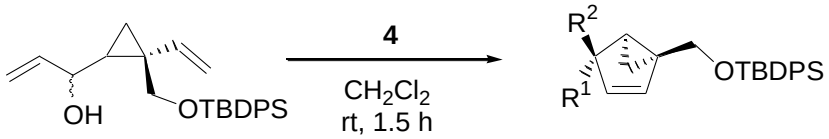


48 **49**

Carbasugars are structurally similar to natural sugars except that a carbon atom replaces the oxygen atom in the ring. A number of five membered ring carbacycles have been synthesized to date, most of these mimicking the carbanucleoside (–)-carbovir. (–)-Carbovir, a reverse transcriptase inhibitor, has been widely used alone or as part of a cocktail in the treatment of AIDS. The advantage of carbaucleosides over other nucleosides (such as AZT) is that they are more resistant to phosphorylation and subsequent degradation.

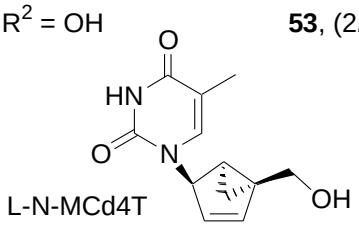
A number of reviews have been published highlighting advancements in the synthesis of these biologically important compounds using ring-closing metathesis as a key strategy.[48] As such, we will only highlight some of the more recent and synthetically challenging syntheses of these analogs here.

In an effort to prepare new anti-HIV compounds with low cytotoxicity, Park and coworker's synthesized L-*N*-MCd4T, a carbocyclic nucleoside containing a fused cyclopropane functionality.[49] Reaction of a diastereomeric mixture of cyclopropyl diene's **50** and **52** in the presence of a catalytic amount of **4** in dichloromethane furnished the corresponding fused carbacycles **51** and **53** in 84% and 85% yield, respectively. Compound **53** was then readily converted to the desired nucleoside analog in three steps.



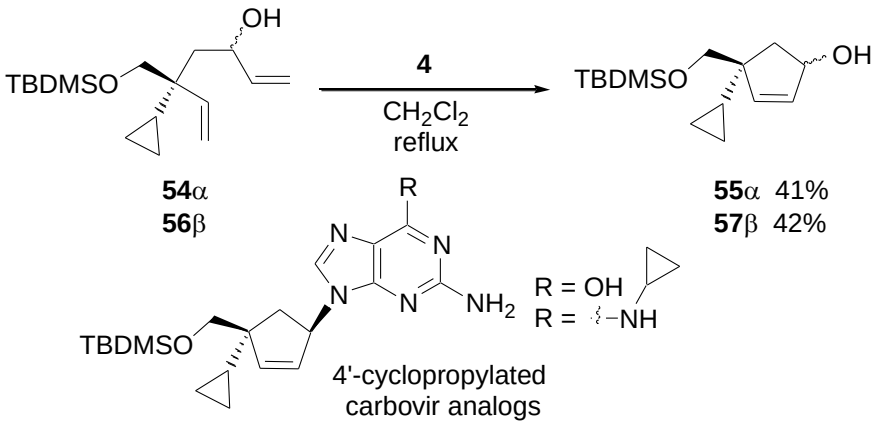
50, (2*S*): R^1 = OH, R^2 = H
52, (2*R*): R^1 = H, R^2 = OH

51, (2*S*): R^1 = OH, R^2 = H 85%
53, (2*R*): R^1 = H, R^2 = OH 84%

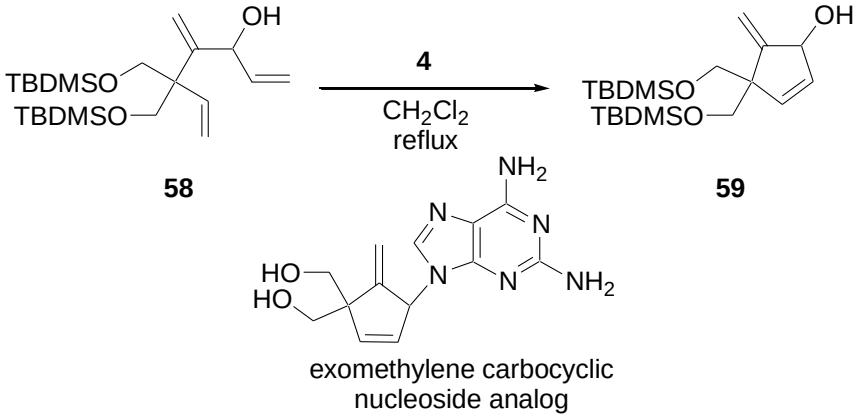


L-N-MCd4T

In a separate study, Liu and co-workers synthesized a nucleoside incorporating a cyclopropyl group at the 4′ position.[50] Reaction of cyclopropyl dienes **54** and **56** with 3 mol% of **4** in refluxing dichloromethane gave a mixture of the corresponding diastereomeric carbasides **55** and **57** in 83% yield overall. Compound **57** was converted to the desired nucleoside analogs in only three additional steps.



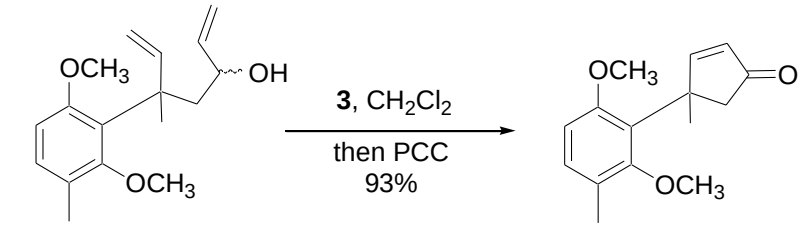
Recently, Li and co-workers reported the synthesis of a 4′ branched exomethylene carbocyclic nucleoside analog as potential mimic of olefinic carbocyclic nucleosides.[51] Treatment of acyclic triene **58** with 10 mol% **4** in refluxing dichloromethane gave the corresponding carbacycle **59** in 74% yield. Surprisingly, isomerisation of the exocyclic double bond, which has been reported with ruthenium-based metathesis catalysts, did not occur under the reaction condition reported.[52] Carbacycle **59** was readily converted to the corresponding exomethylene carbocyclic nucleoside analog.



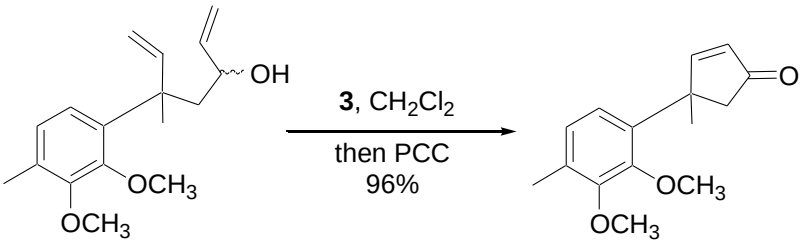
Terpenes are a class of compounds composed of one or more isoprene units. Nearly all organisms produce terpenes, and many of these compounds have interesting biological activities. Many terpenes also exhibit complex architectures that present a considerable challenge for synthetic organic chemists. For these reasons, a number of scientists have set out to synthesize terpenes and terpenoid derivatives, and many recent attempts have incorporated a ring-closing metathesis strategy.

Srikrishna and co-workers used a ring-closing metathesis strategy in the syntheses of HM-3 and HM-4, two aromatic sesquiterpenes with

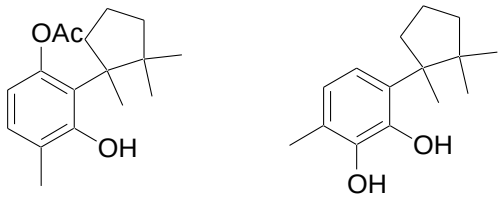
antioxidant and antibiotic activity.[53] Treatment of a diastereomeric mixture of allyl alcohols **60** and **62** with 5 mol% of **3** in dichloromethane, followed by oxidation with PCC, gave the corresponding enones **61** and **63** in 93 and 96% yield, respectively. These compounds were readily converted to the corresponding aromatic sesquiterpenes in just a few additional steps. Srikrishna and co-workers employed a similar strategy for the five membered rings moieties of (±)-12-methoxyherbertenediol dimethyl ether,[54] (±)-laurokamurene B,[55] and (±)-herbertenediol.[56]



60 **61**

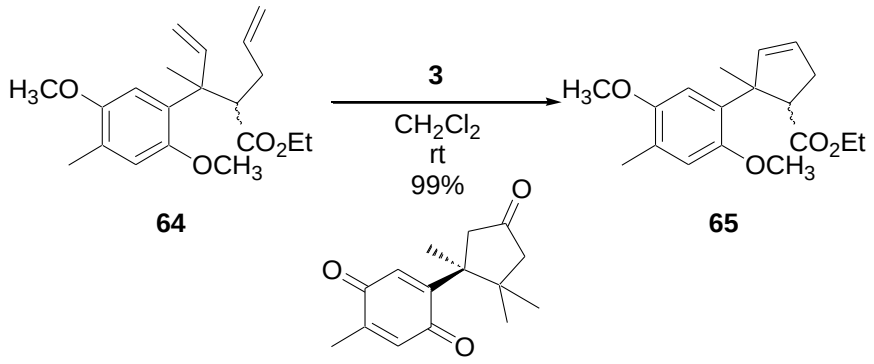


62 **63**



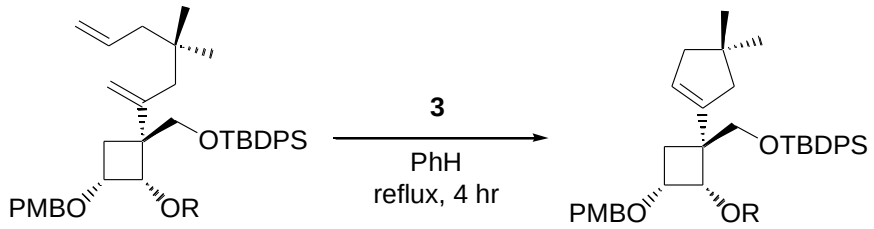
HM-3 HM-4

A ring-closing metathesis strategy was also employed by Srikrishna and workers in the first total synthesis of (±)-lagopodin A.[57] Initially, the authors found the sterically congested 1-aryl-1,2,2-trimethylcyclopentane component especially challenging to construct. However, olefin metathesis of heptadiene **64** in the presence of 5 mol% of **3** in dichloromethane furnished the corresponding cyclopentene ester **65** in quantitative yield. Kulkarni and co-workers applied a similar ring-closing metathesis strategy in their synthesis of (±)-β-cuparenone, a related compound (not shown).[58]



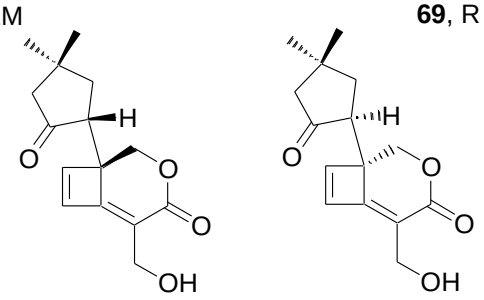
(±)-lagopodin A

Another sesquiterpene, fomannosin, has caused considerable concern in the southeastern United States due to its toxicity, especially towards certain species of pine and select symbiotic bacteria. Paquette and co-workers synthesized both (+)- and (–)-fomannosin in an effort to further investigate its properties.[59] Ring-closing metathesis of cyclobutyl diene precursors **66** and **68** in the presence of 5 mol% of **4** in refluxing benzene furnished the corresponding carbocyclic products **67** and **69** in 77 and 91% yield, respectively.



66, R = MEM
68, R = MEM

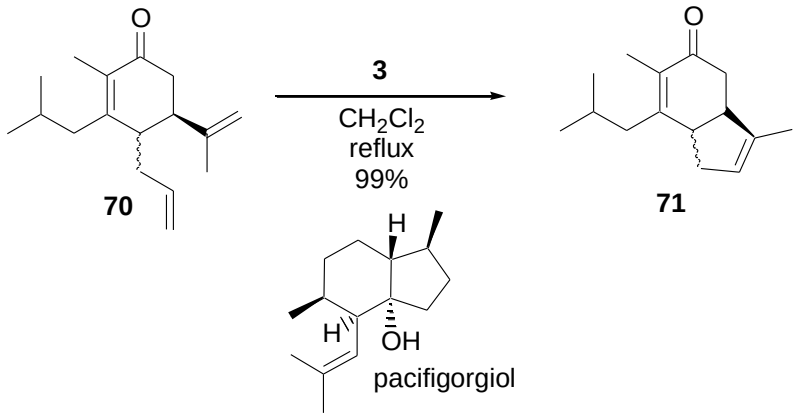
67, R = MEM 77%
69, R = MEM 91%



(+)-fomannosin (–)-fomannosin

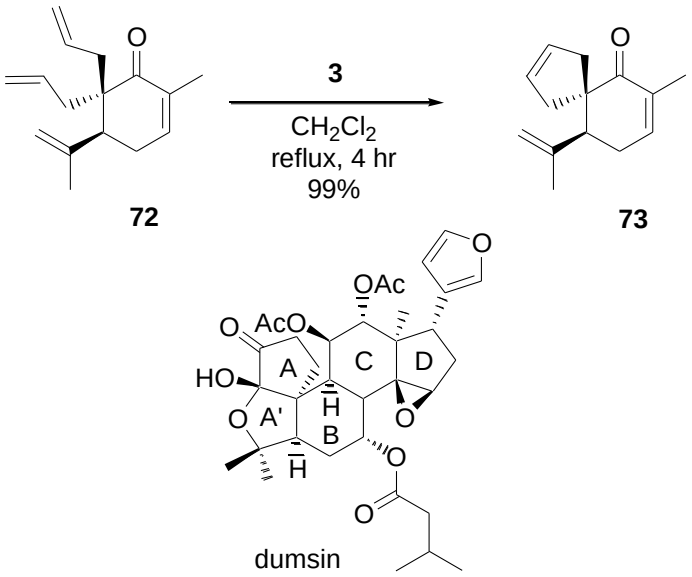
Srikrishna and Dethe employed a ring-closing metathesis strategy in the enantiospecific synthesis of the carbocyclic core of the pacifigorgiane sesquiterpenes. This family of compounds contains a fused 6,5 system.[60]

Treatment of a diastereotopic mixture of **70** with 10 mol% of **3** in refluxing dichloromethane gave the corresponding pacifigorgia-2,7-dien-4-one **71** in quantitative yield. Srikrishna and co-workers later applied this strategy in the synthesis of functionalized bicyclo[4.3.1]decanes for the synthesis of vibsane diterpenoids.[61]

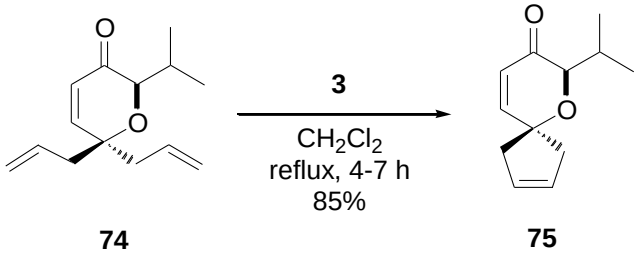


Spirocycles are found in a number of natural products with therapeutic and industrial applications. Until the development of Schrock's and Grubbs' catalysts, the formation of spirocycles was a considerable challenge for synthetic organic chemists. Ring-closing metathesis reactions have made these desirable, rigid compounds easily accessible, and they have been used to synthesize many spirocycles incorporating five-, six-, seven-, and even eight-membered rings.

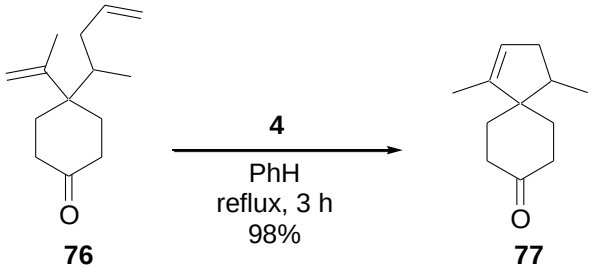
Dumsin is a highly complex tetranortriterpene containing 18 stereogenic centers and spirocyclic system, has recently gained attention due to its potential as a selective and potent pesticide. Srikrishna and co-workers employed a ring-closing metathesis strategy as a key step in the rapid and enantiospecific synthesis of the ABC ring system of dumsin.[62] Treatment of 6,6-bis-allyl carvone **72** with 5 mol% of **3** in dichloromethane provided the corresponding spirocyclic A ring **73** in quantitative yield. Ring-closing metathesis was also employed in the construction of the C ring (not shown) to provide the corresponding tricyclic system.



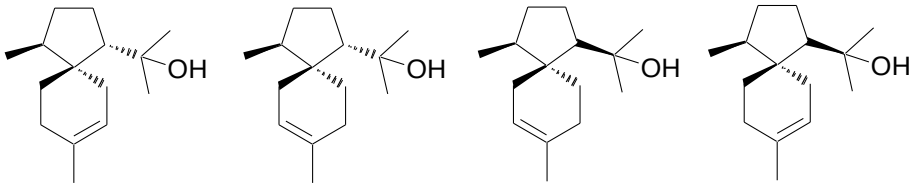
Marquez and Hobson, in an effort to produce spirocycles with functional handles for diversification, synthesized several highly functionalized spirocyclic pyrans using a ring-closing metathesis strategy.[63] Reaction of bis-alkenyl **74** with 5 mol% of **3** in refluxing dichloromethane gave the corresponding spirocycle **75** in 85% yield. Six, seven and eight membered ring spirocycles were also prepared via this method, and the authors noted that yields decreased correspondingly with an increase in the ring size of the spirocycle, presumably due to the lack of conformational constraints.



Srikrishna and co-workers employed a similar strategy in the synthesis of a spirocyclic core, inherent to several acorneols.[64] Treatment of diene **76** with 3 mol% **4** in refluxing benzene gave the corresponding spirocyclic carbocycle **77** in near quantitative yield. Five additional steps were required to produce the corresponding acorneols.

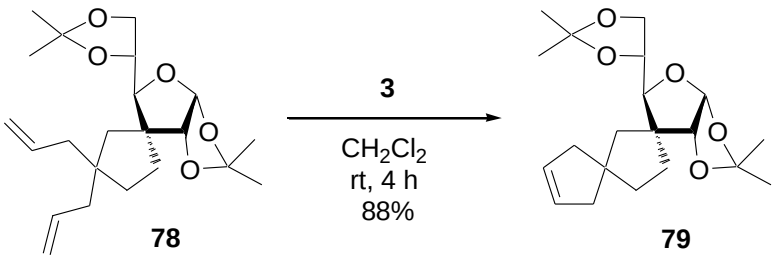


76 **77**

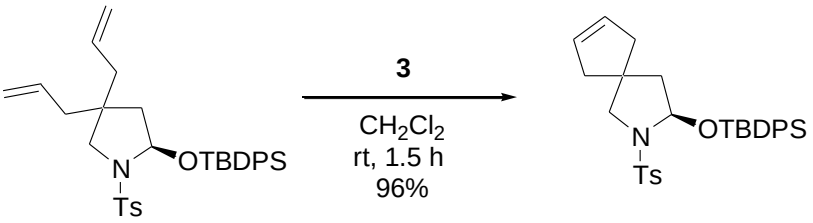


α-acorneol β-acorneol *a-epi*-acorneol β-*epi*-acorneol

Gurjar and co-workers prepared several novel carbohydrate-based spirocycles and a spirocyclic proline derivative for applications in peptide, nucleoside and carbohydrate synthesis.[65] Ring-closing metathesis of carbohydrate diene precursors **78** furnished the corresponding spirocycle **80** in 88% yield using a catalytic amount of **3** in dichloromethane. Reaction of proline derivative **79** under similar conditions gave the corresponding spirocyclic peptide **81** in 96% yield.



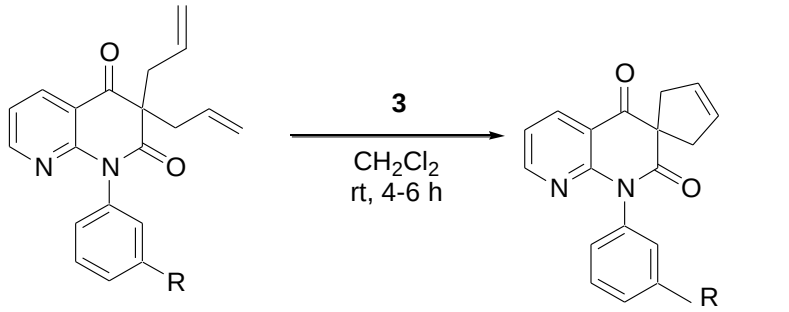
78 **79**



80 **81**

Majumdar and co-workers employed an olefin metathesis strategy in their synthesis of several spironapthyridinone derivatives.[66] Spironapthyridinones are a class of compounds that have shown promise in

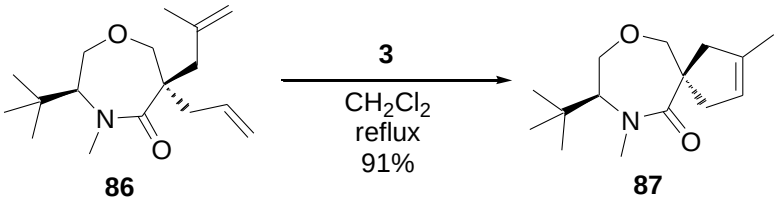
the treatment of autoimmune and other immune disorders. Ring-closing metathesis of **82** or **84** using 10 mol% of **3** in dichloromethane gave the corresponding spirocycles **83** and **85** in 92% and 90%, respectively.



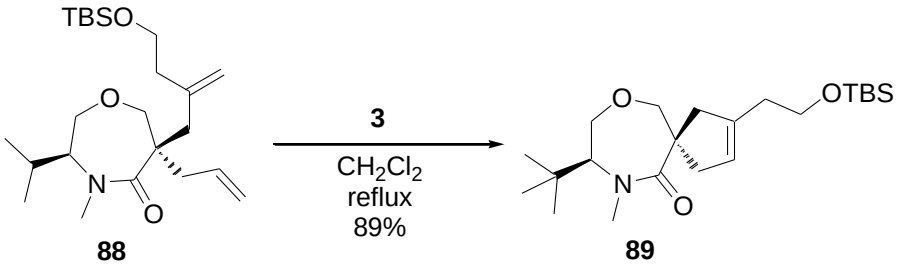
82, R = H
84, R = Cl

83, R = H 92%
85, R = Cl 90%

Hughes and co-workers employed a ring-closing metathesis strategy as a general route to the synthesis of enantiopure five, six and seven membered ring spirocarbocycles using zizaene as a chiral auxiliary.[67] Diene precursors **86** and **88** required 15 mol% catalyst loading of **3** (added in three equal portions every 4-6 hours) in refluxing dichloromethane to achieve the desired five membered ring spirocycles in 91 and 89% yield, respectively. The authors also noted that as the size of the spirocycle increased, the yields obtained from ring-closing metathesis decreased due to conformational constraints.

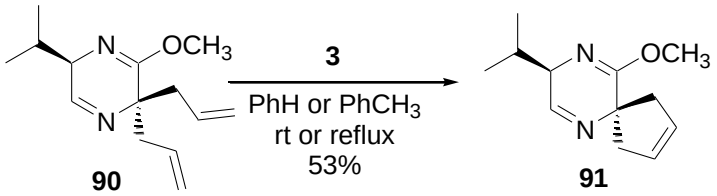


86 **87**



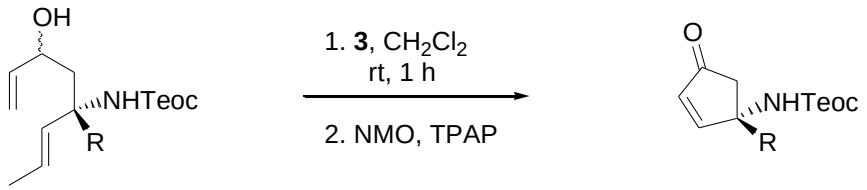
88 **89**

Undheim and co-workers used a similar strategy to prepare and interesting class of five, six, and seven membered ring spirocyclic carbocycles for use as templates in natural product synthesis.[44] Reaction of **90** with 2 mol % catalyst loading of **3** furnished the corresponding spirocycle **91** in 53% yield. The authors were able to produce six and seven membered ring spirocycles of the class, but were unable to access analogous eight membered ring spirocycles.



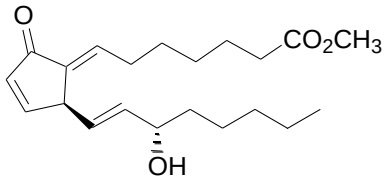
90 **91**

Cyclopentanone prostaglandins have recently garnered attention as potential cancer therapeutics. Unfortunately, native prostaglandins are quickly metabolized, and exhibit limited water solubility. Florent and co-workers, in an effort to prepare metabolically stable and water-soluble prostaglandins, subjected diastereomeric allyl alcohols **92** and **94** to 1 mol% of **3** in the presence of dichloromethane to provide the corresponding allyl alcohols (not shown).[68] These allyl alcohols were subsequently oxidized to the corresponding enones using NMO and TPAP to give **93** and **95** in 89% and 90% yield, respectively. Enones **93** and **95** were selectively functionalized to give the desired prostaglandin derivatives which showed good activity against L1210 leukemia cells.



92, R = $(CH_2)_3OCH_2$-*p*FPh
94, R = $(CH_2)_7CH_3$

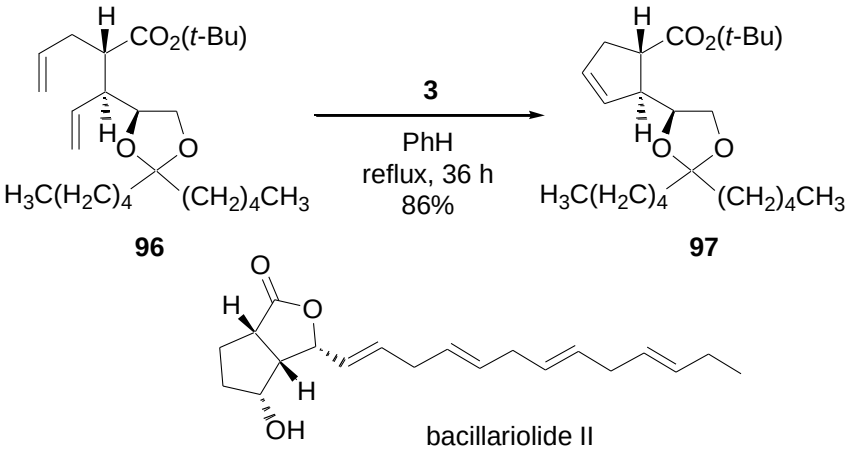
93, R = $(CH_2)_3OCH_2$-*p*FPh 89%
95, R = $(CH_2)_7CH_3$ 90%



Δ^7-PGA_1

Oxylipins are densely functionalized naturally-occurring bicyclic systems with four contiguous stereocenters. Several members of the oxylipin family have shown significant inhibitory activity against phopholipase A_2. Ghosh and co-workers applied an olefin metathesis as a key step in the

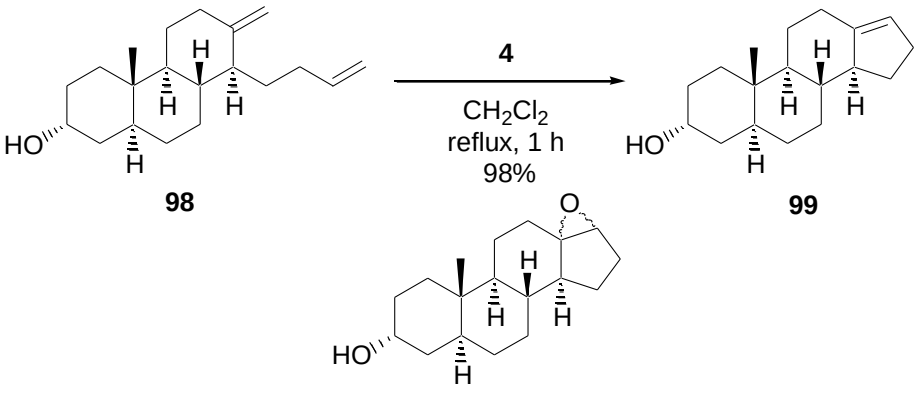
synthesis of the carbocyclic core of *ent*-bacillariolide II, an oxylipin.[69] Reaction of diene **96** with 6 mol% **3** in benzene gave the desired cyclopentene **97** in 86% yield.



96 **97**

bacillariolide II

Covey and co-workers used an abnormal Beckmann fragmentation/ring-closing metathesis strategy in their synthesis of 18-nor-$\Delta^{13(17)}$-androgens, derivatives of 3α-hydroxysteroids.[70] These compounds have been shown to modulate ion channels within the central nervous systems of animals. Treatment of diene **98** in the presence of a catalytic amount **4** in dichloromethane gave the corresponding steroid **99** in 98% yield. Reaction of **99** with mCPBA furnished the desired 18-nor-$\Delta^{13(17)}$-androgens.

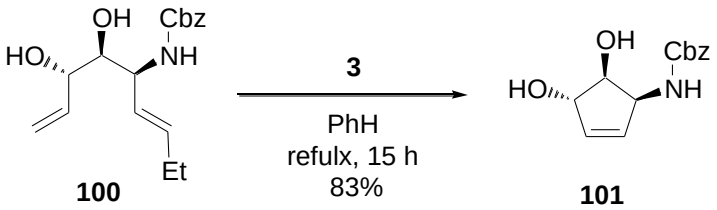


98 **99**

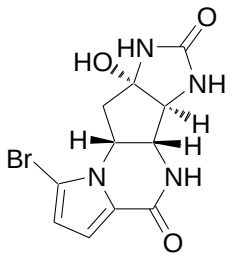
18-nor-$\Delta^{13(17)}$-androgens

(–)-Agelastatin A is an architecturally unusual tetracyclic compound with significant antitumor activity. Ichikawa and co-workers improved on earlier syntheses of (–)-agelastatin A by using a ring-closing metathesis

strategy to synthesize the core five-membered carbocycle.[71] Reaction of diene **100** with 5 mol% **3** in benzene provided the highly functionalized cyclopentene ring **101** in 83% yield. This compound was converted via several steps to provide (–)-agelastatin A.

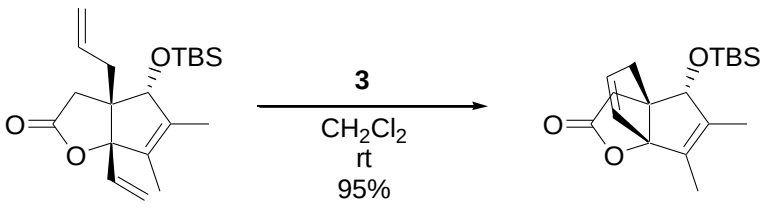


100 **101**

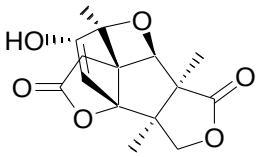


(–)-agelastatin

The sesquiterpene merrilactone A is an important neurotrophic factor of considerable interest for its potential use as a treatment in several neurodegenerative disorders. It remains a considerable challenge synthetically due to its densely oxygenated pentacyclic architecture with seven stereogenic centers, two γ-lactone moieties, and four quaternary carbon atoms. Mehta and co-workers recently reported on the use of a ring-closing metathesis strategy to synthesize the fused tricyclic core of merrilactone A.[72] Treatment of lactone **102** with 10 mol% of **3** in dichloromethane furnished the desired tricyclic system **103** in 95% yield.

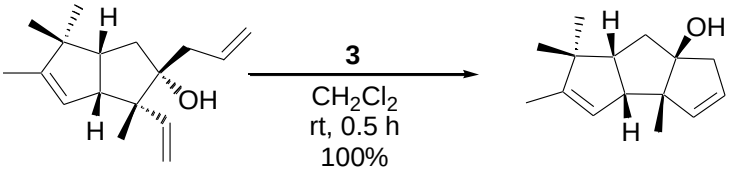


102 **103**



merrilactone A

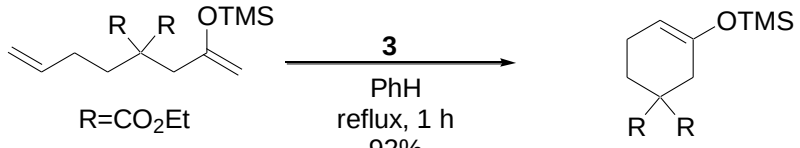
Triquinane natural products have recently gained attention due to their complex architecture, and cytotoxic and antibacterial properties. Recently, Srikrishna and Beeraiah used a ring-closing metathesis strategy in the synthesis of both the *cis*, *syn*, *cis*- and *cis*, *anti*, *cis*-linear triquinanes.[73] Treatment of diene **104** with 5 mol% **3** in dichloromethane provided the corresponding *cis*, *syn*, *cis*-triquinane in quantitative yield. A similar strategy was employed in the synthesis of the *cis*, *anti*, *cis*-triquinane, with the ring closing metathesis proceeding smoothly in 97% yeild (not shown).



104 **105**

5.3.5.3 Six Membered Rings

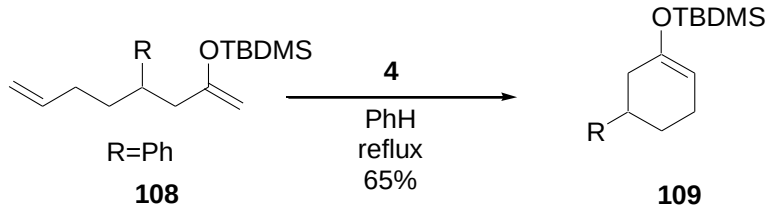
A general example of the versatility of ring-closing metathesis in the formation of six membered ring carbocycles is demonstrated by Shibasaki and co-workers, who employed their ring-closing metathesis strategy for five membered ring carbocyclic enol ethers to six membered ring carbocyclic enol ethers.[37] Carbocyclic enol ethers **107** was readily prepared from the corresponding electron deficient olefin **106** in 92% yield using only 7 mol% catalyst **3** in benzene.



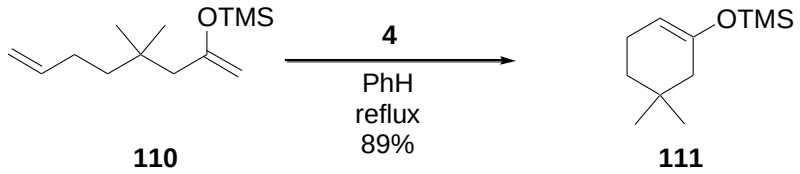
R=CO_2Et

106 **107**

Aggarawa and co-workers also prepared a six membered ring carbocyclic silyl enol ether bearing a phenyl substituent.[38] Their study showed that treatment of **108** with upwards of 20 mol% of **4** in refluxing benzene gave only modest yields of the desired product **109**. The authors attributed the observed yields to the lack of *gem*-substituents. The authors tested this theory by using olefin metathesis to synthesize a cyclic trimethylsilyl enol ether bearing a simple *gem* dialkyl group (**110**). This substrate required only 10 mol% of **4** to provide the corresponding carbocycle **111** in 89% yield.

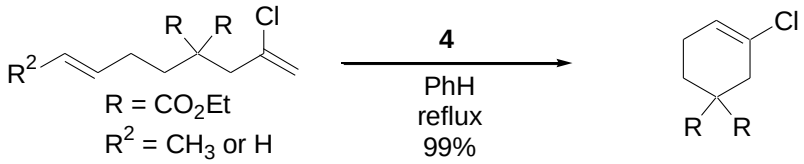


108 **109**



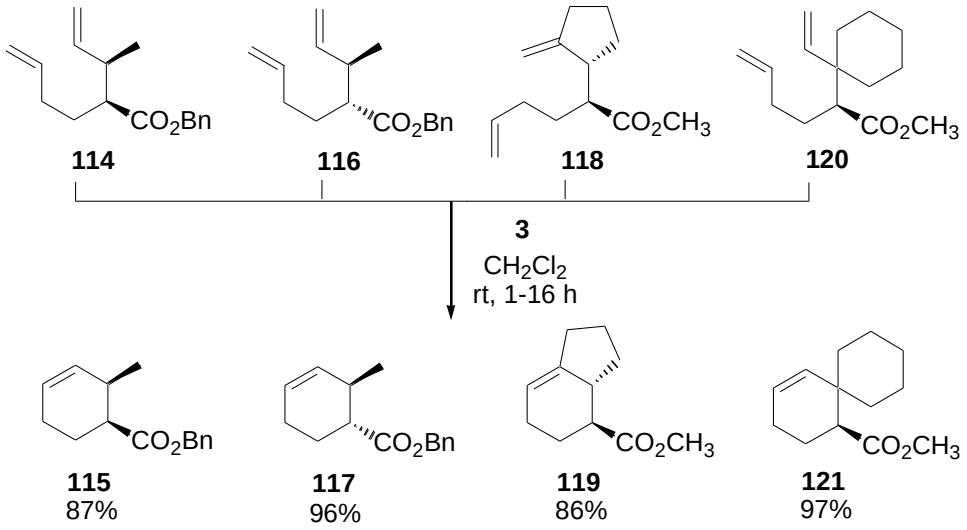
110 **111**

Weinreb and co-workers also employed their ring-closing metathesis strategy to synthesize 1-chloro-1-cyclohexene derivatives.[42] Substituted vinyl chloride **112**, when reacted in the presence of 10 mol% of **4** in benzene, gave the corresponding cyclohexene derivative **113** in quantitative yields.

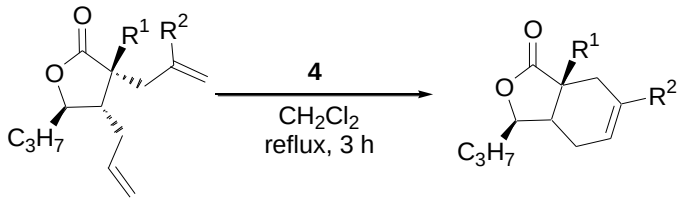


112 **113**

Piscopio and co-workers pioneered a method for the synthesis of functionalized carbocycles via an ester enolate Claisen/ring-closing metathesis strategy.[74] Substrates **114**, **116**, **118** and **120** were subject to olefin metathesis using 2.5 mol% of **3** in the presence of dichloromethane to furnish the corresponding carbocycles, including fused bicyclic (**119**) and spirocyclic (**121**) systems in upwards of 86% yield. Haudrechy and co-workers also applied a similar approach to synthesize a number of highly functionalized carbocycles,[75] and recently Sutherland and co-workers used an analogous aza-Claisen/ring-closing metathesis route in the preparation of functionalized carbocyclic amides.[76]



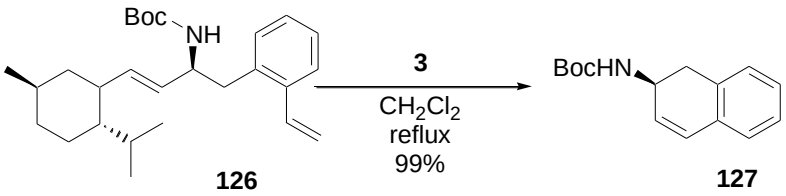
Martin and co-workers applied a ring-closing metathesis strategy in the synthesis of medium sized carbocycles fused to butyrolactones.[77] These systems are ubiquitous in nature and play an important role in the structural integrity and biological activity of many natural products. Butyrolactones **122** and **124** were subject to ring-closing metathesis using 10 mol% **4** in refluxing dichloromethane to produce the corresponding α,β fused γ-lactones **123** and **125** in 85% yield.



122, $R^1 = SO_2Ph$, $R^2 = H$
124, $R^1 = SO_2Ph$, $R^2 = CH_3$

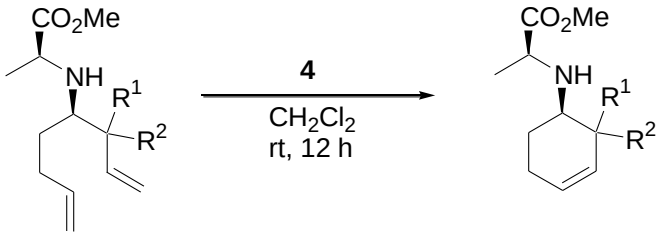
123, $R^1 = SO_2Ph$, $R^2 = H$ 85%
125, $R^1 = SO_2Ph$, $R^2 = CH_3$ 85%

Spino and co-workers employed a relay ring-closing metathesis strategy using a chiral auxiliary in the enantioselective synthesis of several amino carbocycles.[78] Reaction of diene **126**, incorporating a chiral auxiliary, with a catalytic amount of **3** in refluxing dichloromethane gave the corresponding aromatic allyl amine **127** in quantitative yields as a single enantiomer with concomitant loss of the chiral auxiliary.



126 **127**

Loh and co-workers used ring-closing metathesis in the synthesis of a number of homoallylic amines which serve as important intermediates in the synthesis of alkaloid natural products and nitrogen heterocycles.[79] Reaction of several substituted dienes with 10 mol% of **4** in dichloromethane furnished the corresponding amines in high yield (84-86%).



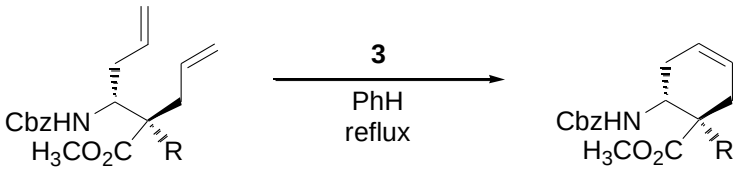
128, $R^1 = CH_3$, $R^2 = H$ **129**, $R^1 = CH_3$, $R^2 = H$ 84%

130, $R^1 = CH_3$, $R^2 = CH_3$ **131**, $R^1 = CH_3$, $R^2 = CH_3$ 92%

132, $R^1 = Ph$, $R^2 = H$ **133**, $R^1 = Ph$, $R^2 = H$ 86%

A number of research groups have used ring-closing metathesis to prepare conformationally constrained α- and β-amino acids containing six membered ring carbocycles. A number of key examples are discussed below.

Abell and co-workers applied their ring-closing metathesis strategy to produce a host of β-amino acid residues constrained by six membered carbocylcles.[46] Treatment of the desired diene (**134, 136** or **138**) with a catalytic amount of **3** in refluxing benzene provided the corresponding constrained β-amino acids (**135, 137** and **139**) in upwards of 90% yield. In a later report, they were able to verify the yields of these compounds using **4** under similar reaction conditions, and expanded their work to include a host of five, six and seven membered ring cyclic β-amino acid analogs.[80]

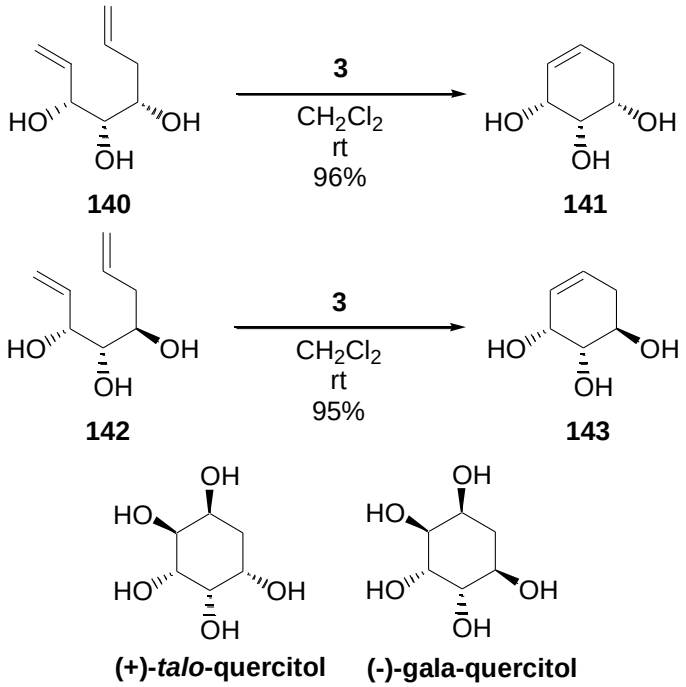


134, R = H
136, R = CH_3
138, R = Et

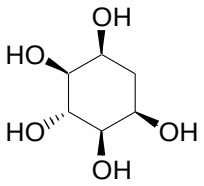
135, R = H 96%
137, R = CH_3 96%
139, R = Et 94%

Ring-closing metathesis has also been applied to the synthesis of a number of carbasugars containing six membered rings. Many of these carbacycles are natural products. Unnatural carbasugars have been prepared and evaluated for their potential to serve as biological mimics of natural pyranose sugars, and several have gained attention as potent glycosidase inhibitors. Other carbasugars have been used as precursors in the synthesis of higher order natural products. Several important examples are illustrated below.

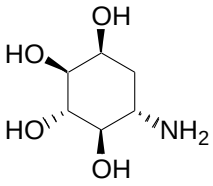
A number of carbocyclic mimetics of pyranose carbohydrates have been prepared using ring-closing metathesis as a key step. One of the first examples by Madsen and co-workers, employed a novel zinc-mediated domino reaction to convert 5-iodo-ribofuranosides to the corresponding diene precursors **140** and **143**.[81] These diene precursors were then subject to ring-closing metathesis using up to 10 mol% of **3** in the presence of dichloromethane to give the corresponding carbasugar derivatives **141** and **143** in near quantitative yields. Compounds **141** and **143** were then subjected to dihydroxylation in the presence of OsO_4 to provide the corresponding carbasugars (+)-*talo*-quercitol and (–)-*gala*-quercitol. Their report signified the shortest enantioselective synthesis of these compounds. The authors reported several additional examples.



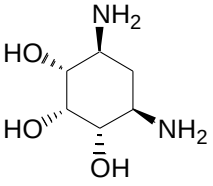
Since Madsen's initial report, several additional pyranose carbacycles have been synthesized. Vankar and co-workers synthesized *muco*-quercitol, (+)-*gala*-quercitol, and 5-amino-5-deoxy-D-*vibo*-quercitol, from D-mannitol using olefin metathesis as a key step.[82] The latter compound, 5-amino-5-deoxy-D-*vibo*-quercitol is especially noteworthy as it an important component of a number of aminoglycoside antibiotics. Van Boom and co-workers used olefin metathesis in the synthesis of 2-deoxystreptamine, a important carbohydrate component of neomycin B and kanamycin B, two aminoglycoside antibiotics.[83] Gallos and co-workers also employed a ring-closing metathesis approach in their synthesis of 6a-carba-β-D-fructopyranose, a potential sweetener.[84] Kumaraswamy and co-workers used a similar ring-closing metathesis approach in their preparation of glucose and galactose based carbacycles as intermediates in drug development.[85] And finally, Cumpstey and co-workers recently published the synthesis of two β-hexosaminidase inhibitors, valienamine and 1-*epi*-valienamine from either D-glucose or L-sorbose,[86] or D-mannose[87] using ring-closing metathesis as the key step in their syntheses.



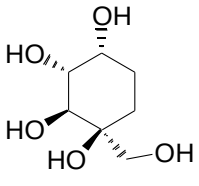
muco-quercitol



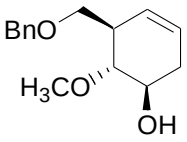
5-amino-5-deoxy-D-*vibo*-quercitol



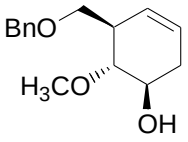
2-deoxy-streptamine



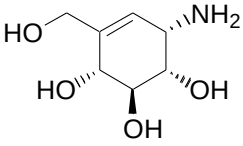
6a-carba-β-D fructopyranose



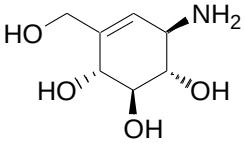
glucose-based carbasugar



galactose-based carbasugar

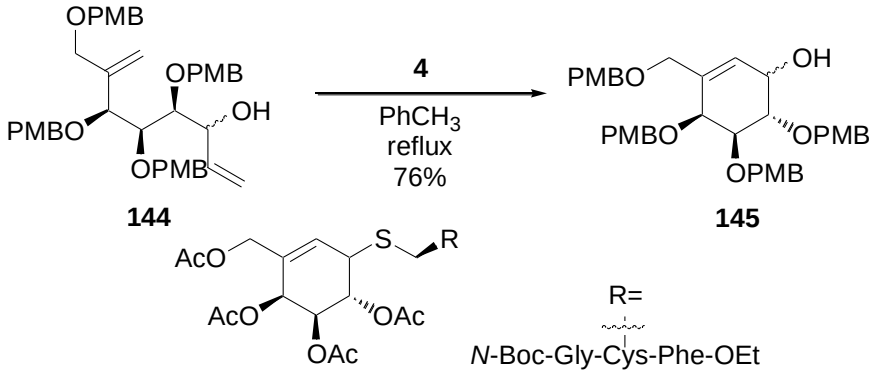


valienamine



1-epi-valienamine

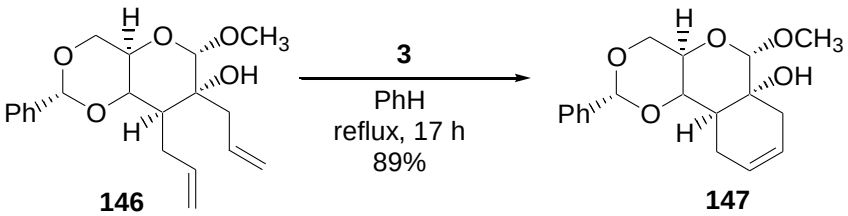
In 2004, Halcomb and co-workers reported the first successful synthesis of an isostere of an *O*-linked glycopeptide in an effort to study how sugar modifications affect protein folding in inflammatory diseases and cancer.[88] Treatment of **144** with 10 mol% of **4** in refluxing toluene gave 78% of the desired product **145** as a mixture of diastereomers.



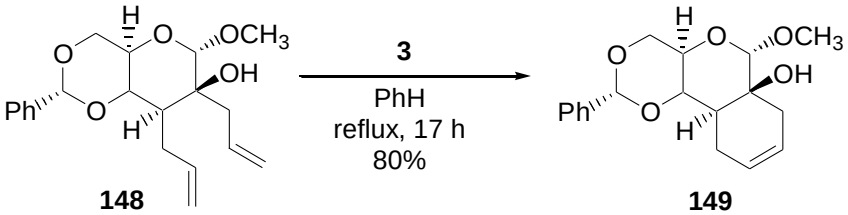
α-galactosyl glycopeptide isostere

Holt and co-workers used ring-closing metathesis strategy to synthesize a number of enantiomerically pure annulated carbohydrate systems containing carbocycles, as precursors for the synthesis of taxoids and other natural products.[89] Treatment of dienes **146** and **148** with a catalytic amount of **3** in benzene gave the corresponding 6,6,6-carbocycles, *cis*-**147** and *trans*-**149**, in 89 and 80% yield, respectively. The authors attempted a similar strategy to prepare 6,6,5-annulated systems, however, they achieved

little to no yield of the desired products, presumably due to ring strain. Holt and co-workers followed up on their initial report, using a similar strategy to prepare a host of 6,6,6-, 6,6,7-, and 6,6,8-, and 6,6,9- membered ring carbocyclic systems as well as several oxygen containing spirocycles.[90]

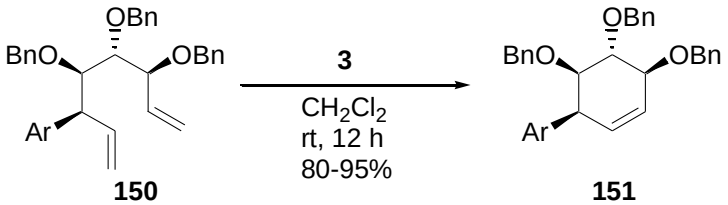


146 **147**



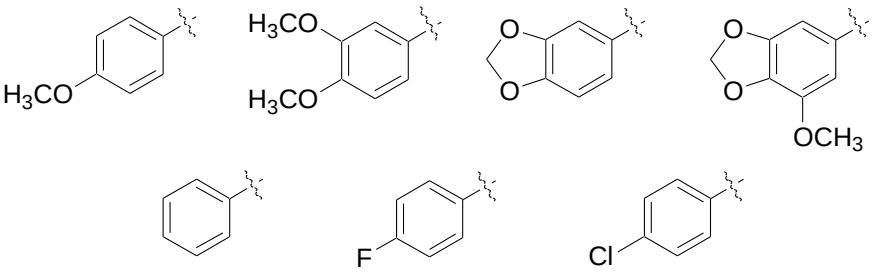
148 **149**

Korinenko and co-workers applied ring-closing metathesis as a key step in the synthesis of several analogs of pancratistatin, a potent anticancer natural product.[91] Dienes with varying aryl substituents (**150**) were prepared and subjected to ring-closing metathesis using 3 mol% of **3** in dichloromethane to furnish the corresponding carbocycles in 80–95% yields.



150 **151**

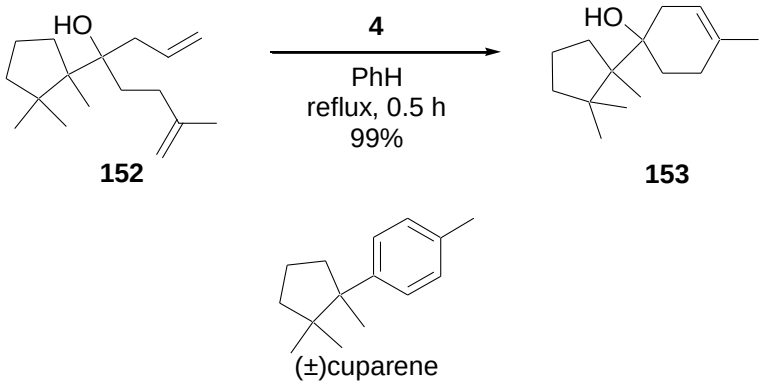
Ar =



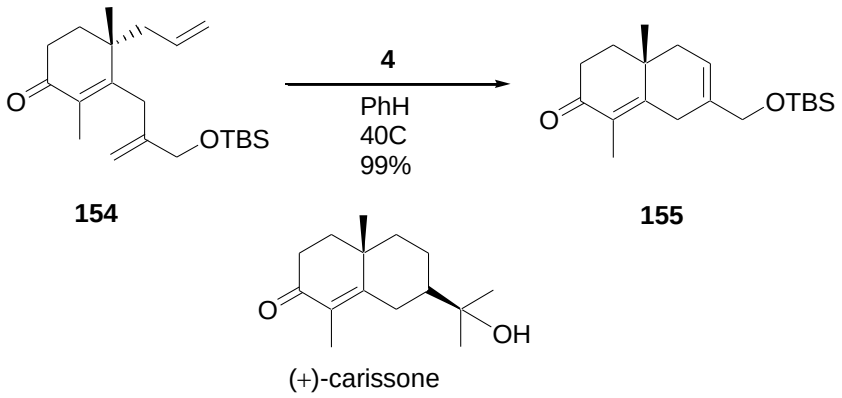
Ring-closing metathesis has also been applied to the synthesis of a number of terpene derivatives containing six membered rings. The terpene

derivatives prepared by this approach have been as simple as cuparene and as complex as tricycloillicinone. Several important examples are illustrated below.

Prasad and co-workers employed ring-closing metathesis as a key step in the synthesis of (±)-cuparene.[92] Diene precursor **152** underwent quantitative conversion to the corresponding carbocycle in refluxing benzene using only 3 mol% of **4**. Dehydration and aromatization of the corresponding tertiary alcohol gave the desired product.

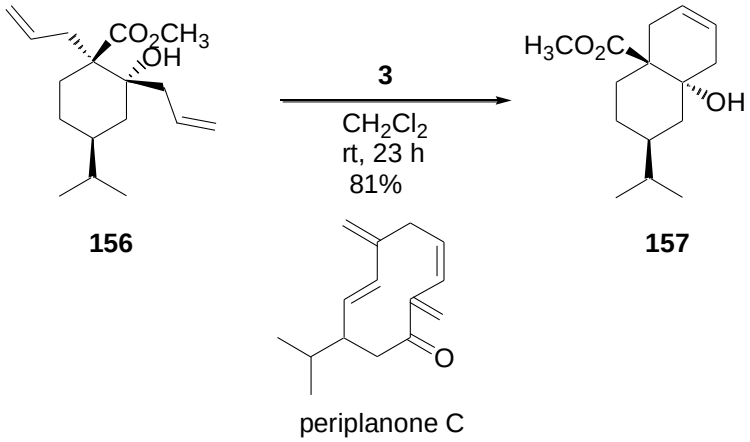


Ring-closing metathesis was also used as a key step in the enantioselective synthesis of (+)-carissone, a eudesmane sesqueterpenoid, by Stoltz and co-workers.[93] Reaction of enone **154** with 3 mol% of **4** in benzene gave the corresponding carbocycle **155** in quantitative yields. Seven additional steps were required to access (+)-carissone.



Saicic and co-workers used ring-closing metathesis as one of the final steps in their synthesis of (±)-periplanone C, a C-macrolide pheromone.[94] α-hydroxy ketoester **156**, when reacted with 3 mol% of **3** in dichloromethane at room temperature over the course of 23 hours gave **157** in 81% yield. The

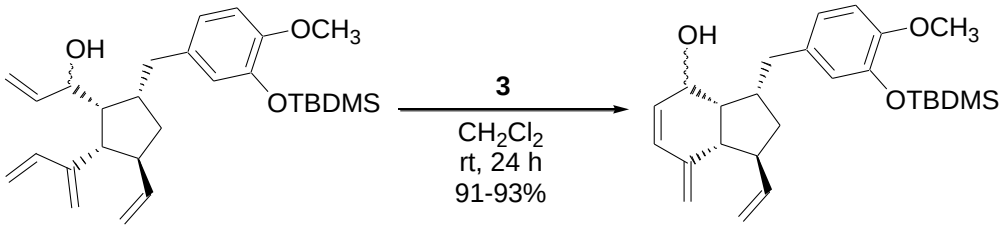
authors applied this methodology to access a number of other intermediates in the periplanone family.



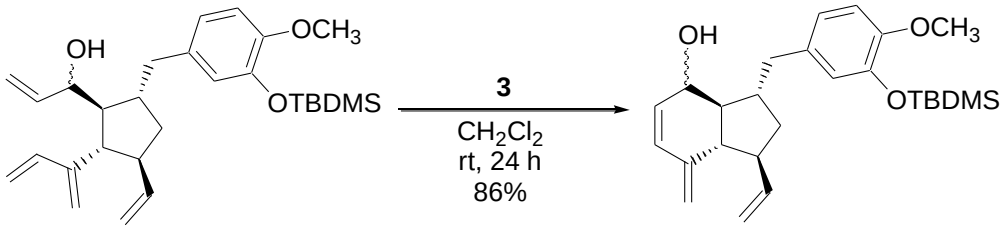
156 **157**

periplanone C

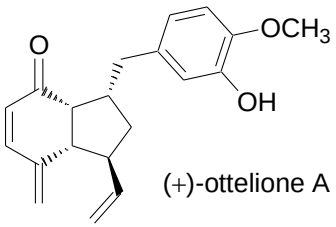
Clive and co-workers used a ring-closing metathesis strategy to access the six member ring carbocyclic components of the anticancer agent's ottelione A and B.[95] Olefin metathesis of **158** or **160** with catalytic amount of **3** in the presence of dichloromethane provided the corresponding carbocycles **159** and **160** in 93 and 86% yields, respectively. The authors also attempted this reaction under similar conditions with **4** but found no improvement in the overall reaction yields.



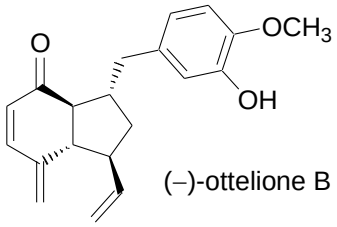
158 **159**



160 **161**

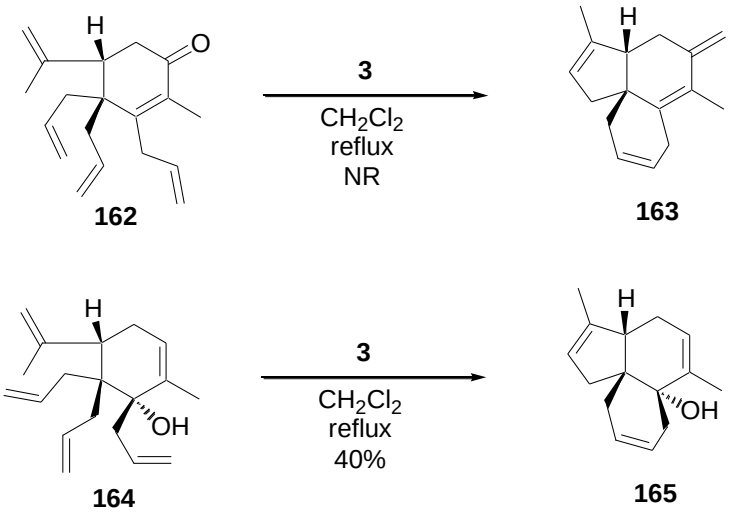


(+)-ottelione A



(–)-ottelione B

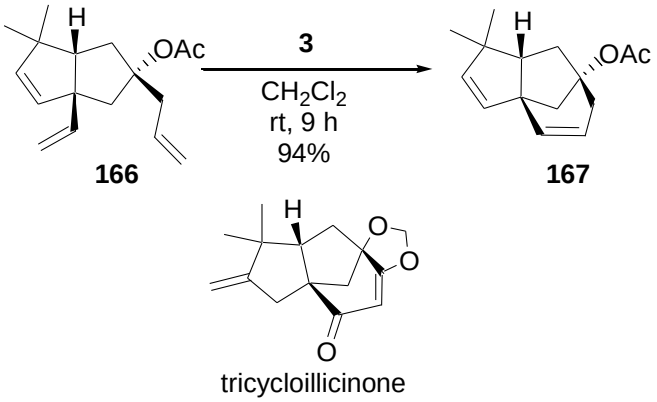
Srikrishna and co-workers employed a ring-closing metathesis strategy in the synthesis of the 6,6,5-tricyclic core of elisabethane diterpenes.[96] These novel diterpenoids have a wide range of biological properties. Interestingly, ring-closing metathesis of 3,4,4-trisallylcarvone **162** in the presence of 5 mol% of **3** in refluxing dichloromethane did not proceed as expected to provide the desired tricycle. However, olefin metathesis of trisallylcarveol **164** proceeded smoothly under similar conditions to give the targeted tricyclic system **165** in 40% yield, highlighting the importance of the allyl alcohol in the activation of the substrate.



162 **163**

164 **165**

Tricycloillicinone is a novel C6-C3 prenylated compound with an interesting 3,4,4-trimethyltricyclo[5.3.1.$0^{1,5}$]undecane ring. This compound has recently attracted attention for its ability to increase choline acetyltranferase activity, and as such may hold promise as a therapeutic for the treatment of neurological disorders. Recently, Srikrishna and co-workers employed a ring-closing metathesis approach to the synthesis of the tricyclic carbocyclic core of tricycloillicinone.[97] Reaction of acetate **166** with 10 mol% **3** in dichloromethane gave the corresponding tricyclic acetate **167** in 94% yield. Ongoing efforts by Srikrishna and cowokers are focusing on the transformation of this compound to the desired tricycloillicinone product.

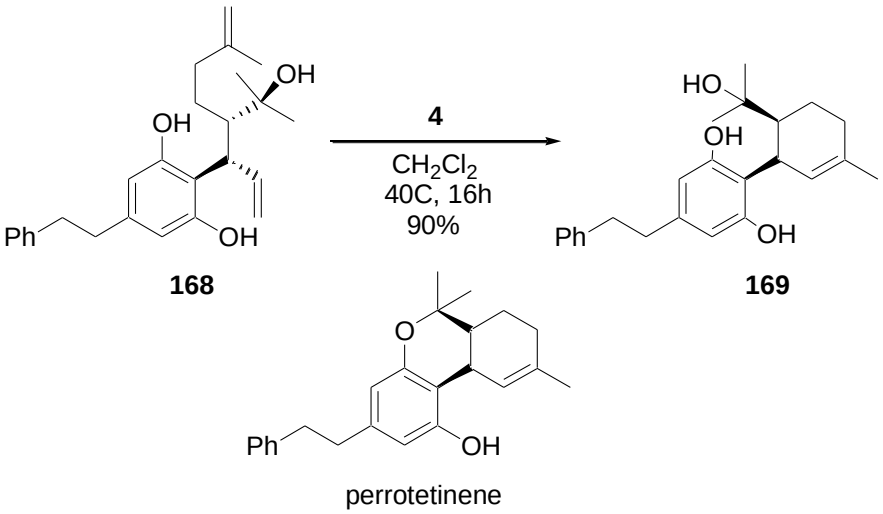


166 **167**

tricycloillicinone

Kim and co-workers applied a ring-closing metathesis strategy in the total synthesis of (–)-perrottentinene, a biogeneic precursor to of (–)-Δ^1-*trans*-tetrahydrocannabinol.[98] Treatment of diene precursor **168** with a catalytic amount of **4** in the presence of refluxing dichloromethane gave the desired

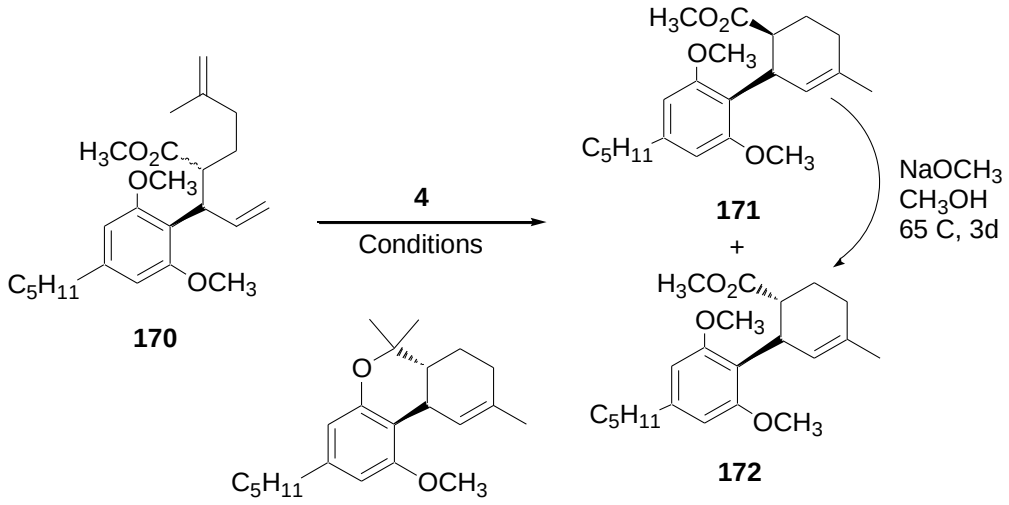
product **169** in 90% yield. The authors noted this was the first successful example of olefin metathesis using a proximal *o*-phenol group.



168 **169**

perrotetinene

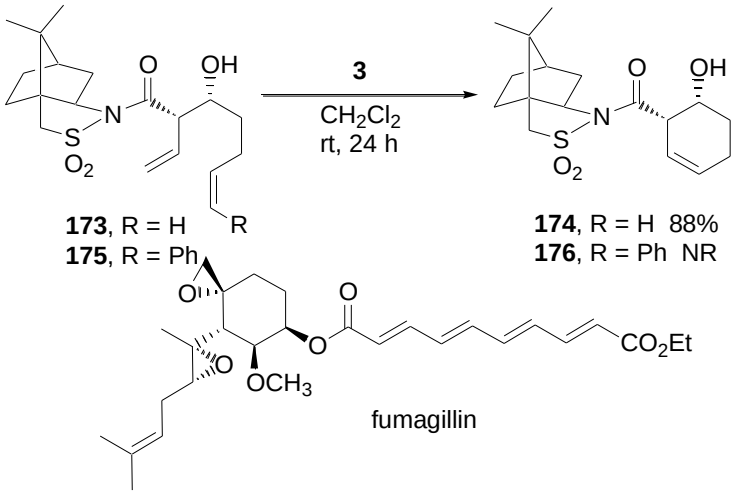
Olefin metathesis was employed by Trost and co-workers in the synthesis of (–)-Δ^9-*trans*-tetrahydrocannabinol, the psychomimetic component of marijuana.[99] A diastereomeric mixture of diene **170**, when treated with catalytic amount of **4**, gave a mixture of the corresponding *anti* **171** and *syn* **172** cyclohexenes. Equilibration in sodium methoxide and methanol over the course of three days gave almost exclusively the *anti* product. Four additional steps were required to achieve (–)-Δ^9-*trans*-tetrahydrocannabinol.



170 **171** **172**

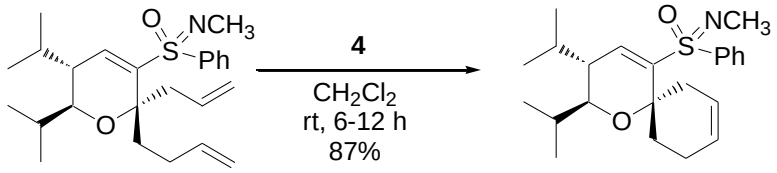
(-)-Δ^9-trans-tetrahydrocannabinol

Fumagillin and several related analogs have been shown to inhibit angiogenesis, and as such are of interest as potential treatments for cancer. Watson and co-workers recently employed a ring-closing metathesis strategy using a chiral auxiliary to enantioselectively construct the cyclohexene core of fumagillin.[100] Diene **173**, where R = H, when subject to ring-closing metathesis using 5 mol% of **3** in dichloromethane gave the corresponding carbocyclic ring **174** in 88% yield as a single enantiomer. Interestingly, diene **175**, where R = Ph, failed to undergo ring-closing metathesis. The authors did not speculate as to why the reaction failed, however it is likely that sterics may played a role.

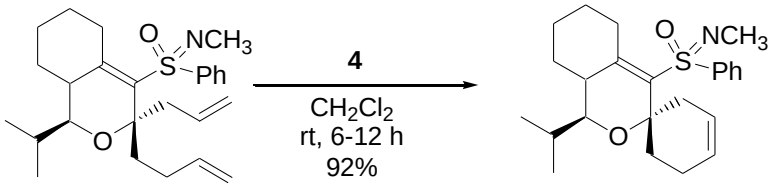


Spirocycles incorporating six membered rings are important synthons in organic synthesis. These structural motifs have also been found in a number of natural products. In general, six membered ring carbocycles are readily prepared in high yields using olefin metathesis. Several key examples are discussed below.

In 2008, Gals and co-workers reported a general method for the asymmetric synthesis of spiroketals.[101] These molecules are important intermediates in the synthesis of a number of biologically active compounds. α,α-dienyl dihydropyrans **177** and **179** were subject to ring-closing metathesis using a catalytic amount of **4** in dichloromethane to provide the corresponding spirocyclic carbocycles **178** and **180**. The authors noted that the Lewis basic sulfoximine group had little effect on the ring-closing metathesis reaction.

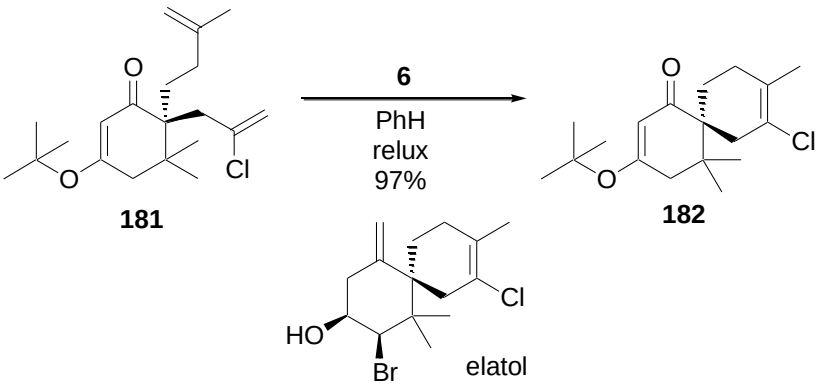


177 **178**



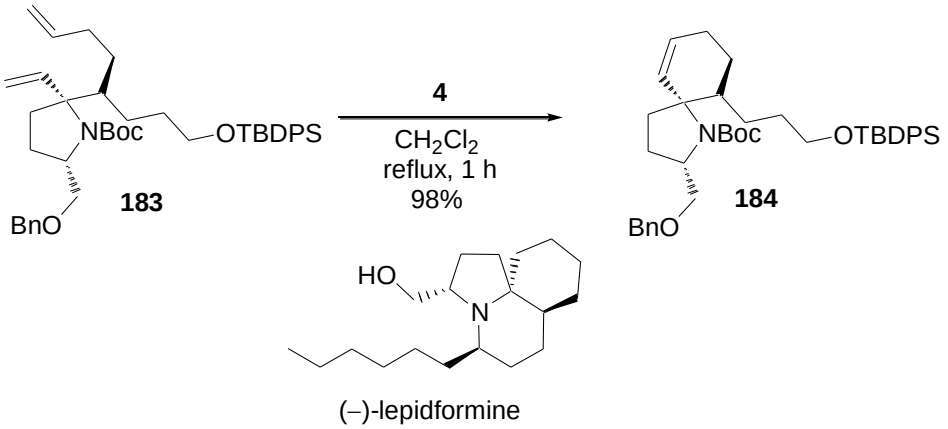
179 **180**

Grubbs, Stoltz and co-workers used olefin metathesis as a key step in the first catalytic asymmetric total synthesis of elatol.[102] Elatol, a chamigrene sesquiterpene with antibiofouling, antibacterial, antifungal, and cytotoxic properties, is a particular challenge synthetically due to its sterically congested spirocyclic system, exocyclic olefin, vinyl halide and *cis*-halohydrin functionality. Reaction of α,ω diene **181** with 5 mol% of **6** in benzene gave the corresponding chloroalkene **182** in 97% yield. Four additional steps were required to access the desired natural product.

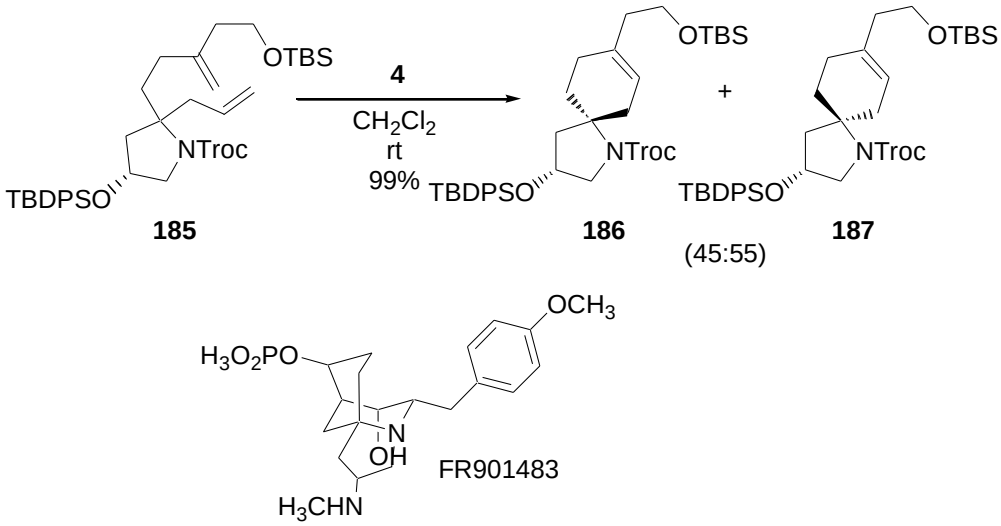


181 **182**

(–)-Lepadiformine is a tricyclic perhydropyrrolo[2,1-*j*]quinolone that has recently gained attention due to its moderate *in-vitro* tumor cell cytotoxicity and positive cardiovascular effects. Kim and and co-workers used ring-closing metathesis to construct the spirocyclic core of (–)-Lepadiformine.[103] Treatment of **183** with **4** in the presence of refluxing dichloromethane gave the corresponding azaspiro cyclohexene **184** in near quantitative yield.

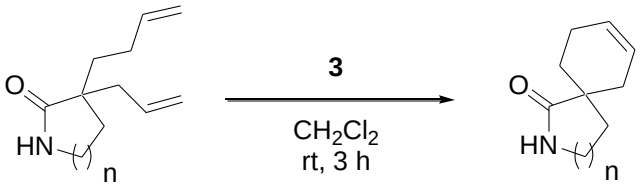


Martin and co-workers employed a ring-closing metathesis strategy in the total synthesis of the azatricyclic skeleton of FR01483.[104] (–)-FR01483 is an immunosuppressant that has been shown to prolong graft survival time by inhibiting purine nucleotide synthesis. Reaction of **185** as a mixture of dienes with 10 mol% of **4** in dichloromethane gave a separable mixture of the corresponding azaspiro cyclohexene derivatives **186** and **187** in near quantitative yield. Unfortunately, the desired diastereomer, **186**, was produced as the minor product. This report constituted slight improvement over their previously work on this compound.[105]



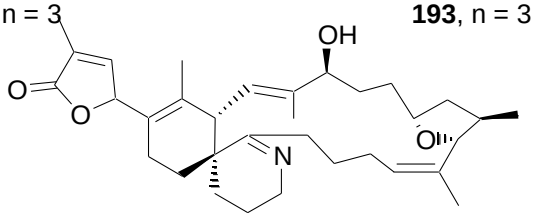
Brimble and co-workers used a double-alkylation/ring-closing metathesis approach in the synthesis of several spiroimines.[106] Spiroimines are important functional moieties in a number of shellfish toxins suchs as gymnodimine. Lactams **188**, **190**, and **192** were subject to ring-closing

metathesis using 5 mol% of **3** in dichloromethane to furnish the corresponding 5,6-(**189**), 6,6-(**191**), and 7,6-(**193**) spirolactams in upwards of 85% yield.



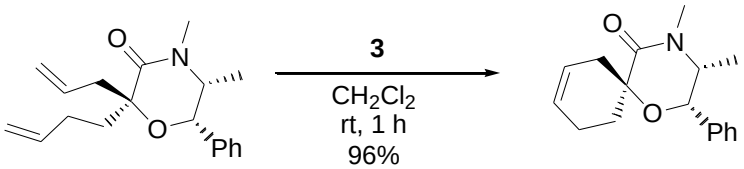
188, n = 1
190, n = 2
192, n = 3

189, n = 1 86%
191, n = 2 90%
193, n = 3 88%

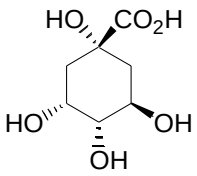


gymnodimine

Pansare and co-workers employed a ring-closing metathesis strategy in the synthesis of (–)-quinic acid.[107] Quinic acid is an important regulator in the biosynthesis of aromatic compounds via the shikimic pathway, and may serve as a potential antifungal, antibacterial and antiparasitic. Ring-closing metathesis of diene **194** using 7 mol% of **3** in dichloromethane at room temperature gave the corresponding spiro-morpholinone **195** in 96% yield.



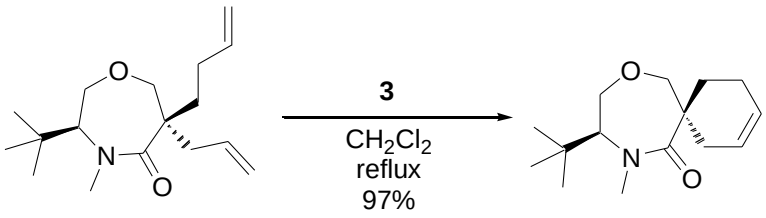
194 **195**



(-)-quinic acid

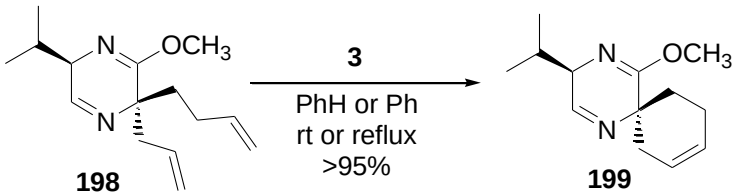
Hughes and co-workers extended their ring-closing metathesis strategy as a general route to the synthesis of enantiopure six membered ring spirocarbocycles using zizaene as a chiral auxiliary.[67] Diene precursor **196**

required 15 mol% catalyst loading of **3** in refluxing dichloromethane to achieve a 97% yield of the desired spirocycle **197**.



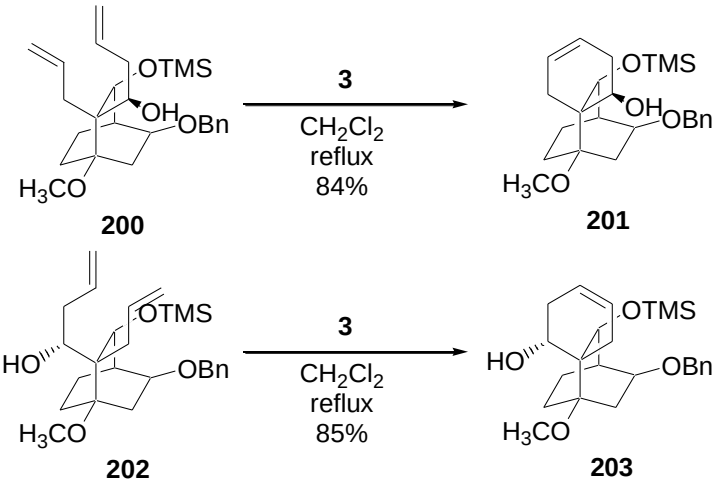
196 **197**

Undheim and co-workers their olefin metathesis strategy to prepare six membered ring spirocyclic carbocycles for use as templates in natural product synthesis.[68] Reaction of **198** with 2 mol % catalyst loading of **3** furnished the corresponding spirocycle **199** in greater than 95% yield.

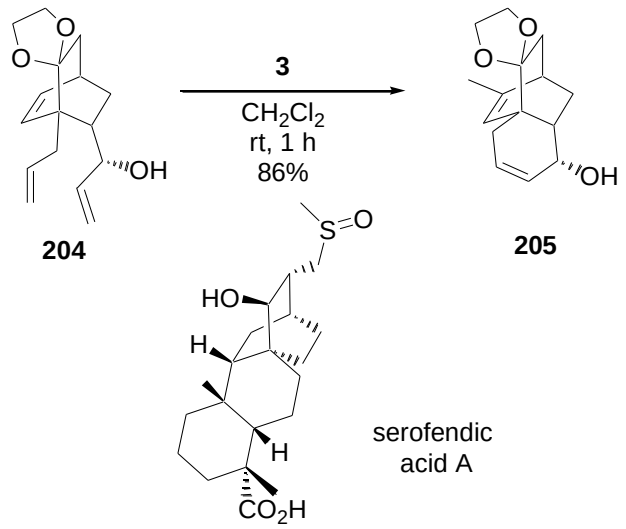


198 **199**

Frejd and co-workers used a ring-closing metathesis strategy as the key transformation in the synthesis of several novel spiro-cyclohexene bicyclo[2.2.2]octane derivatives.[108] Spiro-cyclohexene bicyclo[2.2.2]octane derivatives are rare molecular frameworks, with only a few examples reported to date. Olefin metathesis of diastereomeric homoallylic alcohols **200** and **202** using 10 mol% of **3** in refluxing dichloromethane provided the corresponding spirocyclic carbocycles **201** and **203** in 84 and 85% yields, respectively.

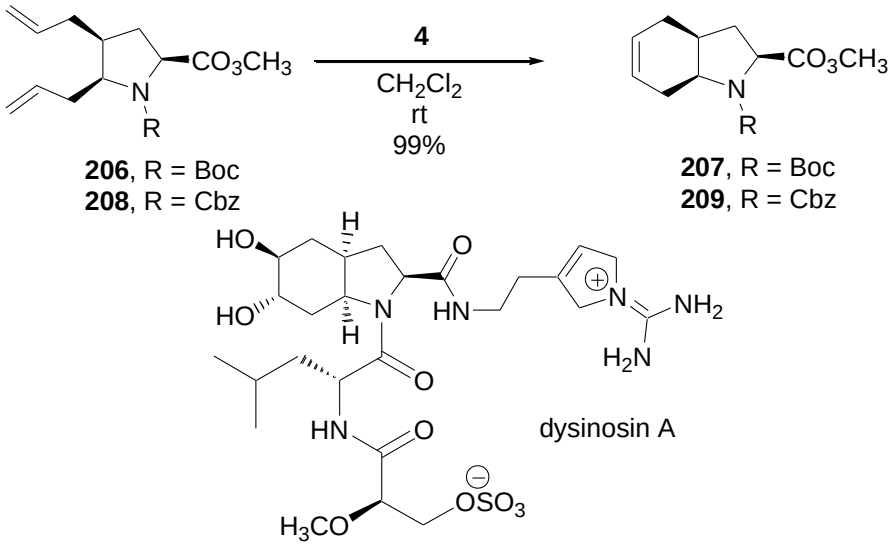


Singh and co-workers recently reported a general method for the synthesis of embellished spiro-fused bicyclo[2.2.2]octane systems using a Diels–Alder cycloaddition/ring-closing metathesis route.[109] These systems, which are key intermediates in the synthesis of atisane diterpenoides such as serofenic acid A, have been shown to possess neuroprotective activity. Treatment of allyl alcohol **204** with 10 mol% of **3** in dichloromethane gave the corresponding spirocyclic carbocycle **205** in 86% yield. The authors also prepared seven and eight membered ring analogs of **205**.

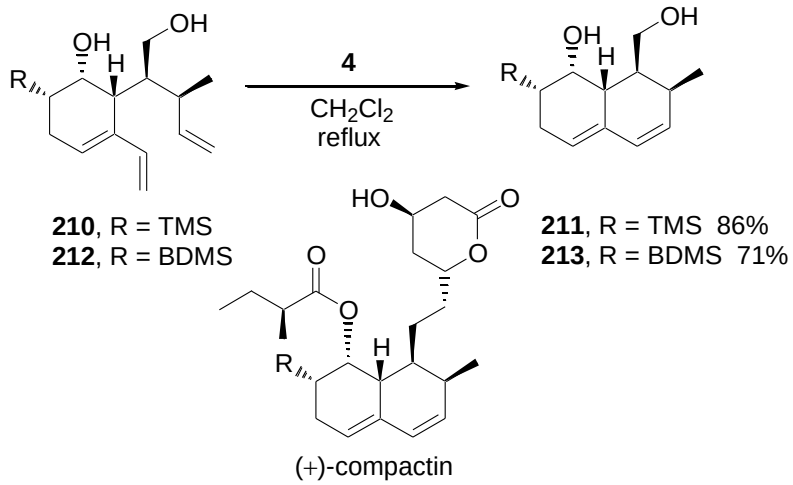


Dysinosin A is a highly oxygenated novel inhibitor of thrombin and factor VIIa. Hanessian and co-workers employed a ring-closing metathesis in the preparation of the 6,5-fused core of dysinosin A.[110] Olefin metathesis of **206** and **208** using only 1 mol% of **4** gave quantitative yields of the

desired carbocycles **207** and **209**. Several additional steps were required for the construction of the final target.

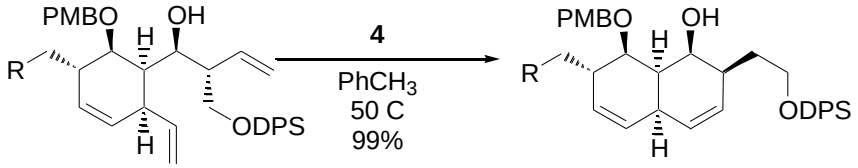


A similar strategy was employed by Robichaud and co-workers in the enantioselective synthesis of (+)-compactin, a hydroxymethyglutaryl coenzyme A (HMG-CoA) reductase inhibitor.[111] Olefin metathesis of trienes **210** and **212** in the presence of a catalytic amount of **4**, provided the corresponding conjugated dienes **211** and **213** in 86% and 71% yield, respectively.



Enev and co-workers employed olefin methathesis in their approach to the *cis*-decalin core of branimycin, an antibiotic.[112] Until their report in 2008, preparations of branimycin almost exclusively employed the use of

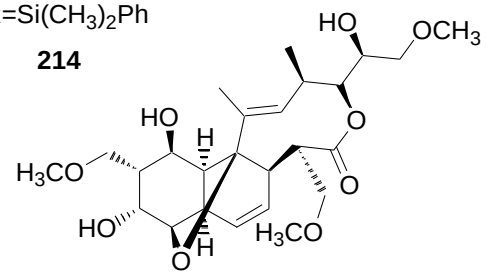
Diels–Alder reactions to construct the *cis*-decalin system. Metathesis of triene **214** in the presence of 5 mol% of **4** in toluene gave the corresponding carbocycle **215** in quantitative yields. The authors reported the ring-closing metathesis of several trienes differing only in the stereochemistry of the groups with similar success.



R=Si(CH$_3$)$_2$Ph

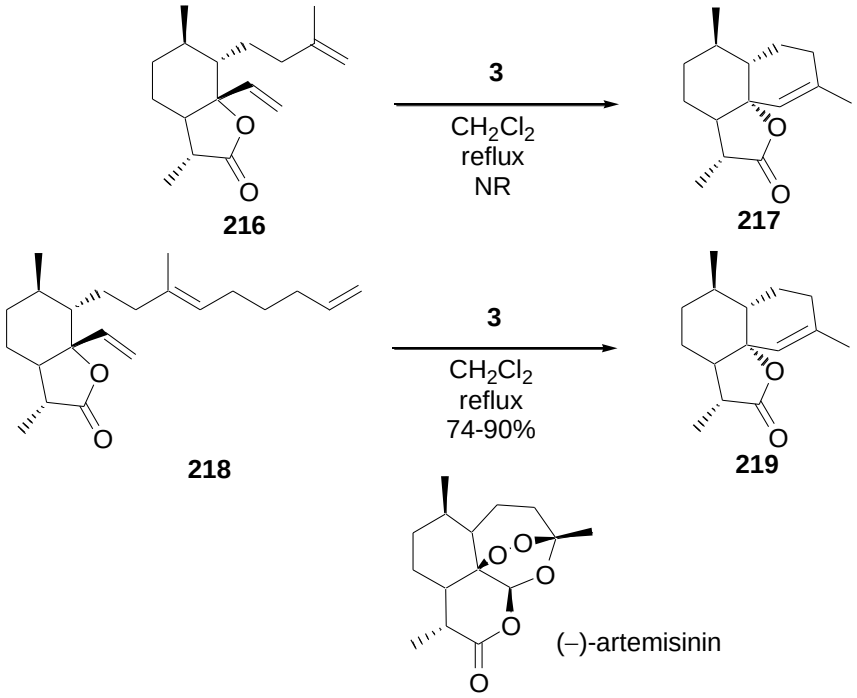
214

215

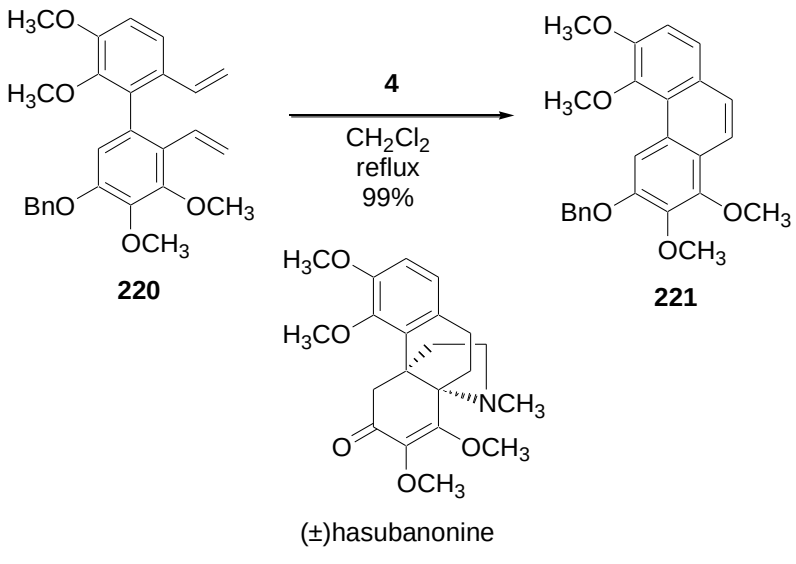


branimycin

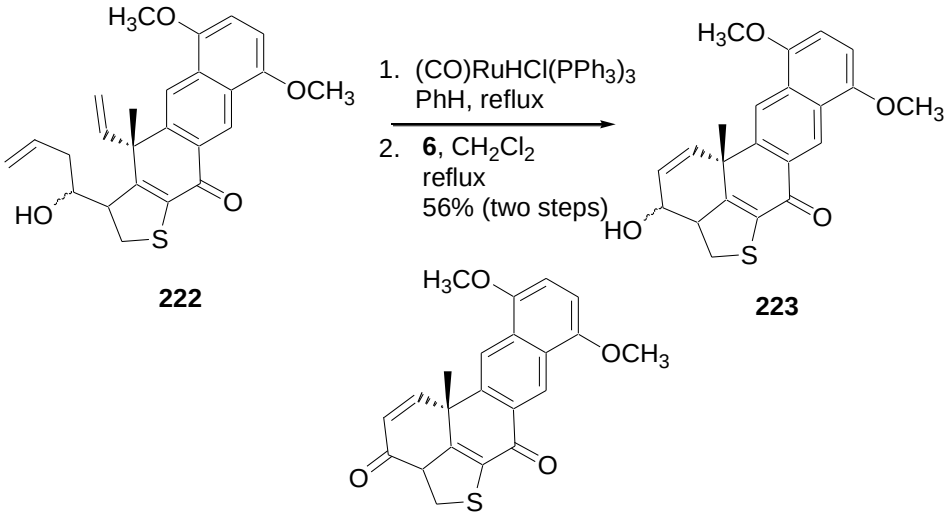
Dudley and co-workers used a relay ring-closing metathesis strategy in the synthesis of (+)-dihydro-*epi*-deoxyarteannuin B, a key biogenic precursor in the synthesis of artemisinin.[113] Initially, Dudley and co-workers attempted olefin metathesis on diene **216** using a catalytic amount of **3**. Unfortunately, the expected product was not obtained. The authors speculated that steric congestion might be the cause of the failed cyclization. However, relay ring-closing metathesis of triene **218** with a catalytic amount of **3** gave the desired tricyclic system **219** in 74-90% yield.



Castle and co-workers used a ring-closing metathesis strategy in the synthesis of the carbocyclic core of (±)-hasubanonine, a member of the hasubanan alkaloid family.[114] Hasubanan alkaloids are of interest due to their structural similarity to morphine alkaloids. Treatment of diene **220** with a catalytic amount of **4** in refluxing dichloromethane furnished the desired phenanthrene core **221** in quantitative yield. Six additional steps were required to obtain the desired natural product.



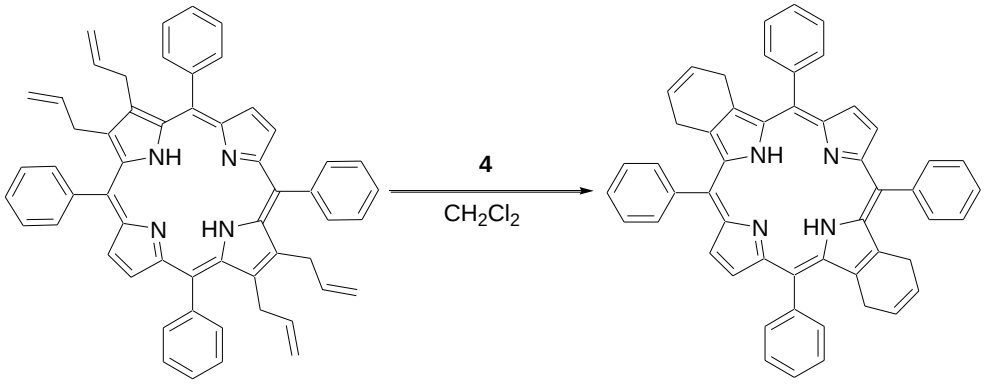
Wipf and co-workers employed ring-closing metathesis strategy in their synthesis of (±)-thiohalenaquinone.[115] (±)-Thiohalenaquinone is of particular interest due to its complex carbocyclic core and wide range of biological activities. In particular it has been shown to inhibit protein tyrosine kinase, phosphatidylinositol 3 kinase, and Cdc25B dual specificity phosphatase. Ruthenium catalyzed bond migration, followed by ring-closing metathesis of diene **222** with 20 mol% of **6** in refluxing dichloromethane gave the corresponding pentacyclic core **223** in 56% yield over two steps. Oxidation of **223** provided (±)-thiohalenaquinone.



222 **223**

(±)thiohalenquinone

Smith and co-workers used a ring-closing metathesis strategy in the synthesis of benzoporphyrins.[116] Benzoporphyrins are particularly attractive in medicine as agents for photodynamic therapy, and in industry for use as electro-optic materials. Treatment of diene **224** with a catalytic amount of **4** in dichloromethane provided the corresponding porphyrin analog **225** in good yield. Oxidation with DDQ furnished the desired benzoporphyrin in nearly quantitative yield. The authors reported the synthesis of several additional mono and tri substituted derivatives as part of their study.

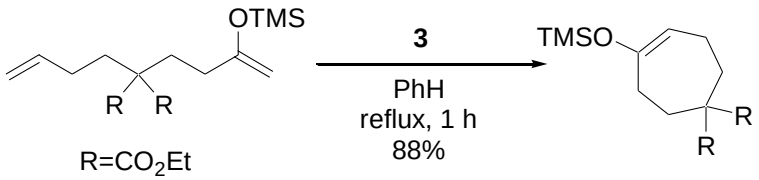


224 **225**

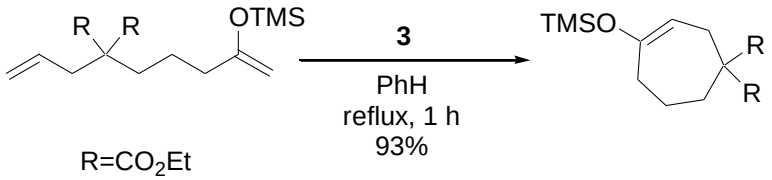
5.3.5.4 Seven Membered Rings

Ring-closing metathesis has been most useful in the synthesis of larger carbocycles, such as seven membered rings. Seven membered ring carbocycles are found in a number of biologically active natural products, but have proven difficult to prepare in high yields using standard ring forming strategies. The development of ring-closing metathesis as a tool for the construction of these systems, has allowed for the synthesis of a number of natural products incorporating seven membered ring systems, often in high yield. The following section provides a number of important examples where ring-closing metathesis has been employed in the synthesis of seven membered ring carbocycles.

Shibasaki and co-workers used a ring-closing metathesis approach to prepare seven membered rings from electron-deficient olefins.[37] Acyclic enol ethers **226** and **228** were subject to ring-closing metathesis with 7 mol% of **3** in benzene to provide the corresponding cyclic enol ethers **227** and **229** in 88 and 94% yield, respectively. Deprotection of the silyl ether resulted in the corresponding cyclic ketone.

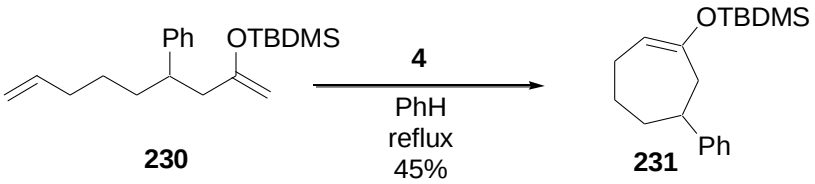


226 **227**



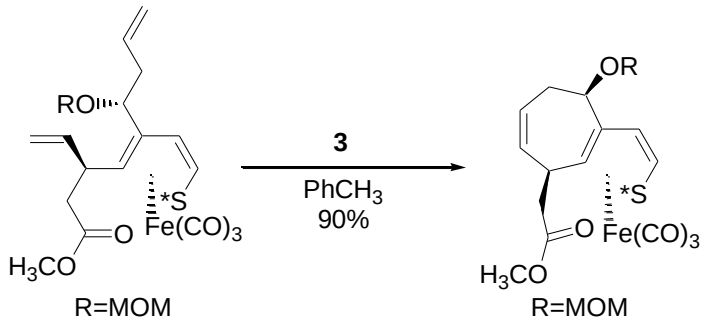
228 **229**

Aggarawa and co-workers applied their strategy in the synthesis of seven membered ring carbocyclic silyl enol ether bearing a phenyl substituent.[38] Substrate **230** required upwards of 20 mol% of **4** in refluxing benzene to achieve modest yields of the desired product **231**.



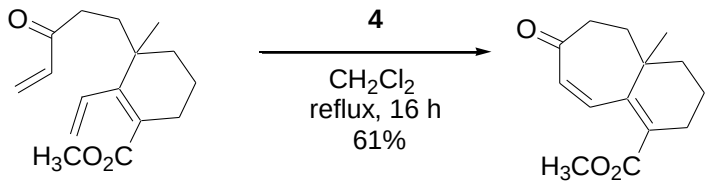
230 **231**

Paley and co-workers used enantiopure n^4-(1-sulfinyldiene)iron(0) tricarbonyl complexes as templates for the enantioselective carbocylic construction via a ring-closing metathesis strategy.[117] Enantiopure homoallyl alcohol adduct **232**, when treated with 8 mol% of **3** in toluene, gave the corresponding seven membered ring carbocyle **233** as a single enantiomer in 90% yield. This strategy was also applied to form eight and nine membered ring carbocycles with similar success.

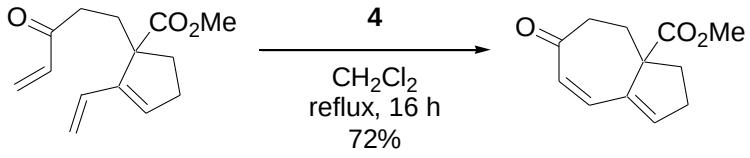


232 **233**

Funk and co-workers employed a ring-closing metathesis strategy in the synthesis of fused haloethyl vinyl ketones.[118] These compounds are important synthetic intermediates in the production of a number of natural and unnatural products. Treatment of the five (**234**) or six (**236**) membered enone with a catalytic amount of **4** in the presence of refluxing dichloromethane gave the corresponding vinyl ketones **235** and **237** in 61 and 72% yield, respectively. The reported yields were based on a two step process with the first step, a retrocycloaddition of the dioxin precursor catalyzed by $ZnCl_2$, giving rise to the enone (not shown).

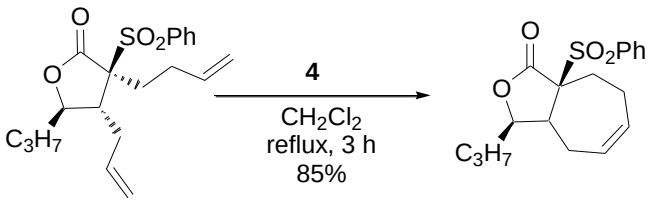


234 **235**

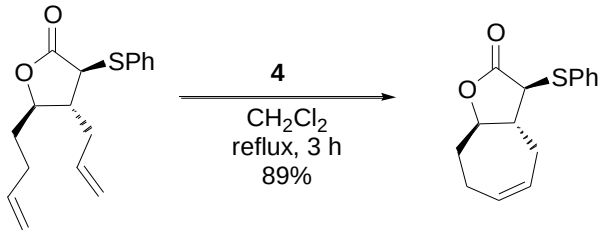


236 **237**

Martin and co-workers applied their ring-closing metathesis strategy in the synthesis of fused-γ-butyrolactones to fused seven membered ring carbocycles.[77] Diene precursors **238** and **240** were subject to ring-closing metathesis using 10 mol% **4** in refluxing dichloromethane to produce the corresponding α,β fused γ-lactones **239** and **241** in 85 and 89% yield, respectively.



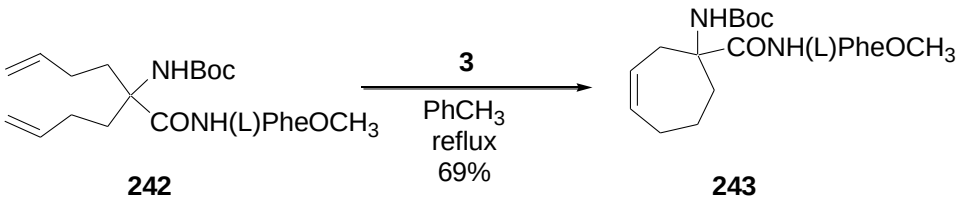
238 **239**



240 **241**

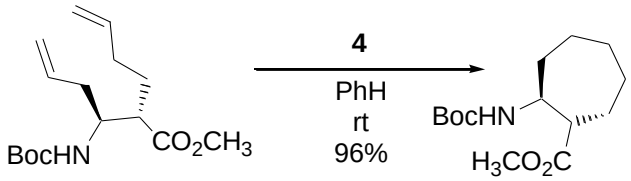
A number of research groups have used ring-closing metathesis to prepare conformationally constrained α- and β-amino acids containing seven membered ring carbocycles. Several examples of the synthesis of these interesting molecules are provided in the following text.

Kotha and co-workers used a ring-closing metathesis strategy in their synthesis of α,α-dialkylated constrained amino acids for the preparation of a conformationally constrained seven membered ring carbocyclic peptide derivative.[43] Treatment of **242** with 10 mol% of **3** in refluxing toluene gave the corresponding carbocycle **243** in 69% yield.



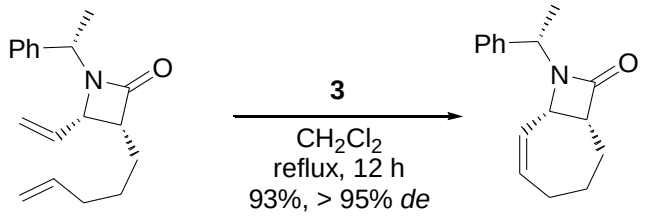
242 **243**

Abell and co-workers used their ring-closing metathesis strategy to synthesize a seven membered ring β-amino acids for incorporation into β-peptide mimetics.[46] Treatment of diene **244** with a 5 mol% of **4** in benzene provided the corresponding β-amino acid derivative **245** in 96% yield.



244 **245**

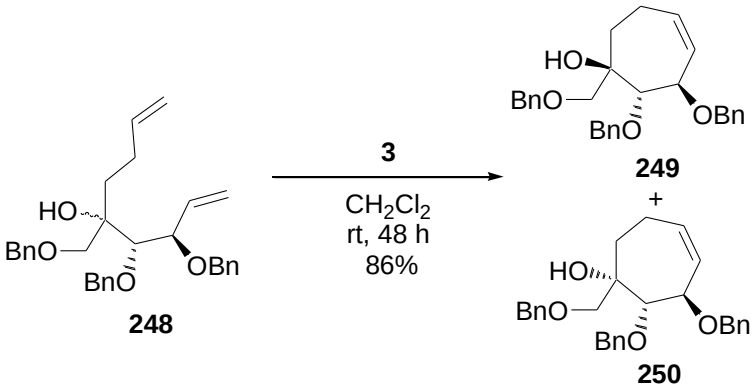
Davies and co-workers also employed a ring-closing metathesis strategy in their preparation of constrained seven membered ring β-amino acid derivative.[119] Treatment of diene precursor **246** with 4 mol% of **3** in refluxing dichloromethane gave the corresponding carbocyclic β-amino acid derivative **247** in 93% yield with greater than 95% diastereoselectivity.



246 **247**

A number of seven membered ring carbacyles have also been prepared using ring-closing metathesis as a key strategy. Many of these carbacycles are natural products or important synthons for the production of natural products. Several examples are highlighted below.

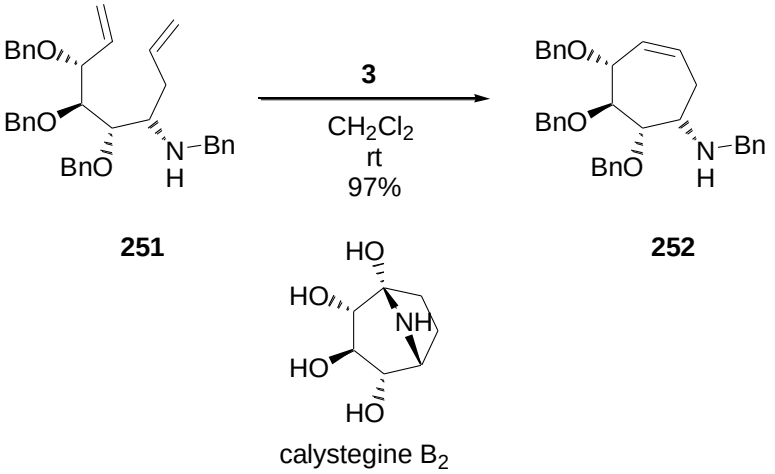
Zhang and co-workers employed a ring-closing metathesis strategy in the preparation of 8-oxa-bicyclo[3.2.1]octane derivatives from D-arabinose.[120] These molecules have been used as important precursors in the synthesis of a number of natural and unnatural products. Treatment of diene **248**, prepared in 3 steps from D-arabinose, with a catalytic amount of **3** in dichloromethane provided corresponding cycloheptene products **249** and **250** in 86% yield as a mixture of diastereomers.



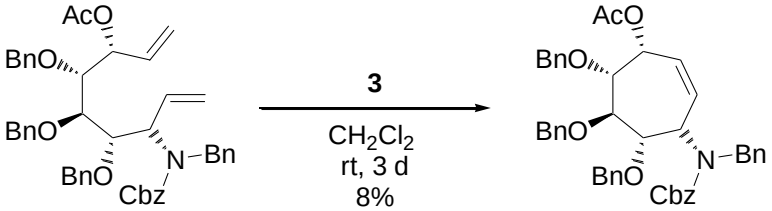
248 **249** **250**

Hanna and Boyer applied an olefin metathesis strategy to the synthesis of (+)-calystegine B_2, a member of a family of compounds known for their nutritional mediation properties in the plant rhizoshpere.[121] Ring closing metathesis of **251** in the presence of 8 mol% **3** in dichloromethane

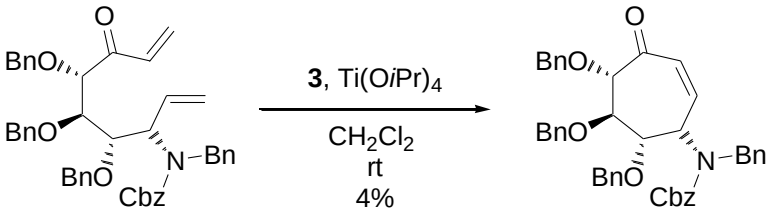
furnished the corresponding carbasugar in near quantitative yield. Oxidation followed by hydrogenolysis and deprotection (not shown) furnished the desired product. Madsen and Skaanderup applied a similar strategy in their synthesis of (+)-calystegine B_2.[122]



Marco-Contelles and Opazo used an alternative ring-closing metathesis strategy in an effort to improve the synthesis of (+)-calystegine B_2.[123] Acetate **253** was subject to ring-closing metathesis using 10 mol% **3** in dichloromethane to furnish the corresponding carbacycle **255** in only 8% yield. In an effort to improve this yield, they reacted ketone **255** under similar conditions using $Ti(OPr)_4$ as a promoter. Unfortunately, the desired carbacycle **256** was produced in only 4% yield. The authors hypothesized that the low yields were due to steric interactions between the catalyst and the functional groups on the carbacycle. Hanna and co-workers used a similar strategy for the preparation of seven-membered ring carbocyclic precursors as to derivatives of the calystegine family from methyl 6-deoxy-6-iodo-3,4-isopropylidene-2-*O*-(tert-butyldimethyl-silyl)-D-galactopyranoside and methyl 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene- D-galactopyranoside with improved yields.[124]

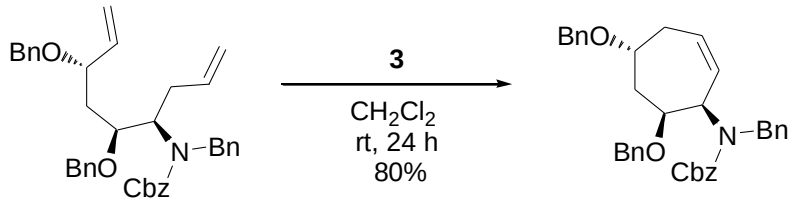


253 **254**

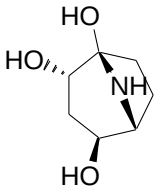


255 **256**

Csuk and co-workers employed a ring-closing metathesis strategy in their total synthesis of calystegine A_7, another member of the calystegine family.[125] Olefin metathesis of **257** using 10 mol% of **3** in dichloromethane gave the desired cycloheptene derivative **258** in 80% yield.

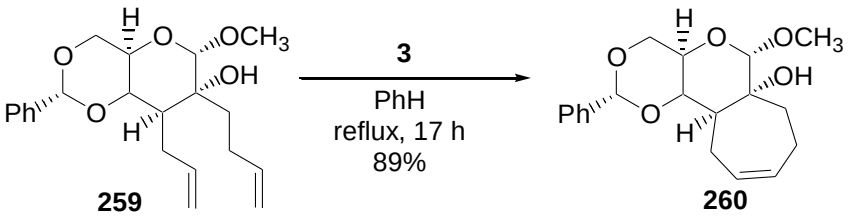


257 **258**

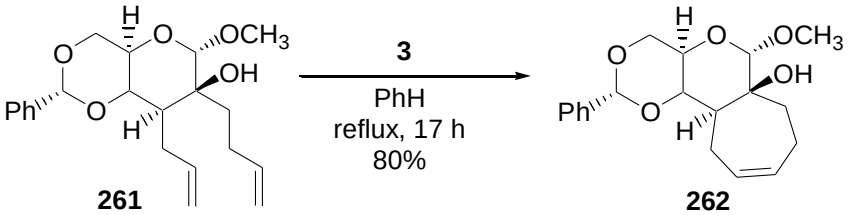


calystegine A_7

Holt and co-workers applied their ring-closing metathesis strategy to synthesize a number of enantiomerically pure annulated carbohydrate systems containing seven membered ring carbocycles.[90] Treatment of dienes **259** and **261** with a catalytic amount of **3** in benzene gave the corresponding 6,6,7-carbocycles, *cis*-**260** and *trans*-**262**, in 89 and 80% yield, respectively.



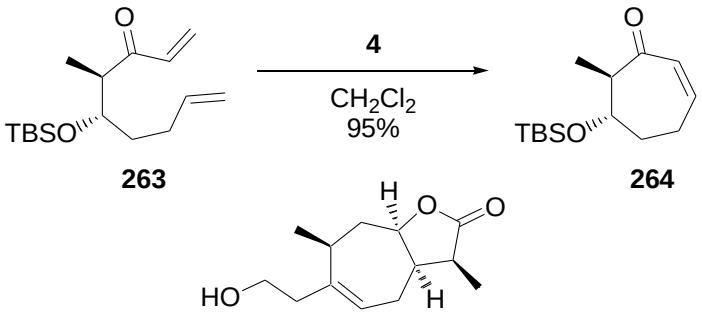
259 **260**



261 **262**

A number of terpenes and terpenoids bearing seven membered rings have also been synthesized using an olefin metathesis strategy. Several examples are provided below.

Ring-closing metathesis was used by Shishedo and co-workers in their total synthesis of (+)-sundiversifolide, a herbicide.[126] Treatment of enone **263** with 5 mol% of **4** in refluxing dichloromethane gave the corresponding α/β unsaturated ketone **264** in 95% yield.

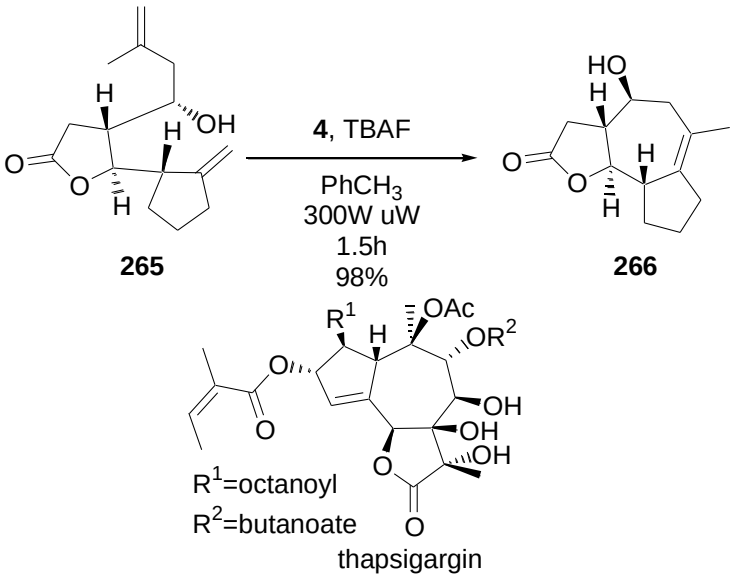


263 **264**

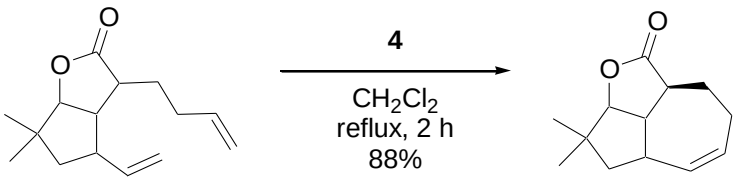
(+)-sundiversifolide

Nosse and co-workers used ring-closing metathesis to synthesize a 5,7,5-fused lactone.[127] These tricyclic frameworks are the core components of the thapsigargin family, a family of sesquiterpene lactones with the ability to restore apoptotic function in cancer cell lines. Thapsigargins are currently under investigation as potential therapeutics for the treatment of prostate cancer. Reaction of lactone **265** with 10 mol% **4** and a catalytic amount of TBAF in toluene while irradiating at 300W gave the corresponding tricyclic system **266** in near quantitative. The authors noted that nitrogen sparging to remove ethylene was used to force the equilibrium of the reaction towards

the products. This presents a slight improvement over Ley's approach to the same tricyclic core using a ring-closing metathesis strategy.[128]



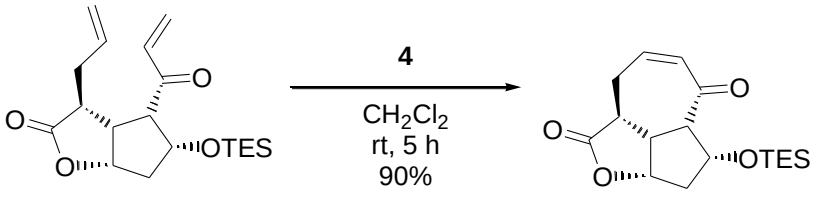
Krafft and co-workers used olefin metathesis to prepare several 'inside-outside' medium sized rings as scaffolds for natural products synthesis.[129] Reaction of bicyclic lactone **267** with 10 mol% of **4** in refluxing dichloromethane gave the corresponding tricyclic lactone **269** in 88% yield after only two hours.



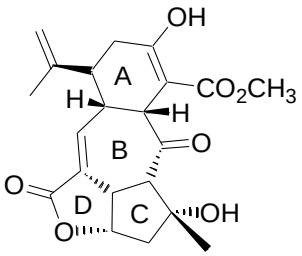
267 **268**

Mehta and co-workers employed a ring-closing metathesis strategy to generate the seven membered ring carbocycle of the tricyclic core of rameswaralide in their ongoing efforts to synthesize this compound.[130] In addition to having a complex and highly functionalized 5,7,6-fused tricarbocyclic core incorporating a stable enol functionality, and six stereogenic centers, ramewaralide is a potential anti-inflammatory compound with activity against TNF-α, IL-15, IL-5, and Cox_2. Treatment of enone **269** in the presence of 10 mol% **4** in dichloromethane gave the corresponding tricyclcic system **270** in 90% yield. Srikrishna and Dethe employed a

similar ring-closing metathesis strategy for the BC and AB rings of rameswaralide (not shown).[131]



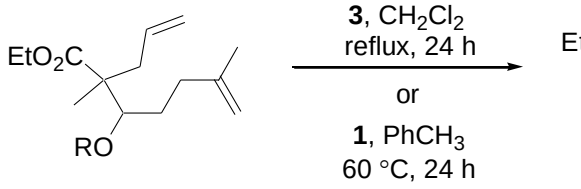
269 **270**



rameswaralide

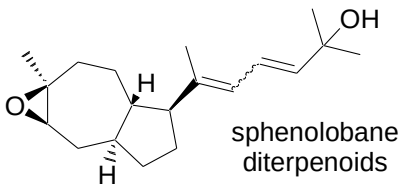
Ring-closing metathesis has also been applied to the synthesis of a number of terpene derivatives containing seven membered rings. Several key examples are illustrated below.

Tori and co-workers used ring-closing metathesis as a key step in the synthesis of several liverwort diterpenes in an effort to determine the absolute configuration of these compounds.[132] Catalysts **1** and **3** were employed in the synthesis of the seven membered ring carbocyclic core of two sphenolobane-type diterpenoids. Higher yields were obtained when **271**, **273**, and **275** were reacted with **1** in comparison to **3**. However, twice the molar percentage of **1** was required.



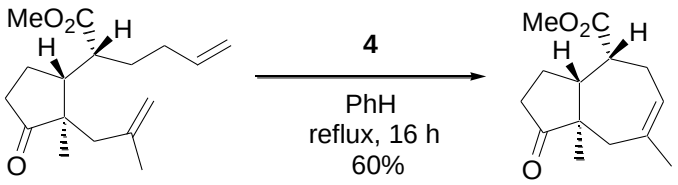
271, R = TES
273, R = OAc
275, R = MOM

272, R = TES **3**-94%, **1**-99%
274, R = OAc **3**-61%, **1**-84%
276, R = MOM **3**-21%, **1**-37%



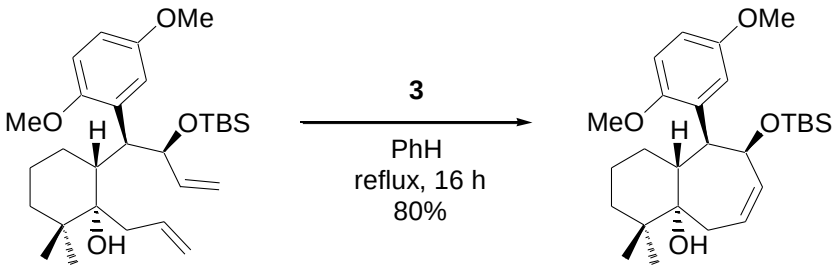
sphenolobane diterpenoids

Olefin metathesis was also used as a key step in Wicha and co-workers synthesis of the carbocyclic core of several di- and sesquiterterpenes.[133] Reaction of **277** with 5 mol% of **4** in refluxing benzene gave the corresponding bicyclic system **278** in 60% overall yield.

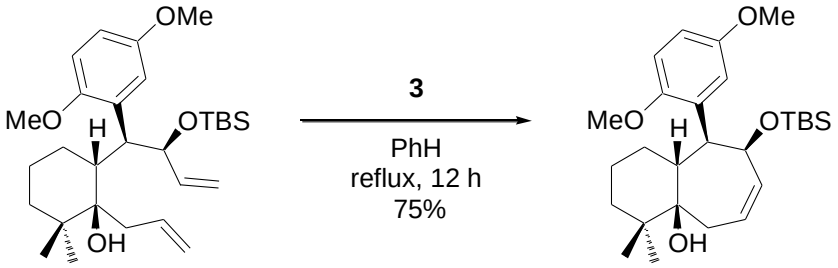


277 **278**

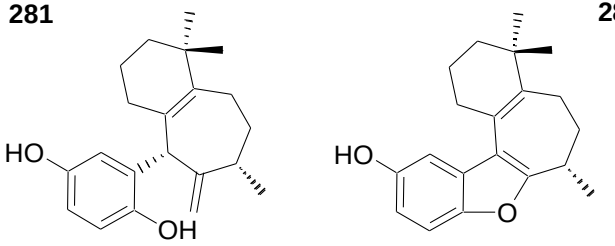
Mehta and co-workers used a ring-closing metathesis approach in the synthesis of (±) frondosins A and B.[134] These novel meroterpenoids have shown promise as therapeutics for the treatment of inflammatory diseases. Ring-closing metathesis of **279** and **281** in the presence of a catalytic amount of **3** in refluxing benzene gave the corresponding bicyclic tertiary alcohols **280** and **282** in 75 and 85% yields, respectively.



279 280

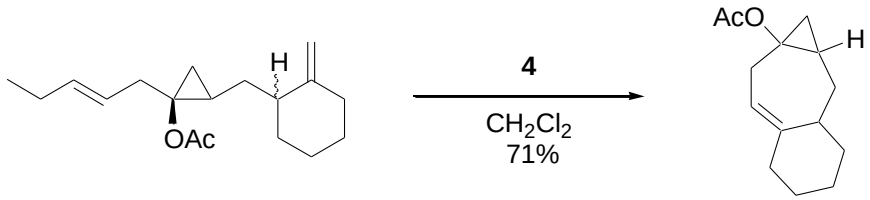


281 282



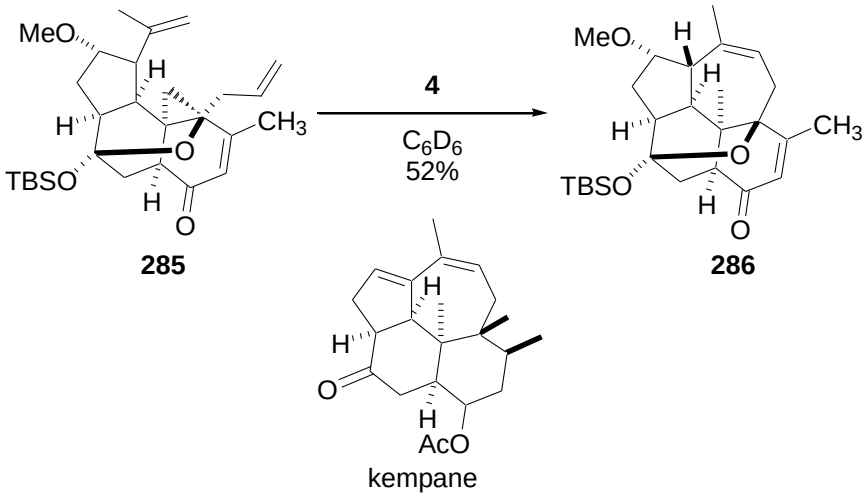
frondosin A **frondosin B**

Building on the work of Llyod-Jones and co-workers,[135] Cha and co-workers used cyclopropanols in an effort to determine the conformational constraints of ring-closing metathesis.[136] Treatment of diene **283** with 10 mol% of **4** in DCM gave the corresponding tricyclic system **284** in 71% yield as a single diastereomer. The authors noted that only one isomer underwent ring-closing metathesis.



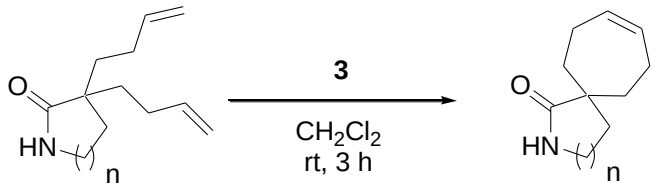
283 **284**

A ring-closing metathesis strategy was employed by Burnell and co-workers in the synthesis of the tetracyclic core found in several kempane derivatives.[137] Kepanes, a group of complex tetracyclic diterpenes, are key defense molecules for many species of termites. The tetracyclic core, incorporating seven contiguous stereocenters, is especially challenging from a synthetic stand point. Most notably, the generation of the cycloheptene ring proved a formidable challenge in a previous synthesis of this compound.[138] A ring-closing metathesis strategy employing diene of **285** and 3 mol% of **4** in deuterated benzene was moderately successful in generating the cycloheptene ring, and subsequently the corresponding tetracycle **286** in 52% yield.



Several spirocyclic compounds incorporating seven membered rings have also been prepared by ring-closing metathesis. These molecules are important structural motifs that have also been found in a number of natural and unnatural products containing seven membered rings.

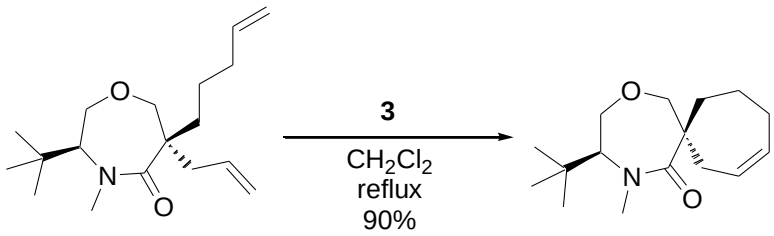
Brimble and co-workers used their double-alkylation/ring-closing metathesis approach in the synthesis of spiroimines containing seven membered ring carbocycles.[106] Lactams **287**, **289** and **291** were subject to ring-closing metathesis using 5 mol% of **3** in dichloromethane to provide the corresponding 5,7-(**288**), 6,7-(**290**) and 7,7-(**292**) spirolactams in upwards of 90% yield. Interestingly, the yields of seven membered ring spirolactams are higher than the analogous six membered ring spirolactams, presumably due to the greater flexibility of the diene precursors of the seven membered rings.



287, n = 1
289, n = 2
291, n = 3

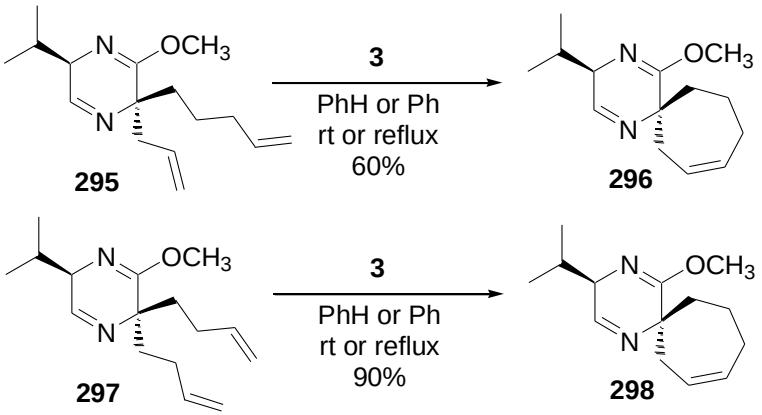
288, n = 1 93%
290, n = 2 95%
292, n = 3 92%

Hughes and co-workers extended their ring-closing metathesis strategy as a general route to the synthesis of enantiopure seven membered ring spirocarbocycles using zizaene as a chiral auxiliary.[67] Diene precursor **293** required 5 mol% catalyst loading of **3** in refluxing dichloromethane to achieve a 90% yield of the desired spirocycle **294**. Several additional seven membered ring carbocycles were prepared using zizaene derivatives.

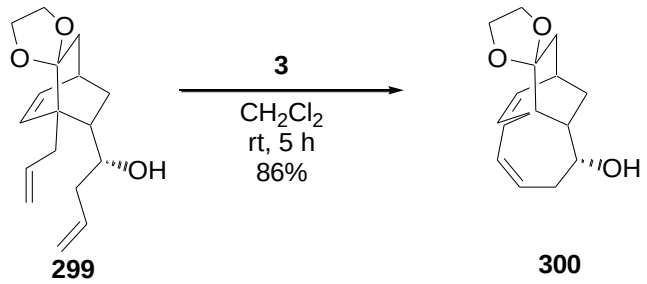


293 **294**

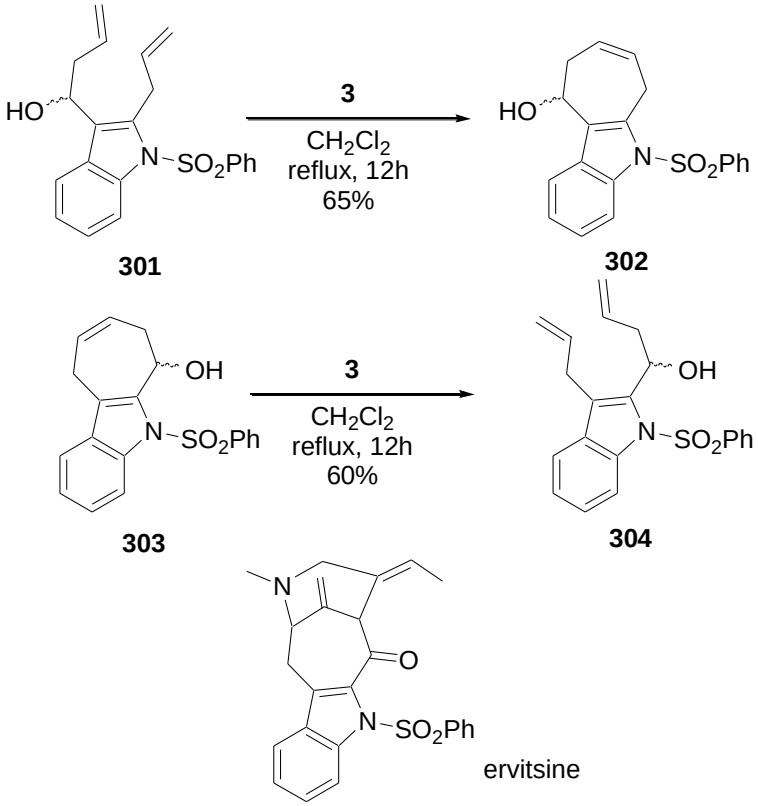
Undheim and co-workers employed their olefin metathesis strategy to prepare seven membered ring spirocyclic carbocycles for use as templates in natural product synthesis.[68] Reaction of diene **295** with 2 mol % catalyst loading of **3** furnished the corresponding spirocycle **296** in only 60% yield. However when diene **297** was reacted under similar conditions, the desired spirocycle **298** was obtained in 90% yield. Although no specific reason is given for the lower yield of **296**, it is possible that conformational constraint may have played a role.



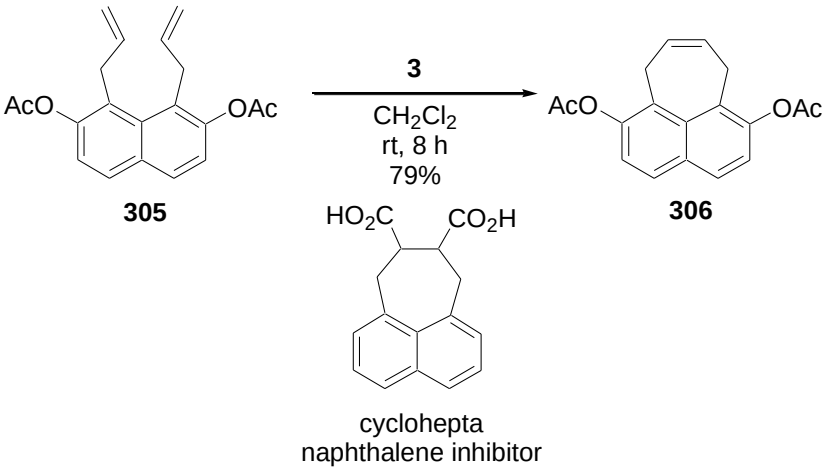
Singh and co-workers applied their methodology for the synthesis of embellished spiro-fused bicyclo[2.2.2]octane systems to the synthesis of seven membered ring carbocyclic derivatives.[139] Treatment of diene **299** with 10 mol% of **3** in dichloromethane gave the corresponding spirocyclic carbocycle **300** in 86% yield.



Bennasar and co-workers used ring-closing metathesis prepare 2,3-fused indole derivatives which are prominent heterocyclic compounds in a number of natural products such as ervitsine.[140] Treatment of dienes and **301** and **303** with 10 mol% **3** in refluxing dichloromethane gave the corresponding cyclohepat[*b*]indoles **302** and **304** in 65 and 60% yields, respectively. The reduced yields in these examples were likely do to conformational constraints.

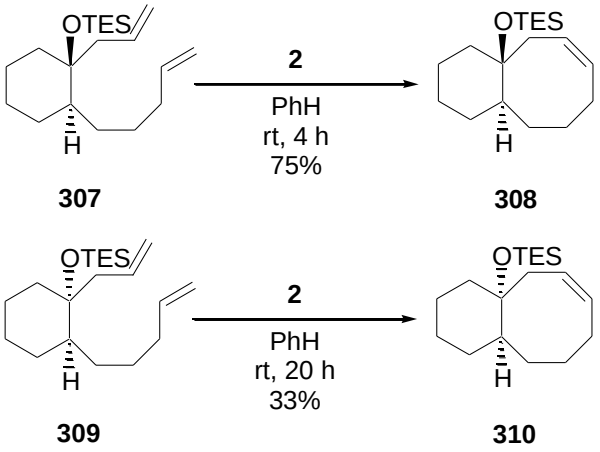


Chattopadhyay and co-workers applied an olefin metathesis strategy in the preparation of a carbocyclic naphthalene derivative containing a seven membered ring carbocycles with goal of generating cycloheptanathalene, an important enzyme inhibitor.[141] Reaction of diene **305**, prepared in two steps from commercially available 2,7-dihydronaphtalne using a double Claisen rearrangement/acetylation sequence, was treated with 5 mol% **3** in dichloromethane to produce the corresponding tricyclic system **306** in 79% yield.



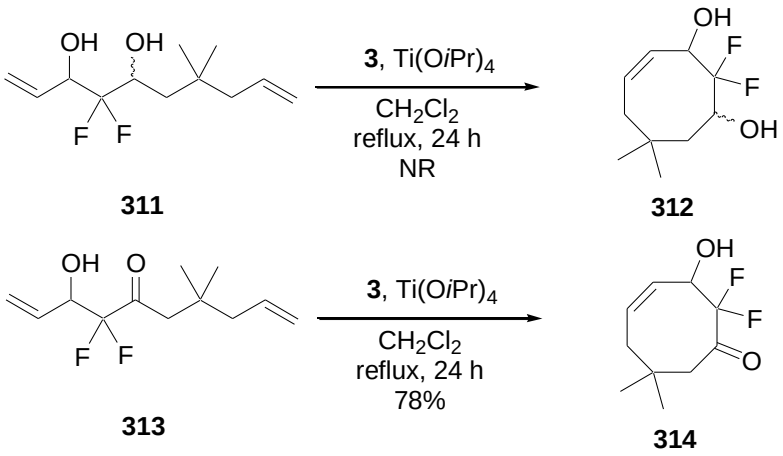
5.3.5.5 Eight Membered Rings

The synthesis of cycloctonoids via ring-closing metathesis strategy was first addressed by Grubbs and co-workers in 1995.[142] Their initial studies showed that intermolecular cross metathesis was favored over the intramolecular ring-closing process. This problem was eventually solved by building conformational constraints into the diene precursors such that preorganization favors ring-closing metathesis. One of the first examples involved a 1,2-*trans*-disubstituted cyclohexane derivative **307**, which, when treated with 5 mol% of **2** in benzene gave the corresponding fused bicyco[6.4.0]dodecane derivative **308** in 75% yield after only four hours. In contrast, treatment of the *cis*-cycohexane precursor **309** under similar reaction conditions gave only 33% yield of the desired product **310** and a number of side products. The authors speculated that the *trans* isomer assisted in orientating the two olefin partners in close proximity resulting in favourable ring closure.

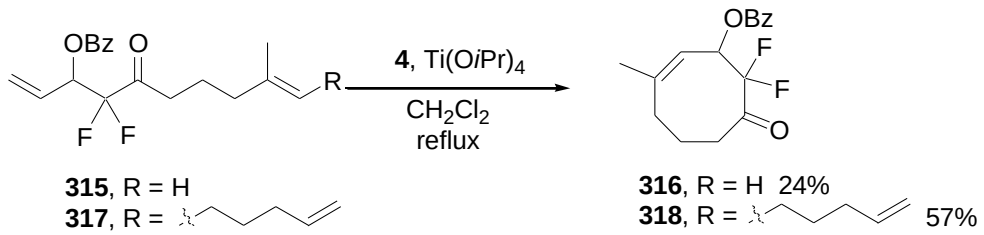


Since Grubbs' initial report, a number of other research groups have prepared eight membered ring carbocycles using ring closing metathesis. Several examples are highlighted below.

Percy and co-workers used a ring-closing metathesis approach to synthesize highly functionalized difluorinated cylooctenones.[143] These compounds have been used in the design and synthesis of protease inhibitors, as they are effective transition state mimetics. In addition, the electrophilic nature of the ring system makes them attractive for targets for adduct formation with active site nucleophiles such as serine. Reaction of allyl alcohol **311** with or without the presence of $Ti(OiPr)_4$ (as a precatalyst) and 5 mol% of **3** failed to provide the corresponding cyclooctenol **312**. However, when β-hydroxy ketone **313** was used instead, the corresponding difluorinated cyclooctenone **314** was produced in 78% yield. The authors later reported a shorter route to a similar difluorinated system using the same ring-closing metathesis approach (not shown). Unfortunately, the substrate required longer reaction times (166 h) and gave lower yields.



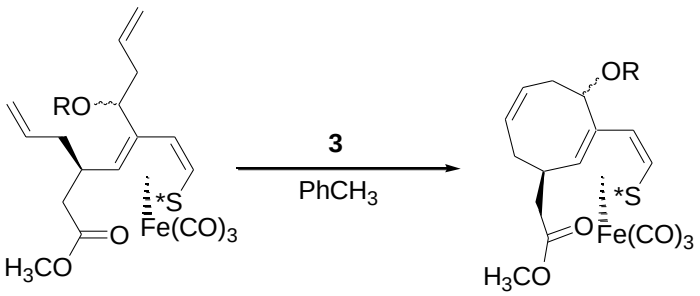
Percy and co-workers applied a similar strategy in the synthesis of a trisubstituted cyclooctene in an effort to determine the limits of relay-ring-closing metathesis.[144] Reaction of diene **315** where R = H in the presence of **4** (three additions: 10 mol%, then 5 mol% then 5 mol% over a 12 day period) in the presence of $Ti(Oi\text{-}Pr)_4$ in refluxing dichloromethane gave the corresponding octocycle **316** in 24% yield. However, when a relay approach was employed, diene **317** (R = $(CH_2)_3CHCH_2$) gave the corresponding cyclooctene **318** in 57% yield, a marked improvement over the previous synthesis.



315, R = H
317, R =

316, R = H 24%
318, R = 57%

Paley and co-workers used enantiopure n^4-(1-sulfinyldiene)iron(0) tricarbonyl complexes as templates for the enantioselective construction of eight membered ring carbocycles.[117] Enantiopure homoallyl alcohol adducts **319** and **321**, when treated with 8 mol% of **3** in toluene, gave the corresponding octocycles **320** and **322** as single diasteromers in 87 and 89% yield, respectively.

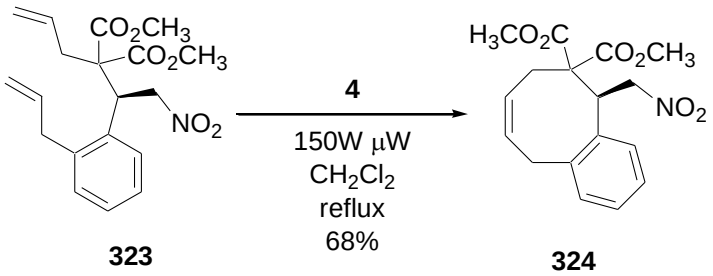


R=MOM
319, *(7S)*
321, *(7R)*

R=MOM
320, *(7S)* 87%
322, *(7R)* 89%

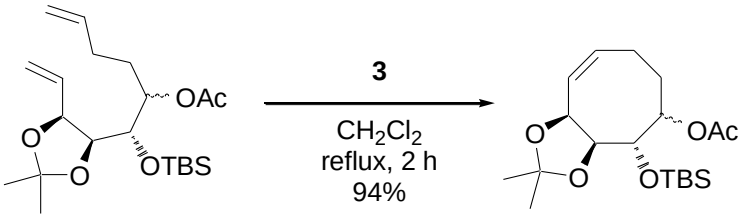
Porco and co-workers used a diversity oriented/ring-closing metathesis approach to construct complex systems as small molecule protein modulators. Microwave irradiation of Michael adduct **323** in the presence of a catalytic amount of **4** in refluxing dichloromethane gave the corresponding

cyclooctene derivative **324** in near quantitative yield. The authors reported several additional examples.



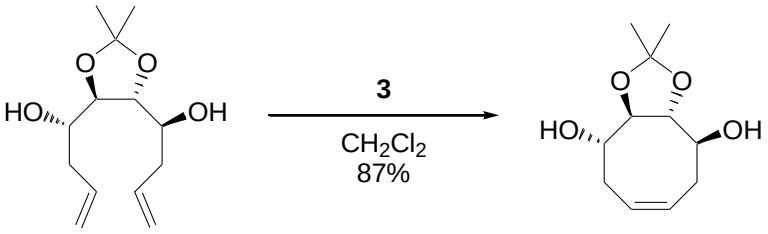
323 **324**

Hanna and co-workers prepared several eight-membered ring carbocyclic rings from methyl 6-deoxy-6-iodo-3,4-isopropylidene-2-*O*-(tert-butyldimethyl-silyl)-D-galactopyranoside and methyl 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene- D-galactopyranoside using a ring-closing metathesis strategy.[124] Diene **325**, prepared in 2 steps from methyl 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene- D-galactopyranoside, gave the corresponding cyclooctene derivative **326** in 94% yield when treated with 5 mol% **3** in refluxing dichloromethane. Several derivatives with various protecting groups were also reported, and the authors applied this strategy to construct a number of carbohydrate-based cyclooctanoids in a later report.[145]



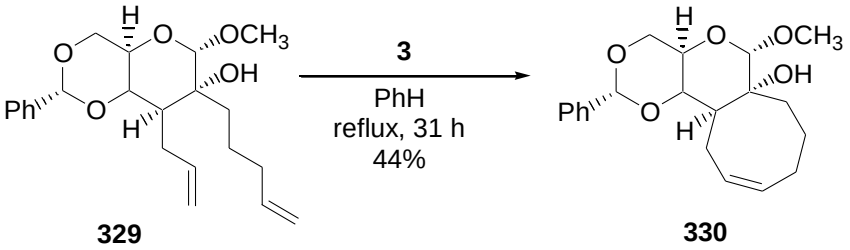
325 **326**

Le Merrer and co-workers employed a ring-closing metathesis strategy to access a number of polyfunctionalized cyclooctane carbasugars as part of their ongoing efforts to prepare new glycosidase inhibitors and non-insulino-based compounds for the treatment of diabetes.[146] Olefin metathesis of **327** using up to 13 mol% in dichloromethane gave the corresponding *cis*-cyclooctene product **328** in 87% yield.

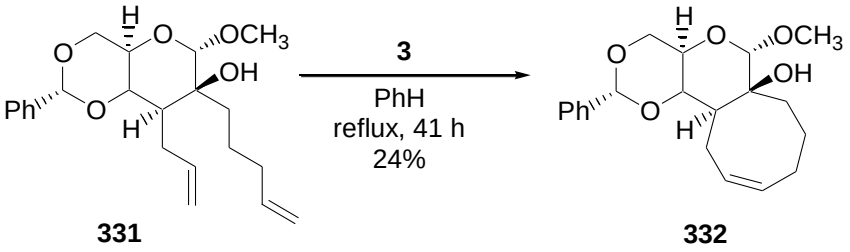


327 **328**

Holt and co-workers employed a ring-closing metathesis strategy in the synthesis of enantiomerically pure annulated carbohydrate systems containing containing eight membered ring carbocycles as precursors for the synthesis of natural products.[90] Treatment of 5-hydroxy-1,9-diene precursors **329** and **331** with a 9 mol% **3** in refluxing benzene gave the corresponding 6,6,8 *cis*-**330** and *trans*-**332** annulated systems in 44 and 24% yield, respectively. The authors noted that in addition to the conformational constraints, the tertiary alcohol likely played a role in transformation.

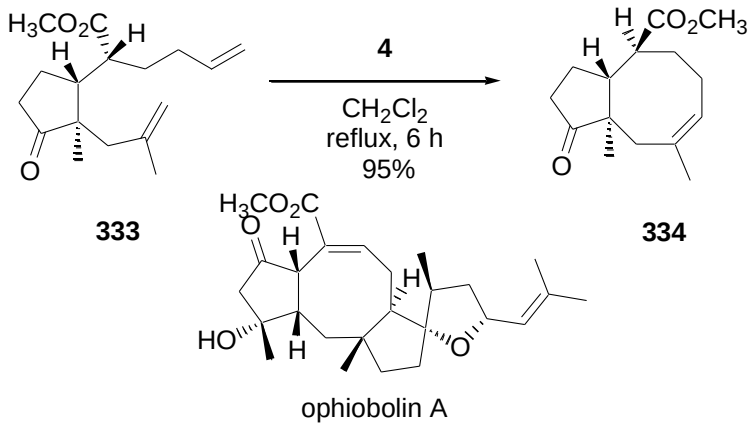


329 **330**

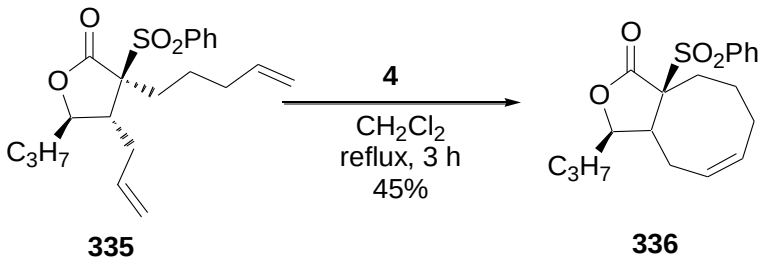


331 **332**

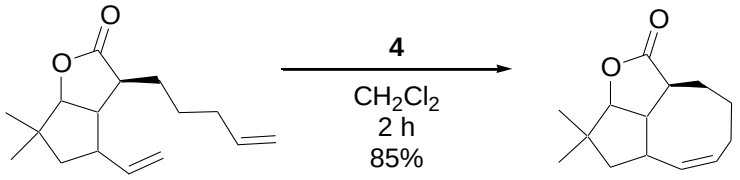
In their continuing efforts to synthesize the carbocyclic core of several di- and sesquiterterpenes such as ophiobolin A, Wicha and co-workers constructed a 5,8-fused carbocyclic system.[147] Reaction of **333** with 5 mol% of **4** in refluxing dichloromethane gave the corresponding bicyclic system **334** in 95% overall yield.



Martin and co-workers applied their ring-closing metathesis strategy to the synthesis of fused eight membered ring γ-lactones.[77] Diene precursor **335** was subject to ring-closing metathesis using 10 mol% **4** in refluxing dichloromethane to produce the corresponding α,β fused γ-lactone **336** in 45% yield.

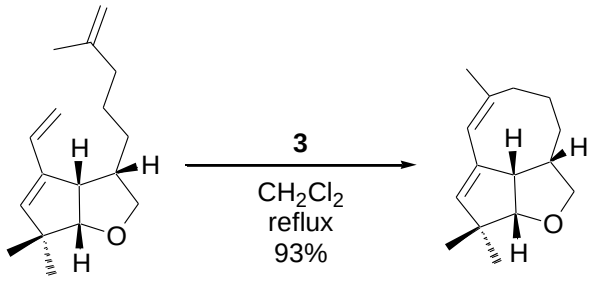


Krafft and co-workers used ring-closing metathesis to prepare several 'inside-outside' medium sized rings, including eight membered carbocycles, as scaffolds for natural products synthesis.[148] Reaction of bicyclic lactone **337** with 10 mol% of **4** in refluxing DCM gave the corresponding tricyclic lactone **338** in 85% yield after only two hours. Additional eight membered ring carbocycles incorporating functional handles were also reported, though significantly higher catalyst loadings (upwards of 50%) were required to achieve high yields. Attempts to apply this strategy to the synthesis of nine membered ring carbocycles led solely to the production of dimeric products.

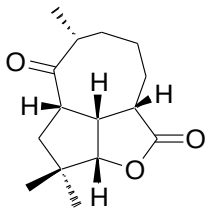


337 **338**

Paquette and co-workers employed a ring-closing metathesis strategy in their total synthesis of (+)-asteriscanolide.[149] The sesquiterpenoid framework of (+)-asteriscanolide consists of a rather uncommon bicyclo[6.3.0]undecane ring system bridged by a butyrolactone fragment. Ring-closing metathesis with 5,5-diene precursor **339** using 10 mol% **3** in refluxing dichloromethane proceeded smoothly to give the corresponding carbocyclic core (**340**) of (+)-asteriscanolide in 93% yield. The authors speculated that limited conformational flexibility of the diene substrate was critical to the high yields achieved in this transformation. Four additional steps were required to access (+)-asteriscanolide. Krafft and co-workers applied a similar ring-closing metathesis strategy in their synthesis of (+)-asteriscanolide.[150]

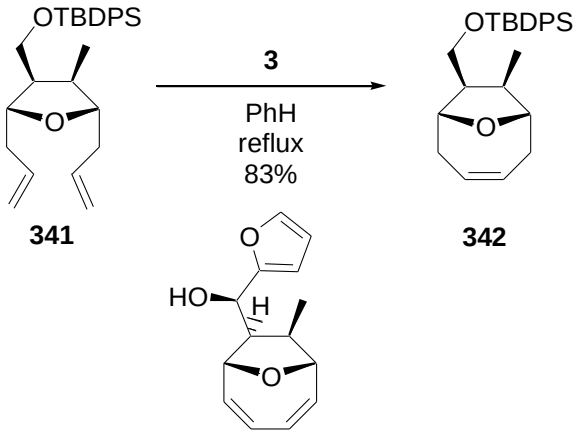


339 **340**

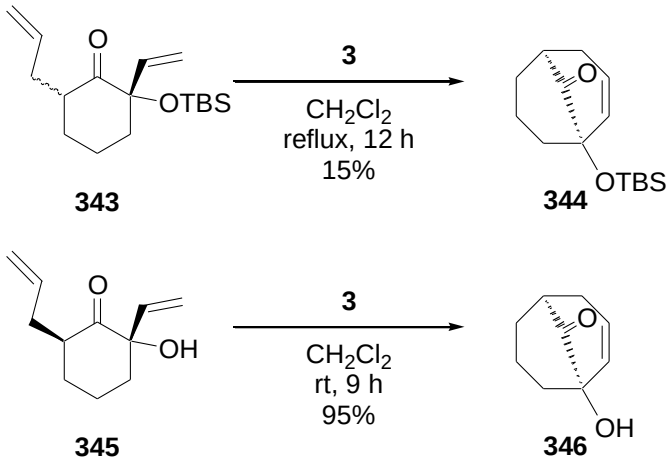


(+)-asteriscanolide

Tadoano and co-workers used olefin metathesis as a key step in their synthesis of (±)-mycoepoxydiene, a novel octacycle with an oxygen-bridged-[4.2.1]nona-2,4-diene core.[151] Reaction of diene **341** with 20 mol% 3 in refluxing benzene gave the corresponding oxygen-bridged cyclooctene derivative **342** in 83% yield. The authors noted that a highly dilute solution was essential in obtaining high yields of the desired product.

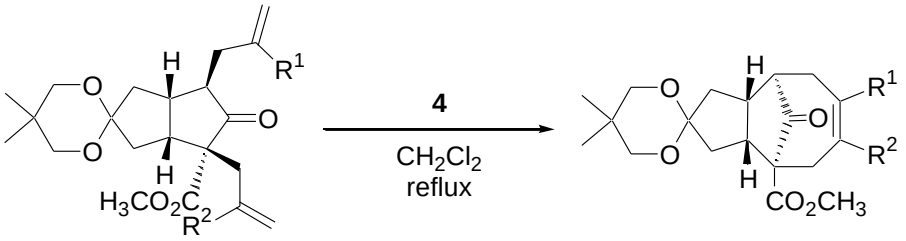


Mascarenas and co-workers employed a similar strategy in the synthesis of eight membered ring carbacycles using a ring-closing metathesis/ring fragmentation strategy.[152] A diastereomeric mixture of dienes **343** were subject to ring-closing metathesis using 5 mol% **3** in refluxing dichloromethane to provide bicyclic **344** as the only product in 15% yield. The unreactive *trans* isomer was easily separated from the bicyclic system after deprotection of the silyl ether. Further studies showed that enantiopure alcohol **345** underwent ring-closing metathesis using 5 mol% **3** in dichloromethane at room temperature in only 9 hours to give the corresponding bicylic system **346** in 95% yield. Treatment with lead acetate gave the corresponding cycloctanoid in near quantitative yields (not shown).



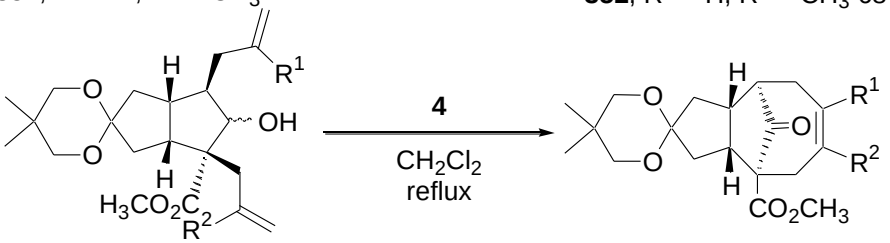
Rodriguez and co-workers applied a similar strategy in the synthesis bicyclo[4.2.1]nonane derivatives as precursors to functionalized

cyclooctanes.[153] Reaction of ketones **347**, **349** and **351** in refluxing dichloromethane with 2 mol% **4** gave the corresponding cyclooctene derivatives in modest yields (68-74%). Unfortunately, these transformations required long reactions times and the decreased yields observed with substrates **350** and **352** were presumably due to sterics and catalyst decomposition. On the other hand, treatment of alcohols **353** and **355** using only 1 mol% of **4** in refluxing dichloromethane furnished the corresponding carbocycles in high yields (92-98%) in only two to three hours, exhibiting the effect of a free hydroxyl group on the ring-closing metathesis reaction.



347, R^1 = H, R^2 = H
349, R^1 = CH_3, R^2 = H
351, R^1 = H, R^2 = CH_3

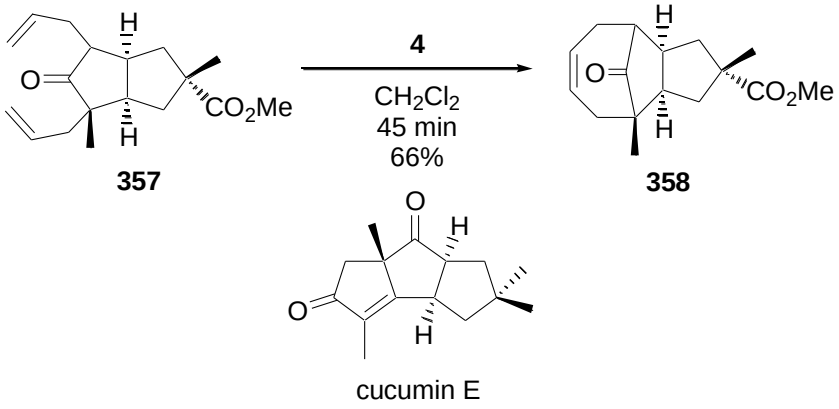
348, R^1 = H, R^2 = H 84%
350, R^1 = CH_3, R^2 = H 70%
352, R^1 = H, R^2 = CH_3 68%



353, R^1 = H, R^2 = H
355, R^1 = H, R^2 = CH_3

354, R^1 = H, R^2 = H 92%
356, R^1 = H, R^2 = CH_3 98%

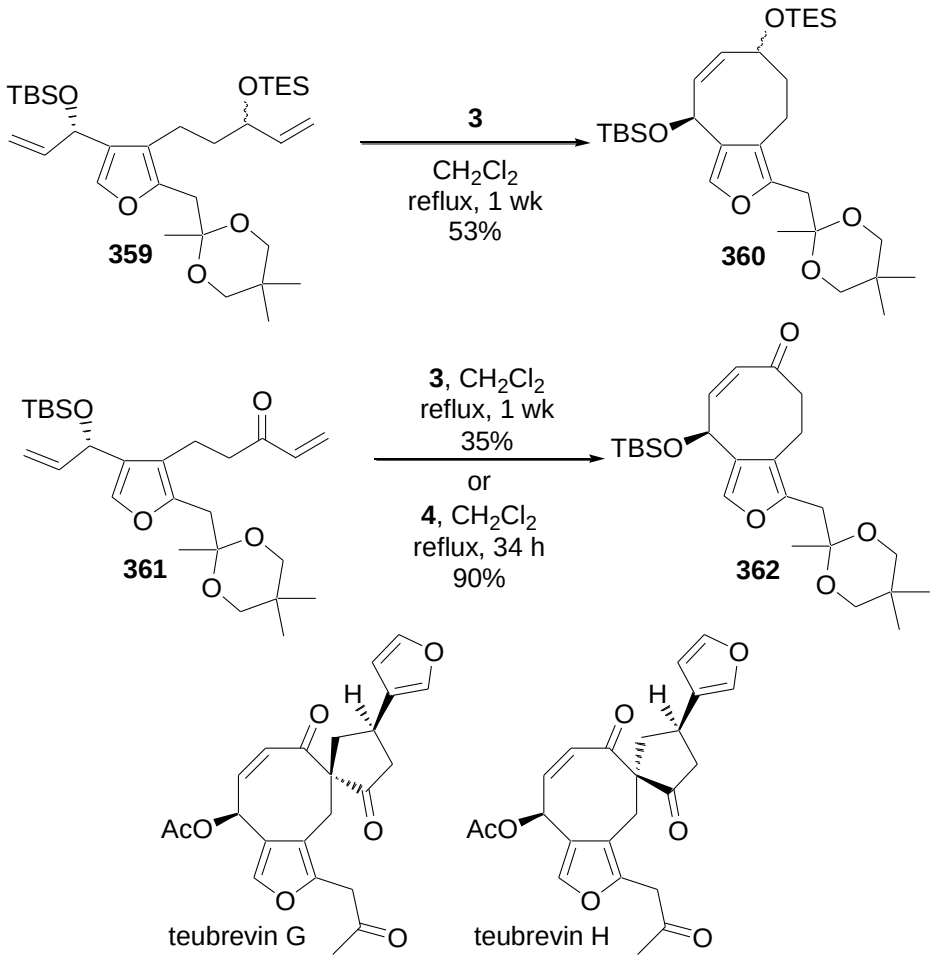
Singh and co-workers employed Rodriquez's ring-closing metathesis strategy in the stereoselective synthesis of expanded homologs of natural products of the cucumin family. These compounds exhibit cytotoxic and antibacterial properties.[154] Diquinane derivative **357** underwent ring-closing metathesis in the presence of **4** in dichloromethane to provide the corresponding tricyclic carbocycle **358** in 66% yield.



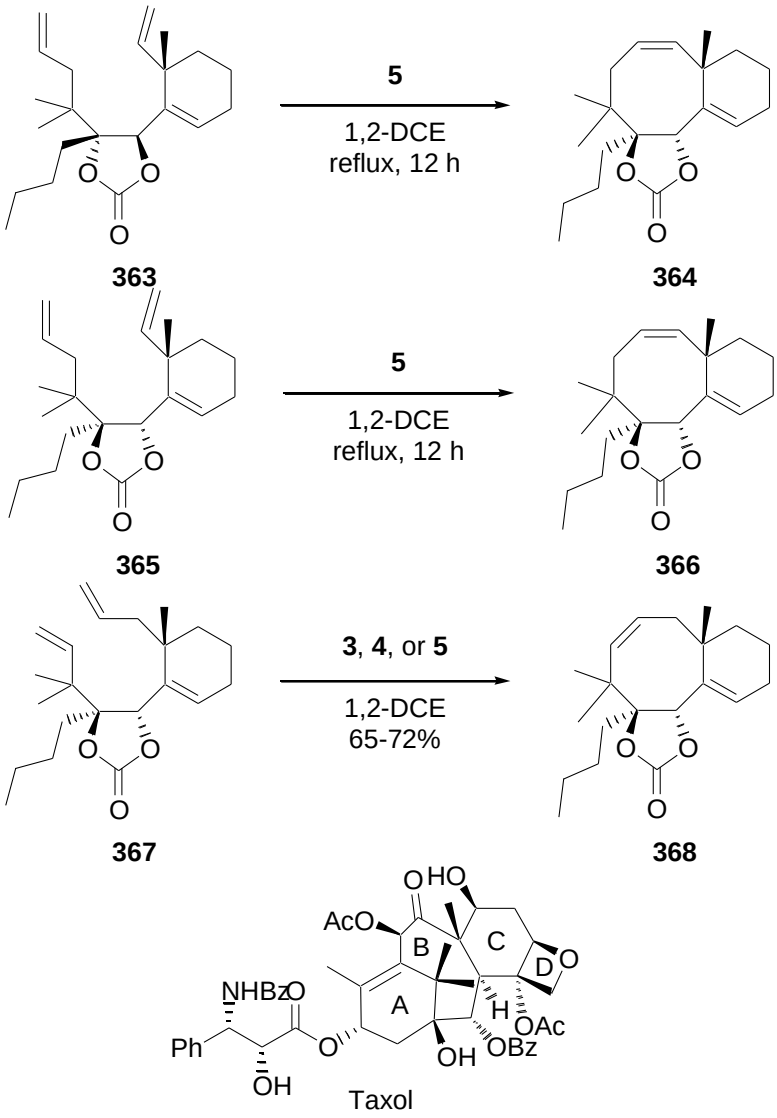
357 **358**

cucumin E

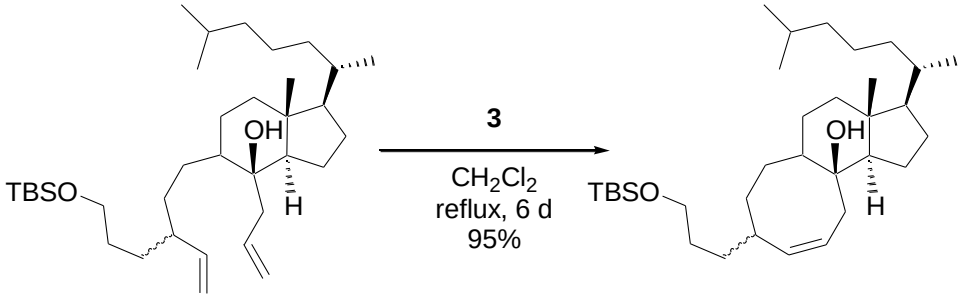
Paquette and Efremov applied a ring-closing metathesis strategy in the first total synthesis of the rearranged neo-clerodanes, teubrevin G and teubrevin H.[155] These compounds feature a cycloctanene core fused and spiroannulated to smaller oxygen containing rings. Treatment of triethylsilyl ether **359** with 30-35 mol% of **3** in refluxing dichloromethane for one week gave the corresponding fused octocycle **360** in 53% yield. Olefin metathesis with enone **361** under similar reaction conditions gave only 35% yield of the desired product **362**. However, reaction of enone **361** with 10 mol% of **4** in refluxing dichlromethane gave the desired product in 90% yield after 34 hours.



Prunet and co-workers applied a ring-closing metathesis strategy in their synthesis of the BC bicycles of Taxol®, a potent treatment for breast and ovarian cancer. Previous research showed that dienes **363** and **365**, when treated with 10 mol% **3** in refluxing benzene or 5 mol% **5** in refluxing 1,2-dichloroethane, gave the corresponding C9-C10 cyclooctene derivatives in good yield.[156] However, in an effort to prepare the cyclooctene system via ring-closing metathesis at the C10-C11 position, the authors noted that only diene **367** underwent ring-closing metathesis, suggesting a higher energy barrier to preorganzation for these substrates.[157] The authors explored a number of catalysts and showed that this strategy could be extended to produce the desired bicyclic C10-C11 system using catalysts **3** (30 mol%), **4** (10 mol%), and **5** (5 mol%) in 65, 69, and 72% yield, respectively.

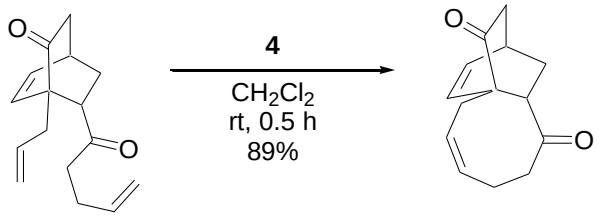


Granja and co-workers employed a ring-closing metathesis strategy in the synthesis of a novel steroid-like polycyclic system incorporating a 6,8,6-fused carbocyclic system.[158] These molecules purportedly mimic the putative transition state structure of the isomerisation reaction of previtamin D_3 to vitamin D_3. Treatment of diene **369** with near quantitative amounts of **3** in refluxing dichloromethane gave the desired tricyclic system **370** as a mixture of diastereomers in 95% yield after six days. In a later report, several additional examples bearing various substitutions patterns were reported.[159]



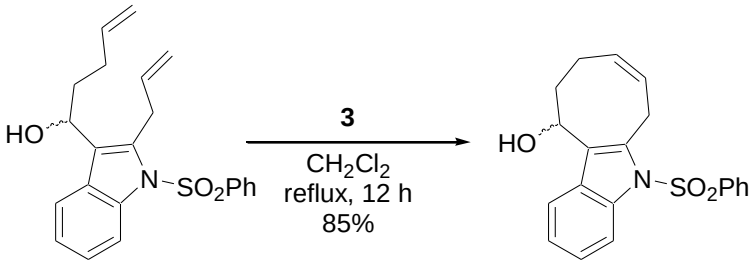
369 **370**

Singh and co-workers also applied their methodology for the synthesis of embellished spiro-fused bicyclo[2.2.2]octane systems to the synthesis of eight membered ring carbocyclic derivatives.[160] Treatment of diene **371** with 5 mol% of **4** in dichloromethane gave the corresponding spirocyclic carbocycle **372** in 89% yield.



371 **372**

Bennasar and co-workers used ring-closing metathesis prepare 2,3-fused indole derivatives containing eight membered ring carbocycles.[161] Treatment of diene and **373** with 10 mol% **3** in refluxing dichloromethane gave the corresponding cyclooctindole derivative **374** in 85 yield.

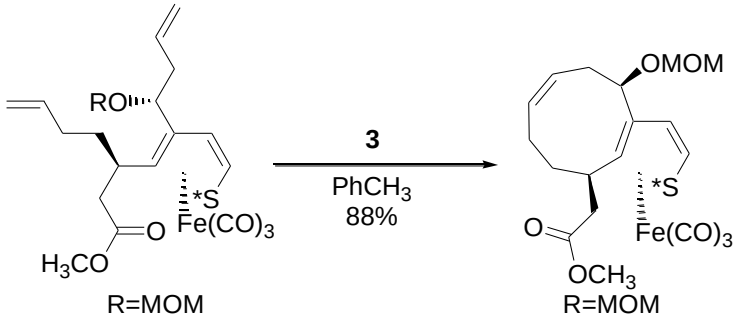


373 **374**

5.3.5.6 Large Membered Rings

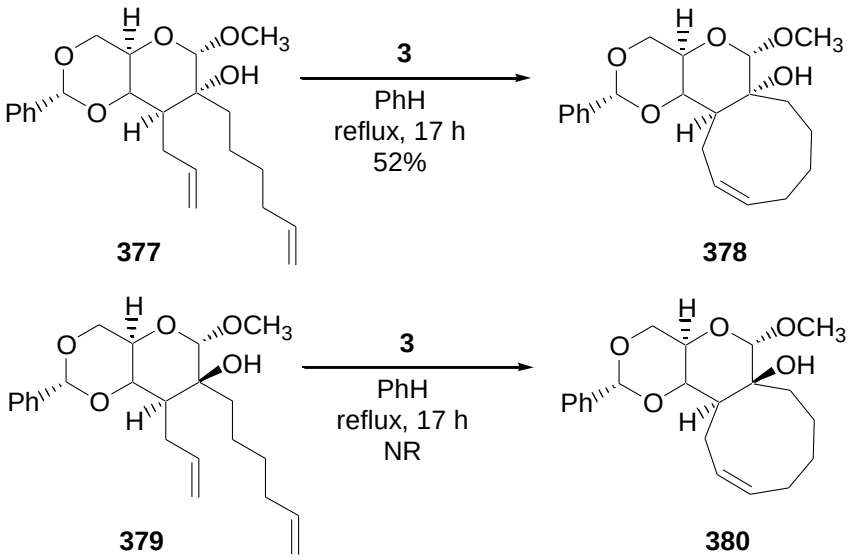
Several large membered ring systems have been synthesized by ring-closing metathesis. This section is organized by ring size and highlights a number of examples including nine, ten, eleven, twelve, thirteen, fifteen, and sixteen membered ring carbocycles.

Paley and co-workers used enantiopure η^4-(1-sulfinyldiene)iron(0) tricarbonyl complexes as templates for the enantioselective construction of nine membered ring carbocycles.[117] Enantiopure homoallyl alcohol adduct **375** and when treated with 8 mol% of **3** in toluene, gave the corresponding nine membered carbocyclic ring in 88% yield with a *cis*/*trans* ratio of 35:1.

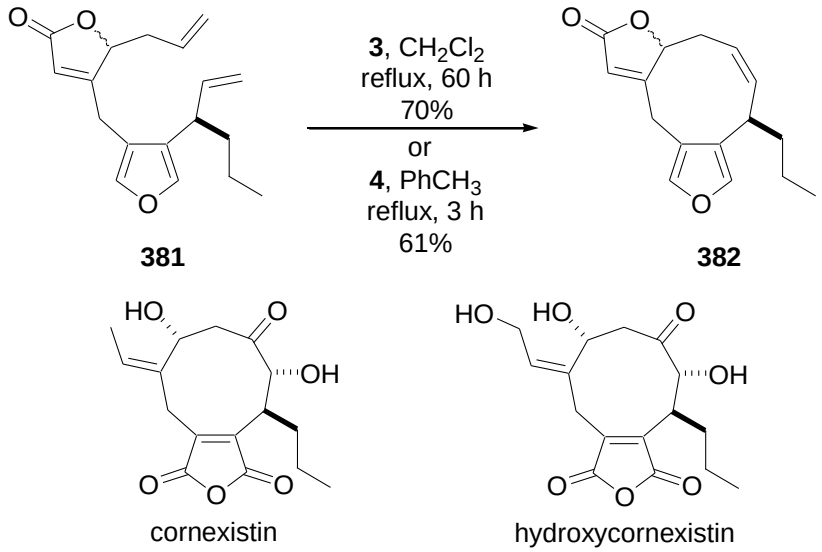


375 **376**

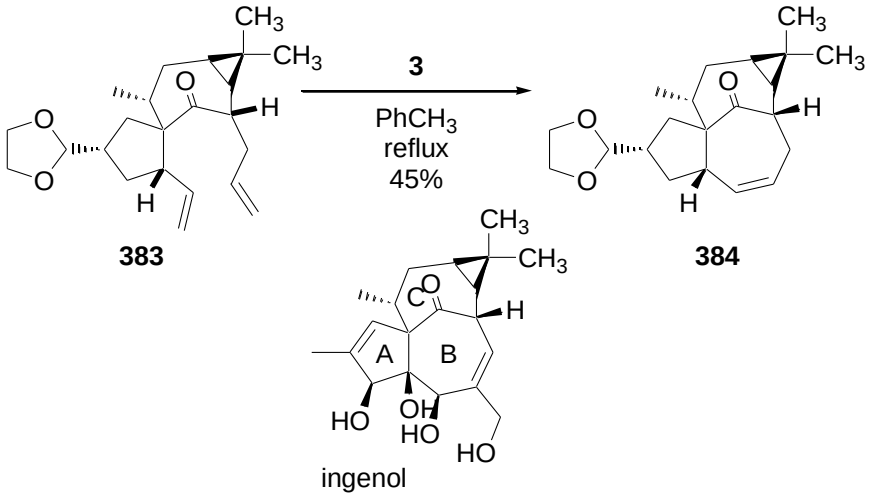
Holt and co-workers applied their ring-closing metathesis strategy to synthesize a number of enantiomerically pure annulated carbohydrate systems containing nine membered ring carbocycles.[90] Treatment of *cis* diene **377** with a catalytic amount of **3** in benzene gave the corresponding 6,6,9-carbocycle **378** in 52% yield. However, treatment of the *trans* diene **379** under similar conditions gave none of the desired product, highlighting the importance of conformational restraints on nine membered ring systems.



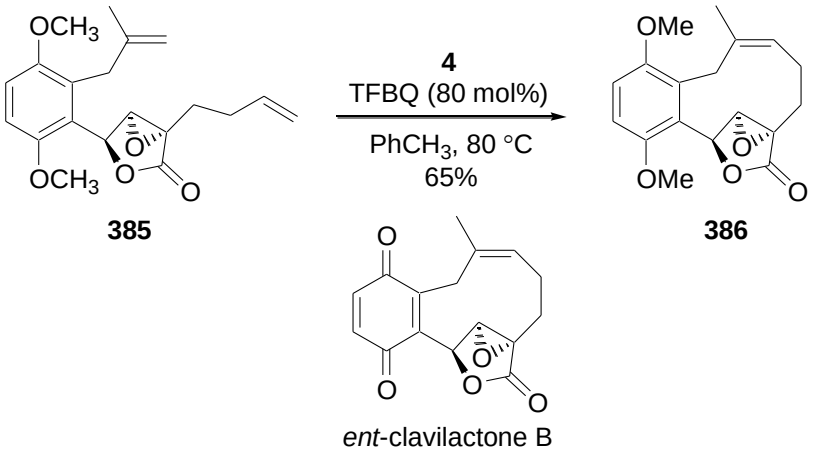
Clark and co-workers[162] employed a ring-closing metathesis, pioneered by Rodriquez and co-workers for the synthesis of eight and nine membered carbocycles,[163] in their synthesis of the nine-membered ring carbocyclic core of the cornextins, a class of herbicidial nonadrides. Their first report in 2003 employed a ring-closing metathesis strategy with diene **381** using either catalytic **3** in refluxing dichloromethane or **4** in toluene to install the nine membered ring carbocycle **382** in 70% and 61% yield as a mixture of diastereomers. In a later report, Clark and co-workers employed a ring-closing fragmentation strategy complete the synthesis of (+/-)-5-*epi*-hydroxycornexistin.[164]



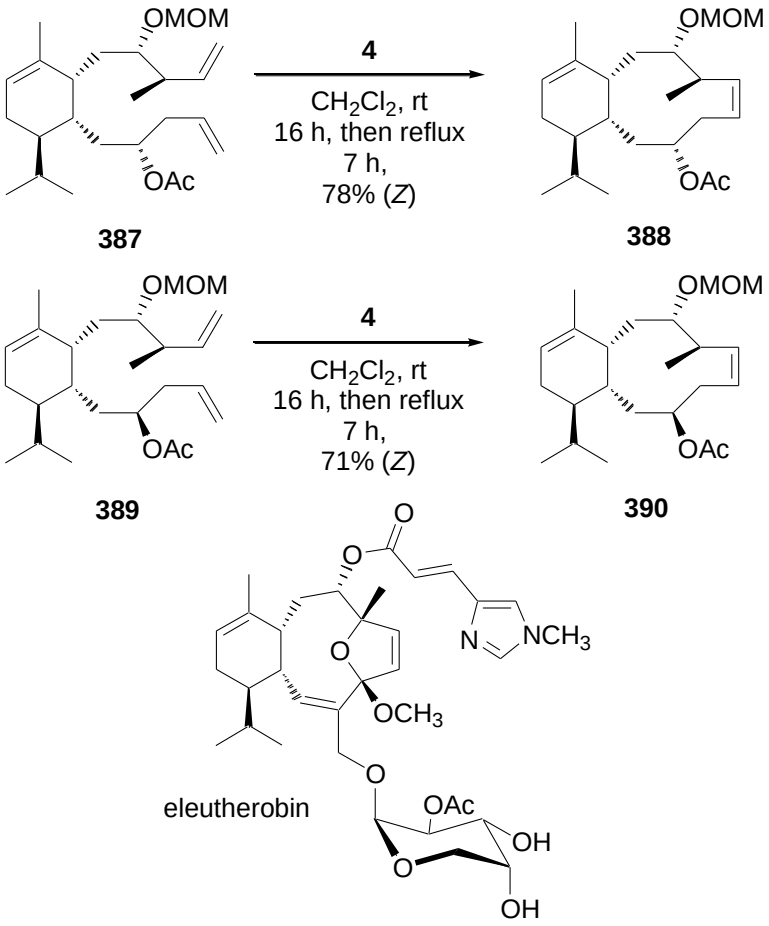
Ingenol esters have been shown to mimic diacylglycerol and function as PKC activators. These compounds pose a particular synthetic challenging due to their high degree of oxygenation, including a *cis*-triol, in addition to a highly strained "inside-outside" BC ring system. Wood and co-workers employed a ring-closing metathesis strategy in the construction of the carbocyclic core of ingenol.[165] Reaction of **383** with a four additions of 20 mol% of **3** every 45 minutes in refluxing toluene gave the corresponding inside-outside ring system **384** in 45% yield. The research groups of Winkler[166] and and Kigoshi[167] have independently applied similar strategies in the synthesis of ingenol.



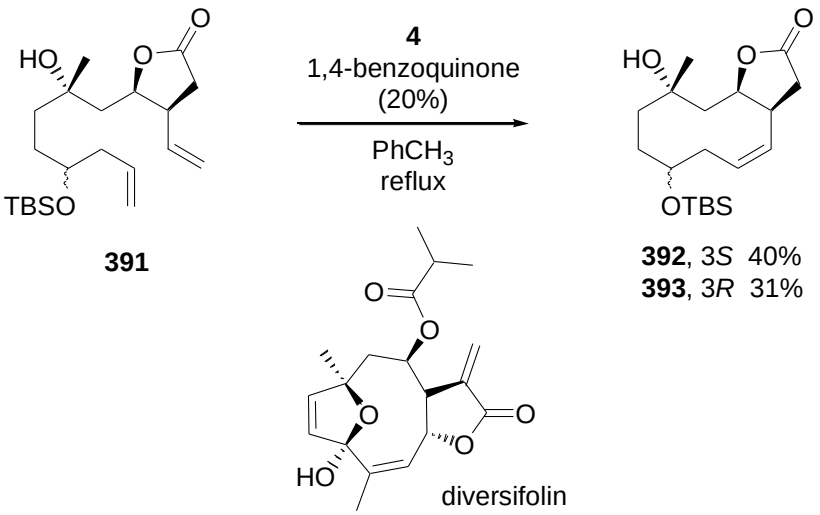
ent-Clavilactone B, a unique compound with antifungal and antibacterial properties, was synthesized by Barrett and co-workers using olefin metathesis as a key step in their synthesis.[168] Extensive optimization led to the slow addition of 40 mol% of **4** to diene **385** in the presence of 80 mol% tetrafluorobenzoquinone in toluene to afford the desired ten-membered ring **386** in 65% yield. The authors noted that the reaction proceeded smoothly without affecting the strained epoxide ring. Treatment of **386** with CAN furnished the desired target.



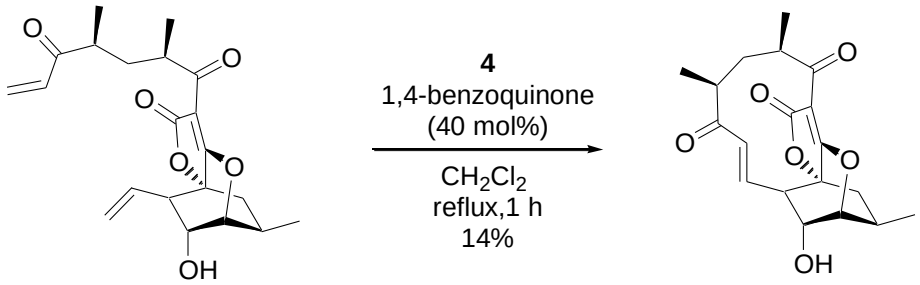
Gennari and co-workers used a ring-closing metathesis strategy in their synthesis of C-7 substituted eleutheside analogs.[169] These molecules are analogs of the sarcodictyin family, which have shown significant microtubule stabilizing activity in tumor cell lines and the ability to inhibit Taxol® resistant tumor cell lines. Eleutheside analogs therefore hold potential as second generation microtubule-stabilizing anticancer agents. Dienes **387** and **389** were subject to ring-closing metathesis using 6 mol% **4** in room temperature and then refluxing dichloromethane to provide the corresponding *Z*-alkenes **388** and **390** in 78 and 71% yields, respectively. A number of additional alkenes bearing various functional handles were also synthesized.



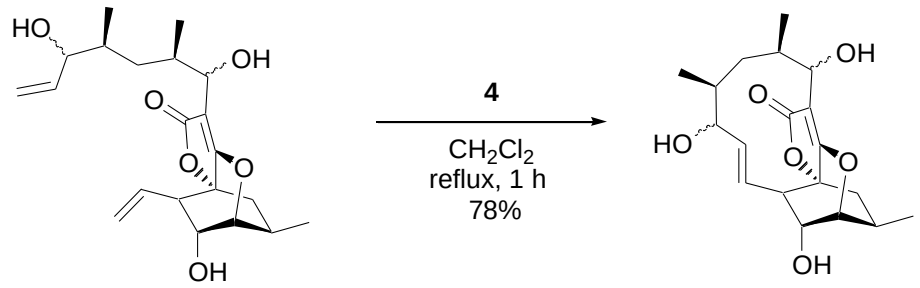
Kobayashi and co-workers employed an olefin metathesis strategy in the synthesis of the 11-oxabicyclo[6.2.1]undec-3-ene core of diversifolin, a densely oxygenated germacrane-type sesquiterpene with the ability to inhibit transcription factor NF-kB.[170] Reaction of **391** as a mixture of diasteromers with 20 mol% **4** in the presence of 20 mol% of 1,4-benzoquinone in refluxing toluene gave the desired bicyclic lactones 3*S* (**392**) and 3*R* (**393**) in 71% yield overall. Interestingly, when only 10 mol% of **4** was used without 1,4-benzoquinone, only the 3*R* isomer **393** was obtained (27% yield) in addition to a trace amount of the 3*S* isomer, recovered starting material, and an isomerisation product (not shown). The reactions were also attempted using **3** with no yield of the desired products.



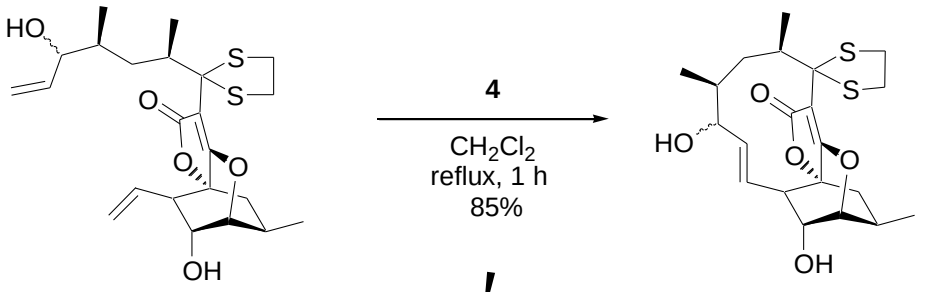
Nicolaou and Harrison used a ring-closing metathesis strategy to construct the carbocyclic core of the abyssomicin C, one of the only compounds to exhibit antibiotic activity via inhibition of the *p*-aminobenzoic acid biosynthetic pathway.[171] The larger, strained 11-membered ring containing four stereogenic centers presented some specific challenges. Treatment of vinyl ketone **394** with 10 mol% **4** and 20 mol% of 1,4-benzoquinone in dichloromethane gave the desired product, abyssomicin C **395** in only 14% yield. However, when a diastereomeric mixture of vinylic triol **396** was treated with 5 mol% of **4** in dichloromethane, the corresponding carbocyclic core **397** was generated in 78% yield. Unfortunately, the authors were unable to find a suitable oxidation strategy to produce abyssomicin C. Speculation that the two additional sp^2 centers of **394** were responsible for the low yield, led to the design of **398** which was devoid of the intramolecular hemiketalization problem. This substrate smoothly underwent ring-closing metathesis using 5 mol% of **4** in dichoromethane to provide the desired carbocycle in 85% yield. **399** could be readily oxidized and deprotected to afford the desired product.



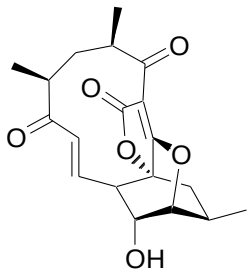
394 **395**



396 **397**



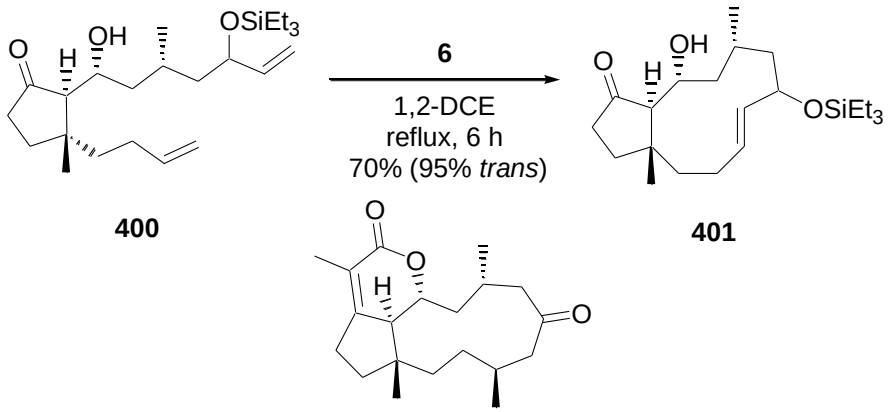
398 **399**



abyssomicin C

Hoyveda and Brown used a ring-closing metathesis strategy in the synthesis of clavirolide C, a member of the dolabellane family of diterpenes.[172] Cavirolide C contains a *trans*-bicyclo[9.3.0]tetradecane core architecture that presents an interesting synthetic challenge. Reaction of diene **400** in the presence of 10 mol% of **6** in refluxing dichloroethane gave the corresponding carbocyclic macrocycle **401** in 70% yield with greater than 95% *trans* selectivity. The authors noted that attempts to employ catalyst **4** led to less

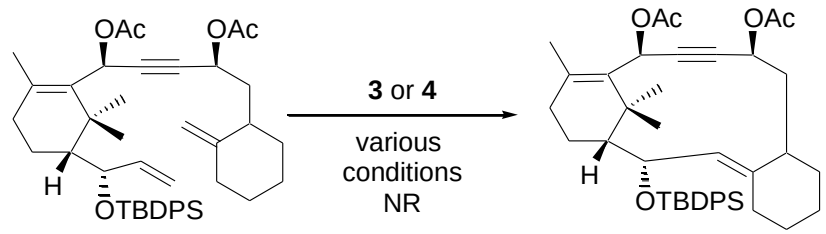
than 10% conversion to the desired macrocycle. In addition, ring-closing metathesis using either the free allylic alcohol or corresponding ketone led to complex mixtures of products.



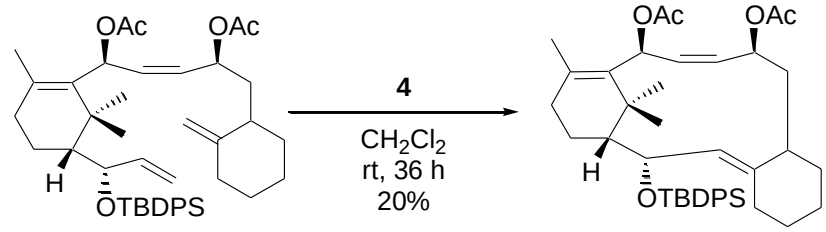
400 **401**

clavirolide C

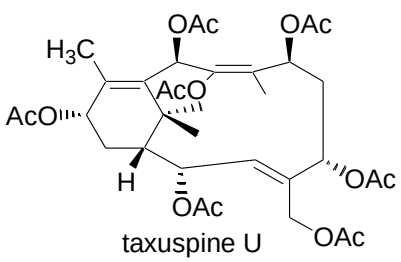
Botta and co-workers employed a ring-closing metathesis strategy in the stereoselective synthesis of advanced intermediates in route to the total synthesis of taxuspines U and X, the biogenic precursors for Taxol®.[173] In addition to an interesting and synthetically challenging architecture, taxuspines U and X are believed to have similar microtubule stabilizing properties to Taxol®, and as such are of current interest for their medicinal properties. Initial attempts at ring-closing metathesis for **402** using either **3** or **4** under a number of conditions failed to provide the desired 3,8-secotaxae diterpenoid **403**. The authors speculated that the failed ring-closing metathesis was the result the catalyst complexing with the more electron-rich alkyne in the presence of the electron rich OTBDPS group. Reduction of the alkyne to the alkene and subsequent treatment of the corresponding ω-ω′-diolefin **404** with 10 mol% **4** gave the corresponding 3,8-secotaxae diterpenoid **405** in 20% yield. In a later report, Botta and co-workers were able to achieve cyclization with a number of alkynyl substrates using 20 mol% **1** in toluene in about 20% yeild. This led the authors to conclude that the molecular constraints for cyclization may require too high of energy barrier for ring-closing metathesis.174



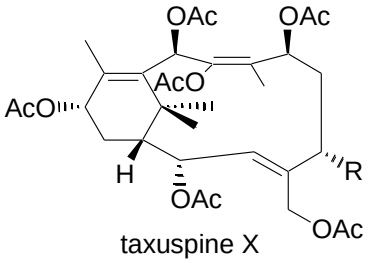
402 **403**



404 **405**

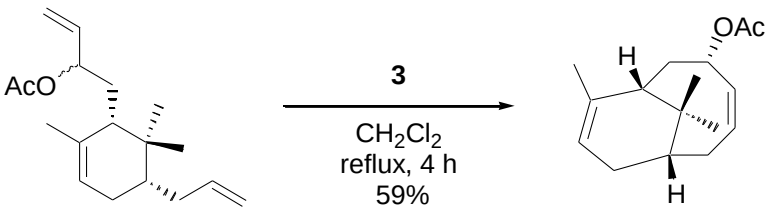


taxuspine U



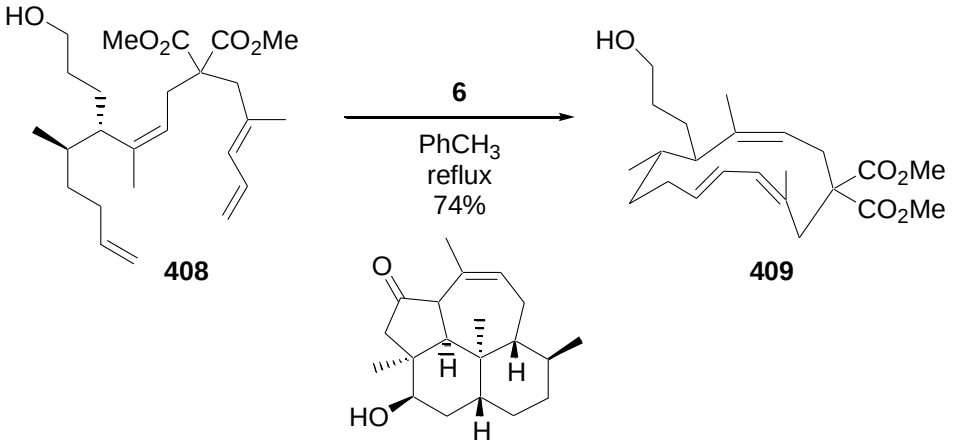
taxuspine X
R=*trans*-cinnamoyl

Blechert and co-workers applied a ring-closing strategy in the synthesis of the central bridged bicyclo[5.3.1]undecane moiety of Taxol®.[175] Diene precursor **406**, prepared in nine steps from commercially available (–)-β-pinene was subject to ring-closing metathesis using 10 mol% of **3** in refluxing dichloromethane to give 58% of the desired substrate **407** in four hours. The authors noted that only one vinyl acetate cyclized to form the desired macrocycle, presumably due to the sterics of the bridgehead.



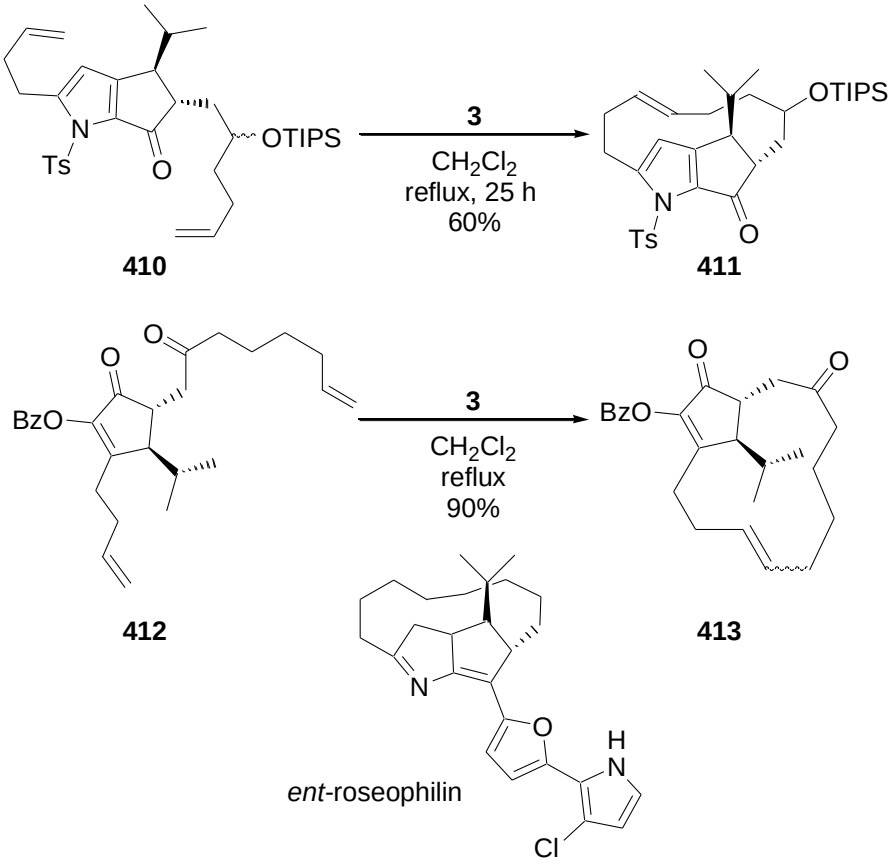
406 **407**

Deslongchaps and co-workers approach to the synthesis of Kempane diterpenes used ring-closing metathesis in the construction of a 13-membered carbocycle, which subsequently subjected to a transannular Diels-Alder reaction to produce the tricyclic core.[176] Treatment of tetraene ester **408** with **6** in refluxing toluene gave the corresponding triene **409** with a *trans-cis-cis* geometry as the only product in 74% yield.



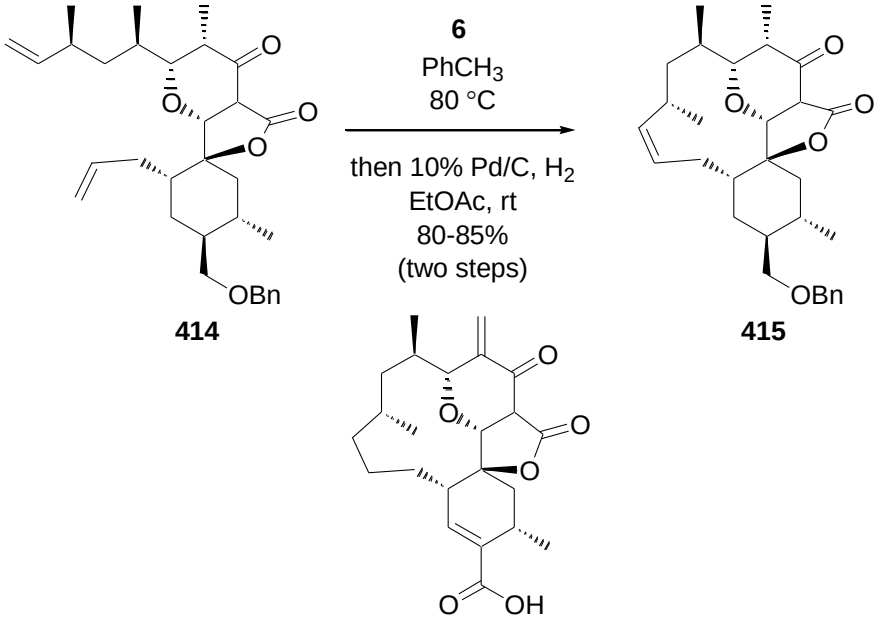
(±)-kempane

A number of ring-closing metathesis strategies have been employed in the the 13-membered core of roseophilin, a novel anti-tumor antibiotic with a unique pentacyclic skeleton. The first synthesis by Fuchs and co-workers employed diene **410** as a mixture of diasteromers.[177] Treatment of **410** with 30 mol% of **3** in dichloromethane gave the corresponding ansa-bridged silylether **411** as a single diastereomer in 60% yield as the major product. The low yield was attributed the conformationally biased diene precursor. The research groups of Furstner,[178] Hiemstra,[179] and Boger[180] used a similar approach in their syntheses of roseophilin in 1999, 2000, and 2001 respectively, although notably Boger achieved an 88% yield of the *ansa*-bridged macrocycle as a 1:1 mixture of the the *E* and *Z* isomers by using a triene analog of **410**.



Tius and co-workers also used a ring-closing methathesis strategy for the construction of the macrocyclic ring of roseophilin.[181] Treatment of the more conformationally flexible olefin **412** with 30 mol% of **3** in refluxing dicholormethane gave the corresponding 13-membered ring carbocycle **413** in 90% yield.

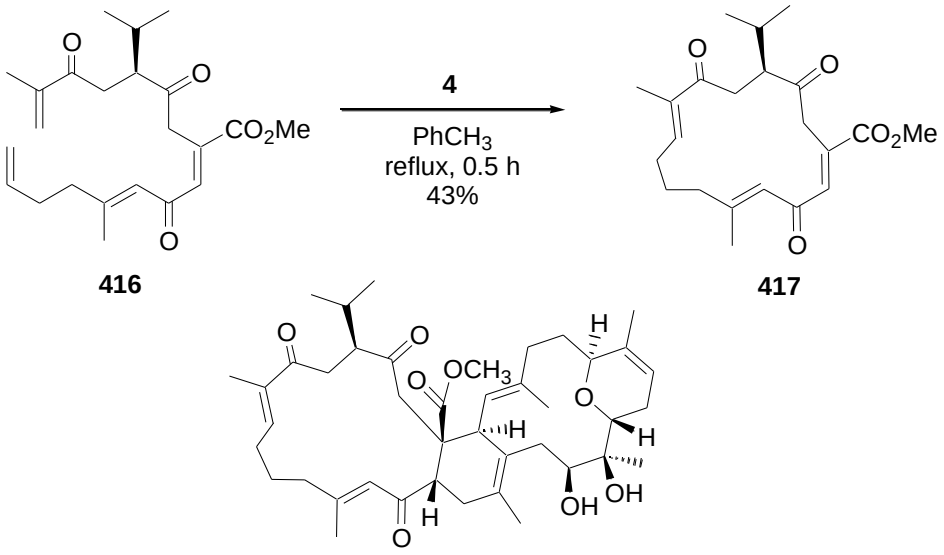
Smith and co-workers used a ring-closing metathesis strategy in the synthesis of the 13 membered ring of (–)-okilactomycin, a novel polyketide antitumor antibiotic.[182] (–)-Okilactomycin is a considerable challenge synthetically due to its highly functionalized cyclohexene ring complete with a spirocenter and a 2,6-cis-tetrahdyropyronone moiety. Ring-closing metathesis of lactone **414** in the presence of 30 mol% of **6** in toluene followed by catalytic hydrogenolysis gave the corresponding *cis*-alkene **415** in 80-85% yield over two steps. The authors noted that in order to achieve optimal yields and prevent dimerization, **6** was decomposed by air prior to concentration. A similar strategy was employed in their most recent report on (–)-okilactomycin.[183]



414 415

(–)-okilactomycin

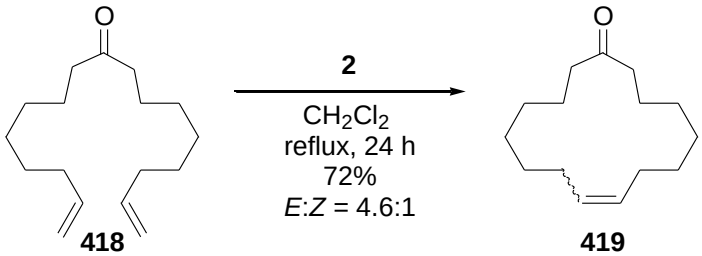
Nakata and co-workers recently employed a ring-closing metathesis strategy to construct the 14 membered carbocyclic core of methyl sarcophytoate, a biscembranoid.[184] Olefin metathesis of diene **416** using a stoichiometric amount of **4** in refluxing benzene gave the corresponding macrocycle **417** in 43 % yield.



416 417

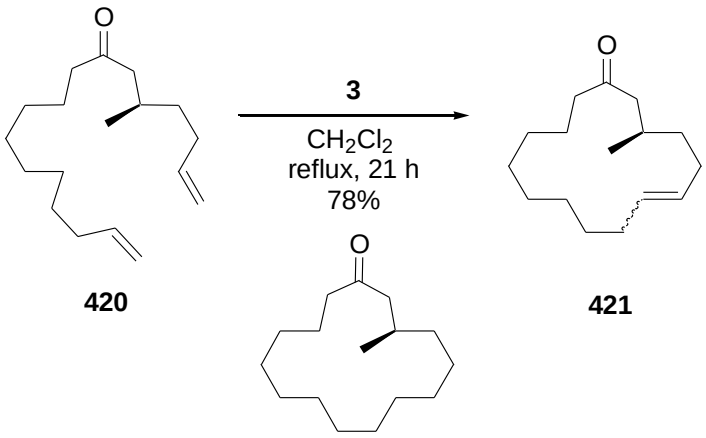
methyl sarcophytoate

A ring-closing metathesis strategy was first employed by Furstner and co-workers in the synthesis of civetone, a macrocyclic musk.[185] Treatment of diene **418** using 5 mol% of **2** in refluxing dichloromethane gave the corresponding 15-member ring macrocycle **419** in 72% yield with *E*:*Z* selectivity of 4.6 to 1.



418 **419**
civetone

Hagiwara and co-workers employed a similar metathesis strategy in their synthesis of (*R*)-(–)-muscone from (+)-citronellal.[186] Treatment of ketone **420**, generated in seven steps from commercially available (+)-citronellal, underwent olefin metathesis smoothly in the presence of 5 mol% of **3** to furnish the corresponding 15 membered ring carbocycle **421** in 78% yield as a mixture of *E* and *Z* isomers. Hydrogenation of **421** led to the final desired product (*R*)-(–)-muscone. A similar protocol was employed in a recent synthesis by the same group.[187]

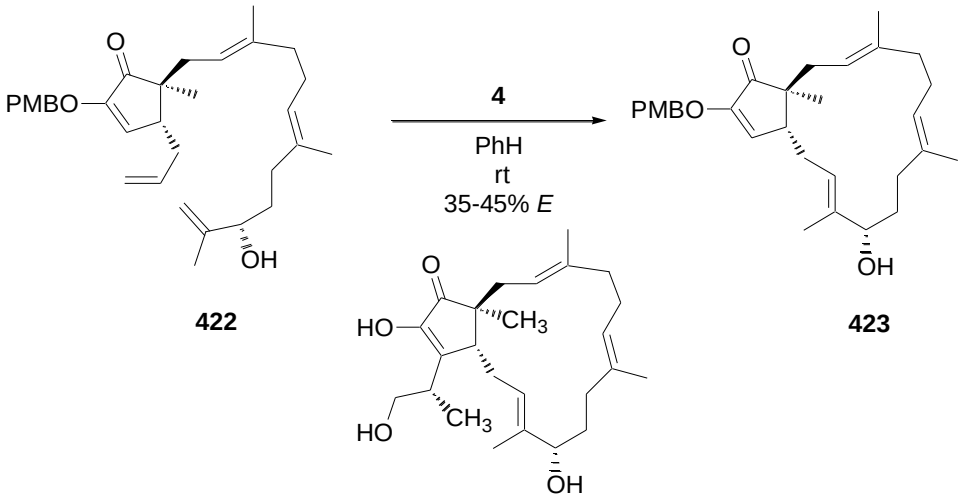


420 **421**

(*R*)-(–)-muscone

Trost and co-workers used ring-closing metathesis in their total synthesis of (–)-terpestacin.[188] Terpetacin is a known inhibitor of syncytia, produced by HIV infected cells, and has also been shown to inhibit angiogenesis. Treatment of **422** with 10 mol% of **4** in benzene gave the

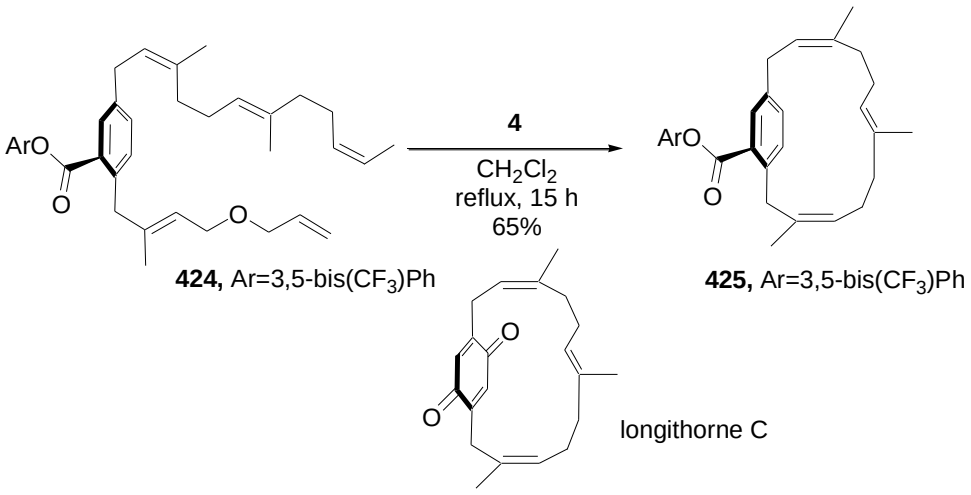
corresponding 15 membered ring carbocycle **423** in 35-45% yields. The authors noted that the low temperatures and allylic alcohol were critical in the chemoselectivity of the ring-closing metathesis.



422 **423**

terpestacin 1

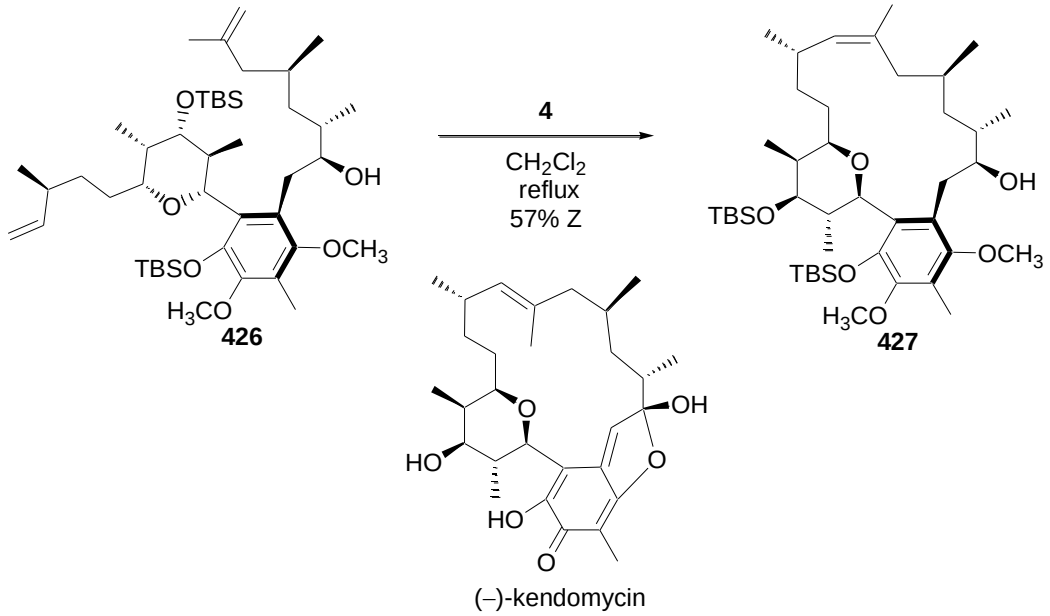
Collins and co-workers used a relay ring-closing metathesis in the preparation of the ring component of longithorone C, a farnesylated quinone with a macrocyclic [12]paracyclophane skeleton.[189] Reaction of ester **424** with 10 mol% of a **4** provided the corresponding macrocycle **425** in 68% yield, based on recovery of starting material. Collins recently expanded on this work using catalyst **8** to provide comparable yields.[190] Several other groups including Smith,[191] Kotha[192] and Suzuki[193] have also employed ring-closing metathesis strategy in the synthesis of related cyclophane derivatives.



424, Ar=3,5-bis(CF_3)Ph **425,** Ar=3,5-bis(CF_3)Ph

longithorne C

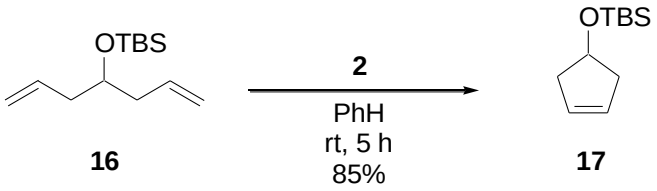
Smith and co-workers recently used a Petasis-Ferrier rearrangement/ring-closing metathesis to construct the 18-membered ring macrocyclic core of (–)-kendomycin, a polyketide macrocyclic endothelin receptor antagonist and antiosteoporotic.[194] Reaction of diene **426**, with 10 mol% **4** in refluxing dichloromethane provided macrocycle **427** in 57% yield as the *Z* isomer, rather than the desired *E* isomer. Despite obtaining the undesired stereioisomer, Smith's report is the first synthesis of an α-branched trisubstituted olefin in a a large macrocylic structure. Compound **427** was cleverly converted to the E isomer in four steps, and three additional steps were required to reach the final product, (–)-kendomycin.



5.3.6 Sample Experimental

The following example, adapted from Grubbs original report on ring-closing metathesis is still considered the standard for most ring-closing metathesis reactions.

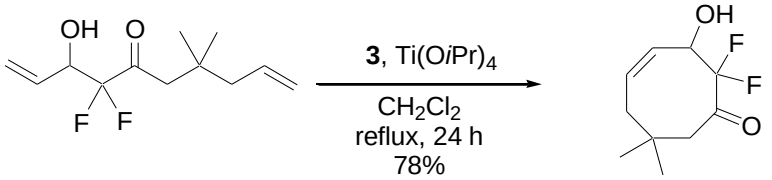
tert-Butyl-(cyclopent-3-enyloxy)-dimethyl-silane[36]



Typical experimental procedure: The diene **16** (0.5mmol) was added to a homogenous orange red solution of **2** (0.01mmol) in dry PhH (15 mL, 0.001M) under argon. The resulting mixture was stirred at 20 °C for 5 h, at which time TLC showed the reaction to be complete. The reaction mixture was quenched by exposure to air, concentrated, and purified by flash chromatography (0 to 6% diethyl ether/hexanes) to give a colorless oil.

The following procedure, adapted from Percy and co-workers synthesis of difluorinated cyclooctenoids, is a useful method when $Ti(OiPr)_4$ is to be used as a precatalyst.

2,2-Difluoro-3-hydroxy-7,7-dimethyl-cyclooct-4-enone[143]



313 **314**

Diene (1.28mmol), $Ti(OiPr)_4$ (0.422mmol), and catalyst (0.064mmol) were dissolved in dried, degassed dichloromethane (512 mL). The solution was allowed to reflux under inert atmospheric conditions for 24 h or until complete as monitored by ^{19}F NMR. The solvent was removed under reduced pressure and the residue was taken up in diethyl ether (5 mL), filtered and concentrated under reduced pressure. The residue was taken up in methanol (1 mL) then eluted through a Stratospheres DPE tube, eluting with methanol (5 x 2 mL). The solution was concentrated under reduced pressure to afford a brown oil, which was purified by flash chromatography (silica gel, 20% diethyl ether/hexane) to give cycloctenone as clear solid.

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Scholarship—Grant Applications

Camille and Henry Dreyfus Faculty Start-up Award Application 2007—Nicole L. Snyder

I. Research Summary

Exploring the Nature of Ligand Binding in the Active Site of Galectin-1 Using Natural and Unnatural Carbohydrates

Introduction

The unique topologies afforded to carbohydrates through their unsurpassed structural diversity have allowed them to play critical roles in a number of biological recognition events including cell trafficking, growth factor recognition, immunological recognition, and metastasis.[1] By studying fundamental interactions between carbohydrates and other biomolecules in these processes, a better understanding of the natural functions of carbohydrates has been realized. This understanding has helped enlighten fundamental biochemical knowledge, and has played a significant role in the construction of designed carbohydrate-based systems that have in turn been used to develop of a variety of therapeutic strategies and new classes of pharmaceutical reagents.

In recent years, the construction of expanded homologs of naturally occurring amino acid and carbohydrate residues has gained considerable attention. The "unnatural" nature and interesting properties these molecules exhibit make them attractive tools for probing biomolecular interactions. For example, Eschenmoser[2] has observed that when the five member furanose sugars of DNA and RNA are expanded by one carbon to pyranoses, an alternative base-pairing and heteroduplex shape is observed. Similarly, Gellman[3] and Seebach[4] studied the homologation of α-amino acids to β-amino acids and found that oligomers constructed from β-amino acids adopt defined conformations that complement natural structures and can selectively disrupt bacterial cell membranes over mammalian cell membranes.[5] More recently, the construction of an entirely new class of ring expanded carbohydrates known as septanose carbohydrates has been introduced.[6] Septanose carbohydrates are unnatural, ring expanded homologs of pyranose carbohydrates. The flexibility of the seven member ring in these sugars allows them to adopt a number of different low energy conformations[7] that make them interesting tools for studying fundamental protein-carbohydrate interactions in conjunction with their natural pyranose homologs.[8]

The aforementioned research has provided a foundation for the design of synthetic glycoconjugates that can be used to explore carbohydrate-protein interactions. As an extension of this idea, the students in my research laboratory at Hamilton College and I will use a host of natural (pyranose) and unnatural (septanose) carbohydrate ligands to explore the ligand-binding interaction of galectin-1. Galectin-1 is a protein that has been implicated in tumor progression, inflammation and immunity, and HIV infectivity. Ligands that selectively target galectin-1 have the potential to serve as effective treatments for cancer, certain inflammatory diseases, immunological disorders, and HIV. Despite recent advances in the preparation and evaluation ligands that target and bind galectin-1, little is known about the nature of the galectin-1 ligand binding interaction. My students and I will work together to design, prepare, and assess a number of lactosyl triazoles based on the natural ligand of galectin-1. The proposed derivatives will be used to further an understanding of galectin-1-ligand binding, and the knowledge gained will serve as a foundation for the design and preparation of agents that selectively target galectin-1. The uniting goals of the interdisciplinary research project described below are:

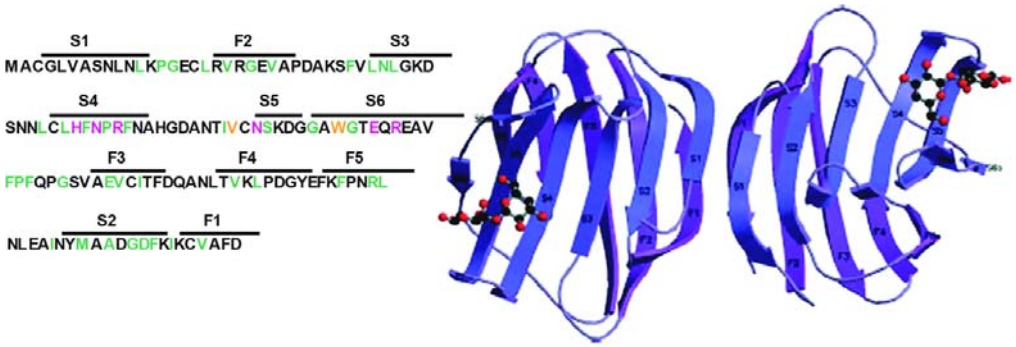
1. To generate a host of rationally designed lactosyl triazoles incorporating natural and unnatural carbohydrate residues that can be used to probe the nature of galectin-1-ligand association.
2. To investigate the structural and functional consequences of the galectin-1-ligand binding interaction using state of the art chemical, biochemical, spectroscopic and computational techniques.

Specifically my students and I will address the following question: ***"What is the nature of the galectin-1-ligand interaction for lactosyl 1,2,3-triazole derivatives incorporating natural and unnatural carbohydrate residues?"***

Background and Significance

Structure and Function of Galectin-1—Galectin-1 is a member of a family of carbohydrate binding proteins that are defined by their affinity for β-galactosides. Members of the galectin family exhibit a significant sequence homology in the carbohydrate-binding site or carbohydrate recognition domain (CRD). The galectin CRD is composed of a two sheet beta-sandwich of 135 amino acids that are slightly bent and antiparallel in nature (Figure 1). Five strands (F1-F5) form the convex portion of the beta-sandwich while six strands (S1-S6) form the concave portion of the protein and provide for a large hydrophobic carbohydrate binding groove long enough to support a linear tetrasaccharide.[9]

Figure 1: Structure of the galectin-1 bound to lactose (Taken from Camby et. al.[10])



Notes: Amino acids highlighted in green illustrate highly conserved residues. Amino acid residues highlighted in pink are known to interact with bound carbohydrates via hydrogen bonding interactions. Amino acids highlighted in orange are known to interact with bound carbohydrates via van der Walls forces.

The most conserved binding region of the galectin family occurs in a subsite between strands S4 and S6 where a galactose residue binds. Hydrophobic packing between the B (hydrophobic) face of the galactose residue and a conserved tryptophan residue (W68) of S6, in addition to extensive hydrogen bonding interactions between basic amino acid residues and the carbohydrate in the binding groove stabilizes the binding interaction. The binding of a saccharide unit in a second subsite between strands S4 and S6 is the next most conserved feature of the galectin family. For galectin-1 this residue is generally a glucose or N-acetyl-glucosamine unit and is bound by hydrogen bonding and van der Waals interactions between the protein and the carbohydrate. Two subsites between strands S1 and S4 are the least conserved between members of the galectin family and can support a wide variety of other groups linked through the 3'OH of the galactose residue. A fifth subsite between strands S5 and S6 is less understood.[11]

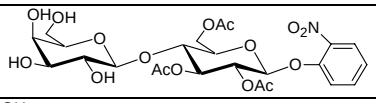
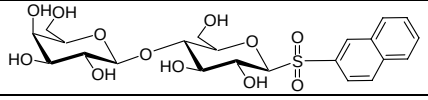
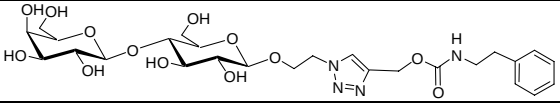
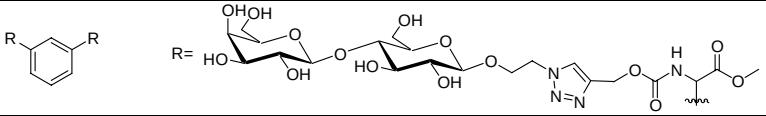
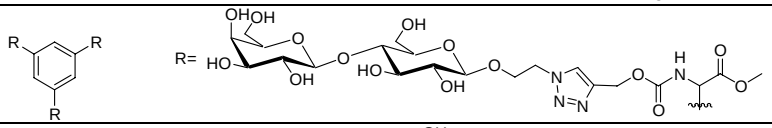
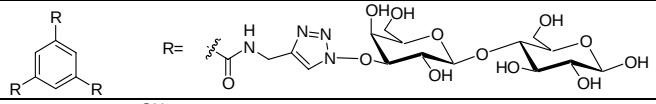
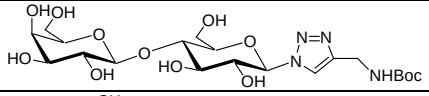
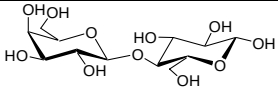
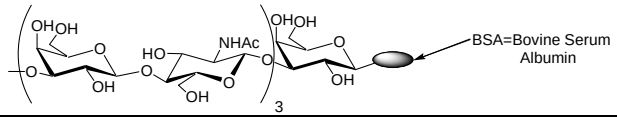
In normal cells, galectin-1 expression is regulated. Galectin-1 is expressed on cell surfaces and in extracellular matrices, and functions in a number of critical process including inflammation, development, mRNA splicing, differentiation, and cell adhesion.[12] Diseased or stressed cells have been shown to over express galectin-1. For example, galectin-1 has been found in unusually high concentrations in and around tumor cells and has been implicated in several aspects of cancer biology including tumor transformation,[13] apoptosis,[14] cell growth regulation,[15] and metastasis.[16] Research has also shown that galectin-1 may play an important role in protecting tumor cells from immune attack,[17] and studies have also suggested that galectin-1 may play an important role in the promotion of HIV infectivity.[18]

Preliminary Studies on Effective Inhibitors of Galectin-1—Because of the role of galectin-1 in cancer, inflammatory diseases, autoimmune disorders and HIV infectivity, considerable research has been devoted to the design and synthesis of specific galectin-1 inhibitors. Most of these efforts are based on the evidence that galectin-1 has an affinity for multiple ligands including lactose, N-

acetyllactosamine, and naturally occurring branched polylactosamine and poly-N-acetyllactosamine derivatives.[19]

Select examples of some of the more effective inhibitors prepared in recent years are summarized in Table 1 below. Of special interest are the highly potent lactosyl triazole-based galectin-1 inhibitors (Table 1, entries 3-7). In most cases, these molecules bind better than lactose (Table, 1 entry 8), and will selectively bind to galectin-1 in the presence of galectin-3 (a lectin with similar binding properties to galectin-1). However, in most cases (exceptions being the multivalent derivatives 4 and 5) these derivatives do not bind as well as the natural ligand, poly-N-acetyllactosamine (Table 1, entry 9).

Table 1: Recent examples of galectin-1 inhibitors and their inhibitory properties.

Entry	Inhibitor	Kd Gal-1 (μM)	Kd Gal-3 (μM)	Ref
1		80	624	20
2		40	313	20
3		24	66	21
4		3.2	27	21
5		7.4	17	21
6		20	250	22
7*		NA	NA	22
8		800	800	20,21,23
9		11.9	--	23

*This derivative was prepared was found to be too insoluble for testing.

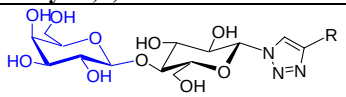
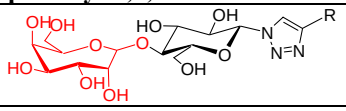
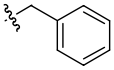
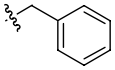
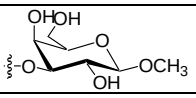
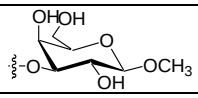
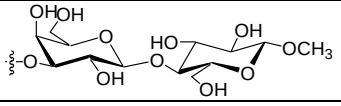
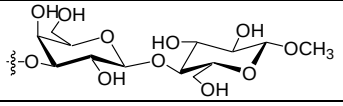
Despite the progress highlighted here, little is known about the molecular mechanism of the galectin-1 ligand interaction for these molecules. The research described in this proposal will be used to provide systematic assessment of the factors that govern galectin-1 ligand binding. My students and I have chosen to use lactosyl 1,2,3-triazoles as models because of the activity that has already been shown for these molecules, and the fact that they are simple to prepare and relatively stable. The general goal of this project is to collect information that can be used to design powerful inhibitors that can be used therapeutically to selectively target overexpressed galectin-1.

Experimental Summary

Design and Rationale—My students and I will focus on the design, preparation, characterization, and biological evaluation of several small lactosyl 1,2,3-triazole derivatives incorporating natural and

unnatural carbohydrate resides. Specific examples of the molecules we wish to prepare are shown below in Table 2. The rationale for the proposed derivatives is based on two parameters that we would like to investigate: (i) how the nature of the reducing galactose residue (natural *versus* unnatural) affects the binding profile for galectin-1, and (ii) how the nature of triazole (flexible versus nonflexible, aromatic versus non aromatic and carbohydrate versus non carbohydrate) influences binding interactions.

Table 2: Target galectin-1 inhibitors.

Entry	Lactosyl-1,2,3-triazoles	Entry	Lactoseptanosyl-1,2,3-Triazoles
			
1		7	
2		8	
3		9	
4		10	
5		11	
6		12	

Lactosyl 1,2,3-triazoles 1-6 (Table 2) incorporate a natural galactose residue (highlighted in blue) at the nonreducing terminus of lactose subunit and will be used as models to assess the binding of galectin-1 to ligands containing only natural carbohydrate residues. Within this series, lactosyl 1,2,3-trizoles 1-4 will be used to address flexibility and hydrophobic interactions within the binding groove. Entries 5 and 6 will be used to determine whether triazoles ligands with additional carbohydrate residues bind more or less specifically than lactosyl 1,2,3-triazoles 1-4 which do not incorporate these structures.

In the case of entries 1-4 (Table 2) it is expected that the more flexible derivatives (2 and 4) will bind more effectively since they will require the least amount of energy to access a suitable binding conformation. It is also expected that the binding affinity for entries 3 and 4, will increase with respect to their non aromatic counterparts (1 and 2) due to stabilizing pi stacking interactions between aromatic residues in the binding groove and the aromatic R groups. Entrie 5 and 6 provide a unique opportunity to asses whether galectin-1 will preferentially bind the terminal non-reducing galactose unit or the 3'O-linked galactose residue.

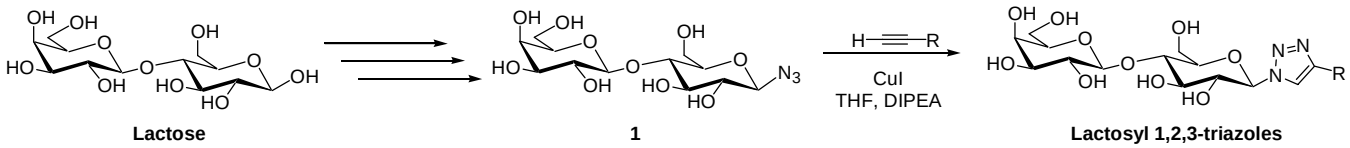
Lactoseptanosyl 1,2,3-traizoles 7-12 (Table 2) are novel derivatives that incorporate an unnatural (septanose) carbohydrate residue (highlighted in red) at the nonreducing terminus of the lactose subunit. Septanose carbohydrates are ring expanded homologs of pyranose carbohydrates and have been shown to adopt several puckered low energy conformations that meet the space filling and polar requirements necessary for binding in a number of protein-carbohydrate interactions.

Lactoseptanosyl 1,2,3-triazoles will be used to assess the ability of galectin-1 to bind ligands containing an unnatural carbohydrate residues in a similar context to those to asses the binding of galectin-1 to lactosyl 1,2,3-trizoles 1-6 (Table 2). It is expected that the increased flexibility of the galactose-based septanose residue of these derivatives may lower the activation energy required for

these molecules to access a conformation that is suitable for binding galectin-1, perhaps even increasing the B-side hydrophobic interactions between the residue and W68. Entries 5 and 6 are especially noteworthy since they provide a useful tool to study competitive interactions between the binding of natural and unnatural carbohydrates to galectin-1.

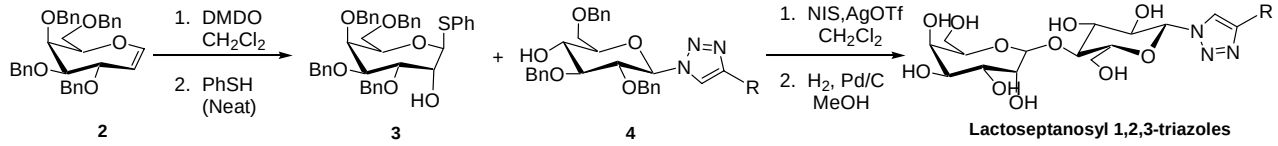
Synthesis of Lactosyl 1,2,3-Triazole Derivatives from Naturally Occurring Lactose—Lactosyl 1,2,3-triazole derivatives incorporating a natural galactose residue at the reducing position of the lactose moiety (Table 2, entries 1-6) can be prepared from commercially available lactose as shown in Scheme 1. Copper I catalyzed 1,3-diploar cycloaddition[24] of lactosyl azide **1** prepared in three steps from lactose,[25] with the appropriately substituted alkyne gives the corresponding lactosyl 1,2,3-triazole derivative. Alkyne derivatives for the preparation of entries 1-4 are commercially available from Aldrich Chemicals. Sugar alkyne derivatives for the preparation of entries 5-7 can be prepared from commercial available methyl-β-D-glucoside (entry 5) and lactose (entry 6) in two and three steps respectively.

Scheme 1: General route for the preparation of lactosyl 1,2,3-triazole derivatives..



Synthesis of Lactoseptanosyl 1,2,3-Triazole Derivatives—A general route for the synthesis of the lactoseptanosyl 1,2,3-triazoles incorporating an unnatural septanose-based galactose residue at the reducing position of the lactose moiety (Table 2 entries 7-12) is shown in Scheme 2 and begins with the stereoselective functionalization of a galactose-based oxepine **2**. The functionally analogous nature of galactose-based oxepine **2** to glycals makes this molecule an especially attractive starting material for the synthesis of galactose based septanosides.[26] Glycals, have been extensively employed as synthons to access a number of important carbohydrate derivatives and glycosylated natural products.[27] Epoxidation of **2** using DMDO followed by nucleophilic addition of benzene thiol provides galactose based thioseptanoside **3**. Activation of thioseptanoside donor **3** using N-iodosuccinamide and silver triflate followed by the addition of a previously prepared glucosyl 1,2,3-triazole acceptor (**4**) gives the protected lactoseptanosyl 1,2,3-triazole precursor (not shown) which, upon hydrogenolysis under standard conditions gives the desired lactoseptanosyl 1,2,3-triazole derivative.

Scheme 2: General route for the preparation of septanose-based lactosyl 1,2,3-triazole derivatives.



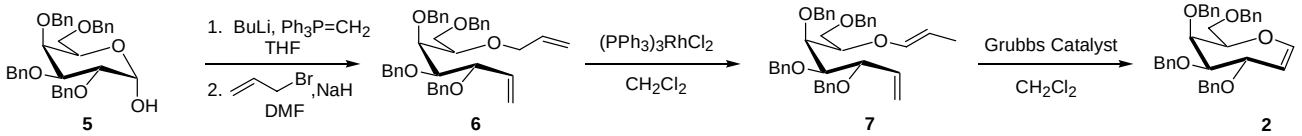
Synthetic approaches to oxepines, including carbohydrate-based oxepines, have recently been reviewed.[28] Approaches to carbohydrate-based oxepines with functional equivalence to glycals generally involve ring closing metathesis and are either restricted to specific functional groups,[29-30] or require the use of expensive catalyst and difficult reaction conditions.[31] In an effort to provide a more globally accessible approach to carbohydrate-based oxepines, and therefore septanose carbohydrates, undergraduate students in my research group will explore two new routes for the production of these molecules. These routes are designed to address the electronic arguments associated with the ring closing metathesis protocols that have been used to prepare these molecules. The specific routes that

will be investigated in this study include: a propenyl ether ring closing metathesis route (Scheme 3) and a tether-based ring closing metathesis route (Scheme 4).

Propenyl Ether Ring Closing Metathesis Route—Recent reports have shown that ruthenium alkylidene species, generated from electron-rich olefins, can be reactive in specific metathesis reactions, but are otherwise unreactive.[32] One method that can be used to impede the formation of these species is to make the vinyl ether sterically inaccessible. The steric effects of the propenyl ether should inhibit formation of the ruthenium alkylidene species facilitating ring closing metathesis.[33]

A general synthetic approach for the production of a diene species incorporating a propenyl ether is shown in Scheme 3. Wittig olefination of the appropriately protected lactol (**5**) followed by the addition of allyl bromide gives allyl ether **6**. Ruthenium mediated isomerization of the allylic ether using tris(triphenylphosphine) ruthenium (II) chloride[34] provides propenyl ether-diene precursor **7**, which undergoes ring closing metathesis in the presence of a catalytic amount of Grubbs catalyst to produce the desired oxepine **2**.

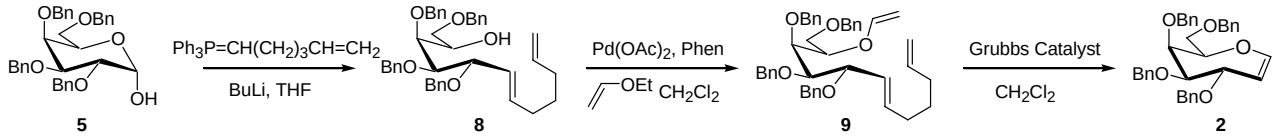
Scheme 3: Propenyl-ether ring closing metathesis route.



Tether-Based Ring Closing Metathesis Route—Hoye has recently demonstrated the use of a tether in order to make the Grubbs' reaction more efficient.[35] The general idea is to use an extender molecule to make one double bond of a polyunsaturated substrate more readily susceptible to chelation by the ruthenium catalyst, thus inhibiting the formation of a ruthenium alkylidene species.

A general approach for the synthesis of a substrate incorporating a tether is outlined in Scheme 5. Initial steps in this sequence involving the preparation of the tether are not shown.[36] Wittig olefination of the appropriately protected lactol (**5**) using the tether-ylide ($Ph_3P{=}CH(CH_2)_3CH{=}CH_2$) under conditions similar to those used in Scheme 3 provides alcohol **8**. Functionalization of the free alcohol using ethyl vinyl ether gives diene precursor **9** which undergoes tandem ring closing metathesis in the presence of a catalytic amount of Grubbs catalyst to produce oxepine **2** and cyclopentene. Cyclopentene (b.p. 44°C) is easily removed from the reaction mixture upon concentration to give the desired oxepine.

Scheme 4: Tether-based ring closing metathesis route.



Characerization of Lactosyl and Lactoseptanosyl 1,2,3-Trizaole Derivatives— Elucidation of the three-dimensional conformations of lactosyl and lactoseptanosyl triazloles is imperative to understanding the chemistry of their interaction with galectin-1. My students and I will conduct experiments aimed at understanding the conformations of these molecules by employing a combination of high-resolution nuclear magnetic resonance (NMR) spectroscopy and computational studies. Specifically, experimental ^3J NMR coupling constants will be obtained for both lactosyl and lactoseptanosyl 1,2,3-triazoles using Hamilton College's Bruker 500MHz NMR Spectrometer. Computational studies[7] will be conducted in parallel using MacroModel and Gaussian software

packages. MacroModel will be used to conduct an initial MonteCarlo search. Gaussian will be used to further optimize structures found within a designated range above the global minimum. NMR coupling constants will be determined from minimized structures using the deMon-NMR software package, and will be compared to the data obtained through experiment.

Binding Assessment of Lactosyl and Lactoseptanosyl 1,2,3-Triazole Derivatives to Galectin-1—The lactosyl and lactoseptanosyl triazoles derivatives prepared in this study will be assessed for binding to galectin-1 via isothermal titration calorimetry (ITC) and saturation transfer difference NMR spectroscopy (STD-NMR) in conjunction with the NMR and molecular modeling experiments described above. Students will learn to use these important techniques to address two specific sub questions aimed at addressing the overall question posed in this study: ***"What is the nature of the galectin-1-ligand interaction for lactosyl 1,2,3-triazole derivatives incorporating natural and unnatural carbohydrate residues?"***

The first question we will address is: *What general factors influence the binding of lactosyl and lactoseptanosyl 1,2,3-triazoles?*

ITC is a thermodynamic technique that allows the study of the interactions of two species.[37] When two species interact, heat is either generated or absorbed and by measuring the interaction heats, binding constants (K_a), reaction stoichiometry (*n*), and thermodynamic parameters including enthalpy (ΔH) and entropy (ΔS) can be accurately determined. My students and I will use the College's Microcal ITC to collect data for galectin-1 in the presence of the lactosyl and lactoseptanosyl 1,2,3-triazole ligands prepared in this study. Experiments will be conducted using parameters established by Brewer et. al.[38] The results will be used to determine which derivatives are more effective at binding galectin-1 and whether or not any trends exist between the molecules evaluated in this study.

The second question we will address is: *What is the nature of the binding interaction between lactosyl and lacosylseptanosyl 1,2,3 trazoles and galectin-1?*

In recent years STD-NMR has become an extremely useful tool for mapping protein-ligand interactions.[39] My students and I will develop an STD NMR assay that will be used to corroborate the ITC data and to collect more specific information about the nature of the galectin-1 ligand interaction. STD-NMR data will be collected for galectin-1 in the presence of the lactosyl and lactoseptanosyl 1,2,3-triazole ligands prepared in this study to determine which atoms play a role in binding to galectin-1. In addition, competitive STD-NMR experiments will be collected for the lactosyl and lactoseptanosyl 1,2,3-trizaole ligands in the presence of galectin-1 and its natural ligand poly-N-acetyllactosamine in order to determine the nature of the binding the binding event.

The results obtained from these experiments will used long term to aid in the design selective and potent inhibitors of galectin-1 that can serve as effective treatments for cancer, certain inflammatory diseases, and the Human Immunodeficiency Virus.

II. Teaching and Service Summary

The entire universe rests on four fundamental components: (i) gravitational forces, (ii) electromagnetic forces, (iii) strong forces, and (iv) weak forces. At lower temperatures, such as those observed on Earth, the four forces appear as separate entities that are somewhat interconnected. At higher temperatures, the electromagnetic force and the weak force appear as one process—the electroweak force. At even higher temperatures the electromagnetic force, weak force and strong force combine to form the strong electroweak force. At temperatures as high as those believed to have created the universe all four forces merge and act as one process—one force. This is the basis for the unified field theory.

Like the four fundamental forces, science education has been heavily compartmentalized into four major disciplines: (i) physics, (ii) chemistry, (iii) biology, and (iv) mathematics. A quick glance from the untrained eye any one of these fields might lead one to believe that these disciplines are relatively autonomous. However, I believe with the appropriate catalyst the traditional energy barriers that have limited the consilience of these disciplines can be overcome. The science departments at Hamilton College have already started to develop such a catalyst with the construction of a new $60 million science center that was designed to encourage collaboration between students and faculty across various disciplines in the sciences.

As a new faculty member at Hamilton College I am excited contribute to the progress that has already been made by my colleagues. Overall, I see my appointment as three fold commitment with teaching, research and service components. I have outlined my commitments in greater detail below. I feel my biggest strength will be my extensive training at the interface of chemistry and biology. My background will allow me to develop a unique and integrated approach to my research and my teaching consistent with the Colleges current mission and the Departments five year plan. Students in all of my classes will learn how chemistry and biology work together *in vivo* and *in vitro*. My second biggest strength will be my unique experience in community outreach. Outreach will play a large role in my career at the College and I look forward to establishing strong ties between the science departments at Hamilton and the surrounding community.

Teaching Commitment—Although my initial teaching commitment will be in the College's core organic chemistry sequence, I hope to work side by side with my colleagues to develop introductory, intermediate, and advanced courses that meet the needs of Hamilton College students across different disciplines. At the introductory level, I would like to offer an integrated science course that focuses on the application of physics, chemistry, biology, and math in the everyday world. In addition, I would like to use my formal training as a carbohydrate chemist to develop courses in immunology and drug design at the intermediate and advanced level that focus on physical, chemical and biological interactions at the molecular and the cellular level. Finally, I would like use my background in patent law to offer courses that focus on the legal aspects of science and technology. This course would serve to draw students from the both the science and the humanities, and would show that the traditional barriers between these two disciplines no longer really exist!

Research Commitment—My main research interest lie in the design, synthesis and evaluation of glycoconjugates that contain natural and unnatural carbohydrate residues and are outlined above. I encourage students from all backgrounds to pursue their interest in science through research, and I offer a nurturing, supportive, and active laboratory environment where the students and I work as a team, often learning from each other. In the past, I have had the pleasure of working with biology, biochemistry, and chemistry majors from every corner of the globe (Hong Kong, China, India, France, Mexico, US)! No student is ever discouraged because there is always space in the Snyder Group!

Service Commitment—In addition to my teaching and commitments at the College, I would like to help expand the College's visibility in the local community through outreach. Specifically, I would like to design outreach programs that introduce chemistry, biology, physics and mathematics through hands on experimentation to middle school students in underprivileged schools in the central New York region. I believe such a program provides an important platform for community engagement, and serves to elevate interests in sciences. I would also like to help expand the College's visibility at the national level by playing an integral role in establishing a nationally recognized charter of the Student Affiliates of the American Chemical Society. This organization supports undergraduate student, their faculty, and the College in promoting professional development, mentoring, and networking in the sciences.

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RESEARCH CORPORATION

A Foundation for the Advancement of Science

COTTRELL COLLEGE SCIENCE AWARDS

*Please confine responses to this form, using one side only; attach **only** pages specified in the instructions. Use no less than an Arial 10 point font or a Times New Roman 11 point font.*

PRINCIPAL INVESTIGATOR:	**Nicole L. Snyder**	PHONE:	**603-888-9198**
ACADEMIC RANK:	**Assistant Professor**	FAX:	**315-859-4807**
DEPARTMENT:	**Chemistry**	E-MAIL:	**NicoleLSnyder@aol.com**
INSTITUTION:	**Hamilton College**		
INSTITUTION ADDRESS:	**198 College Hill Road Clinton, NY 13323**	APPOINTMENT DATE:	**07/01/2007**

EDUCATION AND EXPERIENCE (All degrees, postdoctoral appointments, PhD and postdoctoral mentors, previous employment dates and locations. *See instructions for format.*)

2005-Present	Wellesley College, Wellesley, MA, Visiting Assistant Professor of Chemistry
2000-2005	The University of Connecticut, Storrs, CT, Ph.D. Chemistry, Mark W. Peczuh (Thesis Advisor)
1996-2000	Westminster College, New Wilmington, PA, B.S. Chemistry, Timothy Sherwood (Thesis Advisor)
1996-2000	Westminster College, New Wilmington, PA, B.S. Biology, J. Philip Fawley (Non-Thesis Advisor)

TITLE OF PROPOSAL (Must fit on two lines and not exceed *130 characters*.)

Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates

ABSTRACT: In the following space, provide a summary of your proposal plan. (Maximum 200 words.)

Cisplatin, an effective anti-tumor agent, has been used to treat a number of different types of cancer. Unfortunately, severe toxic side effects and the emergence of resistant tumor cell lines have limited or prohibited the use of this drug in certain patients. This has prompted researchers to search for improved derivatives of cisplatin. The research in this proposal focuses on the synthesis, characterization, and biological evaluation of an entirely new class of cisplatin derivatives that have the potential to reduce unwanted side effects and treat cisplatin resistant tumor strains. The proposed derivatives are novel in that they are the first examples of cisplatin derivatives to incorporate a carbohydrate-based enediyne group. The knowledge gained through the synthesis and biological evaluation of these derivatives will serve to further a general understanding of the anti-tumor properties of cisplatin analogs. Long term objectives for this project will focus the design and synthesis of cisplatin analogs that will be used to target specific cancer types.

PUBLICATIONS OF PRINCIPAL INVESTIGATOR

Peczuh, M.W.; Snyder, N.L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Lett.* **2003**, 44, 4057-4061.

Peczuh, M.W.; Snyder, N.L.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydr. Res.* **2004***, 339(6),* 1163-1171.

DeMatteo, M. P.; Snyder N. L; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *J. Org. Chem.* **2005**, *70*, 24-38

Castro, S.; Duff, M.; Snyder, N.L.; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Org. Biomol. Chem.* **2005**, *3,* 3869-3872.

Snyder, N.L.; Peczuh, M.W. Haines, H.M. "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006***, 62,* 9301-9320.

Castro, S.; Cherney, E. C.; Snyder, N. L.; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydr. Res.* **2007** *(In Press)*

PROPOSED BUDGET (explain, as appropriate, under Budget Rationale)	Year 1 RC	Year 1 Match	Year 2 RC	Year 2 Match
a) ITEMIZED EQUIPMENT *(computers and equip. over $1,000):*				
Biochrom Ultrospec 3300 UVVis ($21,000quote)	$7,000	$14,000	$0	$0
Dedicated computer and software ($3,000estimate)	$1,000	$2,000	$0	$0
b) SUPPLIES *($6,000 per year maximum from RC funds):* Supply costs include chemicals and consumables	$6,000	$12,000	$6,000	$12,000
c) STIPENDS *(not to exceed rates indicated):*				
Faculty Summer Stipend [up to $7,500 for 8 wks.] Weeks: 8	$7,500	$0	$7,500	$0
Undergrad Summer Stipend [up to $3,500 for 10 wks.] Weeks 10 Students per year 1	$3,500	$0	$3,500	$0
FICA/Medicare *(7.65% of stipend):* *(Note: No other benefits or indirect costs are allowed.)*	$570	$0	$570	
d) TRAVEL *(maximum of $2,500 per year from RC funds to conduct research; no conference or meeting travel)*	$0	$0	$0	$0
(keep RC funds separate from match) **TOTALS**	$25,570	$28,000	$17,570	$12,000

TOTAL BUDGET	$83,140
LESS MATCHING FUNDS FROM INSTITUTION	($40,000)
(*must be between $25,000 and $45,000*) REQUESTED FROM RESEARCH CORPORATION	$43,140

ADDITIONAL SUPPORT - List periods and direct cost amounts of all additional support [internal and external] including start-up received and requested for [1] this research, or [2] other research; identify requests that duplicate this one. (Start-up funding should not be listed as matching funds.)

2007-2010—$50,000.00 ([1], [2])—Hamilton College Startup

BUDGET RATIONALE - Include the source of other items [equipment/supplies], which are needed for this research beyond those requested from RC. Explain travel related to research and other budget items that require further clarification. List any items from Proposed Budget above for which a match is being provided and give dollar amount of the match.

NMR studies will be conducted on Hamilton College's 500MHz Bruker NMR Spectrometer.

STATEMENT OF THE PROBLEM AND SCIENTIFIC SIGNIFICANCE OF PROPOSED RESEARCH

Statement of the Problem—Since the serendipitous discovery of the anti-tumor properties of cisplatin (Figure 1) in 1970,[1] cisplatin has become one of the three most widely prescribed anti-tumor drugs in the world.[2] Cisplatin has been highly effective for the treatment of testicular, ovarian and cervical cancer,[3] and has been used as an adjuvant in the treatment of a number of other cancer types.[2]

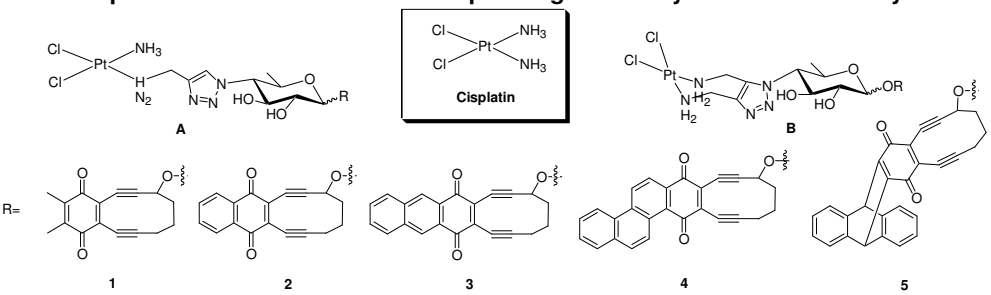
Cisplatin is believed to enter tumor cells through a passive diffusion mechanism. Once inside the cell, cisplatin is activated via hydrolysis and coordinates (platinates) the N7 nitrogen of a guanine base in the major groove of DNA. The 1,2-intrastrand,[4] 1,3-intrastrand[5] or interstrand[6] cross-linking of a second guanine base induces a conformational change in the DNA complex. The conformational change causes bending or unwinding of the helix, and/or widening of the hydrophobic minor groove resulting in loss of helix stability.[7] When DNA repair mechanisms are unable to repair the damaged DNA, apoptosis occurs.

Unfortunately, severe toxic side effects including nephrotoxicity, neurotoxicity, and ototoxicity, and acquired resistance to cisplatin have limited the use of this drug in the clinical setting,[8] prompting researchers to search for improved cisplatin derivatives. In response, over three thousand cisplatin analogs have been prepared and tested for biological activity.[2] Less than 0.1% of these derivatives have entered clinical trials, and of those only a handful of candidates have been proven to be effective.[9] Derivatives including carboplatin,[10] *cis*[$PtCl_2(NH_3)$(2-picoline)],[11] and a host of carbohydrate substituted analogs[12] have been effective at minimizing the side effects associated with cisplatin. However, since mechanism of action for these drugs is the same as the parent compound, only a partial reduction in cellular resistance is observed.

Recently, researchers have shown that cisplatin conjugates containing polycyclic aromatic hydrocarbon moieties can serve as effective anti-tumor agents that do not appear to be plagued by the cross resistance associated with other cisplatin derivatives.[13] These compounds have been shown to platinate DNA in a similar fashion to cisplatin, but provide a second mode of action that involves intercalating within the DNA complex. The mode of binding for these molecules produces a distorted DNA structure different from the structure produced by the parent cisplatin compound, resulting in reduced recognition by the DNA repair system. Unfortunately, many of these derivatives exhibit low solubility, or increased toxicity.

Scientific Significance—Current research in this field continues to focus on the design and preparation of cisplatin derivatives that act synergistically through platination and a second mechanism (usually intercalation or DNA groove binding) to combat resistant tumor lines. The research in this proposal focuses on an entirely new class of cisplatin analogs that incorporate a carbohydrate-linked to an enediyne head group (Figure 1). Carbohydrate-based enediyne anticancer antibiotics have played an important role in cancer treatment since their discovery in the late 1980s.[14] The carbohydrate is important for the solubility and recognition of these molecules, while the enediyne group of acts as a molecular "warhead" [15] that is triggered under physiological conditions to cleave DNA. As a result these molecules are of considerable interest as ligands for cisplatin and may offer an additional mode of attack against cisplatin resistant tumor lines.

Figure 1: Cisplatin and cisplatin derivatives A and B incorporating a carbohydrate-based enediyne moiety.

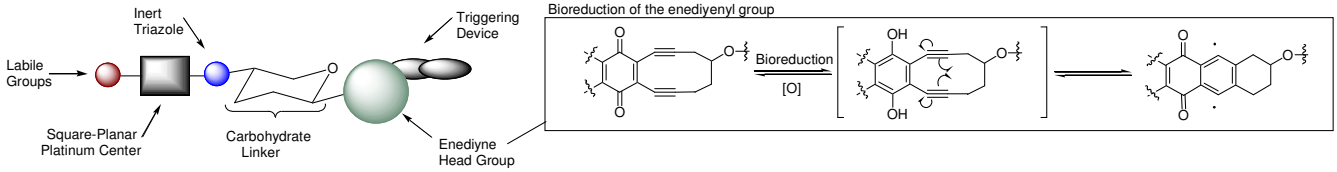


The focus of this study involves the synthesis, characterization, and biological evaluation of ten cisplatin enediyne analogs (Figure 1, A1-A5 and B1-B5). The specific question we will address in this study is: ***"Can carbohydrate-based enediyne cisplatin conjugates effectively combat cisplatin resistant tumor lines?"*** We believe that the compounds illustrated in Figure 1 will be able to effectively serve as platinating agents, selectively targeting guanine rich DNA sequences in similar fashion to cisplatin. In addition, we believe that these molecules will be able to effectively combat resistant tumor lines by interacting with DNA via a second mechanism that involves intercalation and eventual cleavage of double stranded DNA. We anticipate that these molecules will be as effective as similar derivatives that incorporate intercalating groups, but will be less toxic and may be able to more effectively target specific tumor cell lines. The knowledge gained through this study will be used to further a general understanding of the anti-tumor properties of cisplatin derivatives and will provide information on an entirely new class of molecules that contain both platinating and enediyne functional groups.

PLAN OF PROCEDURE

A. Design and Rationale—The general design for the proposed carbohydrate substituted platinum enediyne conjugates is outlined in Figure 2. The rationale for the proposed derivatives is based on three factors: (i) maintenance of the square planar platinum center required for activity, (ii) a flexible carbohydrate linker, and (iii) an enediyne group equipped with a triggering device.

Figure 2: Design and rationale of the proposed carbohydrate substituted platinum enediyne conjugates.



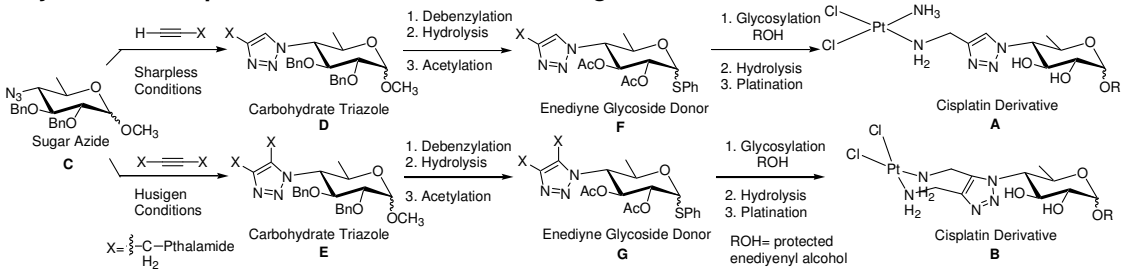
The proposed derivatives are associated with the active square planar platinum complex via an inert aromatic triazole. The triazole was chosen because it is simple to prepare and highly stable under physiological conditions. In this study, different chelation patterns will be used to assess the effects of chelation on activity. In the case of enediyne conjugates derived from **A** (Figure 1), the flexibility of the methylene group of the monosubstituted triazoles should not affect the ability of these analogs to adopt a square planar configuration. For enediyne conjugates derived from **B** (Figure 1), the flexibility of the seven member ring produced through chelation of the disubstituted triazole should allow the molecule to adopt the required square platinum conformation. Examples of highly effective cisplatin analogs chelated to ligands through seven member rings have been demonstrated in the literature.[16]

The flexible carbohydrate linker was inspired by an active platinum-doxorubicin conjugate.[17] Carbohydrates have played an important role in many DNA targeting drugs and have been shown to enhance cell recognition, membrane permeability, and DNA sequence specificity. The carbohydrate linker in this study serves as a flexible tether to the enediyne group. In addition, it is anticipated that the carbohydrate will aid in the solubility of the drug, will help minimize the associated side effects,[11] and may increase the selectivity of the analogs for certain cell types.

The enediyne groups (Figure 1, **1**-**5**) were inspired by a number of designed enediynes that showed significant DNA-cleaving properties and anti-tumor activities.[18] These molecules are relatively simple to prepare from their corresponding commercially available substituted hydroquinones, and are reasonably stable under ambient conditions. Once bound to DNA, we anticipate that the proposed enediyne group will undergo glutathione or NADPH catalyzed bioreduction[19] resulting in the conversion of the enediyne to the corresponding diradical through Bergman cyclization (Scheme 2). The diradical produced in this process will then cleave the DNA sugar-phosphate backbone. Analogs with different substitution patterns will be used in this study to assess the effects of enediyne substitution on platination and DNA cleavage.

B. Synthetic Strategy—The general synthetic strategy for cisplatin analogs derived from **A** and **B** (Figure 1) is shown in Scheme 1 below. Readily available sugar azide **C**[20] undergoes 1,3-dipolar cycloaddition under Sharpless conditions[21] (asymmetric alkyne, copper iodide and diisopropylethylamine) or Husigen conditions[22] (symmetric alkyne, toluene and heat) to produce carbohydrate triazoles **D** and **E** respectively. Carbohydrate triazoles **D** and **E** are then refunctionalized prior to glycosylation to produce enediyne glycoside donors **F** and **G**. Acetylated derivatives **F** and **G** are then glycosylated with alcohol acceptors derived from enediynes **1**-**5** (Figure 1) to produce the corresponding enediyne intermediate (not shown). Base catalyzed hydrolysis of the acetate and amide protecting groups provides the activated enediyne ligand which can be platinated to give the final enediyne analogs **A** and **B**. The synthesis of enediyne glycoside donors **F** and **G**, enediyne alcohols **1-5**, and the general glycosylation strategy for the preparation of enediyne conjugates **A** and **B** are outlined in detail below.

Scheme 1: Synthesis of cisplatin derivatives A and B from sugar azide C.

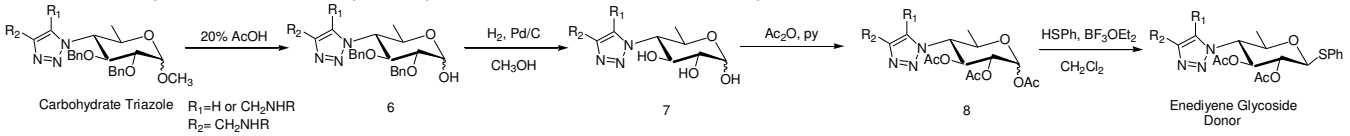


PLAN OF PROCEDURE, continued

I. *Synthesis of Enediyne Glycoside Donors F and G from Carbohydrate Triazoles D and E*: The general synthesis of sugar enediyne glycoside donors **F** and **G** is illustrated in Scheme 2 below. This sequence is necessary for two reasons. First, anomeric thiols are better donors for glycosylation reactions than anomeric ethers. Second, the conditions required to remove the benzyl groups in later steps would also hydrogenate the enediyne head group.

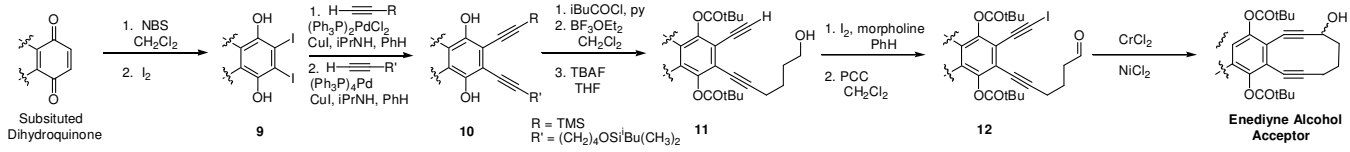
Acid catalyzed hydrolysis of the mono or disubstituted carbohydrate triazole using 20% acetic acid provides lactol **6**. Hydrogenolysis of lactol **6** under standard conditions affords triol **7**. Acetylation of the triol using acetic anhydride and pyridine provides triacyl derivative **8** which can then be readily converted to the corresponding enediyne glycoside donor using benzene thiol in the presence of a Lewis acid catalyst.

Scheme 2: Synthesis of Enediyne Glycoside Donors from Carbohydrate Triazoles



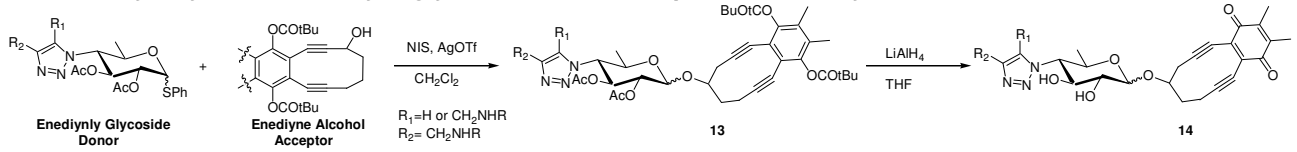
II. Synthesis *of Enediyne Alcohols 1-5*: Enediyne alcohols **1**-**5** can be synthesized via substitution of the corresponding commercially available hydroquinones as described by Nicolaou[18] and outlined in Scheme 3. Bromination followed by iodine exchange of the desired substituted hydroquinone gives **9**. Sequential palladium catalyzed coupling of diiodo hydroquinone **9** using (trimethylsilyl)acetylene and 6-dimethyl-tert-butylsiloxy-1-hexyne provides **10**. Protection of the phenolic alcohols of **10** with t-butoxy acetyl chloride, followed by sequential desilylation gives **11**. Iodination of the terminal acetylene of **11** followed by oxidation of the primary alcohol with PCC gives **12**. The Cr-Ni catalyzed ring closure of **12** gives the targeted protected enediyne alcohol.

Scheme 3: General synthesis of enediyne alcohols 1-5 from the corresponding substituted hydroquinone.



III. *General Glycosylation/Hydrolysis Strategy:* Once the protected enediyne alcohols are prepared (see Scheme 3) they will serve as acceptors in glycosylation reactions with the previously prepared enediyne glycoside donors (see Scheme 2) as outlined in Scheme 4. Activation of the desired enediyne glycoside donor with N-iodosuccinamide and silver triflate, followed by the addition of the protected enediyne alcohol acceptor gives intermediate **13**. Reduction of the ester and amide groups using lithium aluminum hydride provides **14** which can be converted to the subsequent enediyne cisplatin analog (Figure 1 **A** or **B**) via platination (not shown).

Scheme 4: Glycosylation of enediyne glycoside donors with protected enediyne alcohols 1-5.



C. Characterization—Cisplatin analogs derived from **A** and **B** are the first examples of cisplatin derivatives that incorporate sugar enediyne moieties. Full characterization of these compounds in addition to the intermediates created in their preparation will be accomplished using Hamilton College's 500MHz Bruker NMR spectrometer and Jasco P-1020 Polarimeter. Proton and carbon NMR experiments will be used to determine the overall structure and solution conformation. Optical rotations will be collected for any enantiopure compounds synthesized. Samples will also be submitted for mass spectral analysis to support the results obtained from NMR.

D. Biological Evaluation—Interactions between DNA and the cisplatin analogs prepared in this study will be evaluated using agarose gel electrophoresis and UV visible spectroscopy. Electrophoretic mobility experiments[23] using supercoiled DNA will be used to determine changes in tertiary DNA structure. Cleavage patterns for each of the derivatives will be compared to cisplatin and the unsubstituted enediyne alcohol to determine whether the binding mode is dominated by platination, intercalation, or a combination of both. UV visible spectroscopy[24] will be used to evaluate changes in secondary DNA structure. Temperature-dependent UV visible absorption spectra will be collected and used to assess the stability of calf thymus DNA in the presence of each of the analogs. It is anticipated that binding of the proposed derivatives will cause unstacking of DNA base pairs resulting in a hyperchromic effect that will be characteristic for each derivative and different from that of cisplatin or the enediyne alcohols alone. Future work will involve testing active analogs against cisplatin resistant tumor lines.

LIST OF REFERENCES

LIST OF REVIEWERS

The reviewer list should include at least eight "outsiders," individuals with whom you have had no substantive contact, who are experts in your area of research, and at least two "insiders," preferably former mentors. The best outside reviewers are frequently corresponding authors not known to you in the cited references. Do not hesitate to use a combination of reviewers from academia and non-academic laboratories; including scientists from abroad. We may also select reviewers of our choice. Please include complete names (initials are not enough), mailing addresses, phone and fax numbers, and e-mail addresses. **You must note briefly the nature and extent of your interactions, if any, with <u>each</u> of the outside reviewers. Examples: Met at a meeting, interviewed with, no interaction, never met, etc. Do not use more than one page.**

Outside Reviewers (in alphabetical order):

1. Carolyn Bertozzi; Department of Chemistry, University of California, Berkeley, CA, 94720, USA; Phone: 1-510-643-1682; Fax: 1-510-643-2628; E-mail: bertozzi@cchem.berkely.edu
   -I met Carolyn at an ACS meeting in March of 2007, and a former research student of mine from Wellesley College will be conducting research in her laboratory this summer.

2. Ulrich Bierbach; Department of Chemistry, Wake Forest University, Salem Hall, Box 7486, Winston-Salem, NC 27109, USA; Phone: 1-336-758-3507; Fax 1-336-758-4656; Email: bierbau@wfu.edu
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3. Elizabeth Jamieson, Smith College Department of Chemistry, 44 College Lane, Northampton, MA 01063, USA; Phone: 1-413-585-7588; Fax: 1-413-585-3786; Email: ejamieso@email.smith.edu
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4. Stephen Lippard; Department of Chemistry, Massachusetts Institute of Technology, Room 18-498, 77 Massachusetts Avenue, Cambridge, MA 02139, USA; Phone: 1-617-253-1829; Fax: 1-617-258-8150; Email: lippard@mit.edu
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5. Kyriacos Costa Nicolaou; Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA; Phone: 1-858-784-2400; Fax: 1-858-784-2469; Email: kcn@scripps.edu
   -I have never met Kyriacos.

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7. Ernest Wong, AnorMed Inc. 200-20353 64th Avenue, Langley, British Columbia, Canada V2Y 1N5; Phone: 011-1-604-530-1057; Fax: 1-604-530-0976, Email: ewon@anormed.com
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8. Shiganobu Yano; Division of Material Science, Graduate School of Humanities and Sciences, Nara Women's University, Nara 630-8506, Japan; Telephone/Fax: 81-742-20-3392; Email: yano@cc.nara-wu.ac.jp
   -I have never met Shiganobu.

Inside Reviewers (n alphabetical order):

1. Christian Bruckner; Department of Chemistry, University of Connecticut, 55 North Eagleville Road; U 3060; Storrs, CT 06269; Phone: 1-860-486-2743; Fax: (860) 486-2981; Email: c.bruckner@uconn.edu
   -Christian was a former mentor at the University of Connecticut. He was not on my thesis committee.

2. Mark Peczuh; Department of Chemistry, University of Connecticut, 55 North Eagleville Road; U 3060; Storrs, CT 06269; Phone: 1-860-486-1605; Fax**:** 860-486-2981; Email: mark.peczuh@uconn.edu
   -Mark was my doctoral mentor at The University of Connecticut.

ENDORSEMENT PAGE

Conditions of Research Corporation's Cottrell College Science Awards

A RESEARCH CORPORATION AWARD is a contribution to the scientific and academic program of the institution and is to be used for support of work described in the application prepared by the principal investigator and adopted by the institution.

Since research by its very nature is unpredictable and may require adaptations in order to exploit promising leads, the principal investigator should feel free to make changes in the emphasis or direction of the work as it progresses. If major changes are contemplated, prior approval should be obtained.

The amount of the award is the total of the items approved in the budget submitted as part of the application. If it differs from the amount requested, the differences are noted in the letter of notification from the Vice President. Reallocation of awarded funds between budget categories requires prior approval. Faculty salaries not approved in the budget, indirect costs or overhead, secretarial assistance, and other costs not specifically in the budget are not allowed.

Financial and scientific reports prepared on the foundation's forms are absolutely required. The first report is due within 30 calendar days of the 12-month anniversary of the award start date. The final report is due within 30 calendar days of the 24-month anniversary of the award start date. Failure to provide the first annual report may result in suspension of the award and a request to return unspent funds. Failure to provide the final report will result in suspension of the institution from participation in Research Corporation programs. A single 12-month no cost extension is possible, but must be requested by the principal investigator prior to the 24-month anniversary of the award start date. For approved extensions a 24-month report is required and the final report is due within 30 calendar days of the 36-month anniversary of the award start date.

The principal investigator is urged to publish the findings in the appropriate scientific journals, acknowledging the support of Research Corporation and the appropriate donor, if any. One reprint of each publication resulting from the work is to be sent to the Research Corporation office.

Research Corporation awards are true awards to the institution, not contracts for research with the institution or the principal investigator, and Research Corporation disclaims any rights in the results of the research.

APPLICANT: Nicole L. Snyder

PROJECT TITLE: Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates.

SUBMITTED BY (Institution): Hamilton College

Name of institution adopting and assuming responsibility for the above project, believing the principal investigator is qualified to conduct the project, and accepting the Conditions of Award, if an award is approved.

Name and Position of Authorized Financial Officer _____

Signature of Financial Officer _____ Date _____

Name of Chief Executive Officer and Title (President or Chancellor); *signature not required* _____

Signature of Principal Investigator Nicole L Snyder Lee Date 05/10/2007

SCAN THIS SIGNED *ENDORSEMENT* PAGE AS THE LAST PAGE OF YOUR PDF FILE
(NOTE: If awarded, this Endorsement Page – with original signatures – must be *mailed* to Research Corporation)

[1] (a) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. "Platinum Compounds: A New Class of Potent Antitumour Agents." *Nature* **1969**, *222*, 385-386.
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[3] (a) Bosl, G. J; Motzer, R. J. "Medical Progress: Testicular Germ-Cell Cancer**."** *N. Engl. J. Med.* **1997**, *337*, 242-253. (b) Morris, M.; Eifel, P. J.; Lu, J.; Grigsby, P. W.; Levanback, C.; Stevens, R. E.; Rotman, M.; Gershenson, D. M.; Mutch, D. G. "Pelvic Radiation with Concurrent Chemotherapy Compared with Pelvic and Para-Aortic Radiation for High-Risk Cervical Cancer**.**" *N. Engl. J. Med.* **1999**, *340*, 1137-1143. (c) Rose, P.G.; Bundy, B. N.; Watkins, E. B.; Thigpen, J. T.; Deppe, G.; Maiman, M. A.; Clarke-Pearson, D. L.; Insalaco, S." Concurrent Cisplatin-Based Radiotherapy and Chemotherapy for Locally Advanced Cervical Cancer**."** *N. Engl. J. Med*. **1999***, 340*, 1144-1153.
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Subject: FW: 7401 - Excerpts
From: Sandy Champion <sandy@rescorp.org>
Date: Wed, 31 Oct 2007 16:09:39 -0700
To: nsnyder@hamilton.edu

Dear Dr. Snyder:

As you know from our previous email to you, your Cottrell College Science Award proposal was not recommended for funding. Excerpts of pertinent external reviews are included with this email (see below).

In your particular case, significance of the proposed work is high and all reviewers recognize this. They were also generally supportive of the idea of combining the known activity of Pt cancer drugs with enediynes linked with a carbohydrate. However, there were significant concerns expressed about the complexity of the syntheses and the rational for the mechanism by which this combination would have an enhanced effect. The extensive amount of work proposed seemed overly ambitious for a two year award of this scope. Further, general statements like "minimize side effects" and "increase selectivity" were not backed up with a detailed rationale/explanation for why this would be expected. In addition, while case for significance falls on the biological activity of the new materials little was said about what would be done with the compounds if the syntheses were successful.

Nonetheless, there was general support for the significance of the project and some of the aims, and I encourage you, after reading and considering the comments of the reviewers, to consider submitting a revised application for our May 15, 2008 application target.

Please be aware that we anticipate changes in our programs early next year. I suggest that you check our website after February 1, 2008 to learn more about our revised programs.

Best wishes,

Jack Pladziewicz
Research Corporation
4703 East Camp Lowell, Suite 201
Tucson, AZ 85712
Phone: 520-571-1111
Fax: 520-571-1119
E-mail: jrp@rescorp.org

Outside Reviewer Excerpts:

Research Corporation
7401: "Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
(PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)
SCIENTIFIC SIGNIFICANCE:
The PI has chosen a problem with high significance/high impact to human health. The question posed is an important one.
There is an underlying hypothesis in the proposal, but it is not thoroughly developed.

ORIGINALITY
The new cisplatin analogs are highly original. This is a strong point of the proposal. However, the ene-diynes should have a mechanism of action that is essentially the same as cisplatin (DNA strand breaks to initiate apoptosis). There is no clear argument as to why this would be superior to a conjugate that could utilize multiple mechanisms of action.

FEASIBILITY OF THE PROPOSAL:
The synthesis looks feasible. It is straightforward and convergent. The DNA cleavage assays seem feeble and may not provide sufficient information on which analogs to pursue over others. Nonetheless, they will be a first step. Further investigation via collaboration with the new molecules is suggested. Identification of a collaborator would have made this proposal even stronger.

Overall this is an excellent proposal and funding is strongly supported. The proposed research will serve two key purposes: 1 - It has potential to introduce a new class of functional cisplatin analogs; 2 -undergraduates will receive detailed hands-on training. I enthusiastically support funding this proposal.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
7401: "Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
*PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.
This proposal describes Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates.

This proposal is of great interest. I recommend that it be accepted.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
7401: "Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
*PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.
Prof. Snyder proposes to prepare novel glycosylated enediyne structures and conjugate these structures with cis-platin. Both motifs are known structures with great relevance to the treatment of cancer and carbohydrates well known to in part improved biological activity/pharmakinetic properties. There is no question that these are very novel structures, which will be interesting to medicinal chemist. I found this to be an excellent proposal that could be made better is a stronger rationale linking enediynes with cisplatin. Unfortunately, this stronger link may only come from making and testing these structures.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
7401: "Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
*PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.
The idea behind this Cottrell College Science Award proposal, i.e. conjugating a cis-platin-like moiety through a monosaccharide tether to an enediyne, is interesting as the compounds are suggested as potential anti-cancer agents bearing two active groups. The monosaccharide may well enhance solubility and the 1,2,3-triazole linker is indeed easy to install, however there are some concerns regarding the chemistry and the overall construction of the proposal.

The title of the proposal is "Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Conjugates," which implies that the molecules produced during this work will be investigated for their anti-tumor properties as part of THIS proposal. This is not the case; initial biological studies are described but the actual anti-tumor properties are proposed as "future work" and so evidently are not planned here. With this in mind, the proposal of novel chemistry here would make up for that deficiency, however the construction of the targets borrows entirely from literature methods. The syntheses will likely work but the lack of a plan for testing detracts from the overall impact.

In terms of the actual synthetic routes proposed the major concern is feasibility. Taking into account the synthesis of azidodeoxy sugar C (Scheme 1), which is not commercially available, the total number of steps, from purchasable material to the target compounds, approaches 20. This is overly ambitious for a new investigator who does not have the support of graduate students and post-docs. Indeed, a major flaw in the proposal is the request for only one student stipend each summer; that won't be enough of a "workforce" to make serious inroads into this project within the funding period. While the cis-platin and enediyne groups are self-explanatory, the carbohydrate chemistry opens up some obvious questions. Why that particular 4-azido-4,6-doexy sugar? The 6-deoxy portion will not help with solubility and having groups at both C-1 and C-4 of the sugar platform may well reduce its ability to serve as a recognition unit and thus detract from claims that "selectivity" would be enhanced by the sugar's presence. What anomeric selectivities are expected in the glycosylation chemistry? Separation of such diastereomeric mixtures may not be trivial for novice undergraduates and anomeric identity will obviously play a major role on the overall 3-dimensional structure of the target compounds.

Overall this is a good idea but it could be improved by being a little more realistic in terms of the complexity of the syntheses and the way the proposal is constructed with regards to overall goals.

(4) GOOD: Important, lacks imagination. (top 50%)

Research Corporation
7401: "Exploring Platinum Anti-tumor Chemistry Using Carbohydrate-Based Enediyne Cisplatin Conjugates"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
*PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.
This well written and organized proposal by Sneyder deals with the synthesis and DNA testing of a novel, bifunctional compound class: cis-platin-enediyne conjugates. Moreover, the linkage between the two functional moieties is designed to be functional, allowing some flexibility, improving the solubility of the drug, and facilitating the conjugation by means of click chemistry. The design and synthesis of the compounds is clearly laid out, and is deemed absolutely feasible. All steps have precedent in the literature, yet the design of the proposed anti-tumor drugs is novel, incorporating latest findings for the design and functioning of cis-platinum-based drugs. The drugs will be tested in vitro on DNA using standard tests.

Even if the drugs do not fulfill the high expectations the PI has for them, their synthesis is worthwhile, especially within an undergraduate institution as the synthesis combines a number of up-to-date techniques and compound classes that will provide a very broad learning experience for the students involved, touching upon endiynes, carbohydrates, click chemistry, cis-platinum-based drugs, biodistribution and other drug-design-related issues, and the biological evaluation of DNA-targeting drugs. It certainly will provide the

opportunity for publications. Moreover, the convergent design strategy allows the dissection of the total synthesis into many individual projects for undergraduate involved.

In closing, this is an outstanding proposal that can be strongly recommended for funding.

(1) OUTSTANDING: Highly novel. (top 5%)

RESEARCH CORPORATION

A Foundation for the Advancement of Science

COTTRELL COLLEGE SCIENCE AWARDS

(Single Investigator Program)

*Please confine responses to this form, use one side only; attach **only** those additional pages as specified in the instructions. Use an Arial 10 point font or larger.*

PRINCIPAL INVESTIGATOR:	**Nicole L. Snyder-Lee**	PHONE:	**315-859-4742**
ACADEMIC RANK:	**Assistant Professor**	FAX:	**315-859-4807**
DEPARTMENT:	**Chemistry**	E-MAIL:	**nsnyder@hamilton.edu**
INSTITUTION:	**Hamilton College**		
INSTITUTION ADDRESS:	**198 College Hill Road Clinton, NY 13323**	APPOINTMENT DATE:	**07/01/07**

EDUCATION AND EXPERIENCE (All degrees, postdoctoral appointments, PhD and postdoctoral mentors, previous employment dates and locations. *See instructions for format.*)

Education/Training:
1996-2000—Westminster College, Department of Chemistry, New Wilmington, PA, B.S. Chemistry/Biology
2000-2005—University of Connecticut, Department of Chemistry, Storrs, CT, Ph.D. Chemistry (Advisor: M. W. Peczuh)

Experience:
2007-Present—Hamilton College, Clinton, New York, Assistant Professor of Chemistry
2005-2007—Wellesley College, Wellesley, Massachusetts, Visiting Assistant Professor of Chemistry
2001-2002—US Nanocorp, Willington, Connecticut, Research Assistant
2002-2004—University of Connecticut, Storrs, Connecticut, Science Wizards Journeyman Program Coordinator

TITLE OF PROPOSAL (Must fit on two lines and not exceed *130 characters*.)

The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts

ABSTRACT: In the following space, provide a summary of your proposal plan. (Maximum 200 words.)

The recent development of palladium-catalyzed cross-coupling reactions between substituted bromo-porphyrins and amides, amines, alcohols, and thiols has allowed for the preparation of a number of chiral metallo porphyrins that have been used to catalyze several key functional group transformations, including asymmetric cyclopropanation reactions, with good stereoselectivity. Despite the progress that has been made in this field, two key problems still exist. First, the hydrophobic nature of the porphyrin catalysts currently in use makes them relatively insoluble in polar solvents, limiting the utility of these systems as catalysts under polar conditions. In addition, the aromatic nature of the porphyrin ring system often facilitates pi stacking resulting in the aggregation of the catalyst in solution leading to decreased efficiency. To address these problems, we are proposing the synthesis and evaluation of several novel porphyrins bearing carbohydrate ligands. The polarity and conformational nature of the carbohydrate-porphyrin conjugates outlined in this study makes them excellent candidates for addressing the key issues associated with porphyrin catalysis. The ***specific goals*** of this project are: (i) to develop and optimize a palladium-catalyzed cross-coupling reaction to prepare carbohydrate-porphyrin conjugates bearing different carbohydrates, and (ii) to assess the ability of carbohydrate-porphyrin conjugates to serve as asymmetric catalysts.

PUBLICATIONS OF PRINCIPAL INVESTIGATOR (List all during last five years [***no*** abstracts, talks or conference proceedings], include all authors and titles; attach no more than one additional page. For papers with more than 10 authors you may list only the corresponding author(s) and your rank among all authors, e.g., 52 out of 200.)

Ruppel, J. V.; Gauthier, T. J.; Snyder, N. L.; Perman, J. A. ; Zhang, X. P. "Asymmetric Cobalt-Catalyzed Cyclopropanation with Succinimidyl Diazoacetate: General Synthesis of Optically Active Cyclopropyl Carboxamides." *Submitted.*

Markad, S. D.; Xia, S.; Snyder, N. L.; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxdation of Carbohydrate Based Oxepiens." *Journal of Organic Chemistry* **2008**, 73, 6341-6354.

Castro, S.; Cherney, E. C.*; Snyder, N. L.; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007**, *342(10)*, 1366-1372.

Snyder, N.L.; Peczuh, M.W. Haines, H.M.* "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006***, 62,* 9301-9320.

Castro, S.; Duff, M.; Snyder, N. L.; Morton, M.; Kumar, C. V.; Peczuh, M. W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic Biomolecular Chemistry* **2005**, *3,* 3869-3872.

DeMatteo, M. P.; Snyder, N. L.; Morton, M.; Baldisseri, D.; Haddad, C. M.; Peczuh, M. W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38.

Peczuh, M. W.; Snyder, N. L.; Fyvie, W. S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004***, 339(6),* 1163-1171.

Peczuh, M. W.; Snyder, N. L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, 44, 4057-4061.

PROPOSED BUDGET (explain, as appropriate, under Budget Rationale)	Year 1 RC	Year 1 Match	Year 2 RC	Year 2 Match
a) ITEMIZED EQUIPMENT *(computers and equip. over $1,000):* Approximately $24,000.00 is allotted for the purchase of a new digital polarimeter from Jasco.	$8000	$16000	$0	$0
b) SUPPLIES *($6,000 per year maximum from RC funds):* Includes chemicals and minor equipment (under $1,000.00)	$6000	$12000	$6000	$12000
c) STIPENDS *(not to exceed rates indicated):* Faculty Summer Stipend [up to $7,500 for 8 wks.] Weeks: 8	$7500	$0	$7500	$0
Undergrad Summer Stipend [up to $3,500 for 10 wks.] Weeks 10 Students per year 1	$3500	$4000	$3500	$4000
FICA/Medicare *(7.65% of stipend):* *(Note: No other benefits or indirect costs are allowed.)*				
d) TRAVEL *(maximum of $2,500 per year from RC funds to conduct research; no conference or meeting travel)*	$1500	$1500	$1500	$1500
(keep RC funds separate from match) **TOTALS**	$26500	$33500	$18500	$17500

TOTAL BUDGET	$96000
LESS MATCHING FUNDS FROM INSTITUTION	($51000)
(*must be between $25,000 and $45,000*) REQUESTED FROM RESEARCH CORPORATION	$45000

ADDITIONAL SUPPORT - List periods and direct cost amounts of all additional support [internal and external] including start-up received and requested for [1] this research, or [2] other research; identify requests that duplicate this one. (Start-up funding should **not** be listed as matching funds.)

2007-2010—$50,000.00 ([1], [2])—Hamilton College Startup

BUDGET RATIONALE - Include the source of other items [equipment/supplies], which are needed for this research beyond those requested from RC. Explain travel related to research and other budget items that require further clarification. List any items from Proposed Budget above for which a match is being provided and give dollar amount of the match.

NMR studies will be conducted on Hamilton College's 500MHz Bruker NMR Spectrometer.

UV-Vis measurements will be taken on one of the College's Hewlett Packard UV visible Spectrometers.

Travel expenses are for travel to and from the University of South Florida.

A new polarimeter is being requested (see attached quote). The polarimeter is quoted at $24,000.00 with $8.000.00 requested from Research Corporation and a $16,000.00 match coming from Hamilton College.

STATEMENT OF THE PROBLEM AND SCIENTIFIC SIGNIFICANCE OF PROPOSED RESEARCH

Chiral cyclopropane rings are found in a number of biologically relevant natural products. For example, the antifungal nucleoside **1-1**,[1] antitumor curacin A **1-2**,[2] and antifungal ambruticin **1-3**[3] all contain chiral cyclopropane units that are critical to the biological functions of these compounds. Because of the importance of chrial cyclopropane rings, a number of reactions have been developed for their synthesis.[4] However, the development of catalysts that can catalyze asymmetric cyclopropanation with a variety of substrates in high yield and with excellent diastereo- and enantioselectivity is still a major area of research in the field.

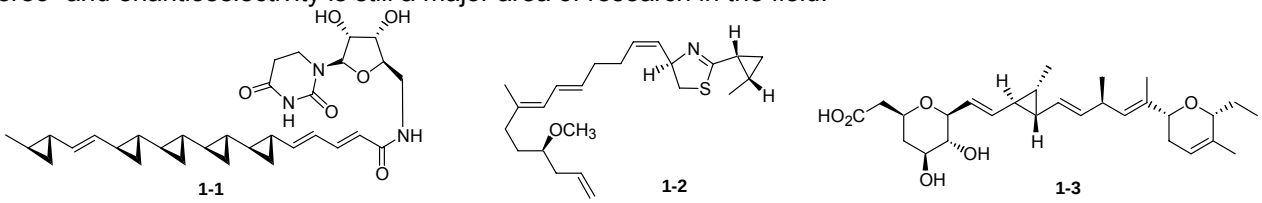
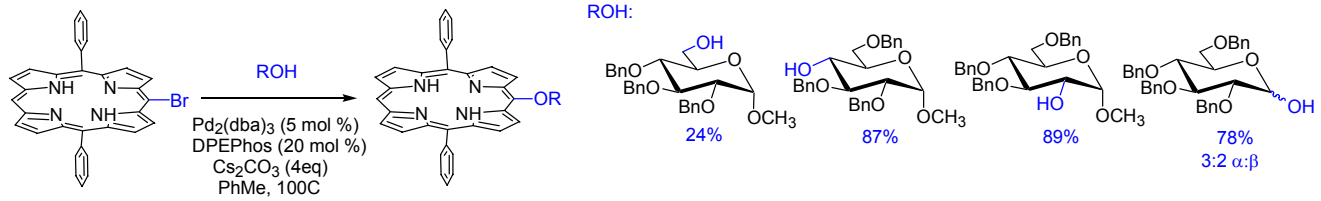


Figure 1. Examples of biologically relevant natural products containing cyclopropane rings.

The recent development of palladium-catalyzed cross-coupling reactions between mono-, di-, and tetrasubstituted bromo-porphyrins and amides, amines, alcohols, and thiols has allowed for the preparation of a number of chiral amino-, amido-, oxo- and mercaptoporphyrins.[5] The cobalt derivatives of these chiral porpyrins have, in turn, been used to catalyze a number of key functional group transformations including the asymmetric cyclopropanation of aromatic and electron-deficient olefins using diazo reagents with good diastereo- and enantioselectivity.[6] Despite the progress that has been made in the development of these catalysts, two key problems still exist. First, the hydrophobic nature of the porphyrin ring makes these systems insoluble in most polar solvents. This limits the utility of these catalysts under polar reaction conditions, further limiting the types of substrates that can be cyclopropanated. In addition, the aromatic nature of the porphyrin ring system facilitates pi stacking with some existing porphyrin catalysts. This results in catalyst aggregation and decreased turnover.

In an effort to address these problems, my undergraduate students and I have started to synthesize novel porphyrins bearing carbohydrate residues in collaboration with Dr. Peter Zhang's group at the University of South Florida. Carbohydrate-porphyrin conjugates or "CarboPhyrins," are chiral molecules that have the potential to serve as asymmetric catalysts. In addition to conferring chirality, we believe the carbohydrate ligands will help improve the overall solubility of the porphyrin catalysts in polar solvents, including water. The conformational nature of the carbohydrate ring should also help decrease the pi stacking effects observed in some systems, without sacrificing stereoselectivity and yield.

The carbohydrate-porphyrin conjugates we have synthesized thus far have been prepared by cross coupling bromo-porphyrin synthons with selectively functionalized carbohydrates using tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) as a source of palladium and bis(2-dipheneylphosphinophenyl) ether (DPEphos) as a ligand source in the presence of cesium carbonate (Cs_2CO_3). An example of the coupling reactions between *meso*-bromodiphenylporphyrin and four carbohydrate analogs derived from glucose is shown in Scheme 1.[7]



Scheme 1. Preparation of carbohydrate-porphyrin conjugates using palladium catalyzed carbon-oxygen coupling reactions.

Through these preliminary studies, we were able to assess the feasibility of performing palladium catalyzed cross coupling reactions with glucose via different carbohydrate linkages. The results obtained from these experiments have provided us with a foundation for the preparation of additional carbohydrate-porphyrin conjugates containing multiple carbohydrate ligands that can serve as effective and efficient catalyst for the asymmetric synthesis of cyclopropane rings. Therefore, the ***specific goals*** of this project are:

(i) To develop and optimize a palladium-catalyzed cross-coupling reactions to prepare carbohydrate-porphyrin conjugates bearing multiple carbohydrate substituents, and

(ii) To assess the ability of carbohydrate-porphyrin conjugates to serve as chiral catalysts in asymmetric cyclopropanation reactions.

The specific question we are trying to answer is: ***"Can carbohydrate-porphyrin conjugates be used as effective catalyst's for asymmetric cyclopropanation reactions?"***

PLAN OF PROCEDURE

I. Synthesis of Carbohydrate Analogs- The carbohydrate analogs required for the work in this proposal are shown in Figure 2 and will be prepared by Hamilton College undergraduate students. Pyranose derivatives **2-1** through **2-3** are synthesized from readily available methyl-α-D-glucopyranoside using standard protecting group chemistry.[8] Pyranose derivative **2-4** is prepared based on a report by McMillan and coworkers.[9] Furanose derivatives **2-5**, **2-6**, and **2-8** are prepared from commercially available methyl-furanosides.[8] Finally, furanose derivatives **2-7** and **2-9** will be prepared based on the procedure for pyranose derivative **2-4**.[9]

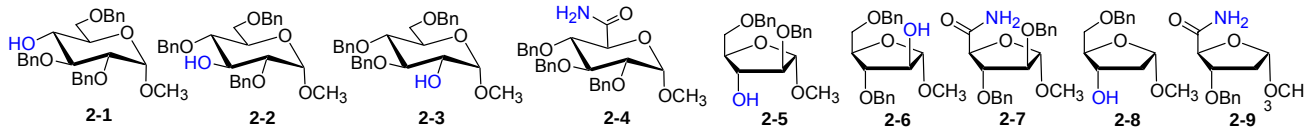


Figure 2. Carbohydrate derivatives for the synthesis of carbohydrate-porphyrin conjugates.

The derivatives in Figure 2 were chosen to study how the size and connectivity of the carbohydrate affects the overall chiral environment of the carbohydrate-porphyrin conjugate. This in turn, will presumably affect the reactivity and selectivity of the resulting catalyst. For example, Spartan modeling studies show that the conformation of carbohydrate-porphyrin conjugates bearing glucopyranose residues linked through the 4-OH (**3-1**) versus the 2-OH (**3-2**) provide for conjugates with different chemical environments (Figure 3).

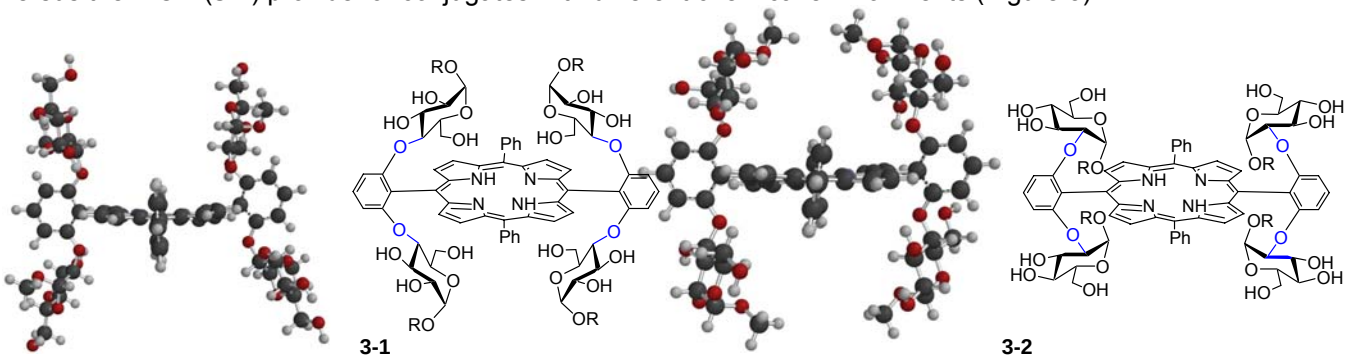


Figure 3. Models of carbohydrate-porphyrin conjugates with glucose residues linked through the 4'OH (**A**) and 2'OH (**B**).

II. Synthesis of Porphyrin-Carbohydrate Conjugates- The porphyrin synthons for this work, shown in Figure 4 below, will be provided by Dr. Peter Zhang's group at the University of South Florida, and as part of their summer research experience, Hamilton College students will have the opportunity to travel to the University of South Florida for two weeks each summer to participate in the construction of these synthons. Synthons **4-1** through **4-4** were chosen to determine (i) how the position (2,6 or 3,5) of the carbohydrate substituents on the A and B rings orthogonal to the porphyrin ring affect the catalytic activity, and (ii) how the functional groups (methoxy or t-butyl) on the neighboring rings C and D rings affect the reactivity. Synthons **4-1** and **4-2** are especially noteworthy for the success that has been already achieved with these systems in asymmetric cyclopropanation reactions.[6]

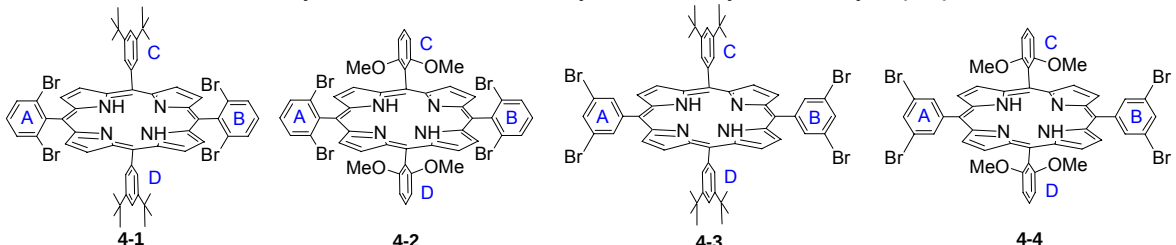
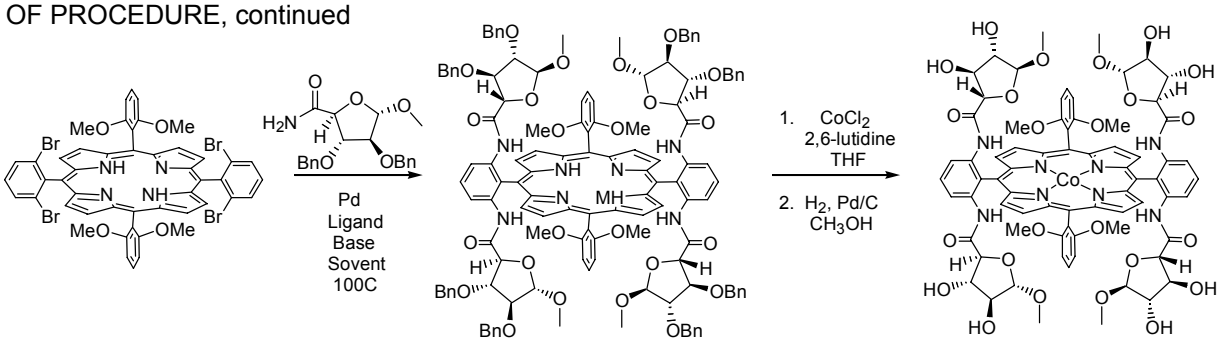


Figure 4. Synthons prepared by Zhang group for use in this study.

A general scheme for the preparation of porphyrin-carbohydrate conjugates is shown in Scheme 2 below. For each of the bromo-synthons investigated in this study, the palladium source, ligand, and carbohydrate will be dissolved in an appropriate solvent using standard Schlenk technique, and heated to 100°C until the starting material is completely consumed. My students and I will use the optimized conditions already in place for the monosubstituted porphyrin derivatives (Scheme 1) as a starting point for the tetrasubstituted derivatives. After purification, the porphyrin will be metalated using cobalt chloride and 2,6-lutidine in THF. Finally, hydrogenolysis to remove the protecting groups provides the corresponding derivative as shown in Scheme 2 below.

PLAN OF PROCEDURE, continued



Scheme 2. General scheme for the synthesis of carbohydrate-porphyrin conjugates.

Several different conditions will be systematically screened in order to achieve the best yields. The sources of palladium used in this study, including palladium (II) acetate ($Pd(OAc)_2$) and tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) will help us determine whether palladium (II) or palladium (0) is more effecient. The ligands employed in this study (Figure 5) will explore the effects of size and flexibility on the synthesis of the carbohydrate-porphyrin conjugates prepared in this study. Ligands that will be used include commercially available bis (2-diphenylphosphinophenyl)ether (DPEphos) **5-1**,9,9-dimethyl-4,5-bis(diphenylphosphino)xanthenes (Xantphos) **5-2**, BINAP **5-3**, 2-(di-*tert*-butylphosphino)-biphenyl **5-4**, 2-(dicyclohexylphosphino)-biphenyl **5-5**, 1,3-bis(2,6-diisoprpylphenyl)imidazolium chloride **5-6**, and readily available 2-(9-phenanthryl)phenyl-dicyclohexylphosphine **5-7**.[10] Finally, a number of different bases including sodium and potassium *tert*-butoxide, sodium, potassium and cesium carbonate, and potassium phosphate will also be employed.

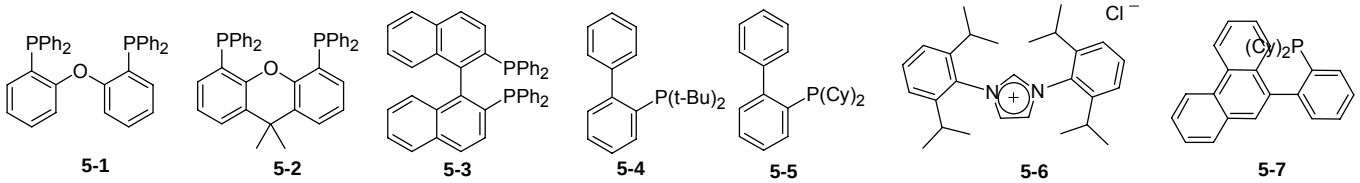
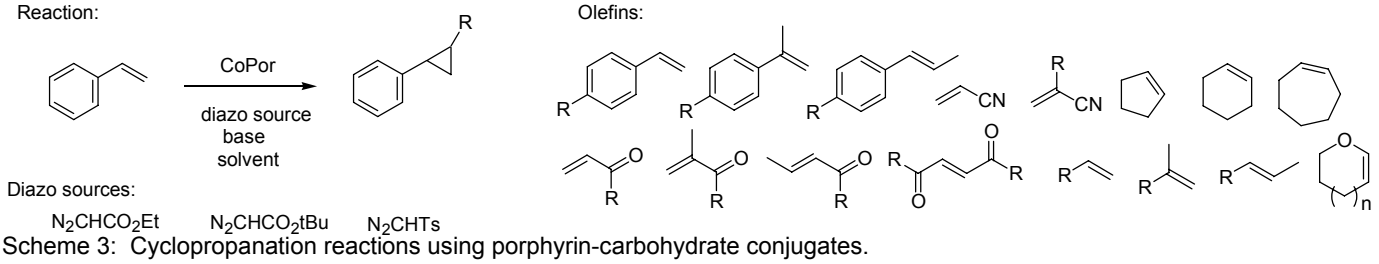


Figure 5. Ligands used for the cross coupling reactions between bromosynthons and carbohydrate derivatives.

III. Asymmetric Catalysis Using Cobalt Porphyrin Complexes- The complexes prepared in this study will be evaluated by Hamilton College undergraduates for their ability to serve as catalysts in asymmetric cyclopropanation reactions. A sample cyclopropanation reaction is shown in Scheme 3 below using styrene.



Scheme 3: Cyclopropanation reactions using porphyrin-carbohydrate conjugates.

A number of different aromatic and aliphatic olefins will be used to explore the scope and limitations of these catalysts under various reaction conditions. The olefins used in this study are commercially available and will be used without further purification. Three different carbene sources, ethyl diazoacetate, *tert*-butyl diazoacetate, and readily available tosyldiazomethane[11] will be evaluated in this study. Finally, polar and non polar solvents will be used to study these reactions with the goal of developing conditions where water can be used as a solvent or cosolvent to perform these reactions.

IV. Future Studies- Future studies will involve the preparation and evaluation of additional carbohydrate-porphyrin conjugates based on the results obtained from this study. For example, if good activity and stereoselectivty are achieved with a glucose-porphyrin conjugate under a specific set of conditions, we will prepare an analogous galactose-porphyrin conjugate and observe the reactivity of this conjugate under similar conditions. My students and I will also explore how other metals (for example, copper, ruthenium or rhodium) can be used to affect the cyclopropanation reactions outline in this study. Long term goals for this project will identify the scope and limitations of carbohydrate-porphyrin conjugates in stereoselective aziridination and olefination reactions.

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Annotate the proposal with a list of references from the primary literature on this page. (Use only one page for your references; font size equal to or one size smaller than proposal body font size. **Include all authors and titles**.)

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LIST OF REVIEWERS

The reviewer list should include at least eight "outsiders," individuals with whom you have had no substantive contact, who are experts in your area of research, and at least two "insiders," preferably former mentors. The best outside reviewers are frequently corresponding authors not known to you in the cited references. Do not hesitate to use a combination of reviewers from academia and non-academic laboratories; including scientists from abroad. We may also select reviewers of our choice. Please include complete names (initials are not enough), mailing addresses, phone and fax numbers, and e-mail addresses. **You must note briefly the nature and extent of your interactions, if any, with each of the outside reviewers. Examples: Met at a meeting, interviewed with, no interaction, never met, etc. Do not use more than one page.**

Outside Reviewers (in alphabetical order):

1. Carolyn Bertozzi; Department of Chemistry, University of California, Berkeley, CA, 94720, USA; Phone: 1-510-643-1682; Fax: 1-510-643-2628; E-mail: bertozzi@cchem.berkely.edu
   -I met Carolyn at an ACS meeting in March of 2007, and a former research student of mine from Wellesley College conducted research in her laboratory in the summer of 2007.

2. David Dolphin, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z1; Phone: 011-1-604-822-4571; Fax: 011-1-604-822-9678, Email: david.dolphin@ubc.ca
   -I have never met David.

3. Danielle H. Dube; Department of Chemistry, Bowdoin College, 6600 College Station, Brunswick, ME 04011, USA; Phone: 1-207-798-4326; Fax: 1-207-725-3017; Email: ddube@bowdoin.edu
   -I have never met Danielle.

4. G. Richard Geier; Department of Chemistry, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA; Phone: 1-315-228-6795; Fax 1-315-228-7935; Email: ggeier@mail.colgate.edu
   -I met Rick on a visit to Colgate to give a talk this year

5. Jennifer L. Koviach; Department of Chemistry, Bates College, 5 Andrews Road, Lewiston, ME, 04240, USA; Phone: 1 207-786-6292; Fax: 207-786-8336; Email: jkoviach@bates.edu
   -I have never met Jennifer

6. Jonathan S. Lindsey; Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Raleigh, NC 27695, USA; Phone: 1-919-515-6406; Fax: 1-919-513-2830; Email: jlindsey@ncsu.edu
   -I have never met Jonathan.

7. Ernest G. Nolen, Department of Chemistry, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA; Phone: 1-315-228-7234; Fax: 1-315-228-7935; Email: enolen@mail.colgate.edu
   -I have met Ernie on two occasions through CHOG (Colgate-Hamilton Organic Groups) and on a visit to Colgate to give a talk this year.

8. Peter H. Seeberger; Eidgenössische Technische Hochschule Zürich, Laboratorium für Organische Chemie ETH Hönggerberg, HCI F315, CH-8093 Zürich; Phone: 41-44-633-2103; Fax: 41-44-633-1235; Email: seeberger@org.chem.ethz.ch
   -I met Peter once when my previous advisor, Mark Peczuh, hosted him when he gave a seminar at The University of Connecticut.

Inside Reviewers (in alphabetical order):

1. Christian Bruckner; Department of Chemistry, University of Connecticut, 55 North Eagleville Road; U 3060; Storrs, CT 06269; Phone: 1-860-486-2743; Fax: (860) 486-2981; Email: c.bruckner@uconn.edu
   -Christian was a former mentor at The University of Connecticut. He was not on my thesis committee.

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   -Mark was my doctoral mentor at The University of Connecticut.

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November 11, 2008

Nicole L. Snyder, Ph.D.
Department of Chemistry
Hamilton College
198 College Hill Road
Clinton, NY 13323

Dear Professor Snyder:

I have studied your proposal entitled *"The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"* with excitement. I am pleased to support your application for a Cottrell College Science Award from the Research Corporation. My laboratory in the Department of Chemistry at the University of South Florida will provide you with the bromoporphyrin synthons described in the proposal. Space and other support will be available to you and your undergraduate students for carrying out summer research in my laboratory at USF. I look forward to continuing our collaboration in research and education.

Sincerely,

Peter Zhang

Peter Zhang

ENDORSEMENT PAGE

Conditions of Research Corporation's Cottrell College Science Awards

A RESEARCH CORPORATION AWARD is a contribution to the scientific and academic program of the institution and is to be used for support of work described in the application prepared by the principal investigator and adopted by the institution.

Since research by its very nature is unpredictable and may require adaptations in order to exploit promising leads, the principal investigator should feel free to make changes in the emphasis or direction of the work as it progresses. If major changes are contemplated, prior approval should be obtained.

The amount of the award is the total of the items approved in the budget submitted as part of the application. If it differs from the amount requested, the differences are noted in the letter of notification from the Vice President. Reallocation of awarded funds between budget categories requires prior approval. Faculty salaries not approved in the budget, indirect costs or overhead, secretarial assistance, and other costs not specifically in the budget are not allowed.

Financial and scientific reports prepared on the foundation's forms are absolutely required. The first report is due within 30 calendar days of the 12-month anniversary of the award start date. The final report is due within 30 calendar days of the 24-month anniversary of the award start date. Failure to provide the first annual report may result in suspension of the award and a request to return unspent funds. Failure to provide the final report will result in suspension of the institution from participation in Research Corporation programs. A single 12-month no cost extension is possible, but must be requested by the principal investigator prior to the 24-month anniversary of the award start date. For approved extensions a 24-month report is required and the final report is due within 30 calendar days of the 36-month anniversary of the award start date.

The principal investigator is urged to publish the findings in the appropriate scientific journals, acknowledging the support of Research Corporation and the appropriate donor, if any. One reprint of each publication resulting from the work is to be sent to the Research Corporation office.

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APPLICANT: Nicole L. Snyder-Lee

PROJECT TITLE: The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts

SUBMITTED BY (Institution): Hamilton College

Name of institution adopting and assuming responsibility for the above project, believing the principal investigator is qualified to conduct the project, and accepting the Conditions of Award, if an award is approved.

Name and Position of Authorized Financial Officer: Amy Lindner

Signature of Financial Officer: Amy Lindner Date: 11/12/08

Name of Chief Executive Officer and Title (President or Chancellor); *signature not required*: Joan Hinde Stewart, President

Signature of Principal Investigator: Nicole L Snyder Date: 11/12/2008

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Date: Mon, 13 Apr 2009 16:22:15 -0700
To: "nsnyder@hamilton.edu" <nsnyder@hamilton.edu>

Dear Nicole,

As you know from our previous email to you, your Cottrell College Science Award proposal was not recommended for funding. Excerpts of pertinent external reviews are included with this email (see below).

In your particular case, there were significant concerns expressed by reviewers regarding your proposed investigations that prevented our Advisory Committee from recommending funding for your proposal. More specifically, our Advisory Committee felt that a new submission of this plan addressing reviewers' comments and contains better justifications for target systems will have substantially increased chances for funding. When you do so, I suggest that you add a strong statement for the anticipated impact of the carbohydrate chemistry on the cyclopropanation reactions and pay special attention to technical detail. For example, what solvents do you have in mind? What is the impact of using tetra-sugar substituted porphyrins? And more importantly, what is the main limitation of currently used compounds and how will these compounds help to overcome that limitation? Lastly, a strong statement describing the intellectual contribution of both the Snyder and Zhang groups to the collaborative effort will make your proposal stronger.

If you choose to reapply to the CCSA program for our next proposal cycle you should begin the reapplication process by taking the eligibility quiz on our website to confirm your eligibility. If eligible, you will be provided the link to submit a brief pre-proposal with a September 15, 2009 deadline. A successful pre-proposal will lead to an invitation to submit the full application by the November 16, 2009 proposal target date.

We encourage you to visit our website, <http://www.rescorp.org/index.php/cottrell-college-science-awards/single-investigator-awards>, for the CCSA program at the link below to review program guidelines and frequently asked questions about the program. If you have further questions you should contact a Program Officer.

Sincerely,

Silvia Ronco
Program Officer
Research Corporation for Science Advancement
e-mail: sronco@rescorp.org <mailto:sronco@rescorp.org>

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL: (PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)
In this proposal, the synthesis of carbohydrates-porphyrin conjugates as ligands for asymmetric catalysts is described. As mentioned by the PI, one of the limitations of porphyrins is related to their hydrophobicity and the possibility of self-aggregation. The introduction of highly polar monosaccharides in these complex structures might solve these problems, still maintaining their catalytic activity.

The PI proposes the synthesis of monosaccharides and their introduction in phorphyrins followed by the evaluation of their catalytic activity. Overall, this synthetic strategy is well structured, original, and is suitable for undergraduate student projects. The students will gain good knowledge not only on the synthesis of monosaccahrides and their introduction in phorphyrins, but also the use of this molecules as

catalysts.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL: (PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)
The overall proposal seems to be original, in that chiral carbohydrate residues will be appended above and beneath the plane of a cobalt porphyrin so as to create an asymmetrical environment that can be used for (initially) cyclopropanation studies. There are a large number of fairly similar studies (ongoing and published) that are probably competitive with that proposed here. The proposal, as written, is certainly feasible, though one can not predict a priori that chiral cyclopropanation will be successful. The PI just needs to find out. The porphyrins that will be obtained from Professor Zhang are fairly trivial, and it might add stature to the overall project if the applicant were to synthesize them herself rather than receive them from elsewhere. Balanced against this suggestion is the fact that students from Hamilton College will get to visit USF. What I like about the proposal is that the cyclopropanation studies will be carried out by undergraduates, and I think this is very appropriate and feasible.
In summary:
significance - very good
originality - good
feasibility - excellent

(3) VERY GOOD: Strong potential. (top 25%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
*PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.
This is a well-written proposal for an interesting research project in the area of designing chiral ligands for metal-based asymmetric catalysis. Specifically, the applicant plans to employ the widely available carbohydrates as chiral building blocks to construct a series of chiral porphyrins with tunable steric and electronic properties. The resulting "CarboPhyrins" are initially targeted as potential ligands to support cobalt-catalyzed asymmetric cyclopropanation reactions. If successful, these "CarboPhyrins" would represent a new family of chiral ligands that may find a wide range of applications in metal-catalyzed asymmetric transformations beyond the proposed cyclopropanation reaction. More important, it offers a great potential to develop asymmetric catalytic processes that can be operated in water and other polar solvents due to the hydrophilic nature of carbohydrates. Furthermore, it is reasonable to expect that the multiple functionalities offered by carbohydrates may be potentially utilized for substrate binding, resulting in enhancement in reactivity and selectivity.

The key to the success of this proposed research is the construction of "CarboPhyrins". The applicant plans to synthesize this class of appealing chiral porphyrins through Pd-catalyzed C-O coupling reactions of appropriate bromoporphyrin synthons with selectively functionalized carbohydrates. The feasibility of this approach is very high as it is well presented in the proposal with support from computer modeling and backup of literature precedents. Plans for accomplishing the goals of the proposal appear to be well laid out and are achievable by undergraduate researchers through collaborations.

(1) OUTSTANDING: Highly novel. (top 5%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL: (PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)

The proposal concerns the synthesis of carbohydrate-porphyrin conjugates using a Pd-catalyzed cross-coupling reactions between bromo-susbtituted porphyrins and suitable carbohydrate derivatives. The cobalt complexes of these chiral molecules will be evaluated with respect to their ability to stereoselectively catalyze the cyclopropanation of olefinic double bonds.

The scientific problem tackled in this well written and edited proposal is significant, and the proposed method chosen varies in key points significantly from that of literature-known methods. The synthesis of the proposed families of porphyrin-carbohydrate conjugates is also generally feasible (for some caveats, see below), whereby the PI will focus on the conjugation of the porphyrins to the carbohydrates - the porphyrins will be provided by Peter Zhang, U of South Florida, a noted expert in the field. The PI presented some preliminary results. The methods chosen promise to generate a large diversity of ligands. The evaluation of the catalytic properties of the porphyrins will also take place in the PI's lab, an enterprise assisted by sending students to Prof. Zhang's group. The work is suitable for an undergraduate institution as the syntheses can be divided up into many well delineated smaller projects. The proposed work can also serve as rich teaching tools. The work is of potential larger impact as a number of other stereoselective metalloporphyrin-catalyzed atom transfer reactions, such as epoxidation and azirination reactions, could potentially benefit from this work.

Overall, this is an excellent proposal but some potential problems cannot be overlooked. Firstly, the number of parameters the PI proposed to be tested are excessive, ranging from the suitability of a number of tetrabromo-substituted porphyrins, the Pd source, the ancillary ligand, base, etc. A full evaluation of this enormous parameter matrix will certainly exceed the time frame of this proposal. On the other hand, it shows that the PI is aware of the many parameters that could be playing a role. Secondly, tetrasubstitution of a porphyrin, particularly in the sterically demanding ortho-positions of the meso-substituents, requires that each step is extremely high yielding, otherwise potentially very large separation problems will arise. The proposal does not make it clear if the PI is aware of this. Again, perhaps the proposal to screen so many different reaction conditions is geared toward overcoming this problem. This reviewer would suggest, if funded - and there is no doubt in this reviewer's mind that the PI will translate the funding into a productive research - to begin optimizing a simple mono- or di-substitution before moving on to the tetrasubstituted systems.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL: (PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)
Dr. Snyder-Lee proposes to develop and optimize the Pd-catalyzed cross-coupling reaction between bromo-porphyrin derivatives and four partially benzylated alpha-methyl glucopyranosides, and five partially benzylated furanosides, and to study the products in asymmetric catalysis. The obtained porphyrin-sugar conjugates, which will have four carbohydrate moieties each, will serve as ligands for cobalt and other metals, and their ability as asymmetric catalysts in propanation reactions with a number of olefins will be tested. The synthetic community has a great interest in the asymmetric propanation because many bioactive natural products contain a cyclopropane moiety with distinct configurations. While chiral porphyrin ligands have been previously synthesized and used in asymmetric catalysis, the P.I. states that their use is limited to non-polar solvents, and that pi-stacking of the porphyrin rings is a problem causing aggregation. Therefore, the basic idea of the proposed research, to develop better catalyst for asymmetric reactions, is a logical extension of the chiral porphyrin arsenal. However, the proposal is not very well developed, and some important questions have not been addressed:

1. The target compounds 3-1 and 3-2 as shown in Figure 3 are achiral meso-compounds because they have D- and L-glucose moieties. I believe that happened accidentally during copying, flipping and pasting, and that all sugars were meant to be D-glucosides.

2. In scheme 1, the starting material has been named meso-bromodiphenylporphyrin. Why is this a meso-compound? It doesn't have any stereogenic centers.

3. Asymmetric catalysis is a very complex topic, and has not been discussed in this proposal, even though the whole purpose of the synthesis of the porphorin-carbohydrate conjugates is their evaluation in the asymmetric cyclopropanation. What is the impact of using a tetra-sugar substituted porphyrin as a chiral catalyst? Does this mean that such a catalyst would have a four-fold greater turnover? Would a porphyrin with only two sugar moieties be inferior? If yes, why? Does each face of the porphyrin produce the same chiral cyclopropane, or could it be that stereoisomers will be produced? How many olefins can coordinate with one cobalt complex? To strengthen her proposal, Dr. Snyder-Lee should demonstrate that she has given this project some deep thought (which she may have, but it is not reflected in this narrative). If the proposed catalysts are expected to do something spectacular, then Dr. Snyder-Lee should stress the asymmetric catalysis aspect of her proposal.

4. Are the chiral porphyrin derivatives that already exist (Reference 5,6) really not soluble in polar solvents such as DMF, DMSO, acetonitrile or HMPA? These solvents usually dissolve polar as well as non-polar compounds well. Protic solvents such as H2O and alcohols can probably not be used because of undesired hydrolysis and alcoholysis. Which solvents does the P.I. have in mind? No specifics are discussed.

5. This reviewer is not convinced that the problem of aggregation will disappear after the porphyrin has been derivatized with sugars. Many organic compounds that suffer from aggregation do so in polar and non-polar environments.

6. It is not clear why chiral (and quite expensive) ligands are suggested for the porphyrin-carbohydrate cross-coupling reactions, since no new chiral centers are being generated in the porphyrin-carbohydrate conjugates.

7. It is certainly an important experience for the P.I.'s undergraduate students to conduct research in the laboratory of collaborator Zhang at the University of South Florida. However, it is this reviewer's opinion that not much can be accomplished in only two weeks. It would be better to send only one student to Tampa, who would then spend two or three months in the collaborator's lab.

In summary, the basic idea of the proposed research is sound, but the proposal is not very well developed. It could turn into a strong proposal, if the P.I. discussed problems and solutions more thoroughly, and by being more specific rather than too general.

(3) VERY GOOD: Strong potential. (top 25%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL: (PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)

General Comments

This is a strong proposal that aims to synthesize several novel porphyrins bearing carbohydrate ligands and evaluate their ability to affect enantioselective cyclopropanation reactions. The key challenge (according to the author) that the new catalysts will address is the solubility of the porphyrins. Addition of carbohydrate appendages is argued to solubilize and create a chiral environment that will favor face selective cyclopropanations. The research as outlined in the plan will provide valuable research experience to the students involved. Moreover, the research is likely to result in a new group of useful catalysts.

SCIENTIFIC SIGNIFICANCE

Stereoselective reactions continue remain a challenge in organic chemistry. Cyclopropanation in particular has been unresponsive (in comparison to reactions such as epoxidations, aziridinations) to conventional strategies for stereoselective reactions. The proposal has identified an active area of research.

The need/significance for polar solvents is underemphasized in the proposal. What are common solvents for Co-porphyrin cyclopropanations? Will the reactions occur in aqueous solvents? The reaction conditions should be more completely discussed.

ORIGINALITY

The approach is original. The "Scheme 1 class" of carboporphyrins is quite unique. The "Scheme 2 class" are akin to those already reported by Zhang, but novel nonetheless. It is puzzling why the scheme 1 catalysts (or analogs with multiple appended carbohydrates) were not developed.

FEASIBILITY

Feasibility of the proposal is high. The Zhang/Snyder-Lee collaboration is up and running. This fact is a strong point. The plan is well crafted to allow access to multiple undergrads to conduct research and to gain success in preparing molecules.

The choice of a polarimeter is not substantiated in the proposal. It is presumed that this will be used to quantify ee's of the cyclopropanation? Are values known for each cyclopropane enantiomer of the alkenes shown in Scheme 3? A chiral GC may be better suited to the project proposed.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL: (PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.)
The applicant plans to conjugate a variety of sugars to porphyrins and after metallation of the porphyrins to use these as asymmetric catalyst, principally in cyclopropanation reactions. The PI has considerable experience in the required carbohydrate chemistry and will work in collaboration with Professor Zhang (University of South Florida) on the porphyrins chemistry. The conjugation chemistry has already been tested by the PI and the porphyrins chemistry is fairly straight forward and will pose no major problems. The sugar-porphyrin constructs will indeed provide chiral environments and there is a reasonable chance that the cobalt complexes will provide a means of producing chiral cyclopropanation.

The proposed research is well suited to a Cottrell College science award in that it is modular and can be broken down into unit of size and complexity appropriate for undergraduates. The fact that some of the students will travel to USF in the summer to better understand the porphyrin chemistry is also appropriate. The request for a polarimeter is well justified and the research cannot effectively proceed without it.

(2) EXCELLENT: Important, original. (top 10%)

Research Corporation
10441: "The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"

COMMENT ON THE SCIENTIFIC SIGNIFICANCE, ORIGINALITY, AND FEASIBILITY OF THE PROPOSAL:
*PLEASE NOTE: YOUR COMMENTS WILL BE SHARED WITH THE APPLICANT.
Significance: Chiral cyclopropane rings are widespread in natural products that display interesting biological activities. Accessing these natural products through synthetic methods requires the ability to

perform asymmetric cyclopropanation reactions. Existing porphyrin-based catalysts are capable of catalyzing the formation of chiral cyclopropane rings. However, these catalysts suffer from two key features: (1) their hydrophobicity, which limits their use in polar solvents with polar substrates, and (2) their aromaticity, which causes aggregation in solution and therefore decreased efficiency. Snyder's proposal aims to address these limitations by attaching chiral hydrophilic molecules, carbohydrates, onto the porphyrin catalyst scaffold. Snyder hypotheses that carbohydrate-porphyrin conjugates will be (1) soluble in polar solvents due to the presence of the polyhydroxylated sugars, and thus increase the utility of these catalysts in polar solvents, and (2) significantly less prone to aggregation due to steric blocking of the core porphyrin by the pendant carbohydrates. This work has the potential to greatly ease the formation of chiral cyclopropane rings on polar compounds.

In addition, this proposed work will provide a unique experience for the undergraduates who work with Snyder. These students will be exposed to two distinct research environments: a small lab at an undergraduate institution and a large lab at a research university.

Originality: Carbohydrates have previously been used as chiral auxiliaries to direct the formation of chiral cyclopropane rings. This work proposes an alternative use of carbohydrates - to alter the solubility and efficiency of an existing catalyst - and represents a novel approach.

Feasibility: This proposal combines the PI's strength, carbohydrate synthesis, with the collaborator's strength, asymmetric catalysis of cyclopropane rings. This strategic alliance will enable the PI to become established in this new field.

Overall, this proposal is built on a set of realistic hypotheses and follows a logical plan of attack. Selectively protected sugars will be accessed using precedented chemistry. These selectively-protected sugars will then be coupled to porphyrin synthons (provided by the collaborator) via palladium-cross coupling. Once the desired compounds are accessed, they will be evaluated for their ability to catalyze chiral cyclopropanation reactions. In the long term, the PI will assess the scope of these carbohydrate-porphyrin conjugates on cyclopropanation, aziridination, and olefination reactions.

There is one exception to the otherwise logical plan of attack. The PI and her students have already established that a model coupling reaction is successful, and have in-hand four carbohydrate-porphyrin conjugates. The PI can immediately test the above-stated hypotheses and assess whether these conjugates have increased solubility in polar solvents and aggregate less than their carbohydrate-less counterparts.

A strength of the proposed work is that it is easy to envision how subprojects could be parceled out to participating undergraduates. The significant matching funds the applicant would receive from Hamilton as part of this proposal will help launch this junior faculty member's career.

(2) EXCELLENT: Important, original. (top 10%)

RESEARCH CORPORATION FOR SCIENCE ADVANCEMENT
Single Investigator Cottrell College Science Award Application

Principal Investigator:	Nicole L. Snyder-Lee	**Phone:**	315-858-4742
Academic Rank:	Assistant Professor	**Fax:**	315-859-4807
Department:	Chemistry	**Email:**	nsnyder@hamiton.edu
Institution Name:	Hamilton College		
Institution Address:	198 College Hill Road Clinton, NY 13323	**Appointment Date:**	07/01/2007

EDUCATION AND EXPERIENCE:

Education/Training:
2000-2005—University of Connecticut, Department of Chemistry, Storrs, CT, Ph.D. Chemistry (Advisor: M. W. Peczuh)
1996-2000—Westminster College, Department of Chemistry, New Wilmington, PA, B.S. Chemistry/Biology

Experience:
2007-Present—Hamilton College, Clinton, New York, Assistant Professor of Chemistry
2005-2007—Wellesley College, Wellesley, Massachusetts, Visiting Assistant Professor of Chemistry
2001-2002—US Nanocorp, Willington, Connecticut, Research Assistant
2002-2004—University of Connecticut, Storrs, Connecticut, Science Wizards Journeyman Program Coordinator

TITLE OF PROPOSAL:

The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts

ABSTRACT:

The recent development of palladium-catalyzed cross-coupling reactions between substituted bromo-porphyrins and amides, amines, alcohols, and thiols has allowed for the preparation of a number of chiral metallo porphyrins that have been used to catalyze several key functional group transformations, including asymmetric cyclopropanation reactions, with good stereoselectivity. Despite the progress that has been made in this field, two key problems still exist. First, the hydrophobic nature of the porphyrin catalysts currently in use makes them relatively insoluble in polar solvents, limiting the utility of these systems as catalysts under polar conditions. In addition, the aromatic nature of the porphyrin ring system often facilitates pi stacking resulting in the aggregation of the catalyst in solution leading to decreased efficiency. To address these problems, we are proposing the synthesis and evaluation of several novel porphyrins bearing carbohydrate ligands. The polarity and predicted conformation of the carbohydrate-porphyrin conjugates outlined in this study render them excellent candidates to obviate the challenges described here. The ***specific goals*** of this project are: (i) to develop and optimize a palladium-catalyzed cross-coupling reaction to prepare carbohydrate-porphyrin conjugates bearing different carbohydrates, and (ii) to assess the ability of carbohydrate-porphyrin conjugates to serve as asymmetric catalysts.

PUBLICATIONS OF PRINCIPAL INVESTIGATOR:

Ruppel, J. V.; Gauthier, T. J.; **Snyder, N. L**.; Perman, J. A. ; Zhang, X. P. "Asymmetric Cobalt-Catalyzed Cyclopropanation with Succinimidyl Diazoacetate: General Synthesis of Optically Active Cyclopropyl Carboxamides." *Organic Letters* **2009***, 11,* 2273-2276.

Markad, S. D.; Xia, S.; Snyder, N. L.; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxdation of Carbohydrate Based Oxepiens." *Journal of Organic Chemistry* **2008**, *73*, 6341-6354.

Castro, S.; Cherney, E. C.*; Snyder, N. L.; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007**, *342(10)*, 1366-1372.

Snyder, N.L.; Peczuh, M.W. Haines, H.M.* "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006***, 62,* 9301-9320.

Castro, S.; Duff, M.; Snyder, N. L.; Morton, M.; Kumar, C. V.; Peczuh, M. W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic Biomolecular Chemistry* **2005**, *3,* 3869-3872.

DeMatteo, M. P.; Snyder, N. L.; Morton, M.; Baldisseri, D.; Haddad, C. M.; Peczuh, M. W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38.

Peczuh, M. W.; Snyder, N. L.; Fyvie, W. S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004***, 339(6),* 1163-1171.

Peczuh, M. W.; Snyder, N. L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, *44*, 4057-4061.

RESEARCH CORPORATION FOR SCIENCE ADVANCEMENT
Single Investigator Cottrell College Science Award Application

PROPOSED BUDGET:

	Year 1		Year 2	
	RCSA	Match	RCSA	Match
Equipment *(Please itemize computers and equipment over $1,000)*	$0	$0.00	$0	$0
Supplies *($6,000 per year maximum from RCSA funds)*	$6,000	$1,800	$6,000	$1,800
Stipends *(not to exceed maximum amounts indicated)*				
Faculty Summer Stipend *(up to $7,500 for 8 weeks)*	$7,500	$0	$7,500	$0
Weeks: 8				
Undergraduate Summer Stipend *(up to $3,500 for 10 weeks)*	$3,500	$4,500	$3,500	$4,500
Weeks: 10 Students per Year: 2				
FICA / Medicare *(7.65% of stipend maximum)*	$0	$0	$0	$0
(Note: No other benefits or indirect costs are allowed.)				
Travel *(maximum $2,500 per year from RCSA funds to conduct research; no conference or meeting travel)*	$500	$1,000	$500	$1,000
TOTALS	**$17,500**	**$7,300**	**$17,500**	**$7,300**

TOTAL BUDGET	**$49,600**
(Matching funds must be at least $10,000) LESS MATCHING FUNDS FROM INSTITUTION	**$14,600**
(Funds from RCSA must add up to $35,000) REQUESTED FROM RCSA	**$35,000**

ADDITIONAL SUPPORT:

2007-2010—$50,000.00 ([1], [2])—Hamilton College Startup

BUDGET RATIONALE:

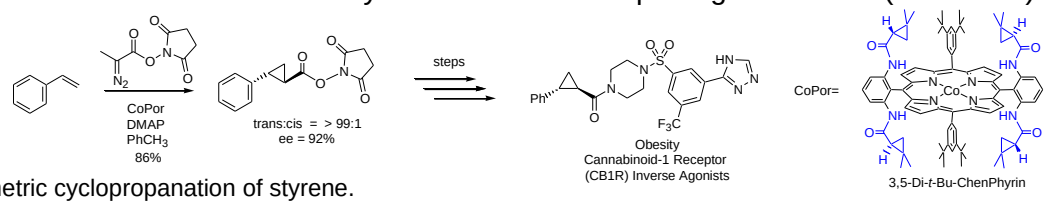
GC studies will be conducted using the College's Shimadzu GCMS instrument. Chiral HPLC will be conducted using the College's Shimadzu SCL-10A HPLC system using a chiral column. NMR studies will be conducted on the College's 500MHz Bruker NMR Spectrometer. IR studies will be conducted on the College's Perkins Elmer Spectrum One IR with ATR attachment. High resolution MS will be conducted at the University of South Florida through collaboration with Dr. X. Peter Zhang. Hamilton College will provide an institutional match in the amount of $14,600.00 including a 30% match on equipment and supplies, a 2:1 match on travel funds ($2,000.00) which will be used for one round trip to the University of South Florida each summer, and funding for at least one additional student per summer ($8,000.00) to conduct the research described in this proposal. Hamilton College will also make up the difference between the student stipend of $3,500.00 allotted by RC, and the standard Hamilton College summer stipend of $4000.00 for ten weeks research.

RESEARCH CORPORATION FOR SCIENCE ADVANCEMENT
Single Investigator Cottrell College Science Award Application

STATEMENT OF THE PROBLEM AND SCIENTIFIC SIGNIFICANCE OF PROPOSED RESEARCH:

Introduction. Chiral cyclopropane rings are found in a number of biologically relevant natural products. For example, the antitumor curacin A[1] and antifungal ambruticin[2] both contain chiral cyclopropane units that are critical to the biological functions of these compounds. The importance of chrial cyclopropane rings has led to the development of a number of reactions for their synthesis.[3] However, the development of catalysts that can catalyze asymmetric cyclopropanation with a variety of substrates in high yield and with excellent diastereo- and enantioselectivity is still a major area of research in the field.

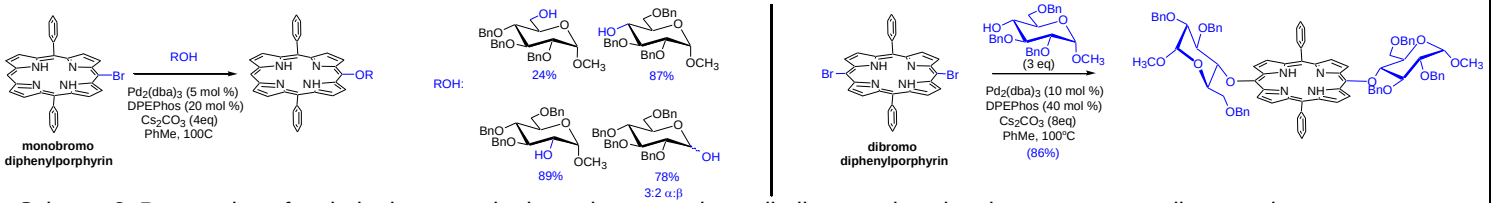
The recent development of palladium-catalyzed cross-coupling reactions between mono-, di-, and tetrasubstituted bromo-porphyrins and amides, amines, alcohols, and thiols has allowed for the preparation of a number of chiral amino-, amido-, oxo- and mercaptoporphyrins.[4] The cobalt derivatives of these chiral porphyrins have been used to catalyze a number of key functional group transformations including the asymmetric cyclopropanation of aromatic and electron-deficient olefins using diazo reagents with good stereoselectivity.[5] For example, we recently demonstrated that 3,5-di-*t*-Bu-ChenPhyrin (Scheme 1) can serve as an effective catalyst for the asymmetric cyclopropanation of aromatic olefins such as styrene, using succinimidyl diazoacetate. [6] These corresponding cyclopropyl carboxamides can serve as important building blocks for pharmaceutics such as the obesity cannabinoid-1 receptor agonist CB1R (Scheme 1).[7]



Scheme 1. Asymmetric cyclopropanation of styrene.

Despite the progress that has been made in the development of these catalysts, two key problems still exist. First, many of the porphyrin catalysts currently in use exhibit low solubility in polar solvents such as water, limiting substrate scope and making them unsuitable for use in green chemistry applications. In addition, the aromatic nature of these catalysts often facilitates pi stacking resulting in metalloporphyrin aggregation in solution, leading to decreased catalytic efficiency. In an effort to address these problems, we have developed a program for synthesizing novel porphyrins bearing carbohydrate residues. The polarity and predicted conformation of the carbohydrate-porphyrin conjugates outlined in this study render them excellent candidates to obviate the challenges described above.

Preliminary Results. The carbohydrate-porphyrin conjugates we have synthesized thus far have been prepared by cross-coupling bromoporphyrin synthons with selectively functionalized carbohydrates using tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) as a source of palladium and bis(2-diphenylphosphinophenyl) ether (DPEphos) as a ligand source in the presence of cesium carbonate (Cs_2CO_3). An example of the coupling reactions between monobromodiphenylporphyrin and four carbohydrate analogs derived from glucose is shown in Scheme 2.[8] We have also been able to generate disubstituted derivatives using dibromodiphenyl porphyrin under similar conditions in upwards of 86% yield (Scheme 2). Solubility studies have shown that these compounds are significantly more soluble than their non-glycosylated counterparts.



Scheme 2. Preparation of carbohydrate-porphyrin conjugates using palladium catalyzed carbon-oxygen coupling reactions.

Through these preliminary studies, we were able to assess the feasibility of performing palladium-catalyzed mono- and di-cross-coupling reactions with glucose via different carbohydrate linkages. The results obtained from these experiments have provided us with a foundation for the preparation of more complex carbohydrate-porphyrin conjugates containing additional carbohydrate ligands that can serve as effective catalysts for asymmetric cyclopropanation. Therefore, the ***specific goals*** of this project are:

(i) To develop and optimize a palladium-catalyzed cross-coupling reactions to prepare carbohydrate porphyrin conjugates bearing multiple carbohydrate substituents, and

(ii) To assess the ability of carbohydrate-porphyrin conjugates to serve as chiral catalysts in asymmetric cyclopropanation reactions.

PLAN OF PROCEDURE:

Synthesis of Carbohydrate Analogs. The carbohydrate analogs required for the work in this proposal are shown in Figure 2 and will be prepared by Hamilton College undergraduate students. Pyranose derivatives **2-1** and **2-2** are synthesized from readily available methyl-α-D-glucopyranoside using standard protecting group chemistry.[9] Pyranose derivative **2-3** is prepared based on a report by McMillan and coworkers.[10] Furanose derivatives **2-4** and **2-5** are prepared from commercially available methyl-furanosides.[9] Finally, furanose derivative **2-6** will be prepared based on the procedure for pyranose derivative **2-4**.

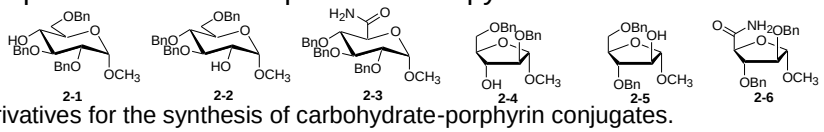


Figure 1. Carbohydrate derivatives for the synthesis of carbohydrate-porphyrin conjugates.

The derivatives in Figure 1 were chosen to probe how the nature, size and connectivity of the carbohydrate affect the overall chiral environment of the corresponding carbohydrate-porphyrin conjugate. This in turn, will presumably affect the reactivity and selectivity of the resulting catalyst. For example, Spartan modeling studies show that the conformation of carbohydrate-porphyrin conjugates bearing glucopyranose residues linked through the 4-OH (**2-1**) versus the 2-OH (**2-2**) provide for conjugates with different chemical environments near the catalytic site as shown in Figure 2.

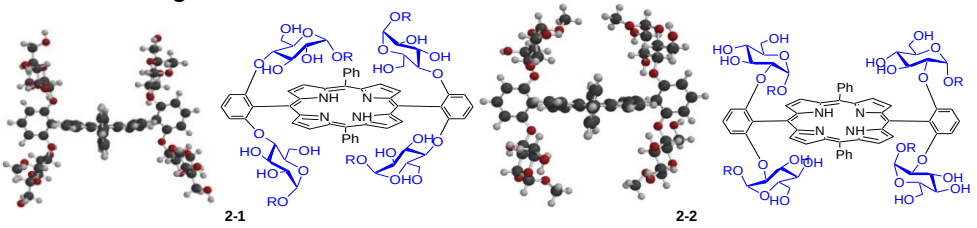


Figure 2. Models of carbohydrate-porphyrin conjugates with glucose residues linked through the 4'OH (**3-1**) and 2'OH (**3-2**).

Synthesis of Porphyrin-Carbohydrate Conjugates. The porphyrin synthons for this work, shown in Figure 3 below, will be provided by Dr. Peter Zhang's group at the University of South Florida. One Hamilton College student, as part of his or her summer research experience, will have the opportunity to travel to the University of South Florida each summer to participate in the construction of these synthons. Synthons **3-1** through **3-4** were chosen to determine (i) how the position (2,6 or 3,5) of the carbohydrate substituents on the A and B rings orthogonal to the porphyrin ring affect the catalytic activity, and (ii) how groups on the neighboring C and D rings affect the reactivity of the corresponding catalysts. Synthon **3-2** is especially noteworthy for the success that has been already achieved with this system (see Figure 1).[6]

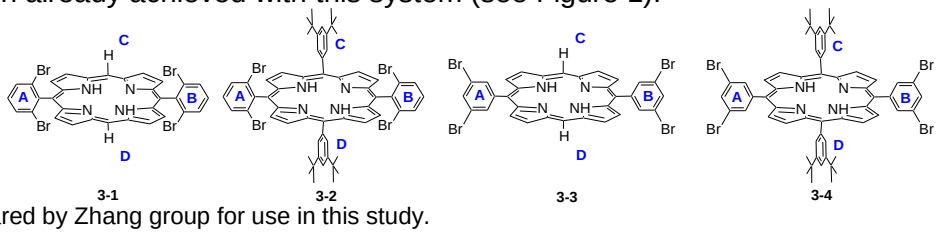
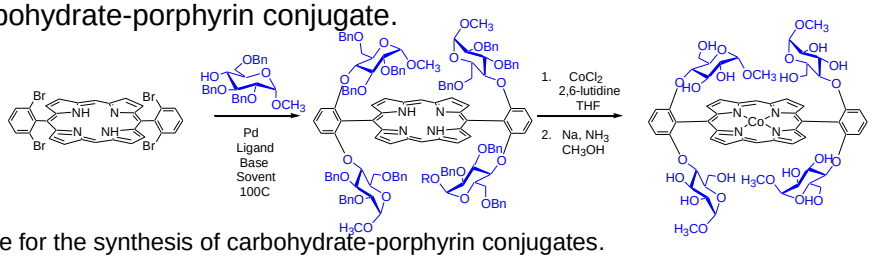


Figure 3. Synthons prepared by Zhang group for use in this study.

The porphyrin-carbohydrate conjugates will be synthesized by Hamilton College undergraduates. A general scheme for the preparation of porphyrin-carbohydrate conjugates is shown in Scheme 3 below. For each of the bromosynthons investigated in this study, the palladium source, ligand, and carbohydrate will be dissolved in an appropriate solvent (toluene, tetrahydrofuran or dioxane) using Schlenk technique, and heated to 100°C until the starting material is consumed as monitored by thin layer chromatography. We will use the optimized conditions already in place for the mono- and disubstituted porphyrin derivatives (Scheme 2) as a starting point for the tetrasubstituted derivatives. After purification, the porphyrin will be metalated using cobalt chloride and 2,6-lutidine in THF. Finally, Birch reduction conditions will be used to remove the protecting groups to provide the corresponding carbohydrate-porphyrin conjugate.



Scheme 3. General scheme for the synthesis of carbohydrate-porphyrin conjugates.

While the optimized reaction conditions work well for the simpler mono- and disubstituted derivatives in our previous studies, the tetrabromoderivatives we are proposing to synthesize are much more sterically encumbered and may require reoptimization of the reaction conditions. Therefore, several experimental conditions will be screened systematically in order to achieve maximum yields. The sources of palladium used in this study, including palladium (II) acetate ($Pd(OAc)_2$) and tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) will help us determine whether palladium (II) or palladium (0) is a more effective entry into the catalytic cycle. The ligands employed in this study (Figure 4) will be used to probe the effects of size, flexibility, and mode of ligand binding on carbohydrate-porphyrin conjugate synthesis. Examples of ligands that will be employed in this study include commercially available R, R-duphos **4-1**, bis (2-diphenylphosphinophenyl)ether (DPEphos) **4-2**, 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthenes (Xantphos) **4-3**, and BINAP **4-4**. Cesium carbonate will be employed in all of the studies conducted based on our previous success with this base.

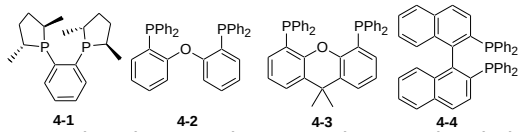
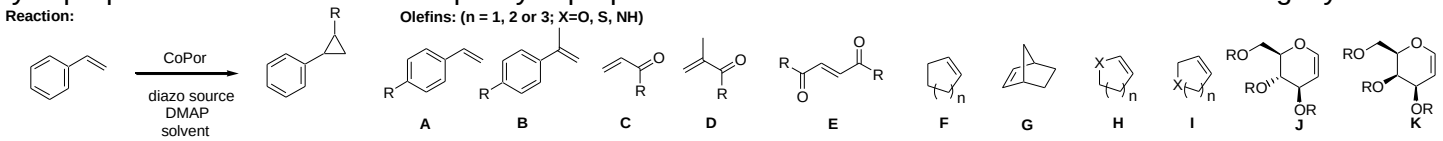


Figure 4. Ligands used for the cross coupling reactions between bromosynthons and carbohydrate derivatives.

Asymmetric Catalysis Using Cobalt Porphyrin Complexes. The complexes prepared in this study will be evaluated by Hamilton College undergraduates for their ability to serve as catalysts in asymmetric cyclopropanation reactions. A sample cyclopropanation reaction is shown in Scheme 4 below using styrene.



Scheme 4. Cyclopropanation reactions using porphyrin-carbohydrate conjugates.

A number of aromatic and aliphatic olefins will be used to explore the scope and limitations of these catalysts under various reaction conditions. The olefins used in this study are commercially available and will be used without further purification. Olefins **A** through **E** will be used to assess the ability of the catalysts prepared in this study to cyclopropanate electron deficient olefins of various sizes in high yield and with good stereocontrol, while olefin's **F** through **K** will be used to study the ability of the catalysts to cyclopropanate a variety of electron rich ring systems. In general, we expect the later reactions to be more challenging, since electron-rich systems tend to be less reactive and can be more difficult to cyclopropanate. Glucals **J** and **K** are especially noteworthy as these compounds are important synthetic intermediates in the preparation of a number of carbohydrate-based natural products, including the C-glycoside ambruticin.[2]

Two different carbene sources, commercially available ethyl diazoacetate and *tert*-butyl diazoacetate, will be evaluated in this study. These reagents will be used to assess the ability of the catalyst to perform cyclopropanation reactions with carbene sources that vary in size. Dimethyamino pyridine will be used as an axial ligand, based on the success of this ligand in our previous work.[6]

Finally, a variety of polar and nonpolar solvents will be used to study these reactions. We are especially interested in determining whether or not the catalysts prepared in this study can be used to cyclopropanate substrates in water, as a green alternative to some of the more common organic solvents employed in cyclopropanation reactions.

The reaction products obtained from each cyclopropanation experiment will be subjected to non-chiral GC to determine the overall yield for each reaction. Enantiomeric and diastereomeric excesses will be determined by chiral HPLC for all chiral products after separation of the desired products from the reaction mixture. Pure compounds will be fully characterized by NMR, IR, and high resolution MS. In addition, absolute configurations will be determined for all of the chiral compounds prepared in this study by GC comparison with the commercially available pure enantiomers when possible.

Future Studies. Future studies will involve the preparation and evaluation of additional carbohydrate-porphyrin conjugates based on the results obtained from this study. For example, if good activity and stereoselectivty are achieved with a glucose-porphyrin conjugate under a specific set of conditions, we will prepare the analogous galactose-porphyrin conjugate and observe the reactivity and selectivity of this conjugate under similar reaction conditions. My students and I will also explore how other metals can be used to affect the cyclopropanation reactions outlined in this study. Long term goals for this project will identify the general scope and limitations of carbohydrate-porphyrin conjugates in epoxidation, aziridination and olefination reactions.

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1. Michael Doyle, Department of Chemistry and Biochemistry, University of Maryland, 0107 Building-9, Park, MD 20742, USA; Phone: 1-301-405-1788; Email: mdoyle3@umd.edu
   -I do not know Michael.

2. Joseph Fox, Department of Chemistry and Biochemistry, University of Delaware, 272 Brown Laboratories, Newark, DE 19716, USA; Phone: 1-302-831-0191; Email: jmfox@udel.edu
   -I do not know Joseph.

3. G. Richard Geier; Department of Chemistry, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA; Phone: 1-315-228-6795; Fax 1-315-228-7935; Email: ggeier@mail.colgate.edu
   -I met Rick on a visit to Colgate to give a talk this year.

4. Karl Kadish, Department of Chemistry, University of Houston, 4800 Calhoun Road, Houston, TX, 77004, USA; Phone: 1-713-743-2740; Fax: 1-713-743-2745; Email: kkadish@uh.edu
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6. Timo Ovaska, Department of Chemistry and Biochemistry, Connecticut College, New London, CT, USA; Phone: 1-860-439-2488; Fax: 1-860-439-2477 ; Email: timo.ovaska@conncoll.edu
   -I met Timo when I gave a talk at Connecticut College this fall.

7. Kevin M. Smith, Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, LA 70803, USA; Phone: 1-225-578-7442; Fax: 1-225-578-3458; Email: kmsmith@lsu.edu
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   -Justin gave a talk at Hamilton College in 2008.

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   -Mark was my doctoral mentor at The University of Connecticut.

RESEARCH CORPORATION FOR SCIENCE ADVANCEMENT
Single Investigator Cottrell College Science Award Application

ENDORSEMENT PAGE

Conditions of Research Corporation for Science Advancement's Single Investigator Cottrell College Science Award

A RESEARCH CORPORATION FOR SCIENCE ADVANCEMENT (RCSA) AWARD is a contribution to the scientific and academic program of the institution and is to be used for support of work described in the application prepared by the principal investigator and adopted by the institution.

Since research by its very nature is unpredictable and may require adaptations in order to exploit promising leads, the principal investigator should feel free to make changes in the emphasis or direction of the work as it progresses. If major changes are contemplated, prior approval should be obtained.

The amount of the award is $35,000 from RCSA plus $10,000 of institutional match for all applicants. Reallocation of awarded funds between budget categories requires prior approval. Faculty salaries are not approved in the budget, indirect costs or overhead, secretarial assistance, and other costs not specifically in the budget are not allowed.

Financial and scientific reports prepared on the foundation's forms are absolutely required. The first report is due within 30 calendar days of the 12-month anniversary of the award start date. The final report is due within 30 calendar days of the 24-month anniversary of the award start date. Failure to provide the first annual report may result in suspension of the award and a request to return unspent funds. Failure to provide the final report will result in suspension of the institution from participation in RCSA programs. A single 12-month no cost extension is possible, but must be requested by the principal investigator prior to the 24-month anniversary of the award start date. For approved extensions a 24-month report is required and the final report is due within 30 calendar days of the 36-month anniversary of the award start date.

The principal investigator is urged to publish the findings in the appropriate scientific journals, acknowledging the support of Research Corporation for Science Advancement and the appropriate donor, if any.

RCSA awards are true awards to the institution, not contracts for research with the institution or the principal investigator, and RCSA disclaims any rights in the results of the research.

APPLICANT NAME: Nicole L. Snyder-Lee

PROJECT TITLE: The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts

SUBMITTED BY: (INSTITUTION) Hamilton College

Name of institution adopting and assuming responsibility for the above project, believing the principal investigator is qualified to conduct the project, and accepting the Conditions of Award, if an award is approved.

NAME AND POSITION OF AUTHORIZED FINANCIAL OFFICER: Amy Lindner, Associate Director of Foundation, Corporate and Government Relations

SIGNATURE OF FINANCIAL OFFICER: Amy Lindner DATE 11/10/09

NAME OF CHIEF EXECUTIVE OFFICER (CEO) Joan Hinde Stewart

TITLE OF CEO (President or Chancellor) President

SIGNATURE OF PRINCIPAL INVESTIGATOR: Nicole L Snyder-Lee DATE 11/09/2009

SCAN THIS SIGNED ENDORSEMENT PAGE AS THE LAST PAGE OF YOUR PDF FILE

(NOTE: If awarded, this Endorsement Page – with original signatures – must be mailed to RCSA.)

X. Peter Zhang, Ph.D.
Associate Professor
Department of Chemistry
University of South Florida
Tampa, FL 33620-5250
Phone: (813) 974-7249
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pzhang@cas.usf.edu

November 14, 2009

Nicole L. Snyder, Ph.D.
Department of Chemistry
Hamilton College
198 College Hill Road
Clinton, NY 13323

Dear Professor Snyder:

I have studied your proposal entitled *"The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"* with excitement. I am pleased to support your application for a Cottrell College Science Award from the Research Corporation. My laboratory in the Department of Chemistry at the University of South Florida will provide you with the bromoporphyrin synthons described in the proposal. Space and other support will be available to you and your undergraduate students for carrying out summer research in my laboratory at USF. I look forward to continuing our collaboration in research and education.

Sincerely,

Peter Zhang

G

THE PETROLEUM RESEARCH FUND
TYPE G PROPOSAL

(Please refer to statement of eligibility, terms, and conditions.)

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This proposal is intended for review exclusively by ACS PRF staff, members of the PRF Advisory Board, and outside reviewers officially asked to furnish scientific comments. It may not be transmitted to other parties, copied, or retained for future reference. Please return to the PRF office, or destroy, in accordance with instructions.

Nicole L. Snyder	July 01, 2007
(Principal Investigator)	(Date of First Faculty Appointment)

Hamilton College	Chemistry Department	Clinton	NY
(Institution)	(Department)	(City)	(State)

Title of Proposed Research: Mechanistic Studies of Immobilized Cellulase

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By signing below, we acknowledge that we have read and understand this scientific misconduct policy.

Principal Investigator: Nicole L Snyder (Signature) 11/27/2007 (Date)

Officer of the Institution Endorsing the Proposal: (Signature) 11/27/2007 (Date)

Payment Schedule Requested: $ 25,0000.00 (First Year) + $ 25,0000.00 (Second Year) = **Total $50,000**

1155 Sixteenth Street, NW, Washington, DC 20036, 202-872-4481, www.acsprf.org

I. EDUCATION AND EXPERIENCE. Indicate all degrees, when and where received. List postdoctoral appointments, previous faculty positions, and other principal positions, when and where, in chronological order.

Education

2000-2005	Ph.D. (Chemistry)	University of Connecticut, Storrs, CT 06268
1996-2000	B.S. (Chemistry)	Westminster College, New Wilmington, PA 16172
1996-2000	B.S. (Biology)	Westminster College, New Wilmington, PA 16172

Experience

2007-Present	Assistant Professor	Hamilton College, Clinton, NY 13323
2005-2007	V. Assistant Professor	Wellesley College, Wellesley, MA 02482

Ph.D. thesis title and supervisor with current mailing address, including email:

Thesis Title: "New Perspectives on the Synthesis and Function of Septanose Carbohydrates"
Thesis Advisor: Mark W. Peczuh, Ph.D., Associate Professor, Department of Chemistry, University of Connecticut, 55 North Eagleville Road, U 3060, Storrs, CT 06268

Postdoctoral research topic and research supervisor with current mailing address, including email: N/A

Is your position at the institution of record for this proposal a tenure-track position? Yes

If not, please explain the nature of your position and attach your Department Chair's letter as page 3a stating that you meet PRF eligibility requirements.

II. STATEMENT OF OPPORTUNITY TO CONDUCT RESEARCH AT GRANTEE INSTITUTION.

A. Highest academic degree awarded to students in your department: Bachelor's Degree

B. Available facilities - space, equipment, and supplies.

Space: Snyder Research Lab (1000 sq ft), Biochemistry Teaching Laboratory (1500sq ft),
Equipment: Agilent 8453 UV/Vis diode array spectrometers (3), Bruker 500MHz Nuclear Magnetic Resonance (NMR) Spectrometer, SoLow –80° Freezers (2), walk-in coldroom, Nanopure water purifier and various biochemical equipment including pH meters, centrifuges, shakers etc.
Supplies: General chemicals supplies are available through the College's stockroom. Additional supplies will be purchased with monies from this grant.

C. Research support by or to be expected from the department or institution, including startup funds.

Start-up Funds: Hamilton College will provide start-up funds in the amount of $50,000.00 over a three year period.
Matching Funds: Hamilton College will also match 2:1 any funds from this grant used to purchase equipment or supplies.

D. Teaching duties - list the courses you are expected to teach next year and give contact hours per week for lectures, recitations, and laboratory.

Fall: Organic Chemistry II (CHEM 255)—3 contact hours per week
Advanced Organic Chemistry (NEW COURSE)—3 contact hours per week

Spring: Organic Chemistry I (CHEM 190)—3 contact hours per week
Senior Project (CHEM 552)—6 contact hours

Percentage of time devoted to research during academic year: 25%

Percentage of time devoted to research during summer:100%

III. PUBLICATIONS. Include titles, co-authors, and literature references. Use separate page(s) if necessary.

Peczuh, M.W.; **Snyder, N.L**. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, 44, 4057-4061.

Peczuh, M.W.; **Snyder, N.L**.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004**, *339(6),* 1163-1171.

Matteo, M. P.; **Snyder, N.L**.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38

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Snyder, N.L.; Peczuh, M.W. Haines, H.M. "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006**, *62,* 9301-9320.

Castro, S.; Cherney, E. C.; **Snyder, N. L.**; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007,** *342(10)*, 1366-1372.

Markad, S.D.; Xia, S.; **Snyder, N.L.**; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxidation of Carbohydrate Based Oxepines."*Submitted*

IV. OTHER RESEARCH GRANTS. Include titles, amounts (*annual direct costs*), sources, time periods of awards, and *relationship to this PRF proposal*. Use separate page if necessary; indicate "none" if applicable.

A. List any previous or current financial support received for research.

Current

Title: Hamilton College Start-up Funds
Amount: $50,000.00
Source: Hamilton College
Award Period: July 01, 2007 through June 01, 2010
PRF Relationship: Approximately one-third of these funds are available for this project.

Previous

The PI has had no previous research support.

B. List any other applications pending.

Pending

Title: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates
Amount: $318,212.00
Source: National Science Foundation—Research at Undergraduate Institutions
Award Period: September 2008 through August 2011
PRF Relationship: No relationship

V. SUGGESTED REVIEWERS.

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I. Abstract

The development of cellulosic ethanol as an alternative fuel source is of increasing importance in today's economy. The complexity of generating biocatalysts that can efficiently convert cellulose to ethanol is a limiting factor in this process. This proposal attempts to address this problem by studying cellulase, a key group of enzymes used in the production of cellulosic ethanol. In the production of cellulosic ethanol, cellulase enzymes convert cellulose into glucose. The glucose produced is then fermented by yeasts to make ethanol. Unfortunately, cellulase is thermally labile and denatures over a short period of time when employed as a free enzyme in solution. Recently, researchers have shown that the thermal stability and activity of many enzymes increases when encapsulated in an appropriate sol-gel matrix. The research described in this proposal aims to study sol-gel encapsulated cellulase from *Trichoderma reesei* with the goal of increasing the thermal stability and activity of this group of enzymes. The **specific aims** of this research are:

1. To design an appropriate sol-gel matrix for the encapsulation of cellulase from *Trichoderma reesei.*
2. To study the activity and stability of immobilized cellulase using two complimentary techniques: UV-visible spectroscopy and nuclear magnetic resonance (NMR) spectroscopy.

The ultimate question we are asking is: *"Can encapsulating cellulase in the appropriate sol-gel complex increase the thermal stability and activity of this collection of enzymes?"*

II. Background and Significance

A. Cellulose and the Production of Cellulosic Ethanol

Cellulose is a linear polysaccharide composed of glucose units that are linked in a 1-4β conformation (Figure 1-A). Individual polysaccharide chains of cellulose adopt a rod-like conformation and associate with one another through hydrogen bonds to form crystalline microfibrils (Figure 1B). These crystalline microfibrils are responsible for the high tensile strength attributed to the primary cell walls of most plants.

A. **B.**

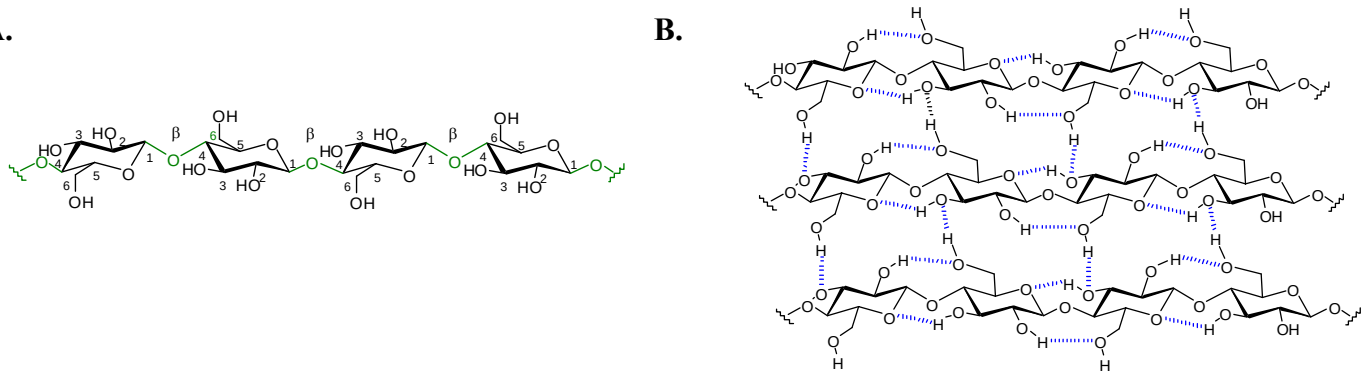


Figure 1: A. Cellulose shown as a linear polysaccharide with β1-4 linkages in green. B. Cellulose microfibrils shown with hydrogen bonds in blue.

Recently, cellulose has gained attention as a potential source of ethanol for use as an alternative fuel.[1] Agricultural residues, municipal solid wastes, herbaceous energy crops and hardwood all provide cheap and easily accessible sources of cellulose. Methods for the production of cellulosic ethanol from cellulosic biomass generally employ three major stages as shown in Figure 2. In Stage 1, cellulosic biomass is pretreated to release the cellulose from lignin. In stage 2, pretreated cellulosic biomass is subjected to enzymatic degradation by a number of different enzymes collectively known as cellulase. In stage 3, yeasts are employed to ferment the sugar resulting in the production of ethanol, which is then recovered by distillation.

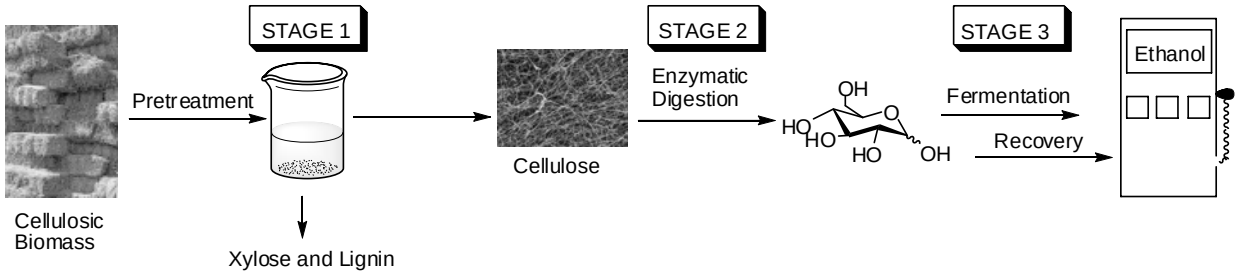


Figure 2: Production of Cellulosic Ethanol

One of the major obstacles associated with the production of cellulosic ethanol is the efficient enzymatic hydrolysis of cellulose into glucose (Stage 2). Methods that commonly employ the cellulotic enzymes required for this process often leave the enzymes susceptible to exposure which results in proteolytic degradation over time, reducing turnover. An additional problem is the efficient recovery of the desired product, in this case glucose, from the enzymes involved in the hydrolysis process.

Non-covalent processes for the immobilization of enzymes in silica gels have gained considerable attention over the past twenty years and a number of enzymes have been successfully immobilized using the sol-gel method.[2] The advantages of this method include biocompatibility, uniformity, thermal and chemical stability, facile recovery, and purification and reuse.[3] One of the major challenges still remaining in this area is the encapsulation of enzymes that target larger substrates, such as cellulase. The microporous nature of most conventional sol-gel systems hinders the ability larger substrates to access the enzymes inside of the sol-gel material.[4]

B. Experimental Goals

The major goal of the research outlined in this proposal is to design an appropriate sol-gel matrix for the encapsulation of cellulase from *Trichoderma reesei*. The success of this experiment will be measured by the ability of the sol-gel matrix to increase the thermal stability and catalytic activity of cellulase. A number of different sol-gel media will be employed to assess these criterion under a variety of encapsulation conditions as described in the Experimental section below. Enzyme assays will be conducted using absorbance and nuclear magnetic resonance spectroscopy.

III. Preliminary Studies

A. Recent Studies on the Sol-Gel Encapsulation of Cellulase from Humicol insolens

In the past year, Knez and coworkers[5] reported the successful hydrolysis of carboxymethyl cellulase using aerogels derived from the sol-gel process. The authors concluded that cellulase from *Humicola inoslens*, when encapsulated in hydrophilic silica aerogels prepared from tetramethyl orthosilicate (TMOS), showed increased thermal stability and high reusability. However the initial reaction rates of the immobilized cellulase were reduced. The authors speculated that the reduced activity was the result of diffusional limitations due in part to the size of the substrate and the highly restricted motion of the enzyme substrate complex. The authors overcame this problem by pre-incubating the aerogels with the substrate and measuring activity after a given time period at which point the activity rose significantly.

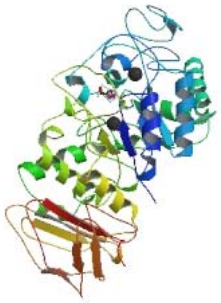
While the process defined by the authors is effective in achieving the desired product, the initial rate of the

reaction is reduced by the system used in this study. In addition, the process defined by Knez and coworkers requires the use of atmospheric and high pressure supercritical carbon dioxide as a solvent. Although supercritical carbon dioxide is readily available and relatively inexpensive, the reactor required for their process is fairly inaccessible. In an effort to overcome these obstacles a new process for the immobilization of cellulase is warranted.

B. Encapuslation of a-Amylases as an Inspiration for the Immobilization of Cellulases

Amylase is an enzyme that is used to convert starch to glucose. Starch is analogous to cellulose, with the major difference between the two being the linkage at the anomeric position (Figure 3). While the nature of these linkages is of considerable importance biologically, the substrate size for amylases and cellulases is similar in nature and so the efforts to overcome the limitations associated with internal mass transfer for sol-gel encapsulated amylases may be applicable to similar issues associated with cellulase.

A



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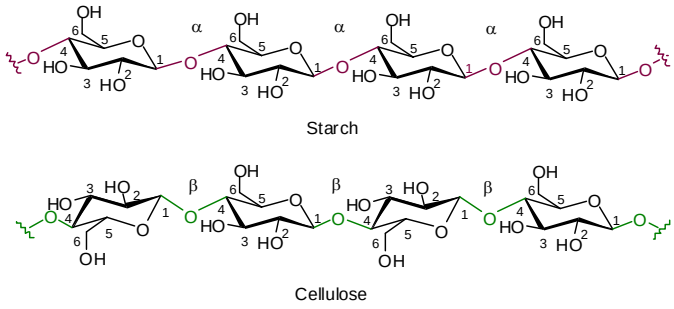


Figure 3: A. α-Amylase (PDB=1amq). B. Starch (polymer of glucose units linked 1-4α) versus cellulose (polymer of glucose units linked 1-4β)

The sol-gel encapsulation of α-amylase has recently been investigated by Vera-Avila and coworkers.[6] Tetraethoxysilane (TEOS) encapsulated α-amylase was shown to have only a little more than 19.7% of the activity of that of the free enzyme. Not surprisingly, the authors speculated that the reduced activity was the result of diffusional limitations, and internal pore collapse of the sol-gel matrix.

Research by Cho and coworkers[7] has shown that sol-gel-processed materials that use biocompatible chitosan as a dispersant in the sol-gel process can be used to prepare sol-gel encapsulated amylase that showed very stable

activity over a thirty day period. Unfortunately, the authors mention nothing about the ability of the dispersant process to increase the initial activity of amylase, nor do they mention whether activity increased in comparison to non-immobilized amylases. However, a recent report by Juang and Chang[8] showed that the relative activities of amylases encapsulated in a chitosan-clay composite were higher than the free enzymes over similar pH and temperature ranges, and maintained greater than 80% of their activity after fifty repeated uses. The outcome of the study suggests that chitosan may enhance the activity of sol-gel encapsulated cellulase.

IV. Experimental Methods

A. Immobilization of Active Cellulase: Sol-Gel Encapsulation Using Free and Chitosan Doped Systems

Cellulase derived from the *Trichoderma reesei* will be investigated in this study. The cellulotic system of *T. reesei* is chosen as a model because of the extensive amount of data already available for cellulase enzymes derived for this organism.[9]

T. reesei is a filamentous fungus that uses a collection of extracellular hydrolytic enzymes to synergistically degrade native cellulose substrates. These are at least three endoglucanases (EC 3.1.2.4), two cellobiohydrolases (EC 3.2.1.91) and one beta glucosidase (EC 3.2.1.21). These enzymes work synergistically to efficiently degrade cellulose as shown in Figure 4.

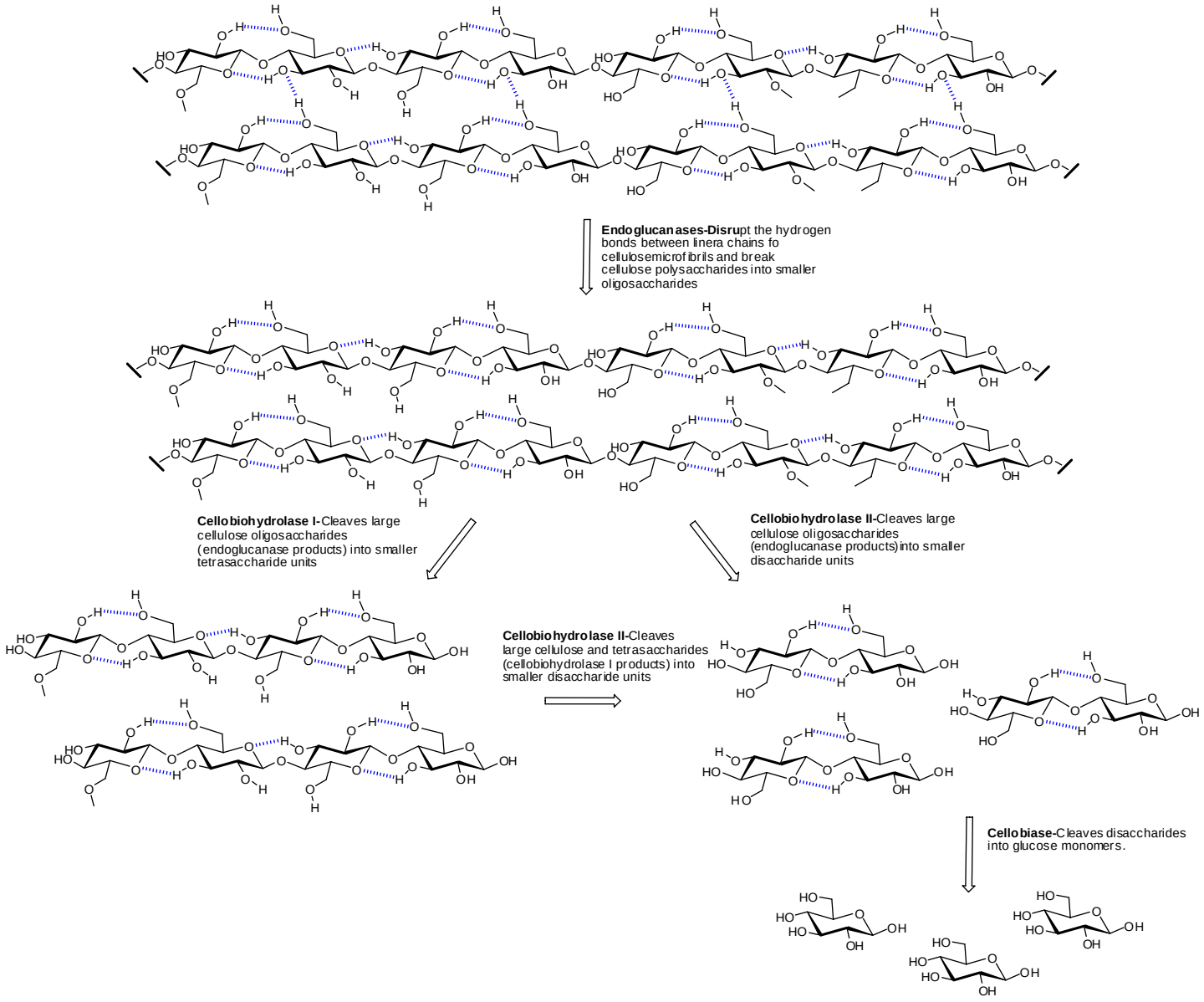


Figure 4: Hydrolysis of cellulose by *Trichoderma reesei*.

Cellulase from *T. reesei* (available commercially through Sigma-Aldrich) will be immobilized in a number of different sol-gel media. Sol-gels derived from tetramethyl orthosiliate (TMOS) and tetraethyl orthosilicate (TEOS) will be used as standards, and the measured activity (described below) will be compared to non-immobilized cellulase from *T. reesei* under native conditions. These initial studies should provide us with a reasonable idea of whether TMOS or TEOS provides a better sol-gel medium for the encapsulation of cellulase.

Once the appropriate sol-gel medium has been determined, the better system (TMOS or TEOS) will be used to prepare sol-gels that incorporate chitosan as an additive. We believe that the appropriate sol-gel system, when doped with chitosan, will result in increased cellulase activity. The hydrophobicity and high porosity of chitosan

should lead to smaller mass transfer resistance.

B. Enzyme Activity Assays

The goal of these experiments will be to determine the optimum sol-gel media, enzyme concentration, and reaction conditions (pH, and temperature) required to obtain the highest activity and stability possible for cellulase derived from *T. reesei*. Two complimentary techniques, absorbance spectroscopy (UV Visible) and nuclear magnetic resonance (NMR) spectroscopy will be used cross-verify the information obtained from these studies.

Absorbance Spectroscopy—UV visible absorbance spectroscopy will be used to measure the concentration of reduced sugar from carboxymethly cellulose (CMC) as a product of time using 3,5-dinitrosalicyclic acid (DNS) reagent.[10] Briefly, a reaction mixture consisting of a buffer system of desired pH and concentration CMC will be prepared and heated to the desired temperature. Addition of the desired sol-gel system will signal the start of the reaction. Samples will be taken from the reaction mixture at defined time periods and quenched with DNS. After a ten minute heating and cooling cycle, the glucose concentration will be determined by measuring the absorbance of the solution at 505 nm.[11] Results from different sol-gel complexes at different pH's and different temperatures will be used to determine the best conditions for encapsulation. Absorbance spectroscopy will also be used to study the stability/reusability of cellulase over time.

Nuclear Magnetic Resonance Spectroscopy—An NMR assay for the assessment of the activity of sol-gel encapsulated enzymes will be developed in conjunction with the procedure already established for the determination of cellulase activity by UV visible spectroscopy and will be used to confirm the results of the experiment. A sol-gel system suitable for assessing the activity of cellulase (as determined by the absorbance spectroscopy experiments above) will prepared directly inside a standard NMR tube. The NMR tube will then be filled with the appropriate buffer containing CMC and the reaction will be conducted at the desired temperature. Signals characteristic of bond cleavage will be monitored to determine the rate of hydrolysis over time.

V. Future Studies

The results obtained from this experiment will be used gain insight into the hydrolysis of cellulose by cellulase from *Trichoderma reesei* using the sol-gel process defined in this proposal. Future studies may involve studying the activity of the individual enzymes involved in this process to help explain the observed activity. Efforts are currently underway to establish a contact at VTT Laboratories in Finland to obtain cellobiohydrolase I and cellobiohydrolase II for future work.

VI. Undergraduate Student Participation

Hamilton College undergraduate students will be involved in every aspect of the research outlined in this proposal. The convergent design strategy of the proposed work allows for the dissection of this project into many challenging individual projects that will provide broad learning experiences for the undergraduate students involved. The principle investigator will provide undergraduate students with opportunities to participate in this research as early as possible in their academic career. In addition, every effort will be made to include students from underrepresented groups. The principle investigator will work alongside the students in the laboratory and will provide extensive training in the techniques and technologies used in the proposed studies. Students will also learn many basic and advanced research skills including experimental design, project development and management. Finally students will have the opportunity to present the culmination of their work in the form of peer reviewed publications and/or public presentations.

VII. Budget

Personnel **–**Hamilton College undergraduate students will be heavily involved in the research outlined in the proposal above. Students conducting research in the laboratory will receive course credit during the regular academic year. Monies from this research grant will be used during the summer to provide undergraduate students with a stipend as outlined in Table 1 below. Hamilton College sets the standard for ten week summer stipends at $4,000.00 per student.

Table 1: Personnel Costs*

Summer	Number of Students	Cost Per Student	PI	Cost Per Summer PI	Total
2009	3	$4,000.00	1	$7,500.00	$19,500.00
2010	3	$4,000.00	1	$7,500.00	$19,500.00
Total =	**6**	**$8,000.00**	**2**	**15,000.00**	**$39,000.00**

*Monies for the principle investigator will be provided by the college or through additional sources of outside support.

Reagents, Equipment and Supplies **--**A breakdown of the costs for reagents (chemical and biological), equipment, and supplies for this project can be found in Table 2 below. Materials for the experiments proposed can be purchased for approximately $24,000.00 with $8,000.00 coming from PRF. ***Hamilton College will provide a 2:1 match on all reagents, equipment and supplies funded with this research grant and will supply the other $16,000.00 required for this project.***

Table 2: Estimated Reagents Equipment and Supply Costs*

Item	Approximate Cost	PRF Funds	Hamilton College Matching Funds
Chemical Reagents	$3,000.00	$1,000.00	$2,000.00
Biological Reagents	$3,000.00	$1,000.00	$2,000.00
Equipment and Supplies	$18,000.00	$6,000.00	$12,000.00
Total=	**$24,000.00**	**$8,000.00**	**$16,000.00**

*Prices are based on vendor list prices and include approximate shipping and handling costs. Itemized costs can be provided upon request.

Summary of the Total Costs—It is estimated that $66,000.00 would be required to start and maintain the proposed

research project over a five year period. The amount of $50,000.00 is kindly requested from the Petroleum Research Foundation with a $16,000.00 match for equipment and supply costs coming from Hamilton College.

Table 3: Total Costs

Category	**Cost**	**PRF Funds**	**Hamilton College Matching Funds**
Personnel	$39,000.00	$39,000.00	$0.00
Reagents, Equipment and Supplies	$34,000.00	$8,000.00	$16,000.00
Travel	$3,000.00	$3,000.00	$0.00
Total=	**$76,000.00**	**$50,000.00**	**$16,000.00**

VIII References

[1] Wyman, C. E. 'What Is (and Is Not) Vital to Advancing Cellulosic Ethanol." *Trends. Biotech.* **2007**, *25*, 153-157 and references therein.

[2] For a recent review see Pierre, A. C. 'The Sol Gel Encapsulation of Enzymes." *Biocat. Biotrans.* **2004**, *22*, 145-170

[3] Han, K.; Wu, Z.; Lee, J.; Ahn, I.; Park, J. W.; Min, B. R.; Lee, K.; "Activity of Glucose Oxidase Entrapped in Mesoporous Gels." *Biochem Eng. J.* **2005**, *22*, 161-166.

[4] Avnir, D.; Braun, S.; Lev, O. Ottolenghi, M. "Enzymes and Other Proteins Entrapped in Sol-Gel Materials" *Chem. Mater.* **1994**, *6*, 1605-1614 and references therein.

[5] Paljevac, M.; Primozic, M.; Habulin, M.; Novak, Z.; Knez, Z. "Hydroluysis of Carboymethyl Cellulose by Cellulase Immobilized on Silica Gels at Loa and High Pressures." *J. Supercritical Fluids* **2007**, *43*, 74-80.

[6] Vera-Avila, L. E., Morales-Zamudio, E.; Garcia-Camacho, M. P. "Activity and Reusability of Sol-Gel Encapsulated a-Amylase and Catalase. Performace in Flow-Through Systems." *J. Sol-Gel Sci and Tech*. **2004**, *30*, 197-204.

[7] Cho, G.; Moon, I.-S.; Lee, J.-S. "Preparation and Characterization of a-Amylase Immoblized Inorganic/Organic Hybrid Membrane Using Chitosan as a Dispersan in the Sol-Gel Process." *Chem. Lett.* **1997**, 577-578.

[8] Chang, M.-Y.; Juang, R.-S. "Activities, stabilities, and Reaction Kinetics of Three Free and Chitosan-Clay Composite Immobilized Enzymes." *Enzyme and Microbial Technology* **2006**, 36, 75-82.

[9] Kubicek, C. P. *The cellulase proteins of Trichoderma reesei: Structure, multiplicity, mode of action and regulation of formation*; Advances in Biochemical Engineering/Biotechnology; Springer: Berlin/Heidelberg, 1992; 45, 1-27.

[10] Miller, G. L. "Use of Dinitorsalicyclic Acid Reagent for Determination of Reducing Sugar." *Anal. Chem*. **1959**, *31*, 426-428.

[11] Assay Procedure 013, Cellulase, Biocatalyst Limited, ISO 90001, July 01, 1999.

ACS PRF
AMERICAN CHEMICAL SOCIETY PETROLEUM RESEARCH FUND

June 6, 2008

Dr. Nicole L. Snyder
Hamilton College
Department of Chemistry
198 College Hill Road
Clinton, NY 13323

PRF# 48693 -GB 4

Dear Dr. Snyder:

We regret to inform you that your research proposal, referred to above, was not recommended for funding by the ACS PRF Advisory Board. During its Spring meeting the Board considered more than 380 proposals. Only a limited number of these could be selected for support.

ACS PRF policy permits us to send excerpts of the technical comments of outside reviewers, edited to insure the anonymity of the reviewer. These reviews are enclosed.

Thank you for your interest in The ACS Petroleum Research Fund and for the opportunity to consider your research proposal. If we can be of assistance with any aspect of the PRF program, please call on us again.

Sincerely,

W. Christopher Hollinsed, Ph.D.
Director

Excerpts From Reviewer Comments

48693-GB4 **Nicole L. Snyder**

The primary goal is to determine optimum sol-gel media, enzyme concentration, and reaction conditions required to obtain the highest activity and stability possible for (purchased) cellulase derived from T. reesei. This will include testing the effect of chitosan doping in the sol-gels to reduce mass transfer resistance. Enzyme activity will be assessed using a standard DNS reagent absorbance assay on CMC, as well as NMR. This research plan builds on prior sol-gel encapsulation work from other labs, which have demonstrated, among other things: 1) improved cellulase stability but not activity, presumably due to diffusion limitations associated with the other sol-gel systems used; and 2) increased amylase activity with a chitosan-clay composite.

The research is important and reasonable, given the requested payment. The plan is straightforward and I see no flaws in the design. The described experiments are not novel or exciting, but rather a primarily empirical approach to improve stability and activity of cellulase. They are relatively simple experiments to carry out and appropriate for undergraduate student participation (as proposed), but that may not be in line with PRF priority.

48693-GB4 **Nicole L. Snyder**

The conversion of biomass into fermentable sugar holds much promise for the production of cellulosic ethanol. Cellulose is one of the main constituents of plant cell walls and is a polymer of Beta-linked glucose. One of the limiting factors for wide-scale implementation of producing cellulosic ethanol is due to the fact that the use of certain enzymes, cellulases, are necessary to break down the cellulose into glucose subunits. These enzymes don't stand up to the harsh industrial conditions often employed due to thermal lability and their instability in solution. Recently, investigators are determining that cellulases and similar enzymes can be encapsulated in sol-gels to increase their thermal stability and reusability. Due to the large size of cellulose it has been determined that introducing chitosan into the sol-gel can increase the pore size and prevent diffusional complications.

The PI is proposing to immobilize cellulases from Trichoderma Reesei in a variety of different sol-gel media. The cellulase activity will subsequently be measured to determine the best medium for immobilization. Sol-gels that incorporate chitosan will then be prepared to determine if cellulase activity is increased. A combination of absorption spectroscopy and NMR spectroscopy will be employed to measure the degradation of cellulose for these studies.

The PI is an assistant professor at Hamilton College and has many publications relating to carbohydrate chemistry from work as a graduate student. Essentially, this proposal draws its hypothesis from studies showing that the activity and stability of amylases can be increased by immobilization in sol-gel doped with chitosan. The only difference between amylases and cellulases is that they cleave glucose polymers of different linkage. These previous studies provide a good framework and show the feasibility of the proposed studies. This may also help in its successful implementation by undergraduate students with time constraints. The main drawback to this proposal is that there is no description of what sol-gels are and how they are made. A description of sol-gel preparation should be included in the experimental methods. The PI's institution is committed to the research and will match 2:1 any funds from this grant used to purchase equipment or supplies.

This proposal is weakened by insufficient experimental methods describing the preparation of sol-gels and the immobilization of the cellulases.

THE PETROLEUM RESEARCH FUND
TYPE UNI PROPOSAL

(Please refer to statement of eligibility, terms, and conditions.)

PRIVILEGED COMMUNICATION

This proposal is intended for review exclusively by ACS PRF staff, members of the PRF Advisory Board, and outside reviewers officially asked to furnish scientific comments. It may not be transmitted to other parties, copied, or retained for future reference. Please return to the PRF office, or destroy, in accordance with instructions.

Nicole L. Snyder-Lee (Principal Investigator)

July 01, 2007 (Date of First Faculty Appointment)

Hamilton College (Institution) | Chemistry (Department) | Clinton (City) | NY (State)

Title of Proposed Research: Mechanistic Studies of Sol-Gel Encapsulated Cellulases for the Production of Cellulosic Ethanol

The ACS Petroleum Research Fund has a "zero-tolerance" policy for scientific misconduct. Scientific misconduct includes, but is not limited to, fabrication, falsification, and plagiarism. Instances of alleged or suspected scientific misconduct will be referred to a committee of the PRF Advisory Board for investigation. Upon the PRF Advisory Board's determination of scientific misconduct, the Board may, in its discretion, take any actions it deems appropriate. Such actions may include: disqualifying proposals from consideration; disqualifying individuals or institutions from submitting future proposals; revoking grant awards; contacting appropriate Officers of the relevant institution(s), such as the Dean, and/or Department Head of the investigator(s); and other such actions that the Board feels are appropriate.

By signing below, we acknowledge that we have read and understand this scientific misconduct policy.

Principal Investigator: Nicole L Snyder-Lee (Signature) 07/25/2008 (Date)

Officer of the Institution Endorsing the Proposal: (Signature) 7/31/08 (Date)

Payment Schedule Requested: $ 25,000.00 (First Year) + $ 25,000.00 (Second Year) = **Total $50,000**

I. EDUCATION AND EXPERIENCE. Indicate all degrees, when and where received. List postdoctoral appointments, previous faculty positions, and other principal positions, when and where, in chronological order.

Education

2000-2005	Ph.D. (Chemistry)	University of Connecticut, Storrs, CT 06268
1996-2000	B.S. (Chemistry)	Westminster College, New Wilmington, PA 16172
1996-2000	B.S. (Biology)	Westminster College, New Wilmington, PA 16172

Experience

2007-Present	Assistant Professor	Hamilton College, Clinton, NY 13323
2005-2007	V. Assistant Professor	Wellesley College, Wellesley, MA 02482

Ph.D. thesis title and supervisor with current mailing address, including email:

Thesis Title: "New Perspectives on the Synthesis and Function of Septanose Carbohydrates"
Thesis Advisor: Mark W. Peczuh, Ph.D., Associate Professor, Department of Chemistry, University of Connecticut, 55 North Eagleville Road, U 3060, Storrs, CT 06268

Postdoctoral research topic and research supervisor with current mailing address, including email: N/A

Is your position at the institution of record for this proposal a tenure-track position? Yes

If not, please explain the nature of your position and attach your Department Chair's letter as page 3a stating that you meet PRF eligibility requirements.

II. STATEMENT OF OPPORTUNITY TO CONDUCT RESEARCH AT GRANTEE INSTITUTION.

A. Highest academic degree awarded to students in your department: Bachelor's Degree

B. Available facilities - space, equipment, and supplies.

Space: Snyder Research Lab (1000 sq ft), Biochemistry Teaching Laboratory (1500sq ft),
Equipment: Agilent 8453 UV/Vis diode array spectrometers (3), Bruker 500MHz Nuclear Magnetic Resonance (NMR) Spectrometer, SoLow –80° Freezers (2), walk-in coldroom, Nanopure water purifier and various biochemical equipment including pH meters, centrifuges, shakers etc.
Supplies: General chemicals supplies are available through the College's stockroom. Additional supplies will be purchased with monies from this grant.

C. Research support by or to be expected from the department or institution, including startup funds.

Start-up Funds: Hamilton College will provide start-up funds in the amount of $50,000.00 over a three year period.
Matching Funds: Hamilton College will also match 2:1 any funds from this grant used to purchase equipment or supplies.

D. Teaching duties - list the courses you are expected to teach next year and give contact hours per week for lectures, recitations, and laboratory.

Fall: Organic Chemistry II (CHEM 255)—3 contact hours per week
Chemical Immunology (CHEM 380)—3 contact hours per week

Spring: Organic Chemistry I (CHEM 190)—3 contact hours per week
Advanced Laboratory (CHEM 371)—6 contact hours per week
Senior Seminar (CHEM 552)—1 contact hour per week

Percentage of time devoted to research during academic year: 25%

Percentage of time devoted to research during summer:100%

III. PUBLICATIONS. Include titles, co-authors, and literature references. Use separate page(s) if necessary.

Peczuh, M.W.; **Snyder, N.L**. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, 44, 4057-4061.

Peczuh, M.W.; **Snyder, N.L**.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004***, 339(6),* 1163-1171.

Matteo, M. P.; **Snyder, N.L**.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38

Castro, S.; Duff, M.; **Snyder, N.L.**; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic Biomolecular Chemistry* **2005**, *3,* 3869-3872.

Snyder, N.L.; Peczuh, M.W. Haines, H.M. "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006***, 62,* 9301-9320.

Castro, S.; Cherney, E. C.; **Snyder, N. L.**; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007,** *342(10)*, 1366-1372.

Markad, S.D.; Xia, S.; **Snyder, N.L.**; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxidation of Carbohydrate Based Oxepines." *Journal of Organic Chemistry* **2008**, in press.

IV. OTHER RESEARCH GRANTS. Include titles, amounts (*annual direct costs*), sources, time periods of awards, and *relationship to this PRF proposal*. Use separate page if necessary; indicate "none" if applicable.

A. List any previous or current financial support received for research.

Current

(1) Title: Hamilton College Start-up Funds
Amount: $50,000.00
Source: Hamilton College
Award Period: July 01, 2007 through June 01, 2010
PRF Relationship: Approximately one-third of these funds are available for this project.

(2) Title: ACS-PRF-SRF—"Bio-inspired Cobalt Catalysts for Carbene and Nitrene Transfer Reactions."
Amount $8,000.00
Source: Petroleum Research Foundation
Award Period: Summer 2008
PRF Relationship: No relationship

Previous

The PI has had no previous research support.

B. List any other applications pending.

Pending

Title: NIH-AREA—"Understanding the Role of the Vancomycin Glycan in Binding Glycosyltransferases: The Synthesis, Characterization, and Biological Evaluation of New Derivatives of Vancomycin with the Potential for Combating Antibiotic Resistance."
Amount: $318,212.00
Source: National Institutes of Health-Academic Research Enhancement Award
Award Period: May 2009 through April 2012
PRF Relationship: No relationship

V. SUGGESTED REVIEWERS.

1. Dr. Sheila Grant; Department of Biological Engineering; University of Missouri; 162 Agricultural Engineering Building; Columbia, MI 65211; email: GrantSA@missouri.edu; phone: (573)-884-9666; fax: (573)-882-1151.

2. Dr. Daryl K. Eggers; Department of Chemistry; San Jose State University; One Washington Square; San Jose, CA, 95192; email: deggers@science.sjsu.edu; phone: (408) 924-4960; fax: (408) 924-4945.

3. Dr. Ryan J. Gilbert; Department of Biological Engineering; Michigan Tech; 304 M and M Engineering Building; 1400 Townsend Drive; Haughton, MI 49931; email: rgilbert@mtu.edu; phone: (906) 487-1740; fax: (906) 487-1717.

4. Dr. William M. Risen, Jr.; Department of Chemistry; Brown University; Providence, RI 02912; email: wrisen@brown.edu; phone: (401)-863-2611; fax: (401)-863-9982.

VI. PROPOSED RESEARCH.

1.0. Abstract

The development of efficient production methods for the generation of biofuels such as cellulosic ethanol is of increasing importance in today's oil-driven economy. However, the complexity of generating biocatalysts that can efficiently convert biomass into fuel is a limiting factor in this process. This proposal attempts to address this problem by studying cellulase, a key group of enzymes used in the production of cellulosic ethanol from cellulotic biomass. Cellulase converts cellulose into glucose, which is subsequently fermented by yeasts to make ethanol. Unfortunately, cellulase is thermally labile and denatures over a short period of time when employed as a free enzyme in solution. Recently, researchers have shown that the thermal stability and activity of many enzymes increases when encapsulated in an appropriate sol-gel matrix. The research described in this proposal aims to study sol-gel encapsulated cellulase from *Trichoderma reesei* with the goal of increasing the thermal stability and activity of this group of enzymes. The **specific aims** of this research are:

1. To design an appropriate sol-gel matrix for the encapsulation of cellulase from *Trichoderma reesei.*
2. To study the biochemical properties of immobilized cellulase including, conformational dynamics, substrate accessibility, reaction kinetics, and stability using a host of biochemical techniques.

The ultimate question we are asking is: *"Can encapsulating cellulase in the appropriate sol-gel complex increase the thermal stability and activity of this collection of enzymes?"*

2.0 Background and Significance

2.1 Cellulose and the Production of Cellulosic Ethanol

Cellulose is a linear polysaccharide composed of glucose units that are linked in a 1-4β conformation (Figure 1-A). Individual polysaccharide chains of cellulose adopt a rod-like conformation and associate with one another through hydrogen bonds to form crystalline microfibrils (Figure 1B). These crystalline microfibrils are responsible for the high tensile strength attributed to the primary cell walls of most plants.

A. **B.**

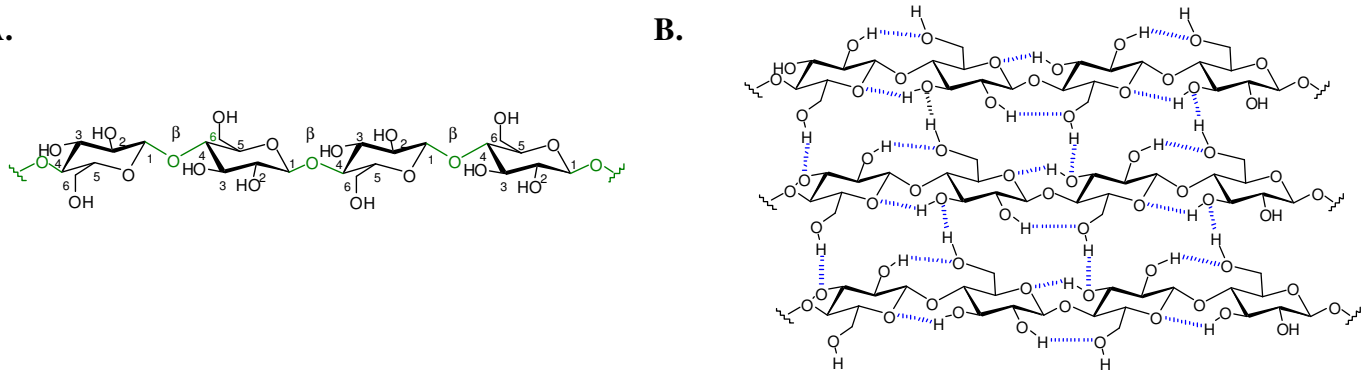


Figure 1: A. Cellulose shown as a linear polysaccharide with β1-4 linkages in green. B. Cellulose microfibrils shown with hydrogen bonds in blue.

Recently, cellulose has gained attention as a potential source of ethanol for use as an alternative fuel.[1] Agricultural residues, municipal solid wastes, herbaceous energy crops, and hardwood all provide cheap and easily accessible sources of cellulose. As shown in Figure 2, cellulosic ethanol is currently produced in three major stages from cellulosic biomass. In the first stage, cellulosic biomass is pretreated to release the cellulose from lignin. In stage two, pretreated cellulosic biomass is subjected to enzymatic degradation by a number of different enzymes collectively known as cellulase to produce free sugars. In the third stage, yeasts are employed to ferment the free sugars into ethanol, which is then recovered by distillation.

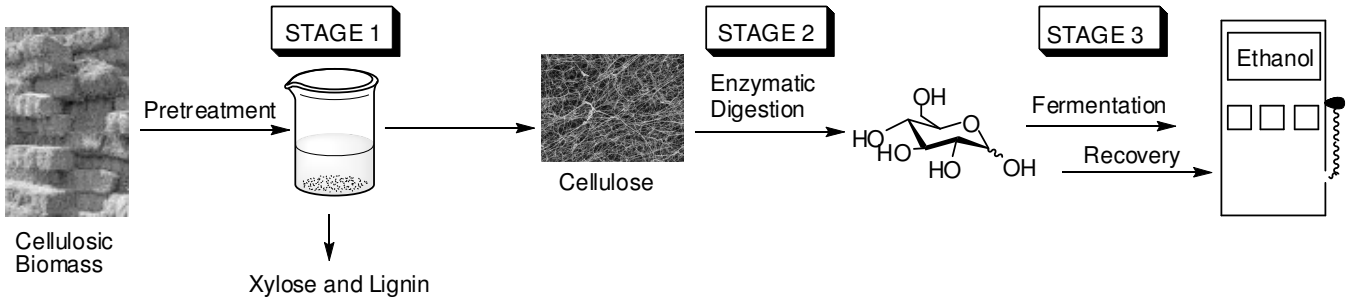


Figure 2: Production of Cellulosic Ethanol

One of the major obstacles associated with the production of cellulosic ethanol is the efficient enzymatic hydrolysis of cellulose into glucose (Stage 2). Methods that commonly employ the cellulotic enzymes required for

this process often leave the enzymes susceptible to exposure which results in proteolytic degradation over time, reducing turnover. An additional problem is the efficient recovery of the desired products, in this case glucose, from the reaction mixture.

Non-covalent processes for the immobilization of enzymes in silica gels have gained considerable attention over the past twenty years, and a number of enzymes have been successfully immobilized using the sol-gel method.[2] As shown in Figure 3, the sol-gel method of encapsulating enzymes involves two major stages. In the first stage, a porous metal oxide material (usually tetraethoxy orthosilicate (TEOS) or tetramethoxy orthosilicate (TMOS)) is hydrolyzed in water using either an acidic or basic catalyst to form a sol solution. In the second stage, an enzyme-buffer solution is introduced to the sol and cast to from an optically transparent sol-gel monolith, thin film, powder, and/or fiber.

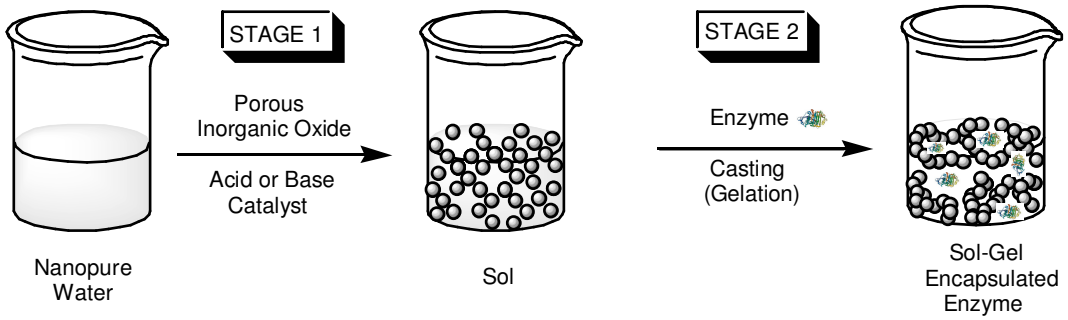


Figure 3: The sol-gel process for encapsulating enzymes.

The resulting sol-gel creates a microporous environment that preserves the structure and function of the encapsulated enzyme. Carefully designed sol-gels have been shown to protect the active site, prevent unfolding, and assist in the stabilization of many key electrostatic interactions important for protein structure and function. These advantages allow most enzymes to retain their activity over prolonged periods of time in comparison to the free enzyme. In addition, the reusability and physical nature of the sol-gel (which provides for facile recovery of the reaction products) makes these materials especially attractive as catalysts for bioconversion.

One of the major challenges still remaining in this area is the encapsulation of enzymes that target larger substrates, such as cellulase. The microporous nature of most conventional sol-gel systems hinders the ability of

larger substrates to access the enzymes inside of the sol-gel matrix.[3] Optimum pore sizes should be large enough to allow for unrestricted transport of substrate molecules and reaction products, but should also be small enough to prevent leakage of the encapsulated enzyme. Theoretically, the ability to control the pore size distribution, geometry, morphology and polarity would aid in the preparation of sol-gel materials that can process larger substrates.

2.2 Experimental Goals

The major goal of the research outlined in this proposal is to design an appropriate sol-gel matrix for the encapsulation of cellulase from *Trichoderma reesei*. A number of different sol-gel media will be screened in order to determine the appropriate conditions for the immobilization of cellulase. The success of this experiment will be measured by the ability of the sol-gel matrix maintain the structural integrity of the encapsulated enzyme, provide adequate access to substrate, exhibit comparable reaction kinetics to the free enzyme in solution, and be stable and reusable. Circular dichroism and UV-visible spectroscopy will be employed in this study to determine whether encapsulating cellulase in the appropriate sol-gel complex can be used to increase the thermal stability and activity of this collection of enzymes

3.0 Preliminary Studies

3.1 Recent Studies on the Sol-Gel Encapsulation of Cellulase from *Humicol insolens*

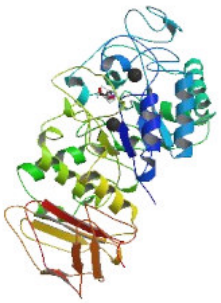
In the past year, Knez and coworkers[4] reported the successful hydrolysis of carboxymethyl cellulose using sol-gel encapsulated cellulase from *Humicola inoslens*. The authors concluded that cellulase, when encapsulated in hydrophilic silica aerogels prepared from tetramethyl orthosilicate (TMOS), showed increased thermal stability and high reusability. However, the initial reaction rates of the immobilized cellulase were reduced in comparison to the free enzyme under native conditions. The authors speculated that the reduced activity was the result of diffusional limitations due in part to the size of the substrate and the highly restricted motion of the enzyme-substrate complex within the sol-gel matrix.

While the process described by the authors is effective in achieving the desired product, the initial reaction rate is significantly reduced by the system used in this study. In addition, the process described by Knez and coworkers required the use of atmospheric and high pressure supercritical carbon dioxide as a solvent. Although supercritical carbon dioxide is readily available and relatively inexpensive, the reactor required for this process is highly specialized and fairly inaccessible. Finally, the authors did not address whether there were any conformational, rotational and/or translational dynamics that led to the reduced activity of the systems they studied. For these reasons, a more accessible, complete and extensive study investigating the sol-gel encapsulation of cellulase is warranted

3.2 Encapuslation of α-Amylase as an Inspiration for the Immobilization of Cellulases

Amylase is an enzyme that is used to convert starch to glucose. Starch is analogous to cellulose, with the major difference between the two being the linkage at the anomeric position (Figure 4). While the nature of these linkages is of considerable importance biologically, the substrate size for amylases and cellulases is similar in nature and so the efforts to overcome the limitations associated with internal mass transfer for sol-gel encapsulated amylases may be applicable to similar issues associated with cellulase.

A



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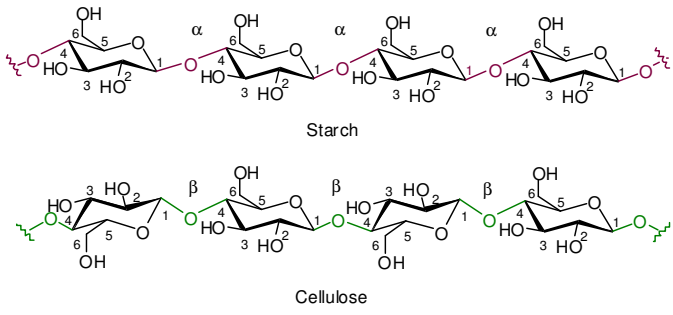


Figure 4: A. α-Amylase (PDB=1amq). B. Starch (polymer of glucose units linked 1-4α) versus cellulose (polymer of glucose units linked 1-4β)

The sol-gel encapsulation of α-amylase has recently been investigated by Vera-Avila and coworkers.[5] In their

pioneering study, tetraethoxy orthosilicate (TEOS) encapsulated α-amylase exhibited 19.7% of the activity of the free enzyme. Not surprisingly, the authors speculated that the reduced activity was the result of diffusional limitations and internal pore collapse of the sol-gel matrix.

Research by Cho and coworkers[6] has shown that sol-gel processed materials that use a biocompatible dispersant such as chitosan can be used to prepare sol-gel encapsulated amylase with high stability over a thirty day period. Unfortunately, the authors mention nothing about the ability of the dispersant process to increase the initial activity of amylase, nor do they mention whether activity increased in comparison to free amylases. However, a recent report by Juang and Chang[7] showed that the relative activity of amylase encapsulated in a chitosan-clay composite was higher than the free enzymes over similar pH and temperature ranges, and maintained greater than 80% activity after fifty repeated uses. The outcome of the study suggests that hybrid sol-gels containing chitosan and similar dispersants may enhance the activity of encapsulated cellulase by assisting in the creation of an environment that optimizes the pore size distribution, geometry, morphology and polarity.

4.0 Experimental Methods

4.1 Immobilization of Active Cellulase: Sol-Gel Encapsulation Using Doped Systems

Cellulase derived from the *Trichoderma reesei* will be investigated in this study. The cellulotic system of *T. reesei* is chosen as a model because of the extensive amount of data already available for cellulase enzymes derived for this organism.[8]

T. reesei is a filamentous fungus that uses a collection of extracellular hydrolytic enzymes to synergistically degrade native cellulose substrates. These are at least three endoglucanases (EC 3.1.2.4), two cellobiohydrolases (EC 3.2.1.91) and one beta glucosidase (EC 3.2.1.21) involved in this process as shown in Figure 5.

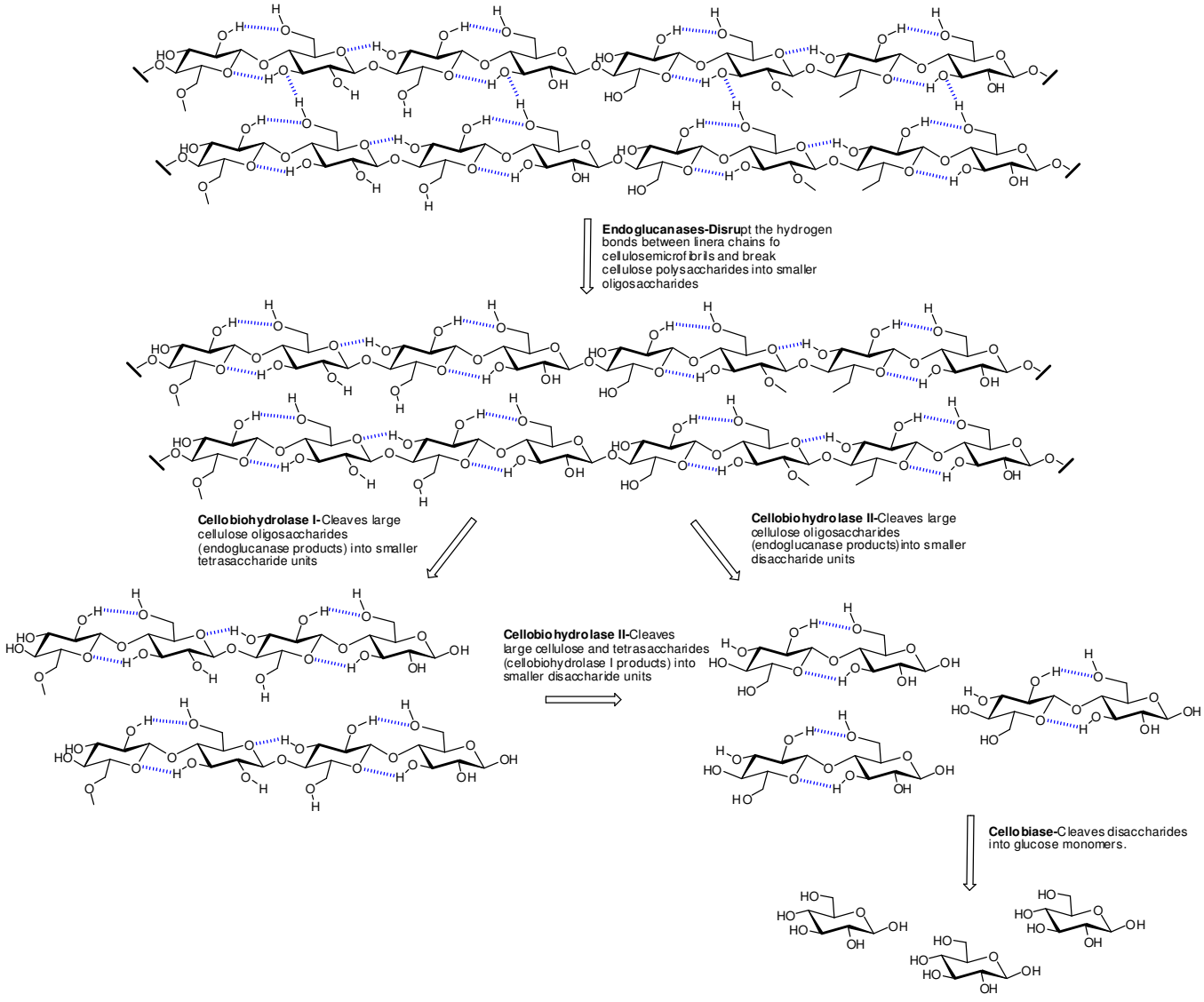


Figure 5: Hydrolysis of cellulose by *Trichoderma reesei*.

Cellulase from *T. reesei* (available commercially through Sigma-Aldrich) will be immobilized in a number of different hybrid sol-gel media. Sol-gels derived from tetramethyl orthosiliate (TMOS) and tetraethyl orthosilicate (TEOS), free of any dispersant will be used as standards, and the measured activity will be compared to non-immobilized cellulase from *T. reesei* under native conditions. These initial studies should provide us with a reasonable idea of whether TMOS or TEOS provides a better sol-gel medium for the encapsulation of cellulase. We hypothesize that TEOS will provide the best medium due the increased pore sizes observed on average for sol-gels prepared from TEOS.

Once the appropriate sol-gel medium has been determined, the better system (TMOS or TEOS) will be used to prepare hybrid sol-gels that incorporate dispersants. We are interested in studying several different dispersants including methyltrimethoxysilane (MTMS)/ethylene glycol (PEG) mixtures[9], poly-vinyl alcohol/4-vinylpyrdine copolymers (PVA-g-PVP)[10], and chiotsan derivatives.[7] These dispersants were chosen due to their ability to improve the activity and stability of the sol-gel encapsulated enzymes that process carbohydrates and other large substrates.

For each sol-gel successfully created in this study we will use enzyme activity assays to determine the appropriate dispersant to sol to enzyme ratio, pH, temperature, and cast (monolith, thin film, or powder) required to maintain substrate accessibility and reaction kinetics in comparison to the free, native enzyme. We will also determine the stability and reusability of the sol-gel encapsulated systems prepared in this study. For optimized sol-gels we will determine the conformational dynamics of the encapsulated enzymes. We believe that the hydrophobicity and high porosity of these hybrid sol-gels should allow us to prepare sol-gels systems with smaller mass transfer resistance and increased activity due to greater conformational flexibility.

4.2 Enzyme Activity Assays

The optimum sol-gel media will be determined by a host of spectroscopic experiments. The ability of the sol-gel matrix to meet four important criteria including: (i) maintenance of the structural integrity of the encapsulated enzyme, (ii) substrate accessibility, (iii) comparable reaction kinetics to the free enzyme in solution, and (iv) stability and reusability will be evaluated. Details for these experiments are given in the paragraphs below.

Conformational Dynamics—The conformation dynamics of the system will be evaluated by two complimentary techniques, circular dichroism (CD) and UV-visible absorbance spectroscopy (UV-Vis). Properly folded proteins under native conditions give distinctive absorbance values which may increase or decrease when the protein is perturbed. The minimum absorbance of the free cellulotic system in solution under native conditions and over a range of several different temperatures will be determined and compared to the absorbance measurements of

immobilized cellulase in various sol-gel media under similar conditions. Since we will be studying a collection of enzymes we will only be able to ascertain whether or not there is a change in the conformation of one or more of the individual cellulotic enzymes. Although we will not be able to determine which particular enzyme (or enzymes) has undergone a change in conformation, these experiments will allow us to explain any observed activity (or inactivity) on the basis of a conformational change.

Substrate Accessibility and Reaction Kinetics—UV visible absorbance spectroscopy will be used to determine the ability of the substrate to access the encapsulated enzyme in each sol-gel system prepared. The concentration of reduced sugar from carboxymethly cellulose (CMC) as a product of time using 3,5-dinitrosalicyclic acid (DNS) as an imaging reagent will be used to determine the initial velocity and rate of the hydrolysis reaction.[11] Carboxymethyl cellulose is chosen as a substrate since it is commercially available and a vast amount of information is available for the hydrolysis of CMC in the presence of cellulase. Results from different sol-gel complexes in different casts (monoliths, thin films, and powders) will be studied over a range of temperature and pH to determine the best conditions for encapsulation and presentation.

Stability and Reusability—Absorbance spectroscopy will also be used to study the stability/reusability of cellulase over time. Experiments will be run on the sol-gels prepared in this study on a routine basis to determine stability of of the encapsulated enzyme as a function of time and usage

5.0. Future Studies

The results obtained from this experiment will be used gain insight into the hydrolysis of cellulose by cellulase from *Trichoderma reesei* using the sol-gel processes defined in this proposal. Future studies will involve studying the activity of the individual enzymes that comprise the cellulose of *T. reesei* to help rationalize the observed activity. Additional studies will involve the production cellulase coencapsulated with yeasts to determine if such systems are capable of producing cellulosic ethanol in a synergistic fashion.

6.0 Budget

Personnel **–**Hamilton College undergraduate students will be heavily involved in the research outlined in the proposal above. Students conducting research in the laboratory will receive course credit during the regular academic year. Monies from this research grant will be used during the summer to provide undergraduate students with a stipend as outlined in Table 1 below. Hamilton College sets the standard for ten week summer stipends at $4,000.00 per student. The Dean of Faculty at College will provide matching funds for one additional student per summer.

Table 1: Personnel Costs*

Summer	Number of Students	Cost Per Student	PI	Cost Per Summer PI	Total	PRF Funds	Hamilton College Matching Funds
2009	3	$4,000.00	1	$7,500.00	$19,500.00	$15,500.00	$4,000.00
2010	3	$4,000.00	1	$7,500.00	$19,500.00	$15,500.00	$4,000.00
Total =	**6**	**$24,000.00**	**2**	**$15,000.00**	**$39,000.00**	**$31,000.00**	**$8,000.00**

Reagents, Equipment and Supplies **--**A breakdown of the costs for reagents (chemical and biological), equipment, and supplies for this project can be found in Table 2 below. Materials for the experiments proposed can be purchased for approximately $48,000.00 with $16,000.00 kindly requested from the PRF. Hamilton College will provide a 2:1 match on all reagents, equipment and supplies funded with this research.

Table 2: Estimated Reagents Equipment and Supply Costs*

Item	Approximate Cost	PRF Funds	Hamilton College Matching Funds
Chemical Reagents	$15,000.00	$5,000.00	$10,000.00
Biological Reagents	$15,000.00	$5,000.00	$10,000.00
Equipment and Supplies	$18,000.00	$6,000.00	$12,000.00
Total=	**$48,000.00**	**$16,000.00**	**$32,000.00**

*Prices are based on vendor list prices and include approximate shipping and handling costs. Itemized costs can be provided upon request.

Summary of the Total Costs—It is estimated that $93,000.00 will be required to start and maintain the proposed research project over a two year period. The amount of $50,000.00 is kindly requested from the Petroleum Research Foundation with a $43,000.00 match for equipment and supply costs coming from Hamilton College.

Table 3: Total Costs

Category	Cost	PRF Funds	Hamilton College Matching Funds
Personnel	$39,000.00	$31,000.00	$8,000.00
Reagents, Equipment and Supplies	$48,000.00	$16,000.00	$32,000.00
Travel	$6,000.00	$3,000.00	$3,000.00
Total=	**$93,000.00**	**$50,000.00**	**$43,000.00**

7.0 References

[1] Wyman, C. E. 'What Is (and Is Not) Vital to Advancing Cellulosic Ethanol." *Trends. Biotech.* **2007**, *25*, 153-157 and references therein.

[2] For a recent review see: (a) Pierre, A. C. 'The Sol Gel Encapsulation of Enzymes." *Biocat. Biotrans.* **2004**, *22*, 145-170. (b) Kandimalla, V. B.; Tripathi, V. S.; Ju, H. "Immobilization of Biomolecules in Sol-Gels: Biological and Analytical Applications." *Critic. Rev. Anal. Chem.* **2006,** *36*, 73-106.

[3] Avnir, D.; Braun, S.; Lev, O. Ottolenghi, M. "Enzymes and Other Proteins Entrapped in Sol-Gel Materials" *Chem. Mater.* **1994**, *6*, 1605-1614 and references therein.

[4] Paljevac, M.; Primozic, M.; Habulin, M.; Novak, Z.; Knez, Z. "Hydroluysis of Carboymethyl Cellulose by Cellulase Immobilized on Silica Gels at Loa and High Pressures." *J. Supercritical Fluids* **2007**, *43*, 74-80.

[5] Vera-Avila, L. E., Morales-Zamudio, E.; Garcia-Camacho, M. P. "Activity and Reusability of Sol-Gel Encapsulated a-Amylase and Catalase. Performace in Flow-Through Systems." *J. Sol-Gel Sci and Tech*. **2004**, *30*, 197-204.

[6] Cho, G.; Moon, I.-S.; Lee, J.-S. "Preparation and Characterization of a-Amylase Immoblized Inorganic/Organic Hybrid Membrane Using Chitosan as a Dispersan in the Sol-Gel Process." *Chem. Lett.* **1997**, 577-578.

[7] Chang, M.-Y.; Juang, R.-S. "Activities, stabilities, and Reaction Kinetics of Three Free and Chitosan-Clay Composite Immobilized Enzymes." *Enzyme and Microbial Technology* **2006**, 36, 75-82.

[8] Kubicek, C. P. "The cellulase proteins of Trichoderma reesei: Structure, multiplicity, mode of action and regulation of formation." Advances in Biochemical Engineering/Biotechnology; Springer: Berlin/Heidelberg, 1992; 45, 1-27.

[9] Soares, C. M. F.; dos Santos, O. A.; Olivio, J. E.; De Castro, H. F.; de Moraes, F. F.; Zanin, G. M. "Influence of the Alkyl Substituted Silane Precursor on Sol-Gel Encapsulated Lipase Activity." *J. Mol. Catal. B. Enzymatic*. **2004**, *29*, 69-70.

[10] Wang, B.; Li, B.; Deng, Q.; Dong, S. "Amperometric Glucose Biosensor Based on Sol-Gel Organic-Inorganic Hybrid Material. *Anal. Chem.* **1998**, *70*, 3170-3174.

[11] Miller, G. L. "Use of Dinitorsalicyclic Acid Reagent for Determination of Reducing Sugar." *Anal. Chem*. **1959**, *31*, 426-428.

VII. WORKFORCE DEVELOPMENT

Hamilton College is a highly selective private liberal arts college devoted entirely to the education of undergraduate students. Hamilton College was originally founded in 1793 as the Hamilton-Oneida Academy by Samuel Kirkland through a grant funded between the Oneida Indian Nation and the State of New York. The purpose of the academy was to provide a first rate co-educational experience designed to foster an environment of learning and cooperation between the Indians and setters.

In 1812 the Hamilton-Oneida Academy was chartered as Hamilton College, the third oldest institution of higher education established in the State of New York. Today, Hamilton College is a coeducation institution of approximately 1,800 students and ranks seventeenth among all National liberal arts colleges. While the name of the college has changed, the overall mission of the college remains the same—to foster an environment of learning and cooperation. Hamilton College's motto "Know thyself" reflects the college's desire to provide an educational experience that enables "young women and men of unusual gifts to realize their fullest capacities, for their own benefit and that of the societies in which they will live." In support of these efforts in the sciences, the College has recently created a $56 million dollar state-of-the-art well equipped science center.

Monies obtained from this grant will be used to provide opportunities for two to four undergraduate Hamilton College students and one rising junior or senior high school student per summer to work as research assistant's in the Department of Chemistry at Hamilton College. Students chosen to work on this project will be heavily involved in every aspect f the research outlined in this proposal. Students will participate fully in the experimental design phase, and will learn first-hand how to master the technical skills required to conduct the experiments outlined in this proposal through a collaborative, hands-on approach. As part of their experience, students will also learn how to properly maintain a research notebook, how to search and interpret the chemical literature, and how to organize and present the data they obtain in the laboratory. Each student participating in this research project will also be given the opportunity to present their data publicly at one or more local or national meetings (for example, an American Chemical Society or Sigma Xi meeting), and will participate fully in manuscript preparation once enough data has been accumulated for publication.

In the first year of the grant, two undergraduate Hamilton College students will be actively recruited for this project from a competitive pool of rising sophomores and juniors. The selection criteria will be based on the students level of interest in the project, and his or her ability to commit to the project for at least two summers, and one full academic semester (for example, as an independent study or senior thesis). This time commitment will provide the student with an adequate opportunity to make a significant physical and intellectual contribution to the project and will ensure continuity of the research conducted. This process also provides an avenue for the initial students trained under this proposal to mentor the new incoming students in the second year via a peer-led team learning approach.

The principle investigator will also work closely with local public high school science educators, especially within the Oneida Indian Nation in Central New York to identify a rising junior or senior high school student interested in pursuing research in chemistry or biochemistry. Qualified students will be asked to submit an essay in the spring of their sophomore or junior year that describes why they want to do research at Hamilton. They will also need to solicit at least three letters of recommendation their behalf, one from a current or previous high school science educator who knows the student intimately through coursework or collaboration, one form the students high school principal, and one from a parent, guardian, or friend familiar with the students interest in scientific inquiry. The student will be provided with a stipend to work in the lab for an eight to ten week period, and the recommending educator will also be invited to participate in the project. The goal of this program is three-fold. First, the program introduces students at an early age to the practice of scientific research and serves to develop in them an interested for scientific inquiry that will hopefully extend beyond the high school years. Secondly, the program provides and avenue for the selected high school student to establish and ongoing relationship with a current undergraduate student pursing a degree in science. Finally, the program provides and opportunity for the professional development of a local public high school teacher who will be able to refresh his or her research skills and network with individuals at the College.

ACS PRF
AMERICAN CHEMICAL SOCIETY PETROLEUM RESEARCH FUND

February 18, 2009

Dr. Nicole L. Snyder
Hamilton College
Department of Chemistry
198 College Hill Road
Clinton, NY 13323

PRF# 49480 -UNI 5

Dear Dr. Snyder:

We regret to inform you that your research proposal, referred to above, was not recommended for funding by the ACS PRF Advisory Board. During its meeting the Board considered more than 140 proposals. Only a limited number of these could be selected for support.

ACS PRF policy permits us to send excerpts of the technical comments of outside reviewers, edited to insure the anonymity of the reviewer. These reviews are enclosed.

Thank you for your interest in The ACS Petroleum Research Fund and for the opportunity to consider your research proposal. If we can be of assistance with any aspect of the PRF program, please call on us again.

Sincerely,

W. Christopher Hollinsed, Ph.D.
Director

Excerpts From Reviewer Comments

49480-UNI5 **Nicole L. Snyder**

After serving as a visiting assistant professor at Wellesley from 2005-2007, the P.I. began her tenure-track career at Hamilton College in 2007. Hamilton has provided start-up funds of $50,000 and will also provide a $43,000 match to this PRF proposal, if funded. At this early date, no independent publications have appeared, but the P.I. is coauthor on seven papers from her graduate work with Mark Peczuh at UCONN. Each summer, three undergraduate students will work on the project, and the P.I. will spend 100% of her time in the lab; in addition, a local high school student and teacher (ideally from the Oneida Indian Nation in Central New York) will also be involved with the project. Hamilton has the facilities required to conduct the proposed research.

The P.I. proposes to encapsulate--and thus stabilize--the cellulase (the term for the mixture enzymes produced by an organism to hydrolyze cellulose) from Trichoderma reesi in a sol-gel matrix and to then study the properties of the immobilized cellulase. Trichoderma reesi cellulase is well-characterized and is commercially available from Sigma-Aldrich. Because cellulase freely dissolved in buffer is quite unstable, success would provide an improved method for hydrolyzing cellulose once it had been freed from lignin. Earlier work in the field has shown that enzymes immobilized in sol-gels [typically prepared from tetraethyl or tetramethyl orthosilicate (TEOS and TMOS, respectively)] have enhanced stability, but a major challenge has been observing good activities with enzymes like cellulase that process larger substrates--the microporous structure of conventional sol-gels hinders substrate diffusion to the immobilized enzyme. This problem was indeed encountered in recent reports with both sol-gel encapsulated cellulase and amylase (which catalyzes the hydrolysis of the related biopolymer starch). The P.I. then notes that sol-gels prepared with biocompatible dispersants such as the polysaccharide chitosan have also been studied and that amylase encapsulated in a "chitosan-clay composite" shows enhanced stability and high activity relative to the free enzyme.

The P.I. thus proposes to prepare a series of sol-gels from TMOS and TEOS, with and without a variety of dispersants. Once prepared, enzyme-activity assays will be employed to determined "the appropriate dispersant to sol to enzyme ratio, pH, temperature, and cast (monolith, thin film, or powder) required to maintain substrate accessibility and reaction kinetics in comparison to the free, native enzyme." Finally, the physical properties of the immobilized cellulase enzymes will be characterized using two spectroscopic techniques (UV-vis and circular dichroism).

Overall, I find this proposal to be unimpressive. As noted above, the key precedent for the work comes from a study using crosslinked clay composites, not sol-gels. (As described in the original reference of Chang and Juang, these composites were "prepared by equal weights of chitosan and activated clay and were cross-linked with

glutaraldehyde.") Moreover, the bulk of the proposed experimental work involves unimaginative combinatorial chemistry: prepare sol-gels under a variety of conditions with a variety of dispersants and see what happens. Finally, I think the spectroscopic-characterization experiments will be uninformative--the UV-vis and CD spectra of encapsulated cellulase are indeed very likely to differ from that of the free enzymes, but drawing concrete structural conclusions will be quite difficult (certainly no details of the analysis to be undertaken are provided in the proposal).

49480-UNI5 **Nicole L. Snyder**

Nicole Snyder-Lee of Hamilton College proposes to encapsulate cellulases into sol-gels and study their thermal stability and activity relative to the free enzymes. The basic question to be addressed is whether these systems can increase both the thermal stability and enzymatic activity. While there seems to be no question that a variety of encapsulation techniques will protect the enzyme, increasing or even maintaining activity will be a substantial reach. The reactivity will depend on the geometry and pore size of the sol gels and the size of the substrates. The proposal addresses these issues and points to a previous study on a related enzyme, amylase, encapsulated in a chitosan-clay composite (reference 7). These authors apparently claim an increase in the activity of amylase, though the current proposal does not mention the level of increase or suggest a mechanism for this unexpected result.

The proposal in its current form provides little support for the notion that the TMOS and TEOS sol-gel systems will show an increase in cellulase activity. The PI proposes a variety of spectroscopic studies using different dispersants and variations in temperature, pH, and sol to enzyme ratios. The techniques to be employed, circular dichroism and UV-visible spectroscopy, clearly will provide information about the folding and perturbation of the enzymes but begs the question about the accessibility of these enzymes to the target carboxymethyl cellulose. These experiments further are complicated by the fact that the commercially available cellulase from T. reesei provides a mixture of different enzymes. The PI might well consider initial studies on purified enzymes and/or use a range of smaller, more reactive substrates (glucose oligomers) that model the chemistry of cellulose. This also would allow a more systematic approach to investigations of the relative size of the sol-gel pores and substrates in understanding the reaction rates of free and encapsulated enzymes.

The PI appears to be well qualified to carry out the proposed research, and there is a strong and supportive environment for undergraduate research at Hamilton College. It is not clear from the proposal whether the Hamilton College start-up funds are contingent on the successful funding of this and other proposals. Nevertheless, there appears to be significant institutional support for the proposed research. In comparison with other Undergraduate New Investigator (UNI) proposals I have read, I rate this proposal in the middle third. This is a promising and important area of research, but the proposal would be improved by considering other approaches to studying the ability of enzymes to function in the protective environment of sol gels.

(Note that the proposal confuses "cellulase" and "cellulose" at the bottom of page 7 and the bottom of page 13.)

49480-UNI5 **Nicole L. Snyder**

Panel Summary Notes:

The main idea in this proposal is to investigate the sol-gel encapsulation of cellulases for enhanced stability. There was considerable discussion in the panel about the advantages/disadvantages of encapsulation. Some panel members felt that enzyme encapsulation should enhance stability, but not necessarily activity, as the reaction with a large polymeric substrate, such as cellulose, may be hampered. It was suggested that work with smaller substrates would be helpful in this regard. This component of the proposal needs to be improved. The PI proposes spectroscopic methods for enzyme characterization upon sequestration, but there is no discussion of what kind of spectroscopic alterations are to be expected. The general panel consensus is that this application could be substantially improved in a resubmission.

UNI

THE PETROLEUM RESEARCH FUND
UNDERGRADUATE NEW INVESTIGATOR PROPOSAL

(Please refer to statement of eligibility, terms, and conditions.)

PRIVILEGED COMMUNICATION

This proposal is intended for review exclusively by ACS PRF staff, members of the PRF Advisory Board, and outside reviewers officially asked to furnish scientific comments. It may not be transmitted to other parties, copied, or retained for future reference. Please return to the PRF office, or destroy, in accordance with instructions.

Nicole L. Snyder-Lee (Principal Investigator)
July 01, 2007 (Date of First Faculty Appointment)

Hamilton College (Institution) | Chemistry (Department) | Clinton (City) | NY (State)

Title of Proposed Research: The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts

The ACS Petroleum Research Fund has a "zero-tolerance" policy for scientific misconduct. Scientific misconduct includes, but is not limited to, fabrication, falsification, and plagiarism. Instances of alleged or suspected scientific misconduct will be referred to a committee of the PRF Advisory Board for investigation. Upon the PRF Advisory Board's determination of scientific misconduct, the Board may, in its discretion, take any actions it deems appropriate. Such actions may include: disqualifying proposals from consideration; disqualifying individuals or institutions from submitting future proposals; revoking grant awards; contacting appropriate Officers of the relevant institution(s), such as the Dean, and/or Department Head of the investigator(s); and other such actions that the Board feels are appropriate.

By signing below, we acknowledge that we have read and understand this scientific misconduct policy.

In addition, we confirm that, should this proposal be funded, the proposed budget will become the approved grant budget and funds will be spent according to the budget amounts and categories approved by ACS PRF. Any revisions to the approved budget require **prior approval** from a program manager.

Principal Investigator: Nicole L Snyder Lee (Signature) 10/28/2009 (Date)

Officer of the Institution Endorsing the Proposal: [signature] (Signature) Associate Director, Foundation Relations (Title) 10/28/09 (Date)

PROPOSED BUDGET — UNDERGRADUATE NEW INVESTIGATOR GRANT

Request: $50,000 for two grant years. Although some budget flexibility can be allowed, with prior approval, after a grant has been awarded, an outline of the projected use of the funds will aid in the evaluation of the proposal. Shifts in budget category allocations, consistent with the terms and conditions outlined on page iv, and time extensions without the commitment of additional funds may be arranged ***with prior approval***. Funds not expended in one budget year may be carried forward into the next.

For the Periods

(Each period must end on August 31 and be of at least twelve months duration.)

Approved budget categories:	Sept. 1, 2010	Sept. 1, 2011
1. Stipends (**includes benefits**):	to Aug. 31, 2011	to Aug. 31, 2012
a. Principal Investigator (not to exceed $7,500 per grant year awarded)	$7500.00	$7500.00
b. Undergraduate Student(s)	$8000.00	$8000.00
c. Master's Student(s)	$0.00	$0.00
2. Expendable Supplies and/or Services, such as Chemicals, Glassware, Analyses, etc.	$7500.00	$7500.00
3. Capital Equipment (Specify item and any match in narrative; see Part VII)	$0.00	$0.00
4. Travel (Maximum: $2,000 per year)	$2000.00	$2000.00
5. Field work	$0.00	$0.00
ANNUAL TOTALS	**$25,000.00**	**$25,000.00**

TOTAL AMOUNT **$ $50,000**

Principal Investigator Nicole L Snyder-Lee 10/28/2009
(Signature) (Date)

Officer of the Institution Endorsing the Proposal [signature] Associate Director, Foundation Relations 10/28/09
(Signature) (Title) (Date)

Grantee Institution Hamilton College

I. EDUCATION AND EXPERIENCE

A. Indicate all academic degrees, when and where received, and Ph.D. thesis title and supervisor. List postdoctoral appointments (if appropriate) and previous positions, in chronological order; significant honors and awards; and other pertinent biographical information.

Education:

1996-2000	B.S. (Chemistry)	Westminster College, New Wilmington, PA 16172
1996-2000	B.S. (Biology)	Westminster College, New Wilmington, PA 16172
2000-2005	Ph.D. (Chemistry)	University of Connecticut, Storrs, CT 06268

Ph.D. thesis title and supervisor with current mailing address:

Thesis Title: "New Perspectives on the Synthesis and Function of Septanose Carbohydrates"
Thesis Advisor: Mark W. Peczuh, Ph.D., Associate Professor, Department of Chemistry, University of Connecticut, 55 North Eagleville Road, U 3060, Storrs, CT 06268, email: mark.peczuh@uconn.edu

Postdoctoral appointments: None

Experience (including previous positions):

2005-2007	V. Assistant Professor	Wellesley College, Wellesley, MA 02482
2007-Present	Assistant Professor	Hamilton College, Clinton, NY 13323

Significant Awards:
*Elsevier Top-50 Most Cited Articles Award (2004-2007) for "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." (*Awarded at EuroCarb 2007 in Lubeck, Germany on September 02, 2007)*

B. Do you currently hold a tenured or tenure-track position? Yes. If not, please explain your eligibility according to PRF criteria, and include a letter from your department chair verifying your eligibility to apply for a PRF Type UNI grant**.**

II. STATEMENT OF OPPORTUNITY TO CONDUCT RESEARCH AT GRANTEE INSTITUTION

A. Highest academic degree awarded to students in your department: Bachelors Degree

B. Available facilities - space, equipment, and supplies.

Space: Snyder Research Lab (1000 sq ft), Organic Teaching Laboratory (1500sq ft),
Equipment: Bruker 500MHz Nuclear Magnetic Resonance (NMR) Spectrometer, Perkins Elmer Spectrum One IR with ATR attachment, Shimadzu SCL-10A HPLC system, Jasco P-1020 Polarimeter, Schlenk apparatus
Supplies: General chemicals supplies are available through the College's stockroom. Additional supplies will be purchased with monies from this grant.

C. Teaching duties - list the courses you are expected to teach in a typical year and give contact hours per week for lectures, recitations, and laboratory.

Fall: Organic Chemistry II (CHEM 255)—3 contact hours per week
Chemical Immunology (CHEM 380)—3 contact hours per week
Advanced Laboratory (CHEM 371)—6 contact hours per week
Spring: Organic Chemistry I (CHEM 190)—3 contact hours per week
Advanced Laboratory (CHEM 371)—6 contact hours per week

Percentage of time devoted to research during academic year: 25%

Percentage of time devoted to research during summer: 100% Please describe any factors that would significantly detract from available summer research time.

III. CURRENT AND PENDING SUPPORT

A. List any active research grants or other current financial support received for research. Give titles, amounts (*annual direct costs; if more than one PI, indicate only your share of the granted amount*), sources, time periods of awards, and *relationship to this PRF proposal*. Use separate page if necessary; indicate "none" if applicable.

None.

B. List any other research grant applications pending. Give titles, amounts requested (*annual direct costs*), sources, *relationship to this PRF proposal,* and date of funding decision for each application. Use separate page if necessary; indicate "none" if applicable.

None.

C. Describe any start-up support or funding. Use separate page if necessary; indicate "none" if applicable.

Current

(1) Title: Hamilton College Start-up Funds
Amount: $50,000.00
Source: Hamilton College
Award Period: July 01, 2007 through June 01, 2010
PRF Relationship: Approximately one-third of these funds are available for this project.

Previous

(1) Title: ACS-PRF-SRF—"Bio-inspired Cobalt Catalysts for Carbene and Nitrene Transfer Reactions."
Amount $8,000.00
Source: Petroleum Research Fund
Award Period: Summer 2008
PRF Relationship: This experience was used to generate the preliminary results reported in this proposal.

IV. PUBLICATIONS

Include titles, co-authors, and literature references. Use separate page(s) if necessary.

Peczuh, M.W.; **Snyder, N.L**. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, 44, 4057-4061.

Peczuh, M.W.; **Snyder, N.L**.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004**, *339(6),* 1163-1171.

Matteo, M. P.; **Snyder, N.L**.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38

Castro, S.; Duff, M.; **Snyder, N.L.**; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic Biomolecular Chemistry* **2005**, *3*, 3869-3872.

Snyder, N.L.; Peczuh, M.W. Haines, H.M. "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006**, *62*, 9301-9320.

Castro, S.; Cherney, E. C.; **Snyder, N. L.**; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007,** *342(10),* 1366-1372.

Markad, S. D.; Xia, S.; **Snyder, N. L**.; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxdation of Carbohydrate Based Oxepiens." *Journal of Organic Chemistry* **2008**, 73, 6341-6354.

Ruppel, J. V.; Gauthier, T. J.; **Snyder, N. L**.; Perman, J. A. ; Zhang, X. P. "Asymmetric Cobalt-Catalyzed Cyclopropanation with Succinimidyl Diazoacetate: General Synthesis of Optically Active Cyclopropyl Carboxamides." *Organic Letters* **2009**, *11*, 2273-2276.

V. SUGGESTED REVIEWERS

1. G. Richard Geier; Department of Chemistry, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA; Phone: 1-315-228-6795; Fax: 1-315-228-7935; Email: ggeier@mail.colgate.edu

2. Michael Doyle, Department of Chemistry and Biochemistry, University of Maryland, 0107 Building-9, Park, MD 20742, USA; Phone: 1-301-405-1788; Email: mdoyle3@umd.edu

3. Joseph Fox, Department of Chemistry and Biochemistry, University of Delaware, 272 Brown Laboratories, Newark, DE 19716, USA; Phone: 1-302-831-0191; Email: jmfox@udel.edu

4. Karl Kadish, Department of Chemistry, University of Houston, 4800 Calhoun Road, Houston, TX, 77004, USA; Phone: 1-713-743-2740; Fax: 1-713-743-2745; Email: kkadish@uh.edu

5. Ernest G. Nolen, Department of Chemistry, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA; Phone: 1-315-228-7234; Fax: 1-315-228-7935; Email: enolen@mail.colgate.edu

6. Kevin M. Smith, Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, LA 70803, USA; Phone: 1-225-578-7442; Fax: 1-225-578-3458; Email: kmsmith@lsu.edu

7. W. Justin Youngblood, Department of Chemistry, University of North Texas, 1155 Union Circle, #305020, Denton, TX 76203; Phone: 940-369-8289; Email: youngblood@unt.edu

VI. COLLABORATIONS

Collaborator: Peter Zhang, Associate Professor, Department of Chemistry, University of South Florida, 4202 E. Fowler Avenue, Tampa, Florida, 33620, U.S.A. Tel: 1-813-974-7249; Email: pzhang@cas.usf.edu
*Peter Zhang no longer holds an active PRF grant. However, this work began when I was a PRF-SRF in his laboratory during the summer of 2008. Our collaboration was inspired by the PRF grant (PRF# 44286-AC1: Bio-inspired Cobalt Catalysts for Carbene and Nitrene Transfer Reactions) Peter held during that summer.

Nature of the Collaboration: This collaboration began in the summer of 2008 and has continued since. Dr. Peter Zhang and coworkers at the University of South Florida will supply the bromoporphyrins outlined in this study (see attached letter of support). He will also provide opportunities for one interested Hamilton College undergraduate students per annum to spend up to eight weeks working in his laboratory preparing porphyrin synthons. Students in my laboratory at Hamilton College will be involved in the synthesis of the monosaccharides outlined in this proposal, as well as the palladium catalyzed cross coupling reactions between the bromosynthons and the carbohydrate analogs. Once the catalysts described in this proposal have been prepared, Hamilton College students will be responsible for performing the catalytic (cyclopropanation) studies.

VII. CAPITAL EQUIPMENT BUDGET EXPLANATION

There will be no capital equipment requested as part of this proposal. However, Hamilton College will provide matching funds totaling $16,900.00 for the work described in this proposal, including a 30% match on equipment and supplies, and additional funds for one undergraduate student per year ($4000.00) to conduct research at Hamilton College.

VIII. SCIENTIFIC EDUCATIONAL IMPACT (Limited to one page)

Hamilton College is a highly selective private liberal arts college devoted entirely to the education of undergraduate students. Hamilton College was originally founded in 1793 as the Hamilton-Oneida Academy by Samuel Kirkland through a grant funded between the Oneida Indian Nation and the State of New York. The purpose of the academy was to provide a first rate co-educational experience designed to foster an environment of learning and cooperation between the Indians and setters.

In 1812 the Hamilton-Oneida Academy was chartered as Hamilton College, the third oldest institution of higher education established in the State of New York. Today, Hamilton College is a coeducation institution of approximately 1,800 students and ranks seventeenth among all National liberal arts colleges. While the name of the college has changed, the overall mission of the college remains the same—to foster an environment of learning and cooperation. Hamilton College's motto "Know thyself" reflects the college's desire to provide an educational experience that enables "young women and men of unusual gifts to realize their fullest capacities, for their own benefit and that of the societies in which they will live." In support of these efforts in the sciences, the College has recently constructed a $56 million dollar, state-of-the-art science center, equipped with many amenities common to a modern chemistry laboratory. The College has also provided significant support in the form of matching funds and summer research stipends for undergraduate Hamilton College students.

Monies obtained from this grant will be used to provide opportunities for three undergraduate Hamilton College students and one rising junior or senior high school student per summer to work as research assistants in the Department of Chemistry at Hamilton College and the University of South Florida. Students chosen to work on this project will be heavily involved in every aspect of the research outlined in this proposal. Students will participate fully in the experimental design phase, and will learn first-hand how to master the technical skills required to conduct the experiments outlined in this proposal through a collaborative, hands-on approach. As part of their experience, students will also learn how to properly maintain a research notebook, how to search and interpret the chemical literature, and how to organize and present the data they obtain in the laboratory. Each student participating in this research project will also be given the opportunity to present their research publicly at one or more local or national meetings (for example, an American Chemical Society or Sigma Xi meeting), and will participate fully in manuscript preparation once enough data has been accumulated for publication.

In the first year of the grant, two undergraduate Hamilton College students will be actively recruited for this project from a competitive pool of rising sophomores and juniors. The selection criteria will be based on the students level of interest in the project, and his or her ability to commit to the project for at least two summers and one full academic semester (for example, as an independent study or senior thesis). Such a time commitment will provide the student with adequate opportunity to make a significant physical and intellectual contribution to the project and will ensure continuity of the research conducted. This process also provides an avenue for the initial students trained under this proposal to mentor the new incoming students in the second year via peer-led approach. In addition, one undergraduate Hamilton College student, chosen from the pool of rising seniors, will have the opportunity to travel to the University of South Florida to work in the laboratory of Professor Peter Zhang. The research conducted in Professor Zhangs laboratory will be used as a foundation for the student's senior thesis in my laboratory at Hamilton College.

I will also work closely with local public high school science educators, especially within the Oneida Indian Nation in Central New York to identify a rising junior or senior high school student interested in pursuing research in chemistry or biochemistry. Qualified students will be asked to submit an essay in the spring of their sophomore or junior year that describes why they want to conduct research at Hamilton or at the University of South Florida. They will also need to solicit at least three letters of recommendation their behalf, one from a current or previous high school science educator who knows the student intimately through coursework or collaboration, one from the student's high school principal, and one from a parent, guardian, or friend familiar with the students interest in science. The student will be provided with a stipend to work in the lab for an eight to ten week period, and the recommending educator will also be invited to participate in the project. The goal of this program is three-fold. First, the program introduces students at an early age to the practice of scientific research and serves to develop in them an interested for scientific inquiry that will hopefully extend beyond the high school years. Secondly, the program provides an avenue for the selected high school student to establish and ongoing relationship with a current undergraduate student pursing a degree in science. Finally, the program provides an opportunity for the professional development of a local public high school teacher who will be able to refresh his or her research skills and network with individuals at the College.

PROPOSED RESEARCH.

1.0 Abstract

The search for new energetic materials over the last decade has led to a number of organic oxidants and fuels for the preparation of propellants and explosives. Recent research has shown that highly strained cyclopropane-fused hydrocarbons, particularly those with good conformational stability, provide a reasonable alternative to many common propulsion fuels. However, methods for the preparation of these materials require the use of rigorous and toxic reaction conditions and excess reagents, and generate products in low yield, with little stereocontrol. Recent studies have shown that chiral metalloporphyrins can be used to catalyze asymmetric cyclopropanation reactions in high yield and with good stereocontrol. Despite the progress that has been made with these catalysts, two key problems still exist. First, many porphyrin catalysts currently in use exhibit low solubility in polar solvents such as water, limiting substrate scope and making them unsuitable for use in green chemistry applications. In addition, the aromatic nature of these catalysts often facilitates pi stacking resulting in metalloporphyrin aggregation in solution, leading to decreased catalytic efficiency. To address these problems, we are proposing the synthesis and evaluation of novel porphyrins bearing carbohydrate substituents. The polarity and predicted conformation of the carbohydrate-porphyrin conjugates outlined in this study render them excellent candidates to obviate the challenges described here. The ***specific goals*** of this project are: (i) to develop and optimize a palladium-catalyzed cross-coupling reaction to prepare carbohydrate-porphyrin conjugates; and (ii) to assess the ability of carbohydrate-porphyrin conjugates to serve as catalysts in the production of highly strained cyclopropane-fused hydrocarbons.

2.0 Background and Significance

2.1 Strained Heterocycles and Cyclopropane-Fused Hydrocarbons as Highly Energetic Materials

The search for new highly energetic materials over the last decade has led to a number of new organic oxidants and fuels for the preparation of propellants and explosives. Many of these compounds contain nitro, azido, and hydrazine groups, and produce energy via oxidation.[1] Recent research has shown that highly strained heterocycles offer a high specific impulse (thrust to propellant mass flow rate), high density, and heat of combustion (ΔH_f) in comparison to the

military's benchmark energetic material octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX) **1-1**. For example, octanitrocubane (ONC) **1-2**,[2] 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20) **1-3**,[3] 3,3-trinitroazetidine (TNAZ) **1-4**,[4] and 1,1,3,3,-tetranitorcyclobutane (TNCB) **1-5,**[5] have recently been shown to be competent energy materials, with densities close to or above that of water, and heats of formation ranging from 10 kcal/mol to well over 100 kcal/mol.

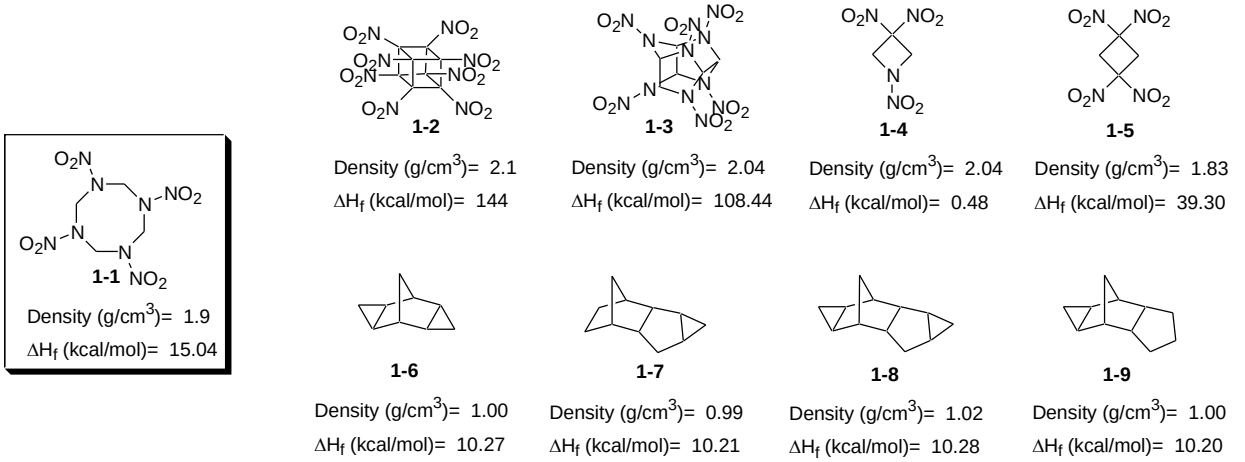


Figure 1. HMX and examples of high energy heterocycles (1-2 through 1-6) and fused hydrocarbons (1-7 through 1-9).

While the byproduct of examples **1-1** through **1-5** are generally environmentally friendly and inert, the conditions required for their preparation often involve a number of toxic reagents and solvents. Another limiting factor is the high cost of the materials required for the synthesis of these compounds. Finally, the yields for many of these compounds are often low and the resulting materials are difficult to separate from other byproducts in the reaction mixtures.

Recently, Han and coworkers have reported that strained cyclopropane-fused hydrocarbons (**1-6** through **1-9**) have the potential to serve as high energetic materials.[6] These materials take advantage of highly strained, fused three, five, and six membered rings which release large amounts of energy upon combustion. In general, these molecules are not as superior as their heteroatom containing counterparts. However, they can be readily prepared by the cyclopropanation of inexpensive starting materials in good yields, providing a reasonable alternative to HMX. These materials are also superior to some of the current fuels commonly used including the jet propellant (JP, average ΔH_f= 8-9 kcal/mol) and rocket fuel (RJ, average ΔH_f= 9-11 kcal/mol) series.

The method reported by Han and coworkers for the preparation of cyclopropane-fused

hydrocarbons requires the use of excess reagents, non-environmentally friendly solvents, and in some cases stoichiometric amounts of catalyst in order to drive the final cyclopropanation reactions to completion. The average yields for these reactions are between 50 and 70 percent. Although the authors do not explicitly mention solubility as a reason for the moderate yields, based on the reaction conditions reported, reagent and/or catalyst solubility may play a role. In addition, the products are generally mixtures of diastereomers that are difficult to separate and characterize. Since conformational stability can play key role in the heat of combustion of a particular compound, the ability to produce cyclopropane-fused hydrocarbons in high yields with excellent stereocontrol is a critical area of development that requires further investigation. Therefore, the development of green catalysts that can catalyze cyclopropanation reactions to produce cyclopropane-fused hydrocarbons with a variety of substrates in high yield, and excellent stereocontrol is of current interest.

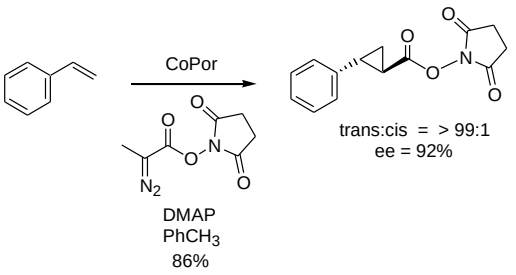
2.2 Experimental Goals

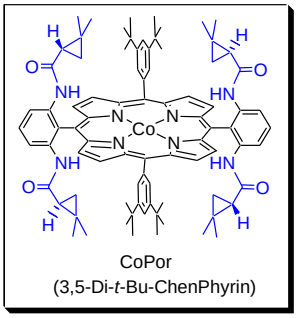
The goals of the experiments outlined in this proposal are two-fold. The first goal is to develop and optimize a palladium-catalyzed cross-coupling reaction that can be used to prepare several porphyrin-carbohydrate conjugates. Metalation of these compounds will provide us with catalysts that can be used to perform cyclopropanation reactions. The success of this goal will rely heavily on our ability to prepare and characterize the aforementioned compounds.

The second goal of this proposal will be to assess the ability of the metalated carbohydrate-porphyrin conjugates prepared in this study to serve as chiral catalysts in asymmetric cyclopropanation reactions, including reactions that will produce highly strained cyclopropane-fused hydrocarbons. The ability to cyclopropanate simple aromatic and aliphatic hydrocarbons using the catalysts prepared in this study will provide initial results that will facilitate this goal. Once a successful protocol for cyclopropanation has been achieved, we will move on to more complex molecules such as bicyclo[2.2.1]hepta-2,5-diene, the precursor to **1-6**, in order to measure our ability to produce highly strained cyclopropane-fused rings.

3.0 Preliminary Studies

A number of reactions have been developed for the synthesis of cyclopropane ring systems.[7] The recent development of palladium-catalyzed cross-coupling reactions between mono-, di-, and tetrasubstituted bromoporphyrins and amides, amines, alcohols, and thiols has allowed for the preparation of a number of chiral amino-, amido-, oxo- and mercaptoporphyrins.[8] The cobalt derivatives of these chiral porpyrins have, in turn, been used to catalyze a number of key functional group transformations including the asymmetric cyclopropanation of aromatic and electron-deficient olefins using diazo reagents with good diastereo- and enantioselectivity as illustrated with styrene in Scheme 1.[9]





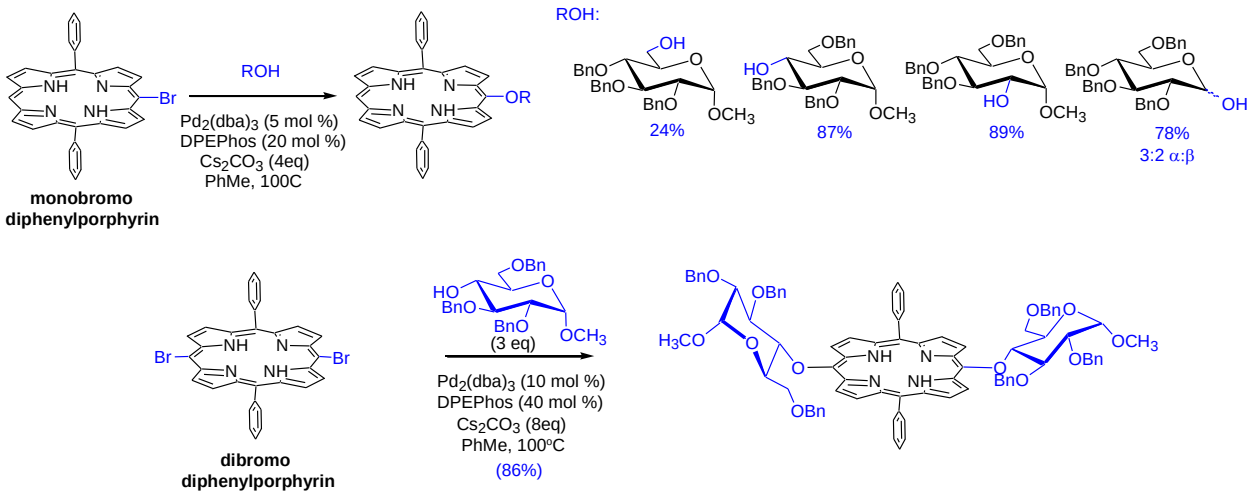
Scheme 1. Asymmetric cyclopropanation of *N*-isopropyl acrylamide.[9a]

Despite the progress that has been made in the development of these catalysts, two key problems still exist. First, the hydrophobic nature of the porphyrin ring makes these systems insoluble in most polar solvents, including water. This limits the utility of these catalysts in the development of green processes for the production of new energetic materials. In addition, solvent limitations decrease the types of substrates that can be cyclopropanated. Finally, the aromatic nature of the porphyrin ring system facilitates pi stacking with many currrent porphyrin catalysts. This results in catalyst aggregation and decreased rate of productive turnover.

In an effort to address these problems, my undergraduate students and I have started synthesizing novel porphyrins bearing carbohydrate residues in collaboration with Dr. Peter Zhang's group at the University of South Florida. Carbohydrate-porphyrin conjugates or "CarboPhyrins," are chiral molecules that have the potential to serve as asymmetric catalysts. In addition to conferring chirality, we believe the carbohydrate ligands will help improve the overall solubility of the porphyrin catalysts in polar solvents. The conformational nature of the carbohydrate ring should also help decrease the pi stacking effects observed in some systems,

without sacrificing stereoselectivity and yield.

The carbohydrate-porphyrin conjugates we have synthesized thus far have been prepared by cross-coupling bromoporphyrin synthons with selectively functionalized carbohydrates using tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) as a source of palladium and bis(2-dipheneylphosphinophenyl) ether (DPEphos) as a ligand source in the presence of cesium carbonate (Cs_2CO_3). An example of the coupling reactions between mono-bromodiphenylporphyrin and four carbohydrate analogs derived from glucose is shown in Scheme 2.[10] We have also been able to generate disubstituted derivatives using dibromodiphenyl porphyrin under similar conditions in upwards of 85% yield.



Scheme 2. Preparation of carbohydrate-porphyrin conjugates using palladium catalyzed carbon-oxygen coupling reactions.

Through these preliminary studies, we were able to assess the feasibility of performing palladium-catalyzed mono- and di-cross-coupling reactions with glucose via different carbohydrate linkages. The results obtained from these experiments have provided us with a foundation for the preparation of carbohydrate-porphyrin conjugates containing multiple carbohydrate ligands that, once metalated, can serve as effective catalysts for the asymmetric synthesis of cyclopropane rings, including highly strained cyclopropane-fused hydrocarbons.

4.0 Experimental

4.1 Synthesis of Carbohydrate Analogs

The carbohydrate analogs required for the work in this proposal are shown in Figure 2 and

will be prepared by Hamilton College undergraduate students. Pyranose derivatives **2-1** and **2-2** are synthesized from readily available methyl-α-D-glucopyranoside using standard protecting group chemistry.[11] Pyranose derivative **2-3** is prepared based on a report by McMillan and coworkers.[12] Furanose derivatives **2-4** and **2-5** are prepared from commercially available methyl-furanosides.[11] Finally, furanose derivative **2-6** will be prepared based on the procedure for pyranose derivative **2-4**.

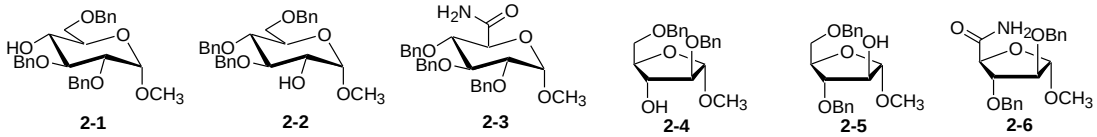


Figure 2. Carbohydrate derivatives for the synthesis of carbohydrate-porphyrin conjugates.

The derivatives in Figure 2 were chosen to probe how the size and connectivity of the carbohydrate affects the overall chiral environment of the carbohydrate-porphyrin conjugate. This in turn, will presumably affect the reactivity and selectivity of the resulting catalyst. For example, Spartan modeling studies show that the conformation of carbohydrate-porphyrin conjugates bearing glucopyranose residues linked through the 4-OH (**3-1**) versus the 2-OH (**3-2**) provide for conjugates with different chemical environments near the catalytic site (Figure 3).

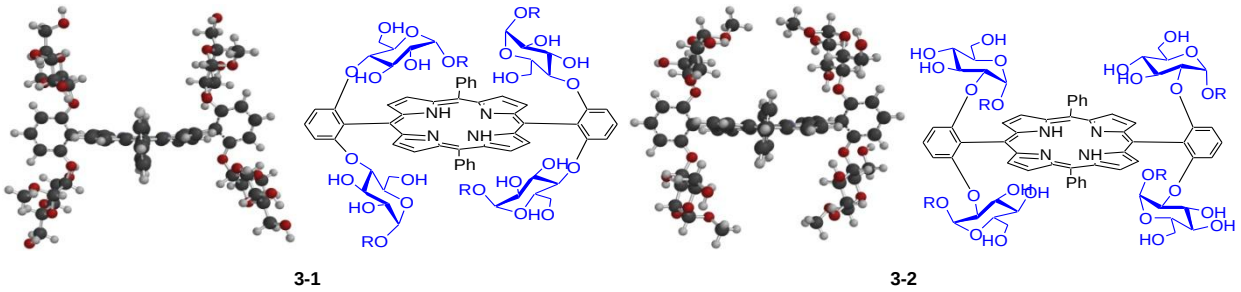


Figure 3. Models of carbohydrate-porphyrin conjugates with glucose residues linked through the 4'OH (**3-1**) and 2'OH (**3-2**).

4.2 Synthesis of Porphyrin-Carbohydrate Conjugates

The porphyrin synthons for this work, shown in Figure 4 below, will be provided by Dr. Peter Zhang's group at the University of South Florida. One Hamilton College student, as part of his or her summer research experience, will have the opportunity to travel to the University of South Florida each summer to participate in the construction of these synthons. Synthons **4-1** through **4-4** were chosen to determine (i) how the position (2,6 or 3,5) of the carbohydrate substituents on the A and B rings orthogonal to the porphyrin ring affect the catalytic activity, and (ii) how groups on the neighboring C and D rings affect the reactivity of the corresponding catalysts.

Synthon **4-2** is especially noteworthy for the success that has been already achieved with this system in asymmetric cyclopropanation reactions (See Figure 1).[9a]

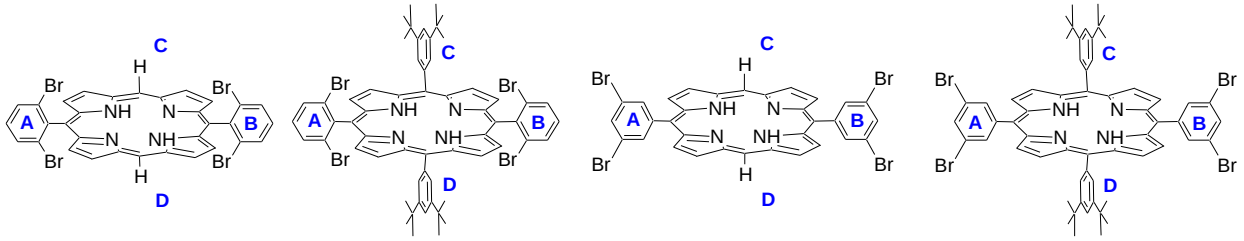
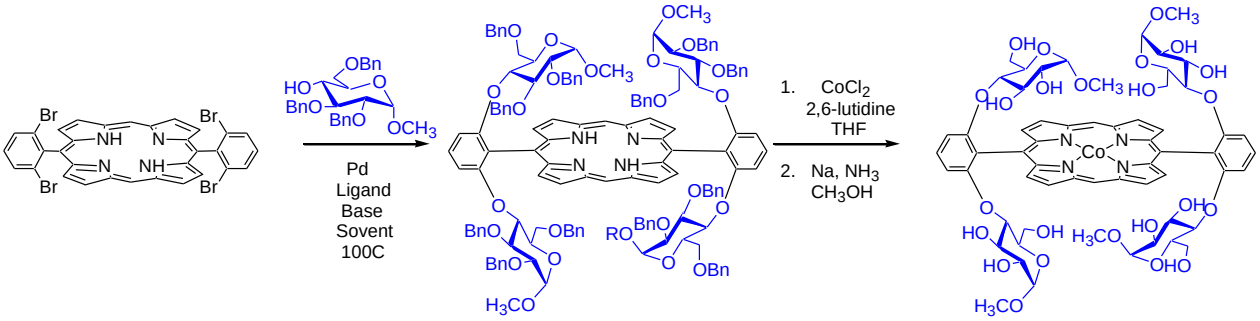


Figure 4. Synthons prepared by Zhang group for use in this study.

A general scheme for the preparation of porphyrin-carbohydrate conjugates is shown in Scheme 3 below. For each of the bromosynthons investigated in this study, the palladium source, ligand, and carbohydrate will be dissolved in an appropriate solvent (toluene, tetrahydrofuran or dioxane) using Schlenk technique, and heated to 100°C until the starting material is consumed as monitored by thin layer chromatography. We will use the optimized conditions already in place for the mono- and disubstituted porphyrin derivatives (Scheme 2) as a starting point for the tetrasubstituted derivatives. After purification, the porphyrin will be metalated using cobalt chloride and 2,6-lutidine in THF. Finally, Birch reduction conditions will be used to remove the protecting groups to provide the corresponding derivative as shown in Scheme 3 below.



Scheme 3. General scheme for the synthesis of carbohydrate-porphyrin conjugates.

While the optimized reaction conditions work well for the simpler mono- and disubstituted derivatives in our previous studies, the tetrabromoderivatives we are proposing to synthesize are much more sterically encumbered and may require reoptimization of the reaction conditions. Therefore, several experimental conditions will be screened systematically in order to achieve maximum yields. The sources of palladium used in this study, including palladium (II) acetate ($Pd(OAc)_2$) and tris-(dibenzylideneacetone)-dipalladium(0) ($Pd_2(dba)_3$) will help us determine

whether palladium (II) or palladium (0) is a more effective entry into the catalytic cycle. The bidentate ligands employed in this study (Figure 5) will be used to probe the effects of size, flexibility, and mode of ligand binding on the resultant carbohydrate-porphyrin conjugates. Examples of ligands that will be employed in this study include commercially available R, R-duphos **5-1**, bis (2-diphenylphosphinophenyl)ether (DPEphos) **5-2**, 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthenes (Xantphos) **5-3**, and BINAP **5-4**. Cesium carbonate will be employed in all of the studies conducted based on our previous success with this base.

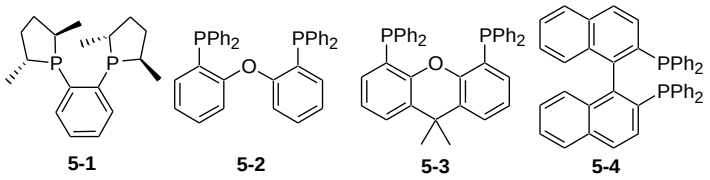
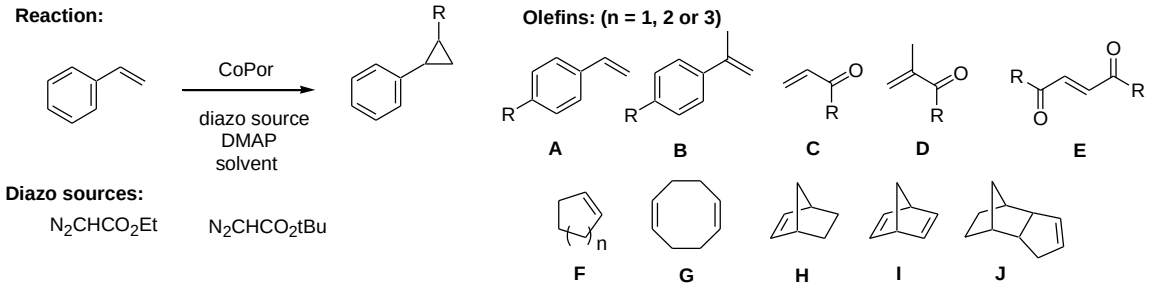


Figure 5. Ligands used for the cross coupling reactions between bromosynthons and carbohydrate derivatives.

4.3 Asymmetric Catalysis Using Cobalt Porphyrin Complexes

The complexes prepared in this study will be evaluated by Hamilton College undergraduates for their ability to serve as catalysts in asymmetric cyclopropanation reactions. A sample cyclopropanation reaction is shown in Scheme 4 below using styrene.



Scheme 4: Cyclopropanation reactions using porphyrin-carbohydrate conjugates.

A number of different aromatic and aliphatic olefins will be used to explore the scope and limitations of these catalysts under various reaction conditions. The olefins used in this study are commercially available and will be used without further purification. Olefins **A** through **E** will be used to assess the ability of the catalysts prepared in this study to cyclopropanate electron deficient olefins in high yield and with good stereocontrol, while olefin's **F** through **J** will be used to study the ability of the catalysts to cyclopropanate electron rich ring systems. We expect the former reactions to be more challenging, since electron-rich systems are less reactive and can

be more difficult to cyclopropanate. Finally, olefins **H** through **J** will be used to assess the ability of the carbohydrate-porphyrin catalysts to cyclopropanate fused ring systems, such as those used to prepare the highly strained cyclopropane-fused hydrocarbons **1.6** through **1.9**.

Two different carbene sources, commercially available ethyl diazoacetate and *tert*-butyl diazoacetate, will be evaluated in this study. These reagents will be used to assess the ability of the catalyst to perform cyclopropanation reactions with carbene sources that vary in size. Dimethyamino pyridine will be used as an axial ligand, based on the success of this ligand in our previous work.[9a]

Finally, a variety of polar and nonpolar solvents will be used to study these reactions. We are especially interested in determining whether or not the catalysts prepared in this study can be used to cyclopropanate substrates in water, as a green alternative to some of the more common organic solvents employed in cyclopropanation reactions.

The reaction products obtained from each cyclopropanation experiment will be subjected to non-chiral GC to determine the overall yield for each reaction. Enantiomeric and diastereomeric excesses will be determined by chiral HPLC for all chiral products after separation of the desired products from the reaction mixture. Pure compounds will be fully characterized by NMR, IR, and high resolution MS. In addition, absolute configurations will be determined for all of the chiral compounds prepared in this study by GC comparison with the commercially available pure enantiomers when possible.

5.0 Future Studies

Future studies will involve the preparation and evaluation of additional carbohydrate-porphyrin conjugates based on the results obtained from this study. For example, if good activity and stereoselectivty are achieved with a glucose-porphyrin conjugate under a specific set of conditions, we will prepare an analogous galactose-porphyrin conjugate and observe the reactivity of this conjugate under similar reaction conditions. My students and I will also explore how other metals can be used to affect the cyclopropanation reactions outlined in this study. Long term goals for this project will identify the scope and limitations of carbohydrate-porphyrin conjugates in epoxidation, aziridination and olefination reactions for the synthesis of highly strained fused heterocycles.

Proposal Narrative = 2,549 words (including abstract)

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October 27, 2009

Nicole L. Snyder, Ph.D.
Department of Chemistry
Hamilton College
198 College Hill Road
Clinton, NY 13323

Dear Professor Snyder:

I have studied your proposal entitled *"The Synthesis and Evaluation of Carbohydrate-Porphyrin Conjugates as Asymmetric Catalysts"* with excitement. I am pleased to support your application for an ACS-PRF Undergraduate New Investigator research grant. My laboratory in the Department of Chemistry at the University of South Florida (USF) will provide you with the bromoporphyrin synthons described in the proposal. Space and other support will be available to you and your undergraduate students for carrying out summer research in my laboratory at USF. I look forward to continuing our collaboration in research and education.

Sincerely,

Peter Zhang

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In addition, if the applicant institution employs more than fifty persons, by electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of the NSF Proposal & Award Policies & Procedures Guide, Part II, Award & Administration Guide (AAG) Chapter IV.A; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be dislosed to NSF.

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Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, ''Disclosure of Lobbying Activities,'' in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, Title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

Certification Regarding Nondiscrimination

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative is providing the Certification Regarding Nondiscrimination contained in Exhibit II-6 of the Grant Proposal Guide.

Certification Regarding Flood Hazard Insurance

Two sections of the National Flood Insurance Act of 1968 (42 USC §4012a and §4106) bar Federal agencies from giving financial assistance for acquisition or construction purposes in any area identified by the Federal Emergency Management Agency (FEMA) as having special flood hazards unless the:

(1) community in which that area is located participates in the national flood insurance program; and
(2) building (and any related equipment) is covered by adequate flood insurance.

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant located in FEMA-designated special flood hazard areas is certifying that adequate flood insurance has been or will be obtained in the following situations:

(1) for NSF grants for the construction of a building or facility, regardless of the dollar amount of the grant; and
(2) for other NSF Grants when more than $25,000 has been budgeted in the proposal for repair, alteration or improvement (construction) of a building or facility.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME		

TELEPHONE NUMBER	ELECTRONIC MAIL ADDRESS	FAX NUMBER

*SUBMISSION OF SOCIAL SECURITY NUMBERS IS VOLUNTARY AND WILL NOT AFFECT THE ORGANIZATION'S ELIGIBILITY FOR AN AWARD. HOWEVER, THEY ARE AN INTEGRAL PART OF THE INFORMATION SYSTEM AND ASSIST IN PROCESSING THE PROPOSAL. SSN SOLICITED UNDER NSF ACT OF 1950, AS AMENDED.

RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates

Project Summary

Galectin-1 is a protein that plays a critical role in a number of biological processes including cell to cell communication, cell-matrix adhesion, cell growth regulation, and inflammation and immunity. Galectin-1 has also been implicated in cancer development and HIV infectivity. Because of the role that galectin-1 plays in a number of critical cellular processes, considerable research has been devoted to the design and synthesis of molecules that can irreversibly bind galectin-1. Such ligands are desirable since they can be used to study how galectin-1 plays a role in these processes. Although a number of ligands that irreversibly bind galectin-1 have been prepared, little has been done to evaluation the specific factors that govern the galectin-1-ligand-binding interaction. The research in this proposal will address this issue by focusing on the synthesis, characterization and evaluation of a host of small lactosyl 1,2,3-triazole derivatives containing natural and unnatural carbohydrates. The proposed derivatives will be used to further an understanding of galectin-1-ligand binding interaction, and the knowledge obtained through the experiments outlined in this proposal will serve as a foundation for the rational design small molecules that can serve as biological probes to study the role of galectin-1 in a number of critical processes. The uniting goals of the research project are:

1. To generate a host of rationally designed lactosyl triazoles incorporating natural and unnatural carbohydrate residues that can be used to probe the nature of galectin-1-ligand association.
2. To investigate the structural and functional consequences of the galectin-1-ligand binding interaction using state of the art chemical, biochemical, spectroscopic and computational techniques.

Specifically, my students and I will address the following question: *"What factors govern the galectin-1 ligand binding interaction?"*

Intellectual Merit—The research described in this proposal focuses on defining the fundamental requirements that govern ligand binding in the carbohydrate recognition domain of galectin-1. The convergent design strategy of the proposed work allows for the dissection of this project into many challenging individual projects that will provide broad learning experiences for the undergraduate students involved. The techniques that will be used to study the nature of the binding interaction between the proposed ligands and galectin-1 are at the forefront of research in chemistry and chemical biology. Finally, the results obtained from the experiments highlighted in this proposal have the potential to open several new avenues of research in synthetic organic and biological chemistry, specifically with the development of designed glycoconjugates that can be used to study the role of galectin-1 and other members of the galectin family in a number of cellular processes.

Broader Impacts—The research projects described in this proposal are ambitious, yet manageable for undergraduate students interested in working at the interface of organic and biological chemistry. The principle investigator will provide undergraduate students with opportunities to participate in this research as early as possible in their academic career. In addition, every effort will be made to include students from underrepresented groups. The principle investigator will work alongside the students in the laboratory and will provide extensive training in the techniques and technologies used in the proposed studies. Students will also learn many basic and advanced research skills including experimental design, project development and management. Finally students will have the opportunity to present the culmination of their work in the form of peer reviewed publications and/or public presentations.

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*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates

1.0 Introduction

The unique topologies afforded to carbohydrates through their unsurpassed structural diversity have allowed these molecules to play critical roles in a number of biological recognition events including cell trafficking, growth factor recognition, immunological recognition, and metastasis. By studying fundamental interactions between carbohydrates and other biomolecules in these processes, a better understanding of the natural functions of carbohydrates has been realized. This understanding has helped enlighten fundamental biochemical knowledge, and has played a significant role in the construction of designed carbohydrate-based systems that have in turn been used to develop new strategies for probing carbohydrate-carbohydrate, carbohydrate-protein, and other carbohydrate interactions of biological interest.

The aforementioned research has provided a foundation for the design of synthetic glycoconjugates that can be used to explore carbohydrate-protein interactions. As an extension of this idea, the students in my research laboratory at Hamilton College and I will use a host of rationally designed carbohydrate ligands to explore the carbohydrate recognition domain of galectin-1. Galectin-1 is a protein that plays a critical role in a number of biological processes including cell to cell communication, cell-matrix adhesion, cell growth regulation, and inflammation and immunity. Galectin-1 has also been implicated in cancer development and HIV infectivity.

Because of the critical role that galectin-1 plays in a number of cellular processes, considerable research has been devoted to the design and synthesis of molecules that can irreversibly bind galectin-1. Such ligands are desirable since they can be used to study how galectin-1 plays a role in these processes. Although a number of ligands that irreversibly bind galectin-1 have been prepared, little has been done to evaluation the specific factors that govern the galectin-1-ligand-binding interaction. The research in this proposal will address this issue by focusing on the synthesis, characterization and biological evaluation of a host of small lactosyl 1,2,3-triazole derivatives containing natural and unnatural carbohydrates. The proposed derivatives will be used to further an understanding of galectin-1-ligand binding interaction, and the knowledge obtained through the experiments outlined in this proposal will serve as a foundation for the design small molecules that can serve as biological probes to study the role of galectin-1 in a number of critical processes. The uniting goals of the research project are:

1. To generate a host of rationally designed lactosyl triazoles incorporating natural and unnatural carbohydrate residues that can be used to probe the nature of galectin-1-ligand association.
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Specifically, my students and I will address the following question: *"What factors govern the galectin-1 ligand binding interaction?"*

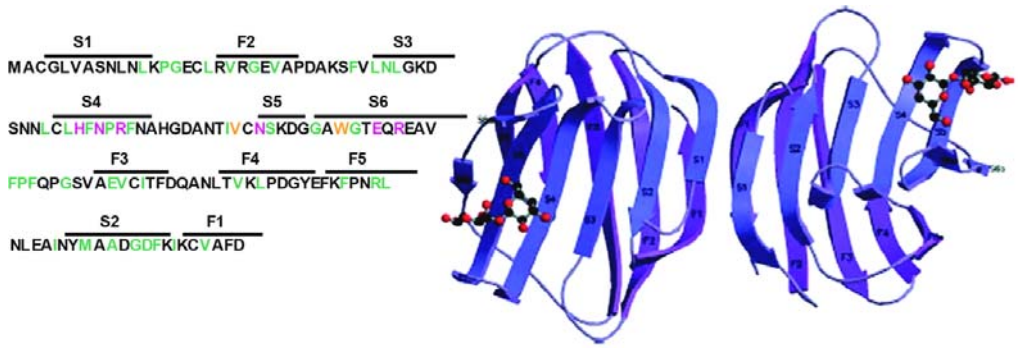
2.0 Background and Significance

2.1 Structure and Function of Galectin-1

Galectin-1 is a member of a family of carbohydrate binding proteins that are defined by their affinity for β-galactosides. Members of the galectin family exhibit a significant sequence

homology in the carbohydrate-binding site or carbohydrate recognition domain (CRD). The galectin CRD is composed of a two sheet beta-sandwich of 135 amino acids that are slightly bent and antiparallel in nature (Figure 1). Five strands (F1-F5) form the convex portion of the beta-sandwich while six strands (S1-S6) form the concave portion of the protein and provide for a large hydrophobic carbohydrate binding groove long enough to support a linear tetrasaccharide.[1]

Figure 1: Structure of the galectin-1 bound to lactose (Taken from Camby et. al.[2]).



Notes: Amino acids highlighted in green illustrate highly conserved residues. Amino acid residues highlighted in pink are known to interact with bound carbohydrates via hydrogen bonding interactions. Amino acids highlighted in orange are known to interact with bound carbohydrates via van der Walls forces.

The most conserved binding region of the galectin family occurs in a subsite between strands S4 and S6 where a galactose residue binds. Two key features help to stabilize the binding interaction between galactose and galectin-1: (i.) hydrophobic packing between the B (hydrophobic) face of the galactose residue and a conserved tryptophan residue (W68) of S6, and (ii.) extensive hydrogen bonding interactions between basic amino acid residues and the carbohydrate.

The binding of a saccharide unit in a second subsite between strands S4 and S6 is the next most conserved feature of the galectin family. For galectin-1 this residue is generally a glucose or N-acetyl-glucosamine unit and is bound by hydrogen bonding and van der Waals interactions between the protein and the carbohydrate.

Two subsites between strands S1 and S4 are the least conserved between members of the galectin family and can support a wide variety of other groups linked through the 3'OH of the galactose residue. A fifth subsite between strands S5 and S6 is less understood.[3]

In normal cells, galectin-1 expression is regulated. Galectin-1 is expressed on cell surfaces and in extracellular matrices, and functions in a number of critical process including inflammation, development, mRNA splicing, differentiation, and cell adhesion.[4] Diseased or stressed cells have been shown to over express galectin-1. For example, galectin-1 has been found in unusually high concentrations in and around tumor cells and has been implicated in several aspects of cancer biology including tumor transformation,[5] apoptosis,[6] cell growth regulation,[7] and metastasis.[8] Research has also shown that galectin-1 may play an important role in protecting tumor cells from immune attack,[9] and studies have also suggested that galectin-1 plays an important role in the promotion of HIV infectivity.[10]

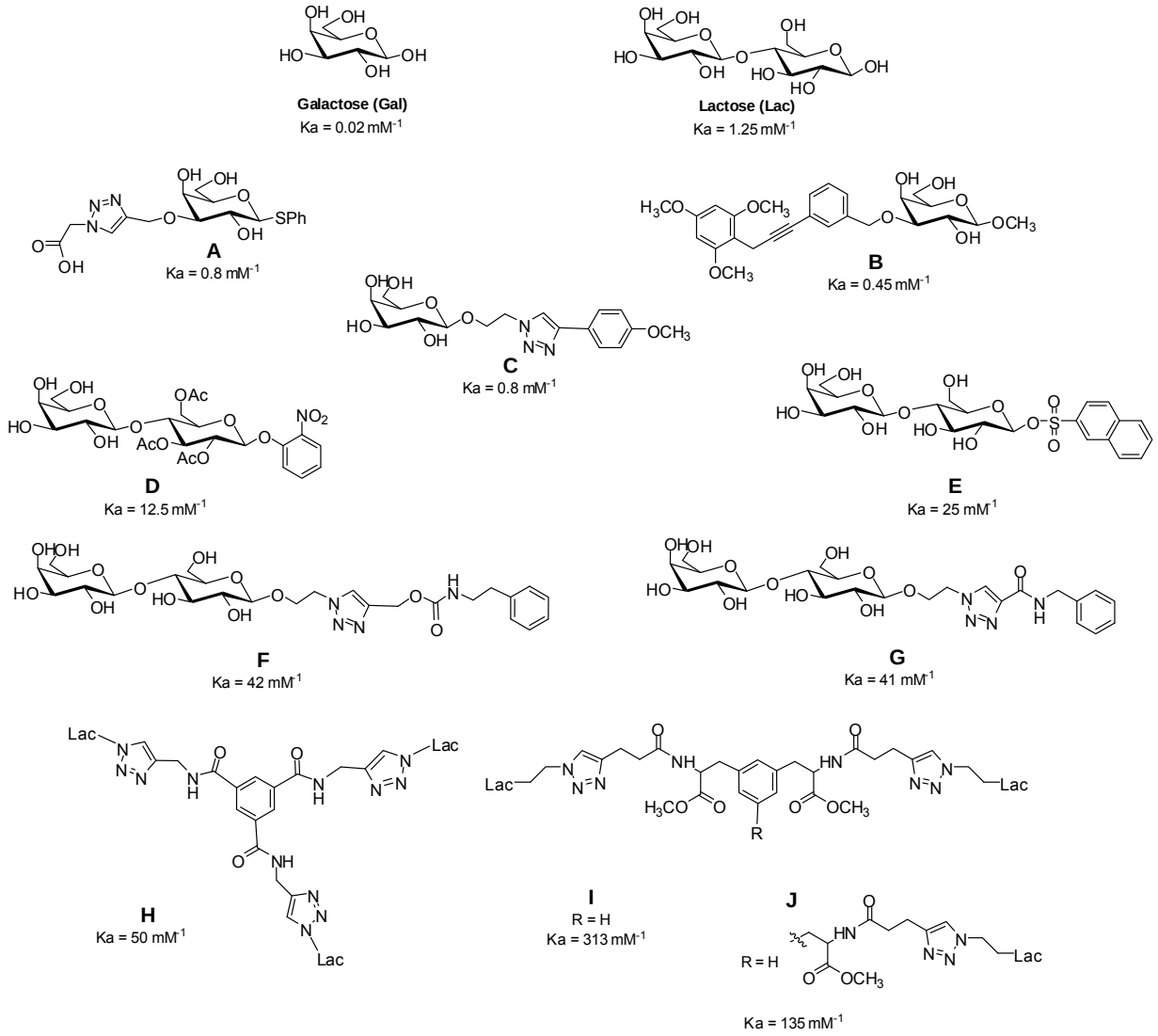
2.2 Preliminary Studies

Because of the role that galectin-1 plays in a number of critical cellular processes, considerable research has been devoted to the design and synthesis of molecules that can irreversibly bind galectin-1. Such ligands are desirable since they can be used to study how

galectin-1 plays a role in these processes. Many recent efforts have capitalized on the fact that galectin-1 is somewhat promiscuous, having an affinity for multiple ligands including lactose, N-acetlyllactosamine, and naturally occurring branched polylactosamine and poly-N-acetyllactosamine derivatives.[11]

Select examples of some of the more effective ligands prepared in recent years are summarized in Figure 2 below. Some general trends can be derived from this data. First, the galactose based derivatives A through C show modest binding affinity for galectin-1 in comparison to lactose derivatives D through J. Galactose derivatives A[12] (3-*O* linked 1,2,3-triazole) and C[12] (1-*O* linked 1,2,3-triazole) are also much more effective at binding galectin-1 than the 3-*O* alkynylbenzyl galactose derivative B.[13] However, there is almost no preference for binding galactose triazoles that are substituted at the 3-*O* position in comparison to the 1-*O* position.

Figure 2: Recent examples of ligands that selectively bind galectin-1.



*Values are expressed as binding association constants (Ka). The higher the Ka the greater the binding affinity.

Of the lactose derivatives, multivalent lactosyl triazoles I[14] and J[14] showed the greatest activity. Interestingly, there was a significant difference between multivalent lactosyl triazole derivatives I and J (which bound with similar affinity to multivalent lactosyl triazole H[12]). This suggests that sterics may play an important role in binding and that mono and divalent derivatives may be more effective at binding galectin-1 than multivalent derivatives. Lactosyl triazoles F[14] and G[14] bound with similar affinity, but bound better than lactose derivatives D[15] and E[15] suggesting that increasing the size of the R group on the triazole may be important, but only until the chain reaches a certain length (presumably that which mimics the length of a tetrasaccharide). It is important to note here that there have been no lactosyl 1,2,3-triazole derivatives prepared to date with the triazole substituent in the 3' position.

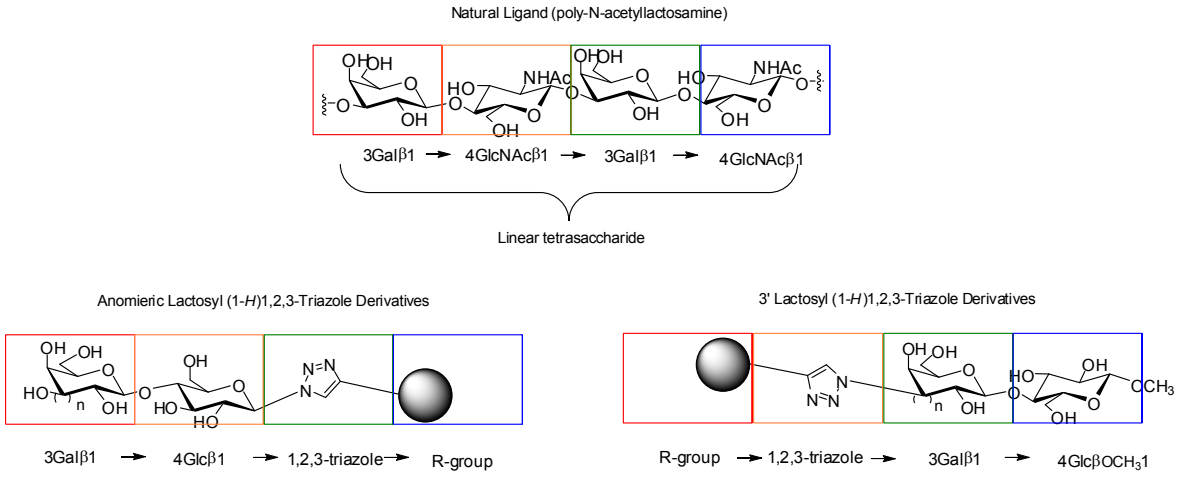
While the derivatives described above have furthered an understanding of the types of molecules that bind galectin-1, the results have been somewhat ambiguous and little has been done to systematically evaluate the specific factors that govern the galectin-1-ligand binding interaction. The research in this proposal will address this issue by focusing on the synthesis and characterization of a host of small lactosyl 1,2,3-triazoles that incorporate natural and unnatural carbohydrates and mimic the natural ligand of galectin-1. My students and I have chosen to use lactosyl 1,2,3-triazoles as models because of they are simple to prepare, relatively stable, and have already been shown to be biologically active. The major goal of this project is to collect information about the proposed derivatives and their interactions with galectin-1 using biochemical (isothermal titration calorimetry), spectroscopic (saturation transfer difference NMR spectroscopy), and computational (molecular mechanics) techniques. This data will be used to understand the factors that influence galectin-1-ligand interactions.

3.0 Proposed Research

3.1 Design and Rationale

My students and I will focus on the preparation, characterization, and biological evaluation of several small lactosyl 1,2,3-triazole derivatives incorporating natural and unnatural carbohydrate residues that mimic poly-*N*-acetyllactosamine, a ligand that shows high affinity for galectin-1 (Figure 3). The proposed derivatives (for specific examples see Table 1) are designed keeping in mind that the carbohydrate recognition domain is large enough to support a linear tetrasaccharide and that the ligands with the best binding affinity tend to mimic a linear tetrasaccharide in length (see Section 2.2).

Figure 3: Rational design of lactosyl 1,2,3-triazoles.



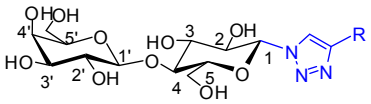
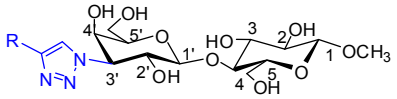
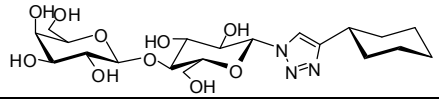
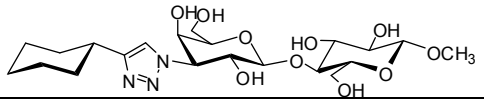
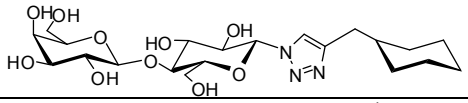
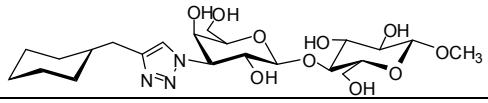
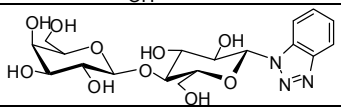
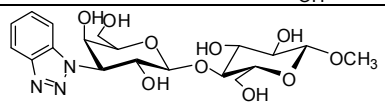
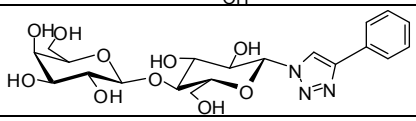
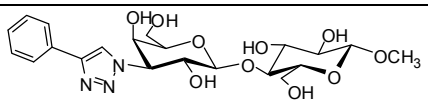
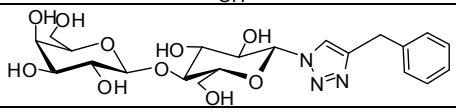
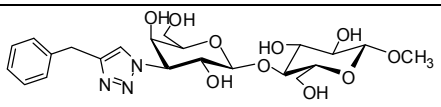
The rationale for the proposed derivatives is based on three parameters that we would like to investigate: (i) how the position of the triazoles (1 vs 3') affects the binding of these molecules; (ii) how the nature of triazole substituent (flexible *versus* nonflexible, aromatic *versus* non aromatic and carbohydrate *versus* non carbohydrate) influences binding interactions and (iii) whether the nature of the reducing galactose residue (natural *versus* unnatural) affects the binding profile for galectin-1. The triazoles we plan to prepare are illustrated in greater detail in the sections below.

A. Lactosyl 1,2,3-triazoles

In the past five years a number of lactosyl 1,2,3-triazoles have been introduced in the literature as effective ligands for galectin 1 (see Section 2.2). These molecules have gained attention because they are ease to prepare and highly stable under a variety of reaction conditions.

Specific examples of the molecules we will initially prepare are shown below in Table 1. Anomeric lactosyl 1,2,3-triazoles A1-5 incorporate a natural galactose residue at the nonreducing terminus of lactose subunit and a 1,2,3-traizole at the 1 position of the β1-4-linked glucose residue. Lactosyl 1,2,3-triazoles B1-5 incorporate a natural galactose residue at the nonreducing terminius of the lactose subunit that is linked a the 3' position to a 1,2,3-triazole. Both of these sets of molecules will be used as models to assess the binding of galectin-1 to ligands containing only natural carbohydrate residues.

Table 1: Target lactosyl 1,2,3-triazoles.

Anomeric Lactosyl 1,2,3-triazoles		3' Lactosyl 1,2,3-triazoles	
 A		 B	
1		1	
2		2	
3		3	
4		4	
5		5	

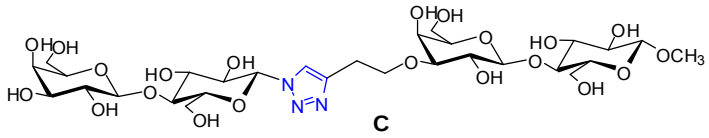
Currently, there is much debate in the literature as to whether galectin-1 prefers to bind ligands substituted at the anomeric position or the 3' position. The binding of lactosyl triazoles A1-5 will be compared to lactosyl triazoles B1-5 to determine whether an actual preference

exists. There is some evidence that substitutents in the 3' position, if properly designed, may confer greater specificity for galectin-1.[16]

The results from the analysis of both sets of triazoles will also be used to address flexibility and hydrophobic interactions within the carbohydrate recognition domain. In the case of entries 1 and 2, and 4 and 5 (Table 1) it is expected that the more flexible derivatives (2 and 5) will bind more effectively than their less flexible counterparts (1 and 4) since they will require the least amount of energy to access a suitable binding conformation. It is also expected that the binding affinity for entries 3-5, will increase with respect to their non aromatic counterparts (1 and 2) due to stabilizing pi stacking interactions between the aromatic R groups and the aromatic residues in the carbohydrate recognition domain.

An additional lactosyl 1,2,3-triazole (C) (shown in Figure 4) will also be prepared and assessed for its ability to bind galectin-1. This molecule will provide us with a unique opportunity to determine whether galectin-1 will preferentially bind the terminal non-reducing galactose unit or the 3'*O*-linked galactose residue and whether or not the polar carbohydrate substituent will have an affect on binding.

Figure 4: Lactosyl 1,2,3-triazole tetrasaccharide C.

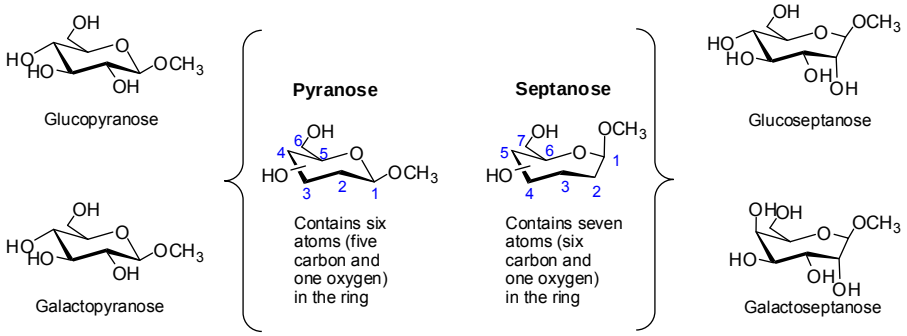


B. Lactoseptanosyl 1,2,3-triazoles

In recent years, the construction of expanded homologs of naturally occurring amino acid and carbohydrate residues has gained considerable attention. The "unnatural" nature and interesting properties these molecules exhibit make them attractive tools for probing biomolecular interactions. For example, Eschenmoser[17] has observed that when the five member furanose sugars of DNA and RNA are expanded by one carbon to pyranoses, an alternative base-pairing and heteroduplex shape is observed. Similarly, Gellman[18] and Seebach[19] studied the homologation of α-amino acids to β-amino acids and found that oligomers constructed from β-amino acids adopt defined conformations that complement natural structures and can selectively disrupt bacterial cell membranes over mammalian cell membranes.[20]

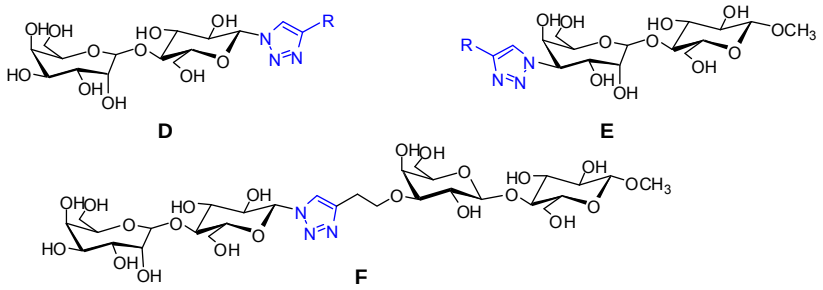
More recently, the construction of an entirely new class of ring expanded carbohydrates known as septanoses carbohydrates has been introduced.[21] Septanose carbohydrates are unnatural, ring expanded homologs of pyranose carbohydrates (Figure 5). The flexibility of the seven member ring in these sugars allows them to adopt a number of different low energy conformations[22] that make them interesting tools for studying fundamental protein-carbohydrate interactions in conjunction with their natural pyranose homologs.[23]

Figure 5: Examples of pyranose and septanoses carbohydrates.



The lactoseptanosyl 1,2,3-triazoles prepared in this study will be based on the results obtained from studies conducted with the lactosyl 1,2,3-triazoles discussed in section 3.1A. General examples of potential derivatives are shown below in Figure 6. Lactoseptanosyl 1,2,3-traizoles are novel derivatives that incorporate an unnatural (septanose) carbohydrate residue at the nonreducing terminus of the lactose subunit. Lactoseptanosyl 1,2,3-triazoles will be used to assess the ability of galectin-1 to bind ligands containing an unnatural carbohydrate residues in a similar context to those to asses the binding of galectin-1 to lactosyl 1,2,3-trizoles A, B (Table 1) and C (Figure 4).

Figure 6: Examples of potential lactoseptanosyl 1,2,3-trizoles.



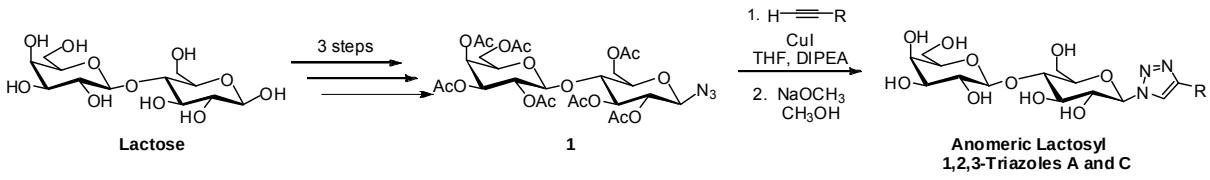
It is expected that the increased flexibility of the galactose-based septanose residue of these derivatives may lower the activation energy required for these molecules to access a conformation that is suitable for binding galectin-1, perhaps even increasing the B-side hydrophobic interactions between the carbohydrate and the tyrosine residue (W68) in the carbohydrate recognition domain (see Section 2.1). Tetrasaccharide F in Figure 6 is especially noteworthy since it will provide a useful tool to study competitive interactions between the binding of natural and unnatural carbohydrates to galectin-1.

3.2 Synthesis of Lactosyl 1,2,3-Triazole Derivatives

A. Synthesis of Anomeric Lactosyl 1,2,3-Triazole Derivatives

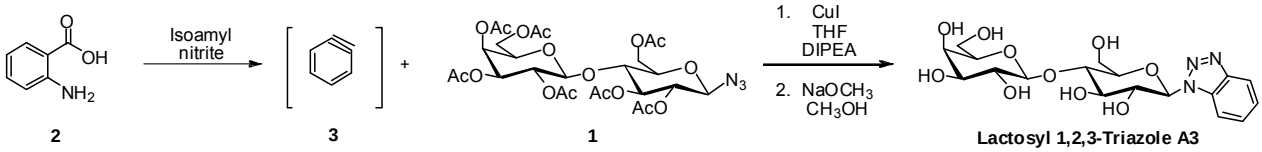
Lactosyl triazoles incorporating a 1,2,3-triazole at the anomeric position (Table1, entries A1-A5 and A5 and Figure 4) can be prepared from commercially available lactose as shown in Scheme 1. Copper (I) catalyzed 1,3-diploar cycloaddition[24] of lactosyl azide **1**, prepared in three steps from lactose,[25] with the appropriately substituted alkyne gives the corresponding anomeric lactosyl 1,2,3-triazole derivative. Alkyne derivatives for the preparation of entries A1-A5 are commercially available through Aldrich Chemicals. The sugar alkyne derivative that would be used for the preparation of lactosyl 1,2,3-triazole C (Figure 4) can be prepared from commercial available lactose in three steps.[13]

Scheme 1: General route for the preparation of anomeric lactosyl 1,2,3-triazole derivatives.



Anomeric lactosyl triazole A3 deserves special attention here. This molecule will be prepared by reaction of azide **1** with benzyne **3** generated from commercially available anthranilic acid **2** and isoamyl nitrite as shown in Scheme 2 below.

Scheme 2: Generation of anomeric lactosyl triazole A3.

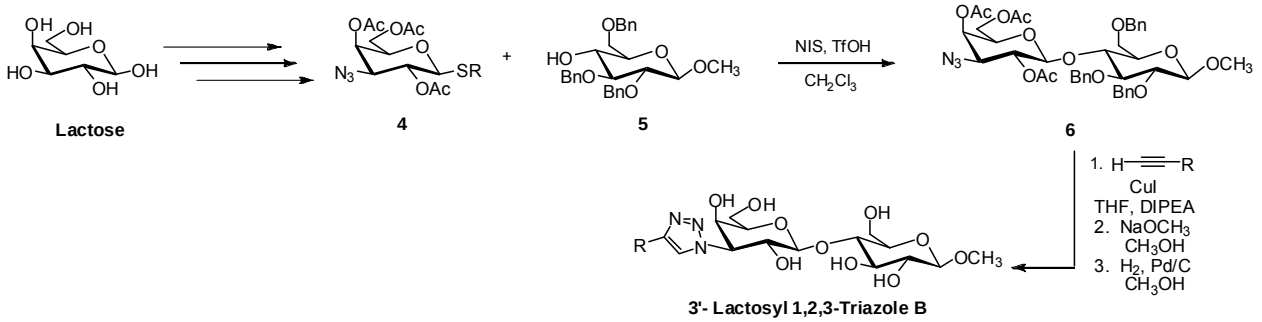


The reaction illustrated in Scheme 2 is novel and to the best of our knowledge has not yet been attempted. This reaction holds promise not only for preparation the lactosyl 1,2,3-triazole derivatives described in this proposal, but also for the construction of nucleoside mimetics.

B. Synthesis of 3' Lactosyl 1,2,3-Triazole Derivatives

Lactosyl triazoles incorporating a 1,2,3-triazole at the 3' position (Table 1, entries B1-5) can be prepared as shown in Scheme 3. The synthesis begins with the preparation of 3-azido-3-dexoy-1-thio-β-D-galactoside **4**. The synthesis of this molecule has been reported in the literature and can be accomplished in six steps from commercially available diacetone glucose.[26] Glycosylation of 3-azido-3-dexoy-1-thio-β-D-galactoside **4** with commercially available glucose derivative **5** gives disaccharide **6**. Copper (I) catalyzed 1,3-diploar cycloaddition with the appropriately substituted alkyne gives the corresponding anomeric lactosyl 1,2,3-triazole derivative.

Scheme 3: General route for the preparation of 3' lactosyl 1,2,3-triazole derivatives.



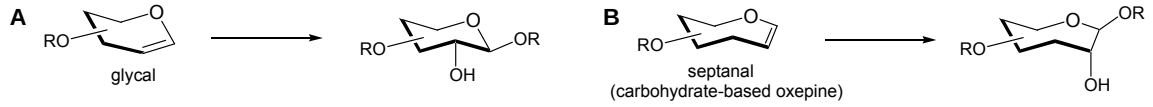
3.3 Synthesis of Lactoseptanosyl 1,2,3-Triazole Derivatives

A Preparation of a Galactose-Based Septanoses Donor—New Routes in the Synthesis of Septanose Carbohydrates

Glycals have been extensively employed as synthons to access a number of important carbohydrate derivatives and glycosylated natural products.[27] Drawing inspiration from glycal chemistry, septanals, or 1,2 unsaturated derivatives of septanose carbohydrates have recently been presented as useful starting materials for the synthesis of septanose sugars. Septanals, more commonly referred to as carbohydrate-based oxepines, are functionally analogous to glycals and show similar reactivity profiles in a number of glycosylation reactions (Figure 7).[28] For this reason, researchers have increasingly focused on methods to produce carbohydrate-

based oxepines for use as building blocks in the preparation of septanose containing oligosaccharides and glycoconjugates.

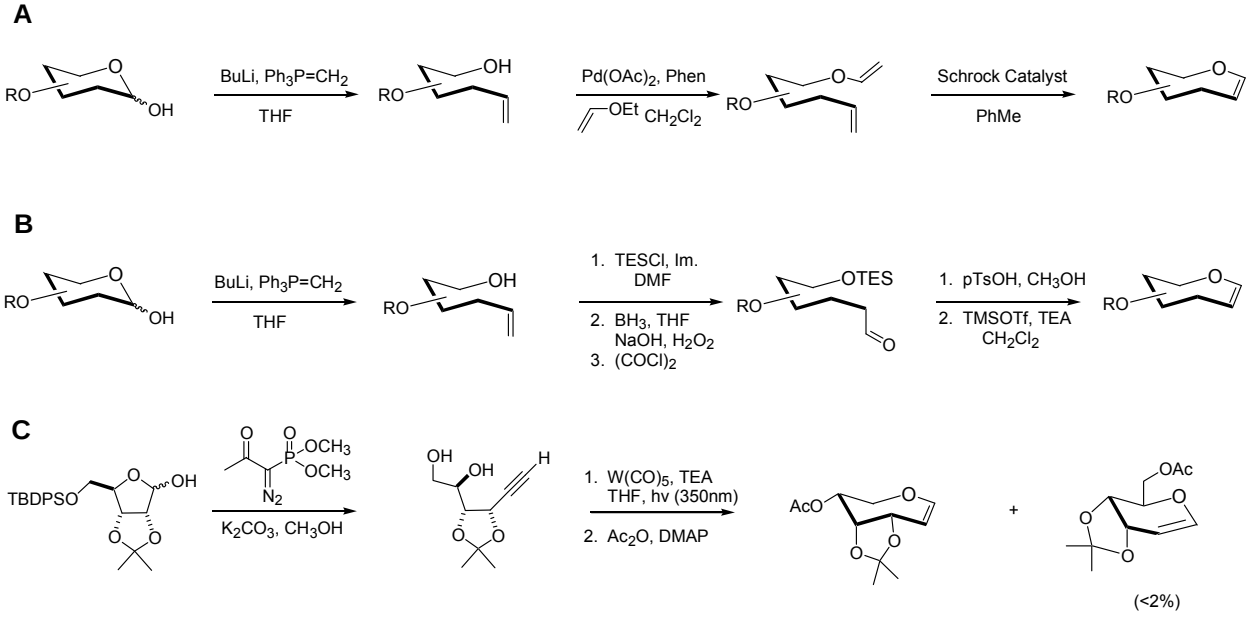
Figure 7: Reactivity of cyclic enol ethers.



Synthetic approaches to oxepines, including carbohydrate-based oxepines, have recently been reviewed.[29] Three syntheses involving the preparation of carbohydrate-based oxepines with functional equivalence to glycals are especially noteworthy.

The first synthesis, reported by Peczuh and Snyder,[21] involves the Schrock catalyzed ring closing metathesis of dienes derived from substituted pyranose lactols (Scheme 4A). This route has been successfully used to prepare a number of carbohydrate-based oxepines in high yields. However, this method is limited by the fact that the catalyst employed requires an inert atmosphere free of oxygen to function properly, and the expired catalyst is difficult to separate from the products once the reaction is complete. The author's attempts to use the more robust Grubbs catalyst with these substrates gave consistently poor results. The low yields were rationalized using steric and electronic arguments.[21,30,31] Reaction of the ruthenium catalyst with the more accessible enol-ether moiety results in the formation of a relatively unreactive ruthenium alkylidene, inhibiting subsequent ring closing metathesis.

Scheme 4: Recent advances in the production of carbohydrate-based oxepines with functional equivalence to glycals.



The second synthesis, reported by Peczuh and Castro,[32] uses a cyclization elimination approach to access carbohydrate-based oxepines (Scheme 4B). This method has been successfully employed to prepare gram quantities of glucose and 2-deoxy glucose based oxepines without the use of expensive organometallic reagents. However, this sequence has

shown limited extension towards the production of carbohydrate-based oxepines other than those reported. The reason(s) for this observation are currently under investigation.

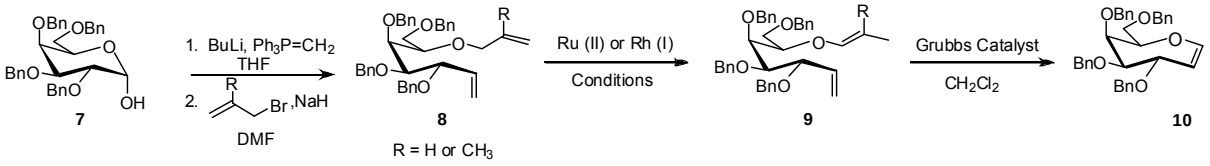
More recently, McDonald and coworkers[33] reported the tungsten catalyzed cycloisomeraization of furanose based alkynols prepared from substituted furanose lactols (Scheme 4C). This route has been proven to be effective for producing carbohydrate-based oxepines deoxygenated at the C-6 position (glucose numbering). However, there are several disadvantages to this method. First, the reaction requires an inert atmosphere free of oxygen in order to inhibit unwanted radically induced side products. Second, the use of a diacetonide protecting group is necessary for cyclization to be efficient. Once cyclization is achieved, any free hydroxyl groups must be capped to stabilize the product prior to isolation. Finally, in some cases the corresponding glycal is produced as minor product of the reaction (< 2%), and can be difficult to separate from the desired oxepine.

In an effort to provide a more globally accessible approach to carbohydrate-based oxepines, and therefore septanose carbohydrates, undergraduate students in my research group will explore two new routes. These routes are designed to address the electronic arguments associated with the ring closing metathesis route to these molecules. Our methodology will allow us to prepare a host of carbohydrate-based oxepines, including the desired galactose-based oxepine, in good yield without the requirement of expensive catalysts or difficult reaction conditions. The specific routes that will be investigated in this study include: (i.) a propenyl ether ring closing metathesis route (Scheme 5), and (ii.) a tether-based ring closing metathesis route (Scheme 6).

i. Propenyl Ether Ring Closing Metathesis Route—Recent reports have shown that ruthenium alkylidene species generated from electron-rich olefins can be reactive in specific metathesis reactions, but are otherwise unreactive.[34] One method that can be used to impede the formation of these species is to make the vinyl ether sterically inaccessible. We believe the steric effects of the propenyl ether should inhibit formation of the ruthenium alkylidene species facilitating ring closing metathesis.[35]

A general synthetic approach for the production of a diene species incorporating a propenyl ether is shown in Scheme 5. Wittig olefination of the appropriately protected lactol **7** (in this case galactose) followed by the addition of a substituted allyl bromide derivative gives allyl ether **8**. Isomerization of the allylic ether using the appropriate ruthenium[36] or rhodium catalyst[37] provides propenyl ether-diene precursor **9**, which undergoes ring closing metathesis in the presence of a catalytic amount of Grubbs catalyst to produce the desired oxepine **10**.

Scheme 5: Propenyl-ether ring closing metathesis route.

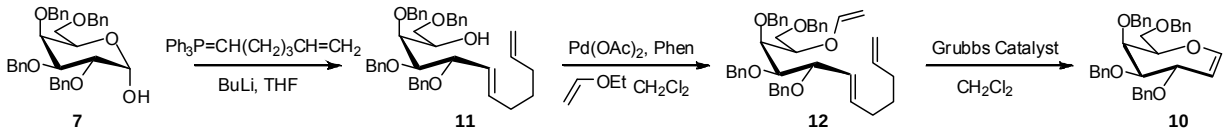


ii. Tether-Based Ring Closing Metathesis Route—Hoye has recently demonstrated the use of a tether in order to make the Grubbs' reaction more efficient.[38] The general idea is to use an extender molecule to make one double bond of a polyunsaturated substrate more readily susceptible to chelation by the ruthenium catalyst, thus inhibiting the formation of a ruthenium alkylidene species.

A general approach for the synthesis of a substrate incorporating a tether is outlined in Scheme 6. Initial steps in this sequence involving the preparation of the tether are not shown.[39] Wittig olefination of the appropriately protected lactol **7** using the tether-ylide

($Ph_3P=CH(CH_2)_3CH=CH_2$) under conditions similar to those used in Scheme 6 provides alcohol **11**. Functionalization of the free alcohol using ethyl vinyl ether gives diene precursor **12** which undergoes tandem ring closing metathesis in the presence of a catalytic amount of Grubbs catalyst to produce oxepine **10** and cyclopentene. Cyclopentene (b.p. 44°C) is easily removed from the reaction mixture upon concentration to give the desired oxepine.

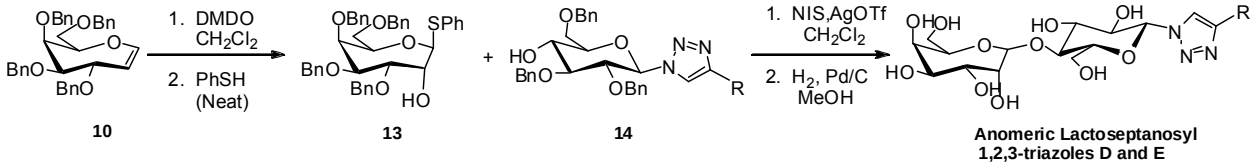
Scheme 6: Tether-based ring closing metathesis route.



B. Synthesis of Anomeric Lactoseptanosyl 1,2,3-Triazoles

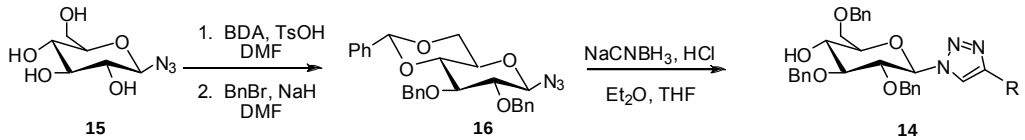
Once the synthesis for carbohydrate based oxepines has been established, the synthesis of anomeric lactoseptanosyl 1,2,3-triazoles is relatively straightforward. As shown in Scheme 7, epoxidation of **8** using DMDO followed by nucleophilic addition of benzene thiol provides galactose based thioseptanoside **13**. Activation of thioseptanoside donor **13** using N-iodosuccinamide promoted by silver triflate followed by the addition of glucosyl 1,2,3-triazole acceptor **14** gives the protected lactoseptanosyl 1,2,3-triazole precursor (not shown). Hydrogenolysis under standard conditions gives the desired anomeric lactoseptanosyl 1,2,3-triazole derivative.

Scheme 7: General route for the preparation of anomeric lactoseptanosyl 1,2,3-triazole derivatives.



Glucosyl 1,2,3-triazole acceptor **14** can be prepared in three steps from readily available 1-azido-1-deoxy-beta-D-glucopyranoside **15** as shown in Scheme 8. Benzylidiene acetal protection using benzaldehyde dimethyl acetal (BDA) followed by benzylation of the corresponding alcohols gives azide **16**. Selective deprotection of the benzylidene acetal of **16** using sodium cyanoborohydride and acid ether/THF to give the free 4-OH followed by copper (I) mediated 1,3-diploar cycloaddition gives the desired acceptor **14**.

Scheme 8: Synthesis of glucosyl 1,2,3-triazole acceptor 14.

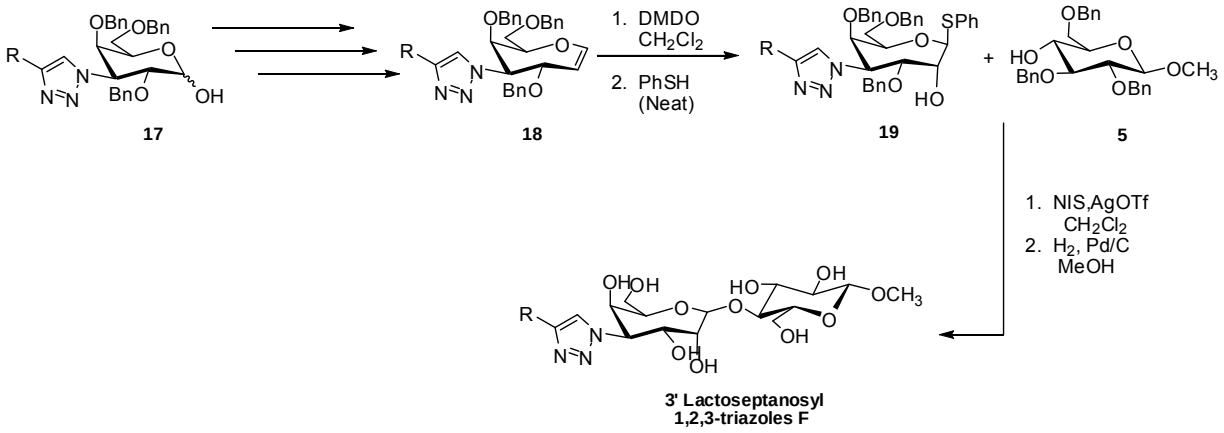


C. Synthesis of 3' Lactoseptanosyl 1,2,3-Triazoles

If 3' lactoseptanosyl 1,2,3-triazoles are desired, the synthesis can be accomplished using a similar process to the one shown for 3' lactosyl 1,2,3-triazoles (Section 3.2B). Lactol **17** (prepared as shown in Scheme 10) can undergo ring expansion to provide oxepine **18** using the procedures outlined in Section 3.3A. Oxepine **18** is then readily converted to thioseptanoside

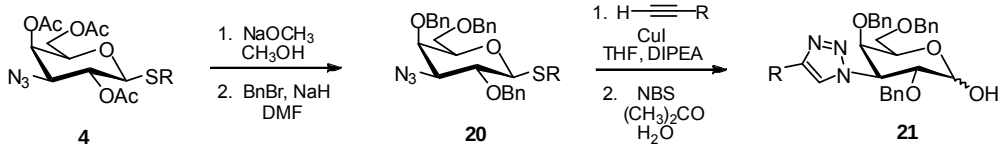
19 using similar reaction conditions for the preparation of anomeric lactoseptanosyl 1,2,3-triazole derivatives in section 3.3B above. Activation of thioseptanoside donor **13** using N-iodosuccinamide and silver triflate followed by the addition of methyl glycoside acceptor **5** gives the protected lactoseptanosyl 1,2,3-triazole precursor (not shown) which, upon hydrogenolysis under standard conditions gives the desired lactoseptanosyl 1,2,3-triazole derivative.

Scheme 9: General route for the preparation of 3' lactoseptanosyl 1,2,3-triazole derivatives.



Lactol **17** can be prepared in three steps from 3-azido-3-dexoy-1-thio-β-D-galactoside **4** as shown in Scheme 10. Deprotection of the acetate groups followed by benzylation of the resulting free hydroxyl groups gives 3-azido-3-dexoy-1-thio-β-D-galactoside derivative **20**. Copper (I) catalyzed dipolar cycloaddition followed by hydrolysis of the thioester gives the desired lactol **17**.

Scheme 10: Preparation of lactol 17.



3.4 Characterization of Lactosyl and Lactoseptanosyl 1,2,3-Trizaole Derivatives

Elucidation of the three-dimensional conformations of the lactosyl 1,2,3-triazlole derivatives prepared in this study is imperative to understanding the chemistry of their interaction with galectin-1. My students and I will conduct experiments aimed at understanding the conformations of these molecules by employing a combination of techniques. The lactosyl 1,2,3-triazole derivatives prepared in this study will characterized by proton and carbon nuclear magnetic resonance (NMR) spectroscopy and high resolution mass spectroscopy (MS). Once the derivatives are completely characterized they will be assessed for binding to galectin-1 via isothermal titration calorimetry (ITC) and saturation transfer difference NMR spectroscopy (STD-NMR) in conjunction with molecular modeling experiments. Details for these experiments are outlined below.

A. Physical Characterization Using NMR and MS

All intermediate and final products prepared in this study will be analyzed by NMR spectroscopy using the College's Bruker 500MHz NMR Spectrometer. Proton and carbon NMR spectra will be collected and, when needed, two-dimensional spectra will also be obtained.

In order to confirm the products of the reactions outlined in this proposal, high resolution mass spectra will be obtained. Since the College does not currently own this instrument, samples will be sent to the analytical laboratory at the University of Illinois-Urbana Champaign for analysis. The College currently has an account with the University of Illinois and samples can be run for a nominal fee.

B. Measuring Galectin-1-Ligand Binding Using Isothermal Titration Calorimetry (ITC)

ITC is a thermodynamic technique that allows the study of the interactions of two species.[40] When two species interact, heat is either generated or absorbed and by measuring the interaction heats, binding constants (K_a), reaction stoichiometry (n), and thermodynamic parameters including enthalpy (ΔH) and entropy (ΔS) can be accurately determined. ITC has become an invaluable tool for understanding the processes that govern many biological interactions. Specifically, ITC has been used to study protein interactions with other proteins and biomolecules such as lipids and carbohydrates,[41] enzyme interactions with coenzymes, inhibitors, substrates, and pharmaceutical compounds,[42] antibody studies and antigen-antibody interactions.[43]

In an ITC experiment, the macromolecular solution, for example a protein, is placed into the sample cell. The reference cell contains the buffer solution used in the experiment (minus the macromolecule) and represents a control. Prior to the injection of the titrant (which includes the ligand to be associated) a baseline signal is generated using the reference cell. The time-dependent input of power required to maintain equal temperatures in the sample and reference cell upon addition of the ligand is measured. Upon titration heat is either taken up or evolved depending on whether the macromolecule-ligand association endothermic or exothermic. If the reaction is exothermic in nature, the temperature in the sample cell will increase. If the reaction is endothermic in nature, the temperature in the sample cell will decrease. The feedback circuit will compensate, increasing or decreasing power to the sample cell to maintain the temperature. The heat absorbed or evolved during a calorimetric titration is proportional to the fraction of bound ligand. As the ligand concentration increases, the macromolecule becomes saturated and less heat is evolved or absorbed on further addition of titrant.

My students and I will use the College's Microcal ITC to collect data for galectin-1 in the presence of the lactosyl and lactoseptanosyl 1,2,3-triazole ligands prepared in this study. Experiments will be conducted using parameters previously established by Brewer et. al.[44] The results will be used to determine which derivatives are more effective at binding galectin-1 and whether or not any trends exist between the molecules evaluated in this study.

C. Mapping Galectin-1-Ligand Binding Using Saturation Transfer Difference NMR Spectroscopy (STD-NMR)

In recent years STD-NMR has become an extremely useful tool for mapping protein-ligand interactions.[45] This technique is advantageous because it allows the binding component to be directly identified even when mixtures of compounds are present. It also allows for mapping of the binding interaction and is highly sensitive using as little as 1 nmol of protein.[46]

STD works by first irradiating a protein in the presence of a ligand (in excess concentration) at a frequency where no ligand signals resonate. This is known as the on resonance frequency and leads to a selective and efficient saturation of the entire protein by spin diffusion. Saturation is then transferred to the binding parts of the ligand by intermolecular saturation transfer. Protons in close contact with the protein receive the highest degree of saturation while protons with minimal or no contact receive little saturation. In essence, the degree of saturation of the

individual protons reflects the proximity of these protons to the protein surface. The frequency is then set to a value different from either of the resonance frequencies of the protein or ligand. This is known as the off resonance frequency. Subtraction of the two spectra gives a spectrum representative of signals resulting from saturation transfer.

My students and I will develop an STD NMR assay that will be used to corroborate the ITC data and to collect more specific information about the nature of the galectin-1-ligand interaction. STD-NMR data will be collected for galectin-1 in the presence of the lactosyl and lactoseptanosyl 1,2,3-triazole ligands prepared in this study to determine which atoms play a role in binding to galectin-1. In addition, competitive STD-NMR experiments will be collected for the lactosyl and lactoseptanosyl 1,2,3-trizaole ligands in the presence of galectin-1 and its natural ligand poly-N-acetyllactosamine in order to determine the nature of the binding the binding event.

D. Evaluation of Observed Binding Activity Using Molecular Mechanics

Conformations of mono- and oligosaccharides play a key role in both the biological functions and applications of these molecules. Like peptides, nucleotides and their respective biopolymers, monosaccharides and oligosaccharides can adopt three-dimensional conformations that may be relatively fixed or flexible in nature. Elucidation of the three-dimensional conformations of carbohydrates and carbohydrate analogs is imperative to understanding the chemistry of these systems. Therefore, a considerable amount of time and effort has been placed on being able to accurately assign and further predict the shape of these molecules.

Currently, our understanding of the conformation of complex carbohydrates remains primitive by comparison with the structures of proteins and nucleic acids. Although this is the case, there is no obvious reason why success using NMR spectroscopy and molecular modeling cannot be duplicated for this challenging problem. Interested undergraduate students in my research group will apply a host of computational methods in order to help rationalize the galectin-1-ligand binding interactions observed in this study. Computational studies will be modeled after work done by Haddad and Peczuh[22,47] and will be conducted using MacroModel, Gaussian, and deMon-NMR software packages. MacroModel will be used to conduct an initial MonteCarlo search. Gaussian will be used to further optimize structures found within a designated range above the global minimum. NMR coupling constants will be determined from minimized structures using the deMon-NMR software package, and will be compared to the data obtained through experiment.

4.0 Conclusions and Future Work

The goal of this project is to use a host of natural (pyranose) and unnatural (septanose) carbohydrate ligands to explore the ligand-binding interaction of galectin-1. My students and I will work together to design, prepare, and assess a number of lactosyl triazoles based on the natural ligand of galectin-1. The work outlined in this proposal is original and will help us gain an understanding of the requirements for galectin-1-ligand binding including (i) how the position of the triazoles (1 *versus* 3') affects the binding of these molecules; (ii) how the nature of triazole substituent (flexible versus nonflexible, aromatic versus non aromatic and carbohydrate versus non carbohydrate) influences binding interactions and (iii) whether the nature of the reducing galactose residue (natural *versus* unnatural) affects the binding profile for galectin-1.

The knowledge gained through the work outlined in this proposal will be used to design molecules that can serve as probes to study the role of galectin-1 in critical process such as inflammation and immunity, cancer progression, and HIV infectivity. In addition, the results obtained from these experiments may be extended to other members of the galectin family which have similar carbohydrate recognition domains but bind different carbohydrate ligands.

5.0 Undergraduate Student Involvement

The projects outlined in this proposal are ambitious, yet manageable research projects for undergraduate students interested in working at the interface of organic and biological chemistry. Hamilton College students will be heavily involved in every aspect of these projects throughout the summer regular academic year. My role as the principle investigator of these projects will be to introduce students to basic research and laboratory skills, including research design, and project management and development.

I will make every attempt to involve students in research as early in their academic career as possible, thus providing them with the opportunity to devote multiple semesters and summers to their research. To reach this goal, attempts will be made to attract at least one of the three students funded from this grant each year from the matriculated pool of incoming freshman. The principal investigator will work with admissions to identify qualified students interested majoring in chemistry or biochemistry and will give those students the opportunity to begin research in the summer before their freshman year. Other student will be chosen from the pool of interested sophomores, juniors, and seniors. Hamilton College will guarantee funding for at least one additional ten week summer stipend.

During the academic year, it is expected that at least three students will work on this project. Students can choose to work in the laboratory during the academic year for course credit (i.e. Independent Research or Senior Project) or for remuneration. Students choosing to work on this project during the academic year for remuneration will be paid by Hamilton College at the rate of $8.50 per hour for a maximum of fifteen hours per week

In addition to learning the synthetic organic and biochemical techniques outlined in this proposal, students will also learn to read the chemical literature critically, and communicate the scientific knowledge they have gained through written reports and presentations (both nationally and internationally meetings). Weekly group meetings throughout the summer and monthly group meetings throughout the academic year will focus on reviewing the current literature in the field. Students participating in the summer research program will have the opportunity to present their results orally at the Colgate-Hamilton Organic Groups (CHOG) meeting in late July and at a poster session at Hamilton College during Family Weekend in the fall. Students will also be given the opportunity to present significant findings at the semiannual National Meeting of the American Chemical Society, the annual American Chemical Society National Organic Symposia, or Eurocarb meetings.

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NICOLE LEIGH SNYDER

Department of Chemistry, Hamilton College, 198 College Hill Road, Clinton, NY 13323, Ph. (315) 859-4742

EDUCATION

- University of Connecticut, Department of Chemistry, Storrs, CT — Ph.D. (chemistry) 2005
- Westminster College, New Wilmington, PA — B.S. (chemistry) 2000
- Westminster College, New Wilmington, PA — B.S. (biology) 2000

PROFESSIONAL EXPERIENCE

- Assistant Professor of Chemistry, Hamilton College, Clinton, NY — 2007-Present
- Visiting Assistant Professor, Wellesley College, Wellesley, MA — 2005-2007

PUBLICATIONS RELATED TO PROPOSED WORK (* undergraduate co-author)

- Peczuh, M.W.; Snyder, N.L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* 2003, 44, 4057-4061.
- Peczuh, M.W.; Snyder, N.L.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* 2004*, 339(6),* 1163-1171.
- DeMatteo, M. P.; Snyder, N.L.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* 2005, *70*, 24-38*.*
- Castro, S.; Duff, M.; Snyder, N.L.; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic Biomolecular Chemistry* 2005, *3,* 3869-3872*.*
- Castro, S.; Cherney, E. C.*; Snyder, N. L.; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* 2007, *342(10)*, 1366-1372*.*

OTHER PUBLICATIONS (* undergraduate co-author)

- Snyder, N.L.; Peczuh, M.W. Haines, H.M.* "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* 2006*, 62,* 9301-9320.
- Markad, S.D; Xia, S.; Snyder, N.L.; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxidation of Carbohydrate Based Oxepines." *Submitted to JOC (November 2007)*

SYNERGISTIC ACTIVITIES

- Reviewer, Scientific Journals International — 2005-Present
- Programming Committee, American Chemical Society, Division of Chemical Education — 2007-Present

COLLABORATORS

- Peter Zhang, Department of Chemistry, University of Southern Florida, Tampa, FL (unrelated project)

GRADUATE AND POSTDOCTORAL ADVISORS

- Mark W. Peczuh, Department of Chemistry, University of Connecticut, Storrs, CT (graduate advisor)

THESIS ADVISOR AND POSTGRADUATE-SCHOLAR SPONSOR

- None

SUMMARY PROPOSAL BUDGET

YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION: **Hamilton College**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: **Nicole L Snyder-Lee**

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Nicole L Snyder-Lee - Summer Salary**	0.00	0.00	2.00	$ **13,708**	$
2.					
3.					
4.					
5.					
6. (**0**) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**1**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	2.00	**13,708**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**0**) POST DOCTORAL SCHOLARS	0.00	0.00	0.00	**0**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**3**) UNDERGRADUATE STUDENTS				**12,000**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**25,708**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**4,092**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**29,800**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
Explosion proof refrigerator $ **5,000**					
TOTAL EQUIPMENT				**5,000**	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				**2,500**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **1,500**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**3**) TOTAL PARTICIPANT COSTS				**1,500**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**5,000**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**0**	
TOTAL OTHER DIRECT COSTS				**5,000**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**43,800**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
Overhead (Rate: 65.5000, Base: 29800)					
TOTAL INDIRECT COSTS (F&A)				**19,519**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**63,319**	
K. RESIDUAL FUNDS				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ **63,319**	$

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME: **Nicole L Snyder-Lee**

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET

YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION: **Hamilton College**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: **Nicole L Snyder-Lee**

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Nicole L Snyder-Lee - Summer Salary**	0.00	0.00	2.00	$ **14,393**	$
2.					
3.					
4.					
5.					
6. (**0**) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**1**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	2.00	**14,393**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**1**) POST DOCTORAL SCHOLARS	12.00	0.00	0.00	**36,000**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**3**) UNDERGRADUATE STUDENTS				**12,000**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**62,393**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**11,833**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**74,226**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				**0**	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				**2,500**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **2,500**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**4**) TOTAL PARTICIPANT COSTS				**2,500**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**5,000**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**0**	
TOTAL OTHER DIRECT COSTS				**5,000**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**84,226**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Overhead (Rate: 65.5000, Base: 62393)**					
TOTAL INDIRECT COSTS (F&A)				**40,867**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**125,093**	
K. RESIDUAL FUNDS				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ **125,093**	$

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME: **Nicole L Snyder-Lee**

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET

YEAR 3

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
Hamilton College

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Nicole L Snyder-Lee

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Nicole L Snyder-Lee - Summer Salary**	0.00	0.00	2.00	$ **15,113**	$
2.					
3.					
4.					
5.					
6. (**0**) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**1**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	2.00	**15,113**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**1**) POST DOCTORAL SCHOLARS	12.00	0.00	0.00	**37,800**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**3**) UNDERGRADUATE STUDENTS				**12,000**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**64,913**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**12,369**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**77,282**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				**0**	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				**2,500**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **2,500**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**0**) TOTAL PARTICIPANT COSTS				**2,500**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**5,000**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**0**	
TOTAL OTHER DIRECT COSTS				**5,000**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**87,282**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Overhead (Rate: 65.5000, Base: 64913)**					
TOTAL INDIRECT COSTS (F&A)				**42,518**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**129,800**	
K. RESIDUAL FUNDS				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ **129,800**	$

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Nicole L Snyder-Lee

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
Hamilton College

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Nicole L Snyder-Lee

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Nicole L Snyder-Lee - Summer Salary**	0.00	0.00	6.00	$ **43,214**	$
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**1**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	6.00	**43,214**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**2**) POST DOCTORAL SCHOLARS	24.00	0.00	0.00	**73,800**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**9**) UNDERGRADUATE STUDENTS				**36,000**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**153,014**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**28,294**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**181,308**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) $ **5,000**					
TOTAL EQUIPMENT				**5,000**	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				**7,500**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **6,500**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**7**) TOTAL PARTICIPANT COSTS				**6,500**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**15,000**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**0**	
TOTAL OTHER DIRECT COSTS				**15,000**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**215,308**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				**102,904**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**318,212**	
K. RESIDUAL FUNDS				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ **318,212**	$

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Nicole L Snyder-Lee

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

C ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

Budget Justification Page

Year 1:

-Summer salaries included for the PI at 2/9 base salary. A five percent cost of living increase in base salary is assumed for each additional year. Fringe benefits are included at 20.1%.

-Summer stipends for undergraduate students are set by the college at $4000.00 per student for ten weeks of research and include a 10% finge benefit. Hamilton College will provide funding for at least one additional student each summer for the duration of this proposal.

-An explosion proof refrigerator ($4,000.00) is requested for the this research. This instrument will reside in the Snyder Lab and will be used to house the chemicals and reagents associated with this proposal.

-Funding is requested for the the PI ($2,500.00) to travel to one annual meeting of the American Chemical Society. Funding will be provided by the College to attend one additional meeting (American Chemical Society or Gordon Conference) per year. Funding is also requested for the undergraduate students ($500.00/student) involved in this proposal to travel to one annual meeting of the American Chemical Society.

-A supply budge of $5000.00 per year is also kindly requested.

Year 2:

-Summer salaries included for the PI at 2/9 base salary. A five percent cost of living increase in base salary is assumed from the previous year. Fringe benefits are included at 21.1%.

-A postdoctoral associate is requested for year two. A five percent cost of living increase in base salary is assumed for each additional year. Fringe benefits for this appointment are included at 21.1%.

-Summer stipends for undergraduate students are set by the college at $4000.00 per student for ten weeks of work and include a 10% finge benefit. Hamilton College will provide funding for at least one additional student each summer for the duration of this proposal.

-Funding is requested for the the PI ($2,500.00) to travel to one annual meeting of the American Chemical Society. Funding will be provided by the College to attend one additional meeting (American Chemical Society or Gordon Conference) per year. Funding is also requested for the postdoctoral associate ($1,000.00) and undergraduate students ($500.00/student) involved in this proposal to travel to one annual meeting of the American Chemical Society.

-A supply budge of $5000.00 per year is also kindly requested.

Year 3:

-Summer salaries included for the PI at 2/9 base salary. A five percent cost of living increase in base salary is assumed from the previous year. Fringe benefits are included at 21.1%.

Budget Justification Page

-A postdoctoral associate is requested for year three. A five percent cost of living increase in base salary is assumed from the previous year. Fringe benefits for this appointment are included at 21.1%.

-Summer stipends for undergraduate students are set by the college at $4000.00 per student for ten weeks of work and include a 10% finge benefit. Hamilton College will provide funding for at least one additional student each summer for the duration of this proposal.

-Funding is requested for the the PI ($2,500.00) to travel to one annual meeting of the American Chemical Society. Funding will be provided by the College to attend one additional meeting (American Chemical Society or Gordon Conference) per year. Funding is also requested for the postdoctoral associate ($1,000.00) and undergraduate students ($500.00/student) involved in this proposal to travel to one annual meeting of the American Chemical Society.

-A supply budge of $5000.00 per year is also kindly requested.

Hamilton college will also provide an additional $48,000.00 to support this research. A portion of this money will be used to purchase a new glove box from MBraun($22,500.00). The glove box will be housed in the chemistry departments synthesis laboratory (due to space issues in the Snyder Research Lab) and is directly related to the research in this proposal. The rest of the funds ($26,000.00) will be used to purchase the software (deMon-NMR software package and NMR Sim) required for the molecular modeling experiments outlined in this proposal and will support equipment maintenance and general supplies.

Current and Pending Support

(See GPG Section II.C.2.h for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: Nicole Snyder-Lee

Other agencies (including NSF) to which this proposal has been/will be submitted.

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: Mechanistic Studies of Immobilized Cellulase

Source of Support: Petroleum Research Foundation

Total Award Amount: $ 50,000 Total Award Period Covered: 09/01/08 - 08/31/10

Location of Project: Hamilton College

Person-Months Per Year Committed to the Project. Cal: 0.00 Acad: 6.00 Sumr: 8.00

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Summ:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

FACILITIES, EQUIPMENT & OTHER RESOURCES

FACILITIES: Identify the facilities to be used at each performance site listed and, as appropriate, indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Use "Other" to describe the facilities at any other performance sites listed and at sites for field studies. USE additional pages as necessary.

Laboratory: -Snyder Research Lab (500sq ft): The lab is located adjacent to the PI's office and houses all the major equipment and supplies necessary to complete the synthesis and purification of the analogs outlined in this proposal.

Clinical: N/A

Animal: N/A

Computer: -Student Computation Center: The Chemistry Department houses a student computation center complete with over twenty computers (both Macintosh and PC) for student use.

Office: -Snyder Office (150 sq ft): This office is directly adjacent to the Snyder Research Lab and central to the organic teaching lab, biochemistry teaching lab and NMR research lab.
-Postdoctoral Office (150 sq ft): There is additional office space

Other: -Two vacuum/intert gas manifolds (Snyder Lab) set up for multistep synthesis.
-Miscellaneus chromatography equipment (Snyder Lab).
-Cold Room (Biochemistry Lab-160sq ft)
-Two SoLow -80 Freezers (Biochemistry Lab)

MAJOR EQUIPMENT: List the most important items available for this project and, as appropriate identifying the location and pertinent capabilities of each.

-Bruker 500MHz Nuclear Magnetic Resonance (NMR) spectrometer: The Chemistry Department houses a Bruker 500MHz NMR on site. This instrument is located less than 100 ft from the Snyder Research Lab and organic teaching lab where all of the synthetic and purification work outlined in this proposal will be peformed. This instrument has multinuclear and two-dimensional capabilites and is suitable for the characterization of the molecules outlined in this proposal.

OTHER RESOURCES: Provide any information describing the other resources available for the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available for the project. Include an explanation of any consortium/contractual arrangements with other organizations.

-All high resolution mass spectra will be obtained from analytical labs at the University of Illinois Urbana Champaign for a nominal fee.

FACILITIES, EQUIPMENT & OTHER RESOURCES

Continuation Page:

LABORATORY FACILITIES (continued):

-Organic Chemistry Teaching Lab (1500 sq ft): This lab is located across from the Snyder Research Lab and houses additional hood space equipped for synthesis and purification.
-Biochemistry Teaching Lab (1500 sq ft): This lab is located 200 feet down the hall from the Snyder Research Lab and houses the most of the major equipment (including the isothermal titration calorimeter) that will be needed for these studies.
-NMR Research Lab (200 sq ft): This laboratory houses the Chemistry Departments Bruker 500MHz NMR which will be used to both characterize the molecules synthesized as part of this proposal.

OFFICE FACILITIES (continued):

located near the Snyder Research Lab for the postdoctoral associate requested with monies from this grant.

OTHER FACILITIES (continued):

-Nanopure Water Purifier (Biochemistry Lab)
-Miscelllaneous equipment including centrifugues, temperature controlled shakers, chromatographic equipment, pH meters, balances and miscellaneous glassware (Biochemistry Lab).

MAJOR EQUIPMENT (continued):

-Microcal VP Isothermal Titration Calorimeter (ITC): The Microcal ITC is located in the biochemistry teaching lab approximately 200 ft from the Snyder Research Laboratory. This instrument is equipped with a microsampling chamber and provides a suitable enviorment for the collection of data associated with protein-ligand binding interactions.
-Jasco P-1020 Polarimeter: The polarimeter will primarily be used for molecular characterization and is located in the general instrumentation room approximately 500 ft from the Snyder Lab.
-Innovative Technologies solvent purification system: The solvent purification system is located in the Snyder Lab and will be used for all of the dry, oxygen free solvents required for the experiments outlined in this proposal.
-Software: Gaussian 03, Macromodel.

RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates

RUI Impact Statement

"In the field of observation, chance favors only the prepared mind."
-Louis Pasteur

Hamilton College is a highly selective private liberal arts college devoted entirely to the education of undergraduate students. Hamilton College was originally founded in 1793 as the Hamilton-Oneida Academy by Samuel Kirkland through a grant funded between the Oneida Indian Nation and the State of New York. The purpose of the academy was to provide a first rate co-educational experience designed to foster an environment of learning and cooperation between the Indians and setters.

In 1812 the Hamilton-Oneida Academy was chartered as Hamilton College, the third oldest institution of higher education established in the State of New York. Today, Hamilton College is a coeducation institution of approximately 1,800 students and ranks seventeenth among all National liberal arts colleges.[1] While the name of the college has changed, the overall mission of the college remains the same—to foster an environment of learning and cooperation. Hamilton College's motto "Know thyself" reflects the college's desire to provide an educational experience that enables "young women and men of unusual gifts to realize their fullest capacities, for their own benefit and that of the societies in which they will live." Over the years the College has become a national leader for teaching students to write effectively, learn from each other and think for themselves.

The chemistry department at Hamilton College has worked to collectively support the primary initiatives of the College and has developed a mission statement to define its role within the context of the college's broader goals:

> *Our mission is to provide the finest possible education in chemistry to a broad spectrum of students, including both majors and non-majors. We are committed to providing an individualized educational experience to our students that allows all students to learn about the role of science and chemistry in the modern world and is characterized by challenge and intellectual stimulation. We aim to accomplish this by teaching small classes, maintaining a culture characterized by extensive out-of-class interactions among students and faculty, and working with students on collaborative research projects. As active scholars, we believe that publishing the results of our research with students improves our teaching and enhances the education of our students. We actively compete for outside funding both for the obvious benefits to our research programs and to provide strong connection to our peers outside the college. As a department, we work closely together to monitor our efforts and to continually improve the education we provide our students.*

Over the past ten years the department has undergone an incredible transformation in an effort to accomplish this mission. In 1996, the department of chemistry contained five full time faculty members and graduated approximately ten (total) chemistry, biochemistry, and chemical physics majors. On average, faculty worked with four research students during the summer months and published 0.2 papers per year. Today, the department boasts eight full time faculty members and is currently conducting a National search for a second biochemist to design and teach courses and conduct high impact research in our expanding biochemistry program. The

[1] Figure according to *U.S. News and World Report Best Colleges 2007*

department now graduates an average of 20 chemistry, biochemistry and chemical physics majors, and the faculty currently publishes on average four papers each year. The number of students conducting research in the department's summer research program has also increased and the faculty now work with an average of 35 students each summer (last summer 41 students took part in summer research).

The chemistry department strives to provide its students with exceptional teaching and research experiences. In addition to the traditional core courses offered in chemistry departments at most colleges, the department of chemistry at Hamilton College offers a number of advanced courses and unique laboratory experiences for students interested in a richer curriculum. Every student also has the opportunity to conduct independent research with a faculty member in his or her research laboratory, and all chemistry, biochemistry, and chemical physics majors are required to complete a senior thesis.

In order to support the continuing teaching and research initiatives of the science faculty at the College, the College recently completed construction on a new 56 million dollar state-of-the-art facility that contains interactive classrooms, spacious teaching and research labs, and numerous student work centers. The chemistry department currently occupies over 10,000 square feet of this facility and houses equipment and facilities for faculty and student use that are comparable to many research universities. Externally funded grants have recently provided for the purchase of a Bruker 500MHz Nuclear Magnetic Resonance Spectrometer, a Thermo Liquid Chromatograph-Mass Spectrometer, a Thermo Electron Almega Raman Microscope, a Rigaku MiniFlex X-Ray Powder Diffractrometer, a Microcal VP Isothermal Titration Calorimeter, and several Agilent 8453 UV/Vis diode array spectrometers. Additional instrumentation available for student use includes: a Jobin/Yvon Fluoromax-3 Spectrofluorometer, Hewlett Packard 8452A Diode Array UV-Visible Spectrometer, Shimadzu UV-2401PC UV-Visible Spectrometer, Perkin Elmer 3100 Atomic Absorption Spectrometer, Mattson Galaxy 6020 Fourier Transform Infrared Spectrometer, MIDAC M2501 Fourier Transform Infrared Interferometer, Perkin Elmer 1310 Infrared Spectrometer, Jasco P-1020 Polarimeter, SPEX 1403 Raman Monochromator with Coherent Argon Ion and Dye Lasers, Spectrophysics Nd:YAG Laser, Perkin Elmer EEG/PAR Potentiostat, Parr Bomb Calorimeter, Shimadzu LC-10AS High-Performance Liquid Chromatograph, Shimadzu QP5050 Gas Chromatograph/Mass Spectrometer, Hewlett Packard 5890A/5970B Gas Chromatograph/Mass Spectrometer, Hewlett-Packard G1602A Capillary Electrophoresis, two SoLow –80° Freezers, Sorvall RC-5B Centrifuge, walk-in Coldroom, Nanopure Water Purifier, Coy Anaerobic Glover Box and a host of computational equipment.

The recent acquisition of a Research Corporation Department Development Award has provided continuing support to help strengthen the chemistry department. As part of this award, the department has developed a five year plan with the overall goal of obtaining national recognition as a leader in education and research in the chemical sciences. The major objectives of this plan are to: (i) increase the number of chemistry majors over the next five years from 20 majors to 30 majors, (ii) increase the number of students conducting summer research from 35 to 45, (iii) publish one paper per faculty member per year with undergraduate students, (iv) obtain and sustain federal research funding, and (v) to integrate postdoctoral researchers into the department's current research program.

The College administration has demonstrated strong support for the chemistry department's five year plan and has guaranteed funding for at least one research student per faculty member per summer. The College also subsidizes housing for students that choose to conduct research at the College during the summer months. During the academic year, the College provides additional support in the form of work-study for student researchers. In addition, the College offers generous support for student and faculty to travel to National meetings and symposia and hosts at least two events each year where students have the opportunity to present their research on campus. The College also provides its faculty with reduced teaching loads (3:2—

labs count as one full load) and course releases to help support faculty research. Additional incentives in the form of monetary support for research supplies and equipment are provided to faculty that submit and receive external funding.

The College has also recently embarked on a new strategic planning process that will continue to support the objectives outlined in the department's five year plan. As part of this process, the College plans to reassess the admissions process and will work to identify ways to increase the number of applications received from students, especially women and members of underrepresented groups wishing to pursue a degree in science. In the long run this will help increase the number of students coming to Hamilton College with the intention of majoring in science and will help us maintain the upward movement we are currently observing in the number of students choosing to major in chemistry, biochemistry, and chemical physics.

As the newest faculty member to join the chemistry department, this award will have a substantial impact on my ability to establish a strong teaching and research program in line with the department's five year plan. I will work hard to encourage students from all backgrounds to pursue their interest in science through research, and I will offer a nurturing, supportive, and active laboratory environment where the students and I work as a team. This award will provide me with the resources to attract students and develop a strong, nationally recognized research group that focuses on the study of carbohydrate-carbohydrate, carbohydrate-protein, and carbohydrate-DNA interactions. The majority of the funds requested in this grant will be used to support nine undergraduate research students (three per year) and one or two postdoctoral associate(s).

Hamilton College undergraduates will be heavily involved in every aspect of this project throughout the summer and regular academic year. The goal will be to involve the students as early in their academic career as possible, giving them the opportunity to devote multiple semesters and summers to their research. To reach this goal, attempts will be made to attract at least one of the three students funded from this grant each year from the matriculated pool of incoming freshman. I will work with admissions to identify qualified students, especially women and members of underrepresented groups interested majoring in chemistry or biochemistry, and will give those students the opportunity to begin research in the summer before their freshman year. Other student will be chosen from a highly qualified and diverse pool of interested sophomores, juniors, and seniors.

In addition to learning the synthetic organic and biochemical techniques outlined in this proposal, students will also learn to read the chemical literature critically and communicate the scientific knowledge they have gained through written reports, publications, and presentations at both National and International meetings. The goal will be to train students to prepare for graduate or professional school. The chemistry department has a strong record of placing students in excellent graduate programs. Some of the more recent examples include: Massachusetts Institution of Technology, Boston College, Boston University, Duke University, University of Michigan, The University of California Santa Cruz, Washington University, The University of Pennsylvania, Dartmouth College, The University of Massachusetts at Amherst, Oregon State University, The University of Vermont, Albert Einstein College of Medicine, Colorado School of Mines, Yale University, George Washington University, The University of Rochester, The University of Wisconsin, The University of Pittsburgh, The University of Toronto, Princeton University, The University of Chicago, Emory University, Johns Hopkins University, The University of Illinois, and Columbia University

Funds are also requested for a full time postdoctoral associate in the second and third years of this award. The postdoctoral associate(s) will have a strong background in chemical biology and an interest in pursuing a career at a small primarily undergraduate institution. He and/or she will provide fresh intellectual insights and continuity for the project during the academic year when I will only be able to work two days per week with students in the laboratory. The postdoctoral associate will also help boost productivity in my laboratory and will allow me to

increase the number of students conducting research during the academic year from two to four. This is especially important considering that our chemistry major now requires all chemistry majors to complete at least one semester of research prior to graduation.

The research outlined in this proposal will also be used to enhance the classroom and laboratory experience for all students studying chemistry and biochemistry at Hamilton College. I hope to incorporate the findings from this research into my teaching and will work hard to integrate the techniques outlined in this proposal into our regular curriculum. For example, the lactosyl triazoles in this proposal are simple enough to prepare that students could synthesize and analyze one in the course of a regular laboratory period in a synthetic organic chemistry course. Biochemistry experiments could be constructed to allow students to use isothermal titration calorimetry (ITC) and saturation transfer difference nuclear magnetic resonance experiments (STD-NMR) to study binding interactions between ligands and macromolecules.

I also hope to use my extensive training at the interface of chemistry and biology to contribute to the department's advanced laboratory course. Currently, this course focuses heavily on organic and inorganic synthesis and spectral characterization. I would like to take the course a step further and provide experiments that allow students not only synthesize and characterize molecules, but to take their research experience to the next step and evaluate them for biological activity. For example, the experimental results obtained from the research described in this proposal could be used to design a semester long project for this course where students use the observations generated in my research laboratory to design additional ligands for galectin-1 or other members of the galectin family. Students can then decide how they would like to investigate the interactions between their ligand and protein/biomolecule of interest. The results obtained from such experiments would significantly impact student learning and could eventually lead to a publication.

In summary, the proposed research will provide opportunities for at least nine undergraduate research students and one or two post doctoral associates. The results obtained from these experiments will have a significant impact on the field of synthetic organic chemistry and lectin biochemistry and the research outlined in this proposal is designed to provide numerous opportunities for students to publish and present their results. The research in this proposal will also help enhance the educational experiences of Hamilton undergraduate students in the classroom and laboratory.

Proposal Status | MAIN

Organization: Hamilton College

Panel Summary #1

Proposal Number: 0809507

Panel Summary:
Panel Summary

What are the intellectual merits?

Most of the proposal is dedicated to the synthesis of target molecules. The syntheses should be do-able by undergraduate students. Concerns are that the work is primarily an extension of the PI's Doctoral work, and the panel would prefer a creative move into newer chemistry. In addition, more specific target molecules and reasons for these targets should be supported. There was some question as to the applicability of her in vitro galectin-1 binary measurements as being predictive of performance in cell cultures to in vivo conditions. Because carbohydrate binding proteins interact with so many other proteins in cells, there is always concern that they behave differently in vitro vs in vivo. A collaboration with a person having experience in protein biochemistry in general, and galectin-1 in particular, would be helpful in improving the overall quality of the science proposed.

Preliminary data would strengthen the proposal, as would a more complete description of the computational studies.

There was no evidence the PI has worked with or purified proteins previously, thus there should be a better explanation for how galectin-1 will be obtained.

What are the broader impacts?

Good impact is possible in biological chemistry if research is successful. No outreach has been proposed, which is a concern for the panel. On the other hand, broad experience would be provided to Undergraduate participants.

Rationale for Panel Summary:

The panel agreed that research on the proposed work would benefit undergraduate researchers. However, the proposal would be stronger with some re-thinking of the research plan, as indicated above.

The summary was read by/to the panel and the panel concurred that the summary accurately reflects the panel discussion.

Panel Recommendation: Fund If Possible

Back to Proposal Status Detail

Proposal Status | MAIN

Organization: Hamilton College

Review #1

Proposal Number:	0809507
Performing Organization:	Hamilton College
NSF Program:	Unimolecular Processes
Principal Investigator:	Snyder-Lee, Nicole L
Proposal Title:	RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates
Rating:	Very Good

REVIEW:

What is the intellectual merit of the proposed activity?

This RUI proposal proposes to synthesize a series of lactosyl triazoles to 'systematically evaluate the specific factors that govern the galectin-1- binding interaction.' The galectin binding protein governs inflammation, cell adhesion and overexpression of this protein has been implicated in tumor transformation and metathesis. Accordingly, the PI will build on a large body of work in preparing irreversible inhibitors of this protein. In that regard, the work proposed in this application will provide a modest advance in knowledge. However, the overall program, which involves computational design, synthesis and determination of the nature and efficiency of binding, is well thought out with regard to the ability of undergraduate students to perform the work. The students will obtain a broad, interdisciplinary research experience with a strong emphasis on synthesis. The proposal would have benefited from the inclusion of a plan to increase/encourage participation from students in traditionally underrepresented groups and from an outreach plan. The synthetic plans are quite reasonable and doable by undergraduates. I suspect that control of the diastereoselectivity of epoxidation/ring opening of the lactoseptanosyl oxepine will not be as selective as indicated in Scheme 7. The inclusion of the isothermal calorimetry and STD-NMR studies represents a strong point of the proposal.

What are the broader impacts of the proposed activity?

This research will provide a modest advance in the design of galectin inhibitors. The education potential for undergraduates is high. The interdisciplinary approach described will provide students with a broad research experience that will allow them to make an informed decision about future career decisions. The proposal would be improved if an outreach plan were included or if a plan to recruit under represented students were proposed.

Summary Statement

Back to Proposal Status Detail

Proposal Status | MAIN

Organization: Hamilton College

Review #2

Proposal Number:	0809507
Performing Organization:	Hamilton College
NSF Program:	Unimolecular Processes
Principal Investigator:	Snyder-Lee, Nicole L
Proposal Title:	RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates
Rating:	Multiple Rating: (Very Good/Good)

REVIEW:

What is the intellectual merit of the proposed activity?

The P.I. proposes to synthesize a library of 1,2,3-triazole derivatives containing natural and unnatural carbohydrates. She then proposes to investigate the binding of these triazoles with galectin-1 in order to investigate the ligand binding interaction.

The synthesis of most of the triazole derivatives is fairly straightforward, and involves well-developed reactions likely to succeed. The proposed synthesis of triazole A3 is novel, and if successful, would be an important contribution to the field. The P.I. also proposes to synthesize carbohydrate oxepines as substrates, and proposes two routes, both of which utilize olefin metathesis, for their synthesis. The first route utilizes the observation that increased steric hindrance of a vinyl ether results in cleaner metathesis reactions, while the second utilizes a previously developed tether/ tandem RCM. At this point, the P.I. does not provide preliminary data for the synthesis of any of the ligands or for the novel reactions she proposes.

Once a library of ligands has been synthesized, the P.I. proposes to study their binding with galectin-1 through isothermal titration calorimetry and saturation difference NMR. Isothermal titration calorimetry will provide binding constants for the ligands as well as stoichiometry, enthalpy and entropy of binding. The NMR work will allow for mapping of the binding domain. No preliminary data on the development of the NMR assay are presented.

Finally, the P.I. proposes to utilize computational chemistry to model ligand binding. The P.I. is rather vague in this area, and it is unclear exactly what will be investigated and how.

What are the broader impacts of the proposed activity?

The projects are very appropriate for undergraduate students, who will gain training in synthesis. In addition, the work will provide new methods for the synthesis of carbohydrate oxepines, and provide useful information on the binding domain of galectin-1.

Summary Statement

The P.I. proposes to synthesize a library of 1,2,3-triazole derivatives containing natural and unnatural carbohydrates. She then proposes to investigate the binding of these triazoles with galectin-1 in order to investigate the ligand binding interaction. Most of the syntheses are well supported in the literature, however, there are several examples of new applications to methods from the literature. No preliminary

data is presented. The work will be performed by undergraduates, and can be carried out with the facilities available.

Back to Proposal Status Detail

National Science Foundation
4201 Wilson Boulevard, Arlington, Virginia 22230, USA
Tel: 703-292-5111, FIRS: 800-877-8339 | TDD: 703-292-5090

Proposal Status | MAIN

Organization: Hamilton College

Review #3

Proposal Number:	0809507
Performing Organization:	Hamilton College
NSF Program:	Unimolecular Processes
Principal Investigator:	Snyder-Lee, Nicole L
Proposal Title:	RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates
Rating:	Very Good

REVIEW:

What is the intellectual merit of the proposed activity?

What are the broader impacts of the proposed activity?

Summary Statement

The PI proposes to synthesize a variety of triazole linked, tailored oligosacharrides in an attempt to identify tight binding galectin-1 ligands. The work builds on the finding that related triazole containing mono and disacharrides are weak ligands. I must say the logic for staying in the triazole series isn't terribly compelling, although I realize the æclick' method is popular and makes life easy.
The chemistry aspects of the proposal are well designed and presented. The PI has previous experience in the area and the likelihood of successful access to the target molecules seems high. In addition, the experiments are well suited for the investigator's school and the training potential for undergraduates is truly excellent.
The only criticisms I have relate to considerations of broader impact. The work is premised on the idea that slective galectin ligands will be of value. The boilerplate tells us that galectin-1 plays a critical role in 'cell to cell communication, cell-matrix adhesion, cell growth regulation' etc. The intention is to synthesize compounds to probe to these effects at a molecular level û and that would be wonderful. But since this is a carbohydrate binding protein, where experience tells us that promiscuity, polyvalency, cooperativity and context are often critical factors governing function, is that a possibly here? And, if so how well might isolated ITC measurements and calculations speak to potential performance? I realize this may asking too much, but a more sophisticated discussion of downstream activities would be helpful. Perhaps a collaboration in the area could be established.

Back to Proposal Status Detail

Proposal Status | MAIN

Organization: Hamilton College

Review #4

Proposal Number:	0809507
Performing Organization:	Hamilton College
NSF Program:	Unimolecular Processes
Principal Investigator:	Snyder-Lee, Nicole L
Proposal Title:	RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates
Rating:	Excellent

REVIEW:

What is the intellectual merit of the proposed activity?

The PI has chosen a very interesting topic of studyùthe nature of carbohydrate ligand binding to galectin-1. Three classes of proposed ligands will be synthesized and tested for binding. The plan is well organized with simpler systems to get things going in the lab, to more carefully designed systems that may offer new knowledge to the field. Specifically, the initial anomeric lactosy triazoles (column 1 of Table 1) are simple synthetic targets and should serve as a nice proving ground for the PI's lab. The more interesting 3'-lactosyl triazoles (column 2 of Table 1) are unique structures that require more effort to prepare, but should yield interesting binding data. Finally, the PI adds some septanosyl triazoles as potential ligands. This last bit grows directly out of the PI's PhD work and frankly seems to be an add-on that may be interesting, but is not critical to the heart of the project and frankly takes up too many pages of the proposal reviewing the literature.

The PI has nicely described plans to measure the binding ability of these synthetic ligands. In particular, the PI will use isothermal titration calorimetry and saturation transfer difference NMR to gain insight into possible binding modes. In addition, computational studies will be employed to support various structural possibilities. [Note: the budget requests software to help with this.]

The PI appears to have the necessary training from her PhD work, with publications in synthesis and characterization of carbohydrate complexes. Given the facilities and the support, the proposed work is doable.

What are the broader impacts of the proposed activity?

This work described will be conducted by the PI with undergraduate student co-workers. These students will learn a tremendous amount as they prepare and analyze these compounds. There seems to be a community in place to foster this development. Further, the proposed work is in a bioorganic field that will be attractive to undergraduates who are deciding between careers in medicine and chemistry. The PI's department has undergone a rather amazing transformation with the help of a Research Corporation Departmental Development grant, and supporting this proposal will certainly help the newest faculty member in that department begin to contribute to their new departmental goal.

Lastly, a post-doctoral student will be trained with this grant during years 2 and 3. There is little description of the duties and training efforts for this position and the PI should detail the plan for this position more clearly. For example, there is only a statement that a post-doctoral student looking for a position at a PUI will be selected. Will the post-doc do any teaching? Will the post-doc be expected to

present this work at national meetings? Will this opportunity truly be a stepping stone for a position at a PUI?

Summary Statement

Overall, this is an excellent proposal from a PUI. The work is interesting and some aspects has potential to open new avenues of study. The work is targeted well for the setting and should produce results for publication.

Back to Proposal Status Detail

National Science Foundation
4201 Wilson Boulevard, Arlington, Virginia 22230, USA
Tel: 703-292-5111, FIRS: 800-877-8339 | TDD: 703-292-5090

Proposal Status | MAIN

Organization: Hamilton College

Review #5

Proposal Number:	0809507
Performing Organization:	Hamilton College
NSF Program:	Unimolecular Processes
Principal Investigator:	Snyder-Lee, Nicole L
Proposal Title:	RUI: Exploring the Nature of Ligand Binding in the Carbohydrate Recognition Domain of Galectin-1 Using Natural and Unnatural Carbohydrates
Rating:	Good

REVIEW:

What is the intellectual merit of the proposed activity?

The PI proposes to synthesize and characterize small lactosyl 1,2,3-triazole derivatives that contain natural and unnatural carbohydrates and explore their binding in the carbohydrate recognition domain of galectin-1. Also, the PI proposes to probe the structural and functional consequences of the galactin-1-ligand binding interaction. The synthesis of the proposed highly stable 1,2,3-triazoles are based on known chemistry and they seem very feasible. However, the lack of more extensive preliminary results and further evidence for the likelihood of desirable binding interactions for those compounds is somewhat lacking. These weaknesses reduce the overall enthusiasm for the proposal.

What are the broader impacts of the proposed activity?

The proposed work is significant because of the importance of galectin-1 in cell communication, cell-matrix adhesion, cell growth regulation, and inflamation and immunity. Therefore, the discovery of novel ligands with high binding affinity and the study of galectin-1-ligand binding interactions is timely. This work will offer undergraduate students a real opportunity and a valuable experience to be involved in organic synthesis, physical characterization, and biological evaluation. However, the proposal could have been strengthened by a more detailed description of how will undergraduates, in particular freshmen, be involved in the research.

Summary Statement

The work proposed is interesting; however, due to the lack of more extensive preliminary data, further evidence for the likelihood of strong binding interactions for the proposed structures, more detailed description of how undergraduate students will be involved in the research, and the many writing mistakes reduce the overall enthusiasm for the proposal.

Back to Proposal Status Detail

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION

❍ Pre-application ● Application
❍ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:**002253540

* Legal Name: Hamilton College

Department: | Division:

* Street1: 198 College Hill Rd. | Street2:

* City: Clinton | County: Oneida | * State: NY: New York

Province: | * Country: USA: UNITED STATES | * ZIP / Postal Code: 13323

Person to be contacted on matters involving this application

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	Nicole		Snyder	

* Phone Number: 315-859-4742 | Fax Number: 315-859-4678 | Email: nsnyder@hamilton.edu

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*:
150532200

7. * TYPE OF APPLICANT
O: Private Institution of Higher Education

8. * TYPE OF APPLICATION: ● New
❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Other (Specify):

Small Business Organization Type
❍ Women Owned ❍ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)*:

* Is this application being submitted to other agencies? ❍ Yes ● No
What other Agencies?

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
NIH-AREA: Understanding the Role of the Vancomycin Glycan in Binding Glycosyltransferases

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*
Clinton, Oneida County, New York

13. PROPOSED PROJECT:

* Start Date	* Ending Date
05/01/2009	04/30/2012

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
24th	24th

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	Nicole		Snyder	

Position/Title: Assistant Professor | * Organization Name: Hamilton College

Department: Chemistry | Division:

* Street1: 198 College Hill Rd. | Street2:

* City: Clinton | County: Oneida | * State: NY: New York

Province: | * Country: USA: UNITED STATES | * ZIP / Postal Code: 13323

* Phone Number: 315-859-4742 | Fax Number: 315-859-4648 | * Email: nsnyder@hamilton.edu

16. ESTIMATED PROJECT FUNDING	
a. * Total Estimated Project Funding	$193,230.00
b. * Total Federal & Non-Federal Funds	$0.00
c. * Estimated Program Income	$0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Ms.	Amy		Lindner	

* Position/Title: Associate Director, Government Relations

* Organization Name: Hamilton College

Department:

Division:

* Street1: 198 College Hill Rd.

Street2:

* City: Clinton

County: Oneida

* State: NY: New York

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 13323

* Phone Number: 315-859-4678

Fax Number: 315-859-4648

* Email: alindner@hamilton.edu

*** Signature of Authorized Representative**

Amy Lindner

*** Date Signed**

06/25/2008

20. Pre-application File Name: Mime Type:

21. Attach an additional list of Project Congressional Districts if needed.

File Name: Mime Type:

424 R&R and PHS-398 Specific
Table Of Contents

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: Hamilton College

* Street1: 198 College Hill Rd.		Street2:
* City: Clinton	County: Oneida	* State: NY: New York
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 13323

File Name	Mime Type

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved?	❍ Yes ● No	
1.a. If YES to Human Subjects		
Is the IRB review Pending?	❍ Yes ❍ No	
IRB Approval Date:		
Exemption Number:	_ 1 _ 2 _ 3 _ 4 _ 5 _ 6	
Human Subject Assurance Number		
2. * Are Vertebrate Animals Used?	❍ Yes ● No	
2.a. If YES to Vertebrate Animals		
Is the IACUC review Pending?	❍ Yes ❍ No	
IACUC Approval Date:		
Animal Welfare Assurance Number		
3. * Is proprietary/privileged information included in the application?	❍ Yes ● No	
4.a. * Does this project have an actual or potential impact on the environment?	❍ Yes ● No	
4.b. If yes, please explain:		
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?	❍ Yes ❍ No	
4.d. If yes, please explain:		
5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators?	❍ Yes ● No	
5.b. If yes, identify countries:		
5.c. Optional Explanation:		
6. * Project Summary/Abstract	6914-Project_Summary.pdf	Mime Type: application/pdf
7. * Project Narrative	1336-Project_Narrative-NIH.pdf	Mime Type: application/pdf
8. Bibliography & References Cited	843-NLS-NIHAREA-FINAL-REF.pdf	Mime Type: application/pdf
9. Facilities & Other Resources	1848-Facilities-NIH.pdf	Mime Type: application/pdf
10. Equipment	6367-Equipment-NIH-AREA.pdf	Mime Type: application/pdf

Project Summary
Vancomycin is a glycopeptide antibiotic used in the clinical setting for the treatment of methicillin-resistant *Staphylcocci* and *Enterococci.* Vancomycin is composed of two bioactive components, a cyclic peptide component (aglycon) and a functionalized peripheral carbohydrate (glycan), that work together to inhibit the biosynthesis of peptidoglycan, a major component of the cell wall of gram-positive bacteria. Over the past twenty years, several vancomycin-resistant strains of bacteria have been detected. This has led researchers to search for new and more potent derivatives of vancomycin. Recent attempts aimed at reversing vancomycin resistance have focused on modifying the glycan component of vancomycin. The glycan is believed to play an important role in inhibiting bacterial cell wall biosynthesis by binding directly to the glycosyltransferases that convert peptidoglycan precursors into mature peptidoglycan, although the exact nature of this event is not well understood. In this proposal we present the design and development of two novel glycan derivatives of vancomycin that incorporate a combination of natural and unnatural carbohydrates. The proposed derivatives will be evaluated for biological activity against penicillin binding protein 2 (PBP2), a membrane bound glycosyltransferase isolated from *S. aureus*, using biophysical (isothermal titration calorimetry), spectroscopic (saturation transfer difference NMR spectroscopy), and computational techniques. The data collected from the studies outlined in this proposal will be used to develop a more specific understanding of the factors that influence the glycan-glycosyltransferase binding interaction. This information will ultimately be used to design more potent derivatives of vancomycin that can be used to combat resistant strains of gram-positive bacteria.

Project Narrative:

Vancomycin is a glycopeptide antibiotic used in the clinical setting to treat methicillin resistant strains of *Staphylcoccus* and *Enterococcus.* This proposal highlights the design, development, and biological evaluation of two novel derivatives of the vancomycin glycan. The proposed derivatives will be used to develop a better understanding of the factors that influence the glycan-glycosyltransferase binding interaction responsible for inhibiting bacterial cell wall biosynthesis. The data collected from the experiments outlined in this proposal will ultimately be used to design more potent derivatives of vancomycin that can be used to combat resistant strains of gram positive bacteria.

Facilities

The PI and her students are housed in the new $65 million state-of-the-art science center, completed in September of 2005. The renovation and expansion of the Hamilton College science facilities provides the campus with an integrated science center that houses the departments of biology, chemistry, geosciences, physics, and psychology, and the faculty with specialties in archaeology. The complex has: 56 offices for faculty and staff; 48 teaching laboratories; 53 research laboratories; 67 support rooms; student study areas; and 11 classrooms.

Equipment

The following equipment is available for use by the PI.

Chemistry and Biochemistry Resources:

-Chemglass inert/vacuum manifolds (3)
-Buchi Rotavapor rotary evaporators (6)
-Innovative Technologies PureSol solvent purification system
(includes dry tetrahydrofuran, diethyl ether, dichloromethane, toluene and dimethylformamide)
-MBraun glovebox
-SoLow –80° freezers (2)
-Sorvall RC-5B centrifuge
-Nanopure water purifier
-Walk-in coldroom
-Miscellaneous equipment including centrifuges, temperature controlled shakers, chromatographic equipment, pH meters, balances and glassware

Instrumentation:

-Bruker 500MHz nuclear magnetic resonance spectrometer
-Microcal VP isothermal titration calorimeter
-Thermo LC-MS
-Shimadzu QP5050 GC-MS
-Agilent 8453 UV/Vis diode array spectrometer (3)
-Perkin Elmer 1310 IR with ATR capability
-Jasco P-1020 polarimeter

Computers and Computational Facilities:

Mercury Consortium Resources:
-32 CPU SGI Origin 300, 32GB Ram, 788Gb of scratch space
-4 CPU SGI Origin 300, 2Gb Ram, 63Gb of scratch space
-Western Scientific Beowulf Cluster: 156 CPU (Dual Core Opteron), 76 Gb Ram, 2TB of scratch space
-In-House Built Beowulf Cluster: 28 CPU, 512mb Ram, 120 Gb of scratch

Workstations and Servers:
Departmental Computer Lab - 20 Apple Macintosh computers, 2 Dell PC’s

RESEARCH & RELATED Senior/Key Person Profile

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Nicole		Snyder	

Position/Title: Assistant Professor

Department: Chemistry

Organization Name: Hamilton College

Division:

* Street1: 198 College Hill Rd.

Street2:

* City: Clinton

County: Oneida

* State: NY: New York Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 13323

*Phone Number	Fax Number	* E-Mail
315-859-4742	315-859-4648	nsnyder@hamilton.edu

Credential, e.g., agency login: nsnyder

*** Project Role:** PD/PI

Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	7270-Biographical_SketchNIH.pdf	application/pdf
Attach Current & Pending Support		

	File Name	Mime Type
ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		
Additional Biographical Sketch(es) (Senior/Key Person)		
Additional Current and Pending Support(s)		

Biographical Sketch—NIH AREA June 23, 2008

Name:
Snyder-Lee, Nicole Leigh

Position Title:
Assistant Professor of Chemistry

Education/Training:
Westminster College, B.S., 2000, Chemistry
Westminster College, B.S., 2000, Biology
University of Connecticut, Ph.D., 2005, Chemistry

A. Positions and Honors

Positions and Employment

2005-2007	Visiting Assistant Professor of Chemistry, Wellesley College, Wellesley, MA
2007-Present	Assistant Professor of Chemistry, Hamilton College, Clinton, NY

Other Experience

2001-2002	Research Assistant, US Nanocorp, Willington, CT
2002-2004	Science Wizards Journeyman Program Coordinator, University of Connecticut, Storrs, CT

Professional Memberships

2001-Present	Member, American Chemical Society
2002-Present	Member, American Association for the Advancement of Sciences
2006-Present	Member, Sigma Xi
2007-Present	Member, Council on Undergraduate Research
2008-Present	District Delegate, Syracuse Section of the American Chemical Society

Honors

2007	Elsevier Top-50 Most Cited Articles Award (2004-2007) for "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine."

B. Selected peer-reviewed publications (in chronological order).

1. Peczuh, M.W.; Snyder, N.L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." Tetrahedron Letters **2003**, 44, 4057-4061.
2. Peczuh, M.W.; Snyder, N.L.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." Carbohydrate Research **2004**, 339(6), 1163-1171.
3 DeMatteo, M. P.; Snyder, N.L.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." Journal of Organic Chemistry **2005**, 70, 24-38.
4. Castro, S.; Duff, M.; Snyder, N.L.; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." Organic Biomolecular Chemistry **2005**, 3, 3869-3872.
5. Snyder, N.L.; Peczuh, M.W. Haines, H.M. "Recent Developments in the Synthesis of Oxepines." Tetrahedron **2006**, 62, 9301-9320.

6. Castro, S.; Cherney, E. C.; Snyder, N. L.; Peczuh, M. W. “Synthesis of Substituted Septanosyl-1,2,3-triazoles.” Carbohydrate Research **2007,** 342(10), 1366-1372.
7. Markad, S.D; Xia, S.; Snyder, N.L.; Hadad, C. M.; Peczuh, M. W. “Stereoselectivity in the Epoxidation of Carbohydrate Based Oxepines.” Accepted to the Journal of Organic Chemistry, May 2008

C. Research Support

Ongoing Research Support

PRF-SRF Zhang (PI) Summer 2008
American Chemical Society
Bio-inspired Cobalt Catalysts for Carbene and Nitrene Transfer Reactions
This study will be used to develop a method for directly attaching carbohydrates to porphyrins using a palladium catalyzed carbon-oxygen coupling reaction. The resulting products will be used to study their applicability in synthetic organic chemistry.
Role: Co-Investigator

Startup Snyder (PI) 07/01/2007-06/30/2010
Hamilton College
Synthesis, Reactivity, and Biological Evaluation of Designed Carbohydrate Systems
These funds were provided to begin an initial research program at the college and are currently used to support the purchase of chemicals and fund student research during the summer months on a variety of projects that are ongoing in the laboratory.
Role: Principle Investigator

Completed Research Support

Facutly Awards Grant Snyder (PI) 10/01/2005-05/31/2005
Wellesley College
The Use of Septanose Carbohydrates to Combat Antibiotic Resistance
This award was used to fund exploratory research on a project that involved the design and synthesis of an unnatural derivative of vancosamine, the nonreducing sugar on the glycan of vancomycin
Role: Principle Investigator

Staley Small Grant Snyder (PI) 01/01/2006-12/31/2006
Wellesley College
Carbohydrate Vaccines Targeted at Galectin-1
This award was used to fund the initial stages of a project focused on designing small inhibitors of galectin-1, a protein involved in tumor transformation and HIV infectivity.
Role: Principle Investigator

Brachman-Hoffman Grant Snyder (PI) Summer 2006
Wellesley College
The Use of Septanose Carbohydrates to Combat Antibiotic Resistance
This award was used to fund a student for the summer of 2006. The student worked a project designing an unnatural derivative of vancosamine, the nonreducing sugar on the glycan of vancomycin.
Role: Principle Investigator

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Nicole
Middle Name:
* Last Name: Snyder
Suffix:

* New Investigator? ○No ●Yes

Degrees: BS | BS | PhD

2. Human Subjects

Clinical Trial? ○No ○Yes

* Agency-Defined Phase III Clinical Trial? ○No ○Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Dr.
* First Name: Nicole
Middle Name:
* Last Name: Snyder
Suffix:

* Phone Number: 315-859-4742
Fax Number: 315-859-4678
Email: nsnyder@hamilton.edu

* Title: Assistant Professor of Chemistry

* Street1: 198 College Hill Rd.
Street2:
* City: Clinton
County:
* State: NY: New York
Province:
* Country: USA:
* Zip / Postal Code: 13323

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ●No ○Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp . Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001
Expiration Date: 9/30/2007

Budget Period: 1

Start Date: 05/01/2009 End Date: 04/30/2012

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	150,000.00
Consortium F&A	
* Total Direct Costs	150,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Salary and Wage Base	65.50	66,000.00	43,230.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services, Robert Aaronson, 212-264-2069

Indirect Cost Rate Agreement Date: 07/01/2006

Total Indirect Costs: 43,230.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 193,230.00

Budget Period: 2

Start Date: End Date:

A. Direct Costs

	Funds Requested ($)
* Direct Cost less Consortium F&A	
Consortium F&A	
* Total Direct Costs	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number):

Indirect Cost Rate Agreement Date:

Total Indirect Costs:

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($):

PHS 398 Modular Budget, Periods 3 and 4

OMB Number: 0925-0001
Expiration Date: 9/30/2007

Budget Period: 3

Start Date: End Date:

A. Direct Costs

Funds Requested ($)

* Direct Cost less Consortium F&A
Consortium F&A
* Total Direct Costs

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

Budget Period: 4

Start Date: End Date:

A. Direct Costs

Funds Requested ($)

* Direct Cost less Consortium F&A
Consortium F&A
* Total Direct Costs

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

PHS 398 Modular Budget, Period 5 and Cumulative

OMB Number: 0925-0001
Expiration Date: 9/30/2007

Budget Period: 5

Start Date: End Date:

A. Direct Costs

Funds Requested ($)

* Direct Cost less Consortium F&A
Consortium F&A
* Total Direct Costs

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

Cumulative Budget Information

1. Total Costs, Entire Project Period

* Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$	150,000.00
Section A, Total Consortium F&A for Entire Project Period	$	
* Section A, Total Direct Costs for Entire Project Period	$	150,000.00
* Section B, Total Indirect Costs for Entire Project Period	$	43,230.00
* Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$	193,230.00

2. Budget Justifications

Personnel Justification: 7852-Budget_Justification_personnel%5B
Consortium Justification:
Additional Narrative Justification: 3288-Budget_Justification_additional_narr

Attachments

PersonnelJustification_attDataGroup0

File Name	Mime Type
7852-Budget_Justification_personnel%5B1%5D.pdf	application/pdf

ConsortiumJustification_attDataGroup0

File Name	Mime Type

AdditionalNarrativeJustification_attDataGroup0

File Name	Mime Type
3288-Budget_Justification_additional_narrative%5B1%5D.pdf	application/pdf

Budget Justification:Personnel

Year 1:
-Summer salaries are included for the PI at 2/9 base salary. Fringe benefits are included at 10%.
-Summer stipends for undergraduate students are set by the college at $4000.00 per student for ten weeks of research. Fringe benefits are included at 10%. Hamilton College will provide funding for at least one additional student each summer for the duration of this proposal.

Year 2:
-Summer salaries are included for the PI at 2/9 base salary. Fringe benefits are included at 10%.
-Summer stipends for undergraduate students are set by the college at $4000.00 per student for ten weeks of research. Fringe benefits are included at 10%. Hamilton College will provide funding for at least one additional student each summer for the duration of this proposal.

Year 3:
-Summer salaries are included for the PI at 2/9 base salary. Fringe benefits are included at 10%.
-Summer stipends for undergraduate students are set by the college at $4000.00 per student for ten weeks of research. Fringe benefits are included at 10%. Hamilton College will provide funding for at least one additional student each summer for the duration of this proposal.

Undergraduate Student Involvement

Hamilton College undergraduate students will be heavily involved in every aspect of the projects outlined in this proposal. Students interested in synthetic organic chemistry will synthesize the glycans highlighted in this study. Students interested in a more biochemical approach will be involved in the production and purification of PBP2, as well as the binding studies that will be performed with this protein. Students interested in computational chemistry will play a key role in the computational analysis of the glycans prepared in this study and will work closely with the students performing synthetic organic and biochemical studies to rationalize any observed activity and generate new leads. Students will also be involved in manuscript preparation and will have the opportunity to present their work at national and international meetings.

Budget Justification: Additional Narrative Justification

Year 1:

-A Waters High Performance Liquid Chromatography system (cost ~$90,000.00) is requested for this research. Approximately $30,000.00 is requested from the NIH for this system with an additional $60,000.00 match coming from the College. This instrument will be dedicated to purification of PBP2 required for the experiments outlined in this proposal.

-An upgrade for the Colleges Microcal VP-ITC system (cost ~60,000.00) is also requested for this research. Approximately $20,000.00 is requested from the NIH for this system with an additional $40,000.00 match coming from the College. This instrument will reside in biochemistry suite and will be used to conduct PBP2 binding studies with the glycan derivatives outlined in this proposal.

-Funding is requested for the PI ($1,300.00) to travel to one annual meeting of the American Chemical Society. Additional funding ($2,600.00) will be provided by the College to attend one or more additional meetings per year. The College provides funding for student travel to one annual meeting per year.

-A supply budget of $7,500.00 is also kindly requested. The College will match this budget 2:1 to provide a total budget for chemicals and supplies of up to $22,500.00 for year one.

Year 2:

-Funding is requested for the PI ($1,300.00) to travel to one annual meeting of the American Chemical Society. Additional funding ($2,600.00) will be provided by the College to attend one or more additional meetings per year. The College provides funding for student travel to one annual meeting per year.

-A supply budget of $7,500.00 is also kindly requested. The College will match this budget 2:1 to provide a total budget for chemicals and supplies of up to $22,500.00 for year two.

Year 3:

-Funding is requested for the PI ($1,300.00) to travel to one annual meeting of the American Chemical Society. Additional funding ($2,600.00) will be provided by the College to attend one or more additional meetings per year. The College provides funding for student travel to one annual meeting per year.

-A supply budget of $8,500.00 is also kindly requested. The College will match this budget 2:1 to provide a total budget for chemicals and supplies of up to $25,500.00 for year three.

OMB Number: 0925-0001
Expiration Date: 9/30/2007

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

● New ❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	3250-NLS-NIHAREA-FINAL-SPAIM.pdf
3. Background and Significance	9778-NLS-NIHAREA-FINAL-BKDSIG.pdf
4. Preliminary Studies / Progress Report	1382-NLS-NIHAREA-FINAL-PRESTUD.pdf
5. Research Design and Methods	8244-NLS-NIHAREA-FINAL-RESDES.pdf
6. Inclusion Enrollment Report	
7. Progress Report Publication List	

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects	
9. Inclusion of Women and Minorities	
10. Targeted/Planned Enrollment Table	
11. Inclusion of Children	

Other Research Plan Sections

12. Vertebrate Animals	
13. Select Agent Research	
14. Multiple PI Leadership	
15. Consortium/Contractual Arrangements	
16. Letters of Support	
17. Resource Sharing Plan(s)	

18. Appendix

Attachments

IntroductionToApplication_attDataGroup0

File Name	Mime Type

SpecificAims_attDataGroup0

File Name	Mime Type
3250-NLS-NIHAREA-FINAL-SPAIM.pdf	application/pdf

BackgroundSignificance_attDataGroup0

File Name	Mime Type
9778-NLS-NIHAREA-FINAL-BKDSIG.pdf	application/pdf

ProgressReport_attDataGroup0

File Name	Mime Type
1382-NLS-NIHAREA-FINAL-PRESTUD.pdf	application/pdf

ResearchDesignMethods_attDataGroup0

File Name	Mime Type
8244-NLS-NIHAREA-FINAL-RESDES.pdf	application/pdf

InclusionEnrollmentReport_attDataGroup0

File Name	Mime Type

ProgressReportPublicationList_attDataGroup0

File Name	Mime Type

ProtectionOfHumanSubjects_attDataGroup0

File Name	Mime Type

InclusionOfWomenAndMinorities_attDataGroup0

File Name	Mime Type

TargetedPlannedEnrollmentTable_attDataGroup0

File Name	Mime Type

InclusionOfChildren_attDataGroup0

File Name	Mime Type

VertebrateAnimals_attDataGroup0

File Name	Mime Type

SelectAgentResearch_attDataGroup0

File Name	Mime Type

MultiplePILeadershipPlan_attDataGroup0

File Name	Mime Type

ConsortiumContractualArrangements_attDataGroup0

File Name	Mime Type

LettersOfSupport_attDataGroup0

File Name	Mime Type

ResourceSharingPlans_attDataGroup0

File Name	Mime Type

Appendix

File Name **Mime Type**

2.0 Specific Aims

Vancomycin is a glycopeptide antibiotic used in the clinical setting for the treatment of methicillin-resistant *Staphylcocci* and *Enterococci.* Vancomycin is composed of two bioactive components, a cyclic peptide component (aglycon) and a functionalized peripheral carbohydrate (glycan), that work together to inhibit the biosynthesis of peptidoglycan, a major component of the cell wall of gram-positive bacteria. The aglycon binds to peptidoglycan precursors terminating in the amino acid sequence D-ala-D-ala. This essentially blocks the approach of several key enzymes involved in the transglycosylation and transpeptidation steps of peptidoglycan synthesis. The role of the glycan is less understood, but it is thought that the glycan is involved in a direct binding event with the glycosyltransferases involved in peptidoglycan biosynthesis.

The emergence of several vancomycin resistant strains of *Staphylcocci* and *Enterococci* has led researchers to search for new and more potent derivatives of vancomycin. Initial attempts to overcome vancomycin resistance focused on the modification of several key functional groups on the aglycon scaffolding. While these attempts were generally successful, the chemical manipulations required to modify the peptide backbone were rather complex. More recent attempts aimed at reversing vancomycin resistance have focused on modifying the glycan. To date, a small number of vancomycin derivatives with modified glycan's have been prepared. These derivatives have been shown to inhibit the glycosyltransferases involved bacterial cell wall biosynthesis, however the specific role of the glycan in combating resistant strains of bacteria is not well understood.

The research in this proposal will be used to develop a more specific understanding of the factors that influence the glycan-glycosyltransferase binding interaction by focusing on the synthesis, characterization and biological evaluation of two novel glycan derivatives containing natural and unnatural carbohydrates. The knowledge obtained through the experiments outlined in this proposal will serve as a foundation for the design and preparation of new derivatives of vancomycin that can be used to combat resistant strains of gram-positive bacteria. The uniting goals of the research project are:

1. To generate a host of rationally designed natural and unnatural carbohydrate residues that can be used to probe the nature of glycan-glycosyltransferase interaction.
2. To investigate the binding interaction between the glycan's generated in this study and penicillin binding protein 2 (PBP2), a membrane bound glycosyltransferase isolated from *S. aureus*, using state of the art chemical, biochemical, spectroscopic and computational techniques.

Specifically, my students and I will address the following question: *"What factors govern the binding interaction between the glycan of vancomycin and the glycosyltransferases employed in bacterial cell wall biosynthesis?"*

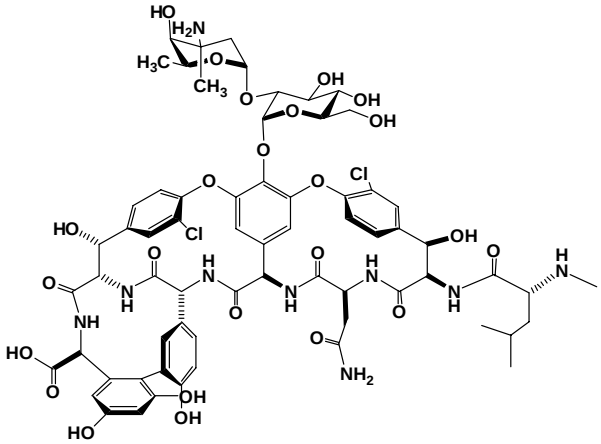
3.0 Background and Significance

3.1 Vancomycin: The antibiotic of Last Resort

In the 1940s, penicillin was introduced as a highly effective antibiotic that could be used to treat gram positive bacterial infections. For many years penicillin was the antibiotic of choice because it was shown to be highly effective against a broad spectrum of bacteria including *Staphylococcus aureus*. Unfortunately, repeated and continued misuse of the drug and its derivatives created selection pressures that favored the growth of antibiotic-resistant mutants.

In the mid 1950s scientist introduced vancomycin (Figure 1) in order to combat strains of *Staphylococci* that were growing resistant to penicillin. Vancomycin was highly effective at targeting *Staphylococci* and many other gram-positive bacteria including *Enterococci*. The drug eventually became known as the "antibiotic of last resort" and was often employed under circumstances in which no other drug could be used to effectively treat a gram-positive bacterial infection.

Figure 1. Vancomycin.



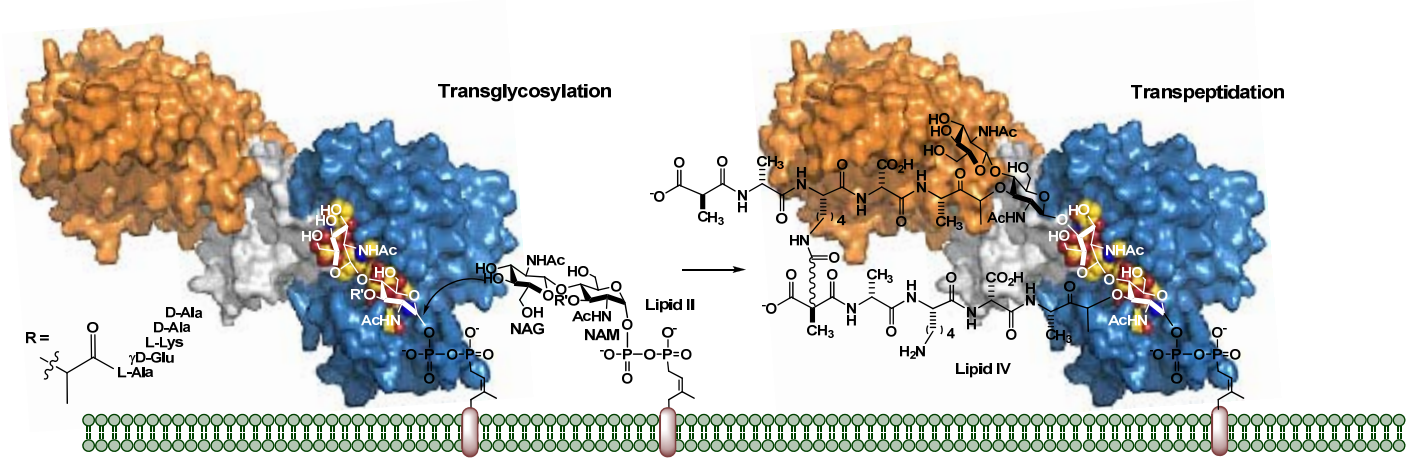
The hope that vancomycin would be a cure-all antibiotic ended in 1986 when vancomycin-resistant *Enterococci* (VRE) began to appear in hospitals.[1] Since then, several vancomycin-resistant strains of *Enterococci* have been detected, and in 2002 the first strain of vancomycin resistant *Staphylococcus aureus* (VRSA) was reported.[2] This has raised serious concerns within the scientific and health care communities as researchers scramble to search for new and more potent compounds to act as weapons in the war against bacteria.

3.2 Vancomycin: Biological Activity and Antimicrobial Resistance

Vancomycin is a glycopeptide antibiotic composed of two bioactive components: a cyclic peptide component known as the aglycon and a functionalized peripheral carbohydrate component known as the glycan. These two components work together to inhibit the glycosyltransferases and transpeptidases involved in the biosynthesis of peptidoglycan, a major component of the cell wall of gram positive bacteria.[3]

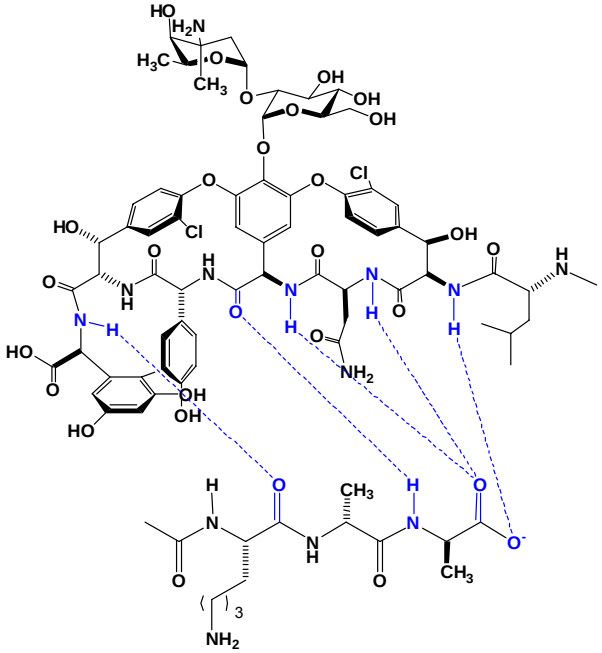
The biosynthesis of peptidoglycan occurs in three major stages. In the first stage, recurring units of backbone structure are synthesized as derivatives of peptidoglycan in the cytoplasm. In the second stage, which takes place on the inner surface of the cytoplasmic membrane, derivatives from the first stage are modified to form a complete nascent peptidoglycan-lipid subunit known as Lipid II. Lipid II is composed of a lipid component, two sugars (N-acetly-D-glucosamine (NAG) and N-acetlymuramic acid (NAM)), and a pentapeptide terminating in D-alanine residues. Stage two ends with the translocation of the completed Lipid II subunit to the exterior of the cytoplasmic membrane. The third stage takes place on the exterior surface of the bacterial cell membrane and is shown in Figure 2. In the final stage of peptidoglycan synthesis, the disaccharide unit of the nascent Lipid II subunit is polymerized to form higher order lipid precursors such as Lipid IV (immature peptidoglycan) by glycosyltranferases. These subunits are then crosslinked by transpeptidases to form mature peptidoglycan.

Figure 2. Stage 3 of peptidoglycan synthesis (adapted from Strynadka *et. al*.[4]). The glycosyltransferase domain (blue) of penicillin binding protein 2 (PBP2) from *S. aureus*, polymerizes Lipid II into higher order peptidoglycan precursors (immature peptidoglycan units) such as Lipid IV. Immature peptidoglycan units are then cross linked by transpeptidases (orange) to form mature peptidolglycan. PBP2 is shown with an inhibitor overlay above which the NAM-NAG disaccharide of Lipid II is superimposed.



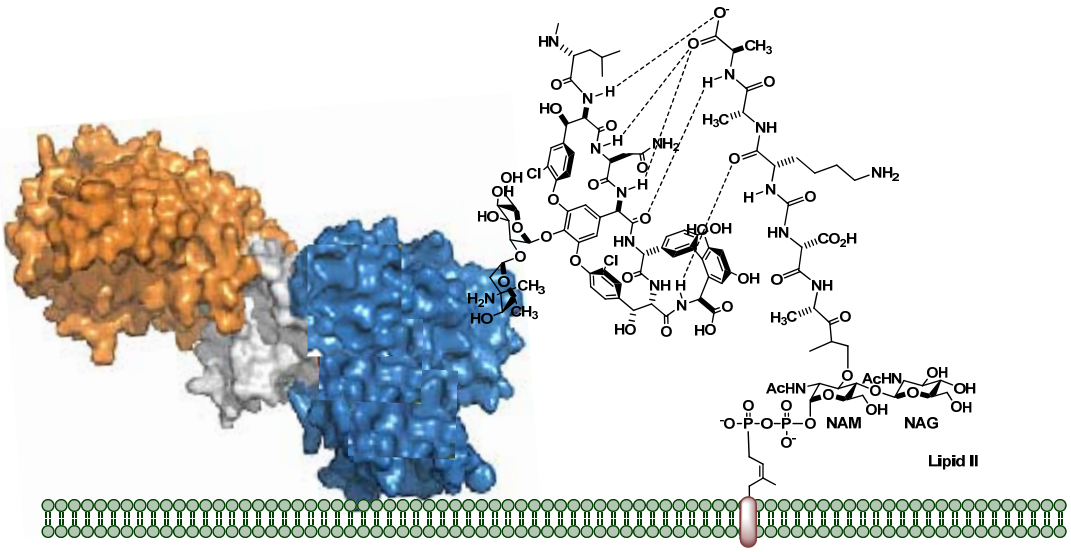
The role of the aglycon in the inhibition of peptidoglycan biosynthesis is well understood. The aglycon, featured in Figure 3, forms a binding pocket in which five key amino acid residues in the pocket hydrogen bond to peptidoglycan precursors terminating in the amino acid sequence D-alanyl-D-alanine (D-ala-D-ala).[5] Once bound, the aglycon creates an obstruction that impedes the processing of immature peptidoglycan precursors into mature peptidoglycan. The result is the loss of mechanical strength in the cell wall of the bacteria and ultimately death by osmotic shock.

Figure 3. The role of the aglycon in the inhibition of gram positive bacteria. The aglycon binds to immature peptidoglycan precursors terminating in D-ala-D-ala through five key hydrogen bonds, thus inhibiting the association of these units into mature peptidoglycan.



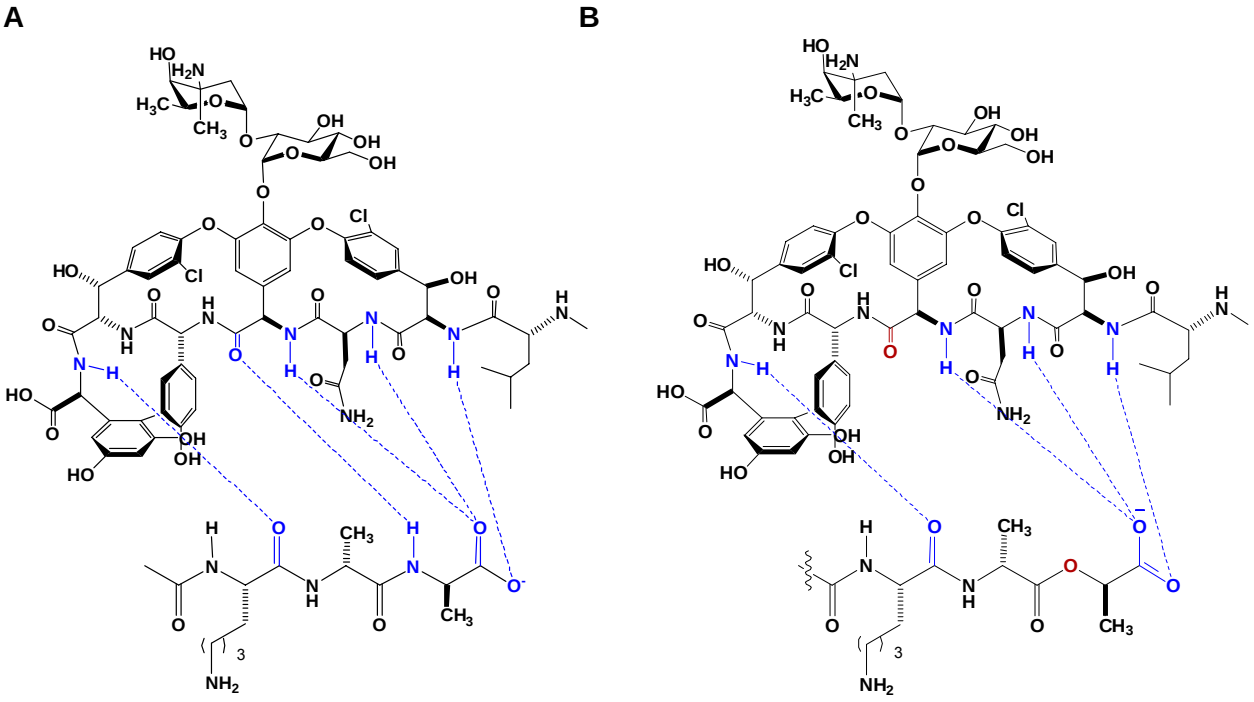
The role of the glycan is less understood. Experimental evidence suggests that the glycan plays an important role in the conformational maintenance of the aglycon.[6] The glycan is also believed to assist the aglycon in dimerization and membrane anchoring events that act cooperatively to create a chelating effect that increases the affinity of the aglycon for D-ala-D-ala.[7] Recent research has also revealed that the glycan may be involved in a direct binding event with the glycosyltransferases involved in transglycosylation, regardless of a petidoglycan binding event (Figure 4).[8] However, the exact nature of this binding event is still unknown.

Figure 4. The role of the glycan in the inhibition of gram positive bacteria. The glycan is believed to be involved in a direct binding event with the glycosyltransferases involved in bacterial cell wall biosynthesis (adapted from Strynadka *et. al.*[4]).



Bacteria that have developed a resistance to vancomycin produce peptidoglycan precursors that terminate in D-alanyl-D-lactate (D-ala-D-lac) instead of the native D-ala-D-ala sequence (Figure 5).[9] This structural modification results in the loss of a critical hydrogen bond between an amide carbonyl in the binding pocket of vancomycin and the peptide substrate (Figure 15-**B**). The electrostatic repulsion between the carbonyl oxygen on the backbone of the aglycon and the oxygen on the lactate residue, combined with the loss of a critical hydrogen bond, decreases the effectiveness of the antibiotic by three orders of magnitude. There are currently no examples of resistant strains of bacteria exhibiting glycosyltransferase modifications.

Figure 5. Binding of vancomycin to D-ala-D-ala **A** versus D-ala-D-lac **B**.



The emergence of several vancomycin-resistant strains of *Staphylcocci* and *Enterococci* over the past fifteen years has led researchers to search for new and more potent derivatives of vancomycin. The first

attempts focused on modifying the aglycon to bind more effectively to peptidoglycan precursors terminating in D-ala-D-lac rather than the native D-ala-D-ala. More recently, however, researchers have focused on modifying the glycan to observe how certain modifications impact the ability of vancomycin to bind to the glycosyltransferases involved in bacterial cell wall biosynthesis. The results of this research are summarized in the preliminary studies section below.

4.0 Preliminary Studies

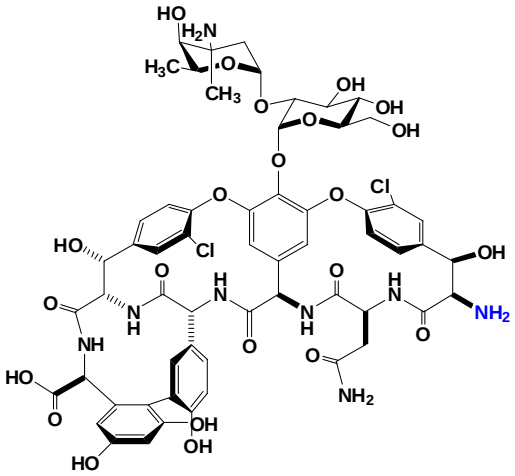
4.1 Modifications to the Aglycon

Initial attempts to overcome vancomycin resistance focused on the modification of several key functional groups on the aglycon scaffolding. The overall goal was to design aglycon scaffolds that could bind peptidoglycan precursors terminating in the modified D-ala-D-lac, rather than the native D-ala-D-ala sequence.

In one of the first examples, Jain and coworkers[10] prepared a damaged vancomycin analog in which the terminal leucine residue was deleted (Figure 6-**A**). The authors thought that the deletion of this amino acid residue might help reduce the steric interactions between the analog and peptidoglycan precursors terminating in D-ala-D-lac. Unfortunately, their derivative showed almost no activity against vancomycin-resistant bacteria in inhibitory studies. However, dimers of their derivative showed enhanced activity at 5.8 MIC (μg/mL). The authors attributed the increase in activity to multivalency.

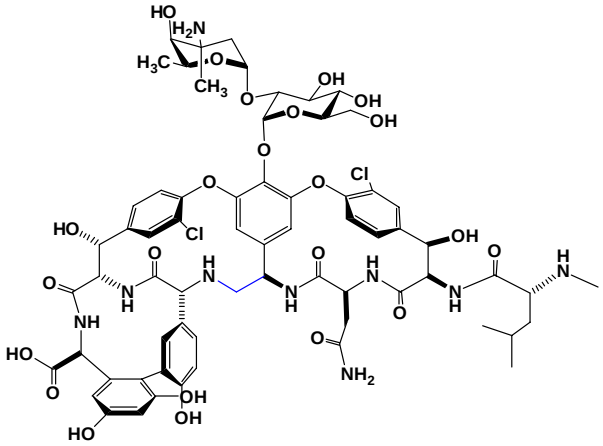
Figure 6. Modifications of the aglycon of vancomycin: damaged vancomycin **A** and reduced vancomycin **B**.

A



Required greater than 1200 MIC to reverse vancomycin resistance.

B



Exhibited a 40-fold increase in affinity for vancomycin resistant *Enterococci*.

Recently, Boger and Crowley[11] were able to produce a vancomycin analog in which a key amide residue was reduced to provide the corresponding amine (Figure 6-**B**). This analog exhibited a 40-fold increase in affinity for vancomycin-resistant *Enterococci*. The authors believed that the reduction of the amide to the amine relieved the destabilizing lone pair interaction between the carbonyl at that position and the D-ala-D-lac sequence, thus restoring the binding affinity of the antibiotic for the modified ligand.

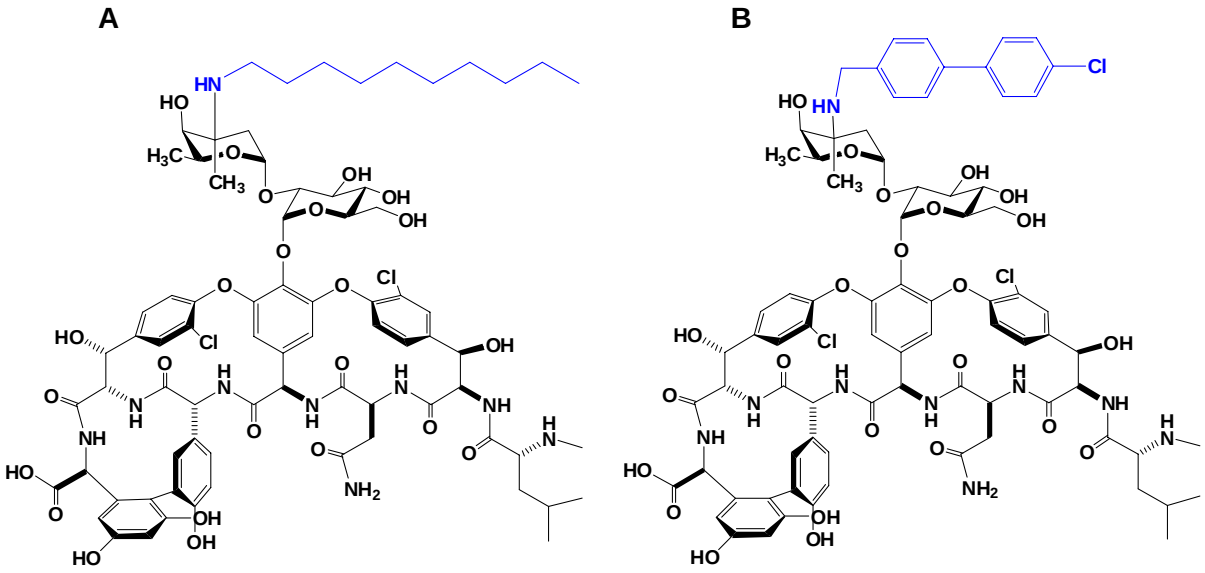
While attempts to modify the aglycon have met with some success, the chemical manipulations required to modify the peptide backbone are often complex and difficult, at times requiring the complete synthesis of an entirely new aglycon scaffold. Therefore, a number of recent attempts aimed at reversing vancomycin resistance have focused on modifying the glycan component of vancomycin.

4.2 Modifications to the Glycan

Vancomycin analogs prepared by selective functionalization of the N-terminus of the vancosamine subunit have shown reasonable activity against strains of vancomycin-resistant of bacteria. While the reasons for this are still unclear, it is believed that the substituent plays a role in binding directly to the glycosyltransferases involved in peptidoglycan synthesis.

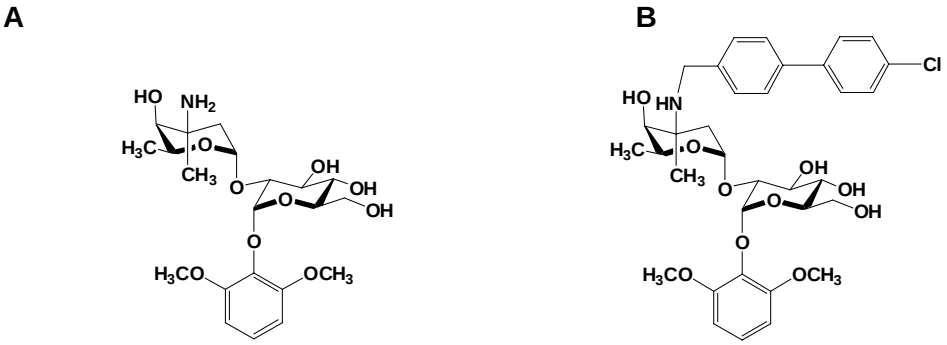
In 1993, Nagarajan and coworkers at Eli-Lilly prepared the first derivative of vancomycin incorporating a long hydrocarbon chain on the nitrogen of the vancosamine sugar of the glycan[12] (Figure 7-**A**). The N-decyl derivative required 4.8 MIC (μg/mL) in inhibition studies to reverse vancomycin resistance.

Figure 7. Modifications of the glycan of vancomycin: *N*-decyl vancosamine derivative **A** and chlorobiphenyl vancomycin (CBP-V) **B.**



Inspired by Nagarajan's work, Kahne and coworkers[8a] prepared the chlorobiphenyl vancomycin derivative (CBP-V) shown in Figure 7-**B**. This analog required only 16 MIC (μg/mL) to reverse vancomycin resistance in inhibition studies. In order to obtain more specific insight into the role of the glycan, Kahne and coworker prepared a number of glycan derivatives separate from the aglycon and analyzed them for activity against vancomycin resistant bacteria (Figure 8). A glycan analog bearing a chlorobiphenyl substituent (Figure 8-**B**) was shown to be almost ten times more effective than the natural vancomycin glycan against resistant strains of bacteria (Figure 8-**A**). Using radio labeling experiments, Kahne and coworkers were able to determine that the chlorobiphenyl group was directly involved in a number of complex interactions involving immature peptidoglycan, Lipid II, and the proteins involved in transglycosylation.

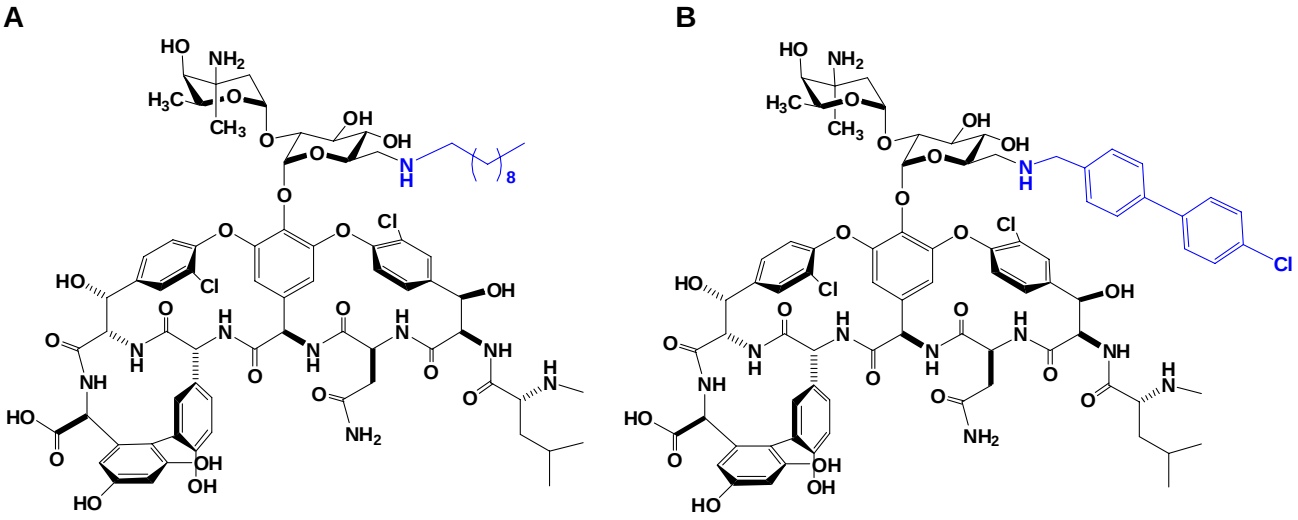
Figure 8. Glycan analogs used to study glycan inhibition of peptidoglycan biosynthesis.



In a separate report, Kahne and coworkers conducted inhibition studies to determine the biological function of the hydrophobic substituents on vancomycin glycan derivatives. They chose to study the influence of the position of the substituent on the biological activity of the derivative (Figure 9).[8b] The first derivative they prepared was similar in nature to the derivative prepared by Nagarajan and coworkers[12] (Figure 7-**A**) but involved the substitution of a long hydrophobic alkyl chain on the C6 carbon of the glucose residue of the glycan (Figure 9-**A**) rather than on the amino group of the vancosamine sugar. This derivative showed activity against vancomycin-resistant bacteria at 512 MIC (μg/mL). The second derivative they prepared incorporated

a chlorobiphenyl substituent on the C6 carbon of the glucose residue of the glycan (Figure 9-**B**). This derivative also showed activity against vancomycin-resistant bacteria, but required twice as much of the derivative (1024 MIC) to inhibit growth under similar conditions.

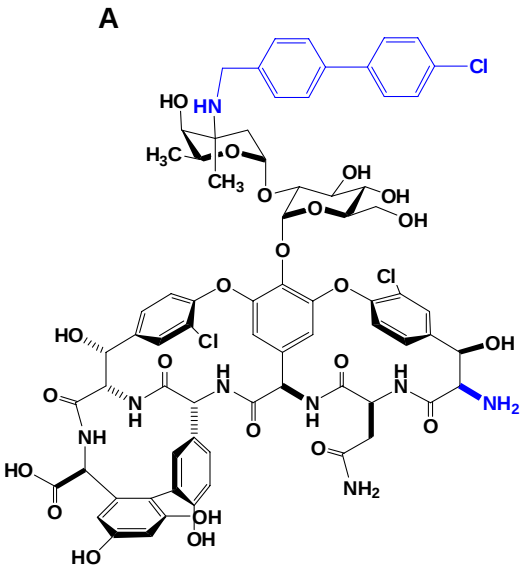
Figure 9. Modifications of the glycan of vancomycin: glucose substituted *N*-decyl **A** and chlorobiphenyl **B** derivatives.



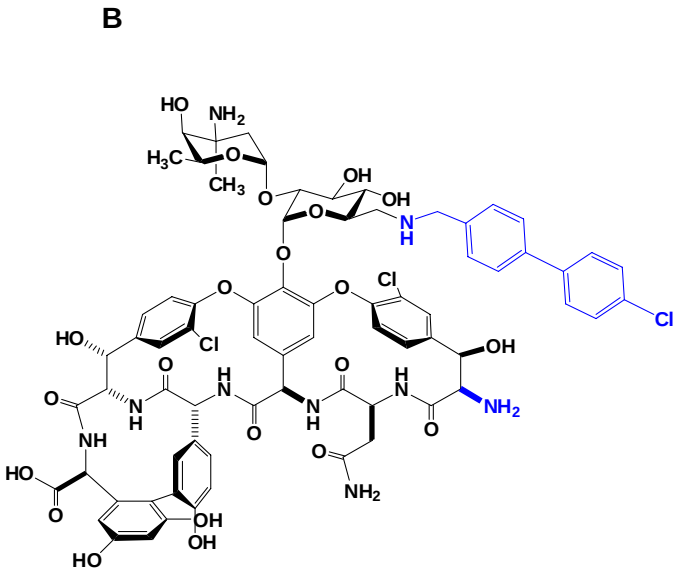
Kahne and coworkers compared the derivatives shown in Figure 9 against the derivatives in which the hydrophobic substituent was on the vancosamine sugar (see Figure 7) and found that the presence of a substituent affects the mechanism of action of the antibiotic.[7b] Derivatives of vancomycin incorporating a substituted vancosamine or glucose were shown to block transglycosylation, while the natural vancomycin (unsubstituted) was shown to block transpeptidation. At the time the authors hypothesized that all of the derivatives bound at some point to D-ala-D-ala, and that compounds containing hydrophobic substituents served to anchor the derivatives to the bacterial membrane, enhancing binding to Lipid II via proximity effect, blocking the approach of the glycosyltransferases involved in cell wall biosynthesis.

In order to test their hypothesis, Kahne and coworkers used damaged derivatives of compounds containing the chlorobiphenyl substituents to see if these derivatives were still active against vancomycin resistant bacteria (Figure 10).[7b] Since the aglycon plays a key role in binding D-ala-D-ala, it was expected that derivatives with damaged binding pockets would no longer inhibit cell wall biosynthesis. Surprisingly, the damaged derivative of CBP-V with chlorobiphenyl group at the vancosamine position (Figure 10-**A**) maintained activity, while the damaged derivative of CBP-V with the chlorobiphenyl substituent on the glucose residue (Figure 10-**B**) no longer displayed activity against vancomycin resistant strains of bacteria. Therefore, they concluded that derivatives with substituents on the vancosamine residue operated under a second mechanism of action that did not involve binding to peptidoglycan precursors, while those with substituents on the glucose residue required binding to peptidoglycan precursors to inhibit cell wall biosynthesis. Goldman and coworkers confirmed the observations reported by Kahn and workers in a separate report in 2000.[13]

Figure 10. A damaged derivative of CBPV with the chlorobiphenyl group at the vancosamine position **A** and damaged CBPV with the chlorobiphenyl group at the C6 position of glucose **B**.



Required 16 MIC to reverse vancomycin resistance.

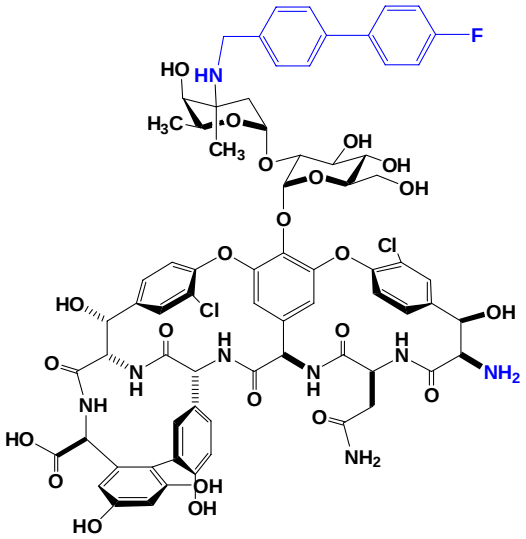


Required 1024 MIC to reverse vancomycin resistance.

In 2001, Roy and coworkers used affinity chromatography to show CPB-V bound several membrane proteins, including the glycosyltransferase penicillin binding protein 1b (PBP1b), from *E. coli*.[14] This was the first direct evidence for the binding of a vancomycin derivative directly to a glycosyltransferase, and was followed by a 2003 report by Kahne and coworkers who used inhibition studies to show that vancomycin analogs (damaged and undamaged) containing hydrophobic groups interacted directly with PBP1b derived from *E. coli* to inhibit cell wall biosynthesis.[15] In 2005, Kahne and coworkers completed an analogous inhibition study with PBP2 isolated from *S. aureus* and obtained similar results.[16]

Early this year, Schafer and coworkers[17] used rotational-echo double-resonance spectroscopy to show that a desleucyl(fluorophenyl)-benzylvancomycin (DFPB-V-Figure 11) bound directly to cell wall peptidoglycan of *S. aureus*, interfering indirectly with cell wall biosynthesis without the direct formation of an enzyme complex. This is in contrast to what Kahne and coworkers reported in their 2005 study of CBP-V.[15] The results of this study are interesting because the derivative prepared by Schafer and coworkers is almost identical in structure to damaged CBP-V with the only difference being the substitution of the chloro group with a fluoro substituent.

Figure 11. Desleucyl(fluorophenyl)-benzylvancomycin.



While the derivatives described above have furthered an understanding of the role of the glycan in inhibiting bacterial cell wall biosynthesis, the specific factors that govern the glycan-glycosyltransferase binding interaction are still unknown. The research in this proposal will begin to address this issue by focusing on the synthesis, characterization, and biological evaluation of two novel glycan derivatives that incorporate natural and unnatural carbohydrate residues. The major goal of this project is to collect information about the proposed derivatives and their interactions with penicillin binding protein 2 (PBP2), a membrane bound glycosyltransferase isolated from *S. aureus*, using biochemical (isothermal titration calorimetry), spectroscopic (saturation transfer difference NMR spectroscopy), and computational techniques. This data will be used to understand the specific factors that influence the glycan-glycosyltransferase binding interaction. Ultimately, we plan to use the results of this research to design more potent derivatives that can be used to combat resistant strains of bacteria.

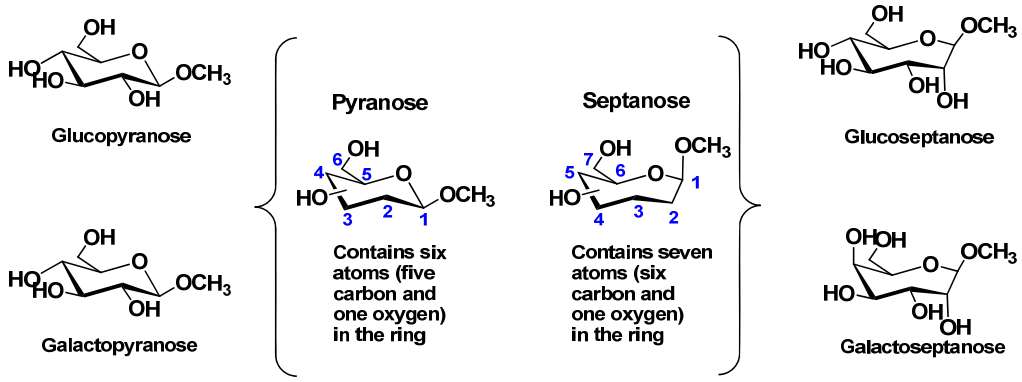
5.0 Research Design and Methods

5.1 Design and Rationale

In recent years, the construction of expanded homologs of naturally occurring amino acid and carbohydrate residues has gained considerable attention. The "unnatural" nature and interesting properties these molecules exhibit make them attractive tools for probing biomolecular interactions. For example, Eschenmoser[18] has observed that when the five member furanose sugars of DNA and RNA are expanded by one carbon to pyranoses, an alternative base-pairing and heteroduplex shape is observed. Similarly, Gellman[19] and Seebach[20] studied the homologation of α-amino acids to β-amino acids and found that oligomers constructed from β-amino acids adopt defined conformations that complement natural structures and can selectively disrupt bacterial cell membranes over mammalian cell membranes.[21]

More recently, the construction of an entirely new class of ring expanded carbohydrates has been introduced.[22] Septanose carbohydrates, shown in Figure 12, are unnatural, ring expanded homologs of pyranose carbohydrates. The flexibility of the seven member ring in these sugars allows them to adopt a number of different low energy conformations[23] that make them interesting tools for studying fundamental protein-carbohydrate interactions in conjunction with their natural pyranose homologs.[24]

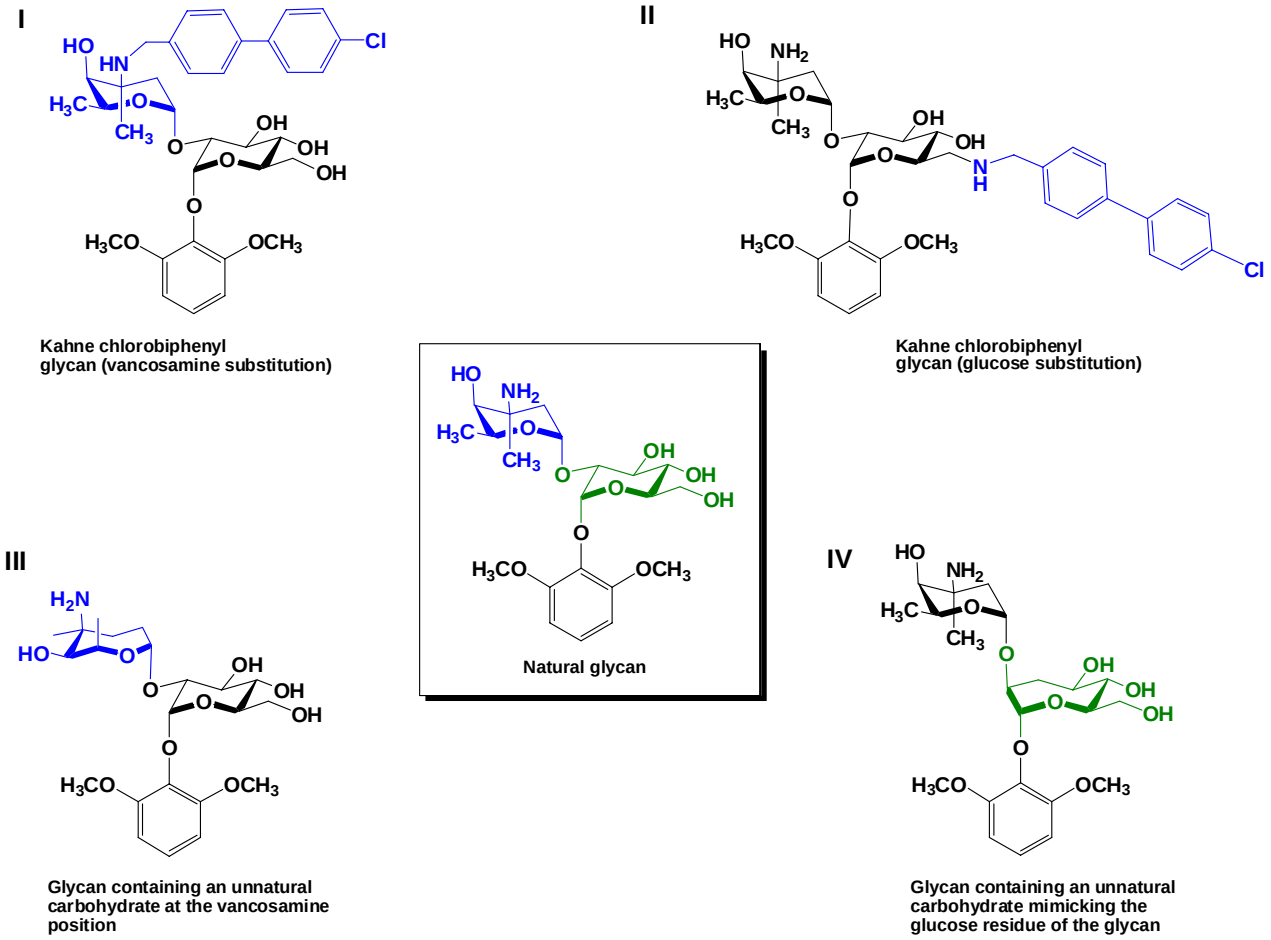
Figure 12. Examples of pyranose and septanoses carbohydrates.



My students and I will focus on the preparation, characterization, and biological evaluation of two new derivatives of the glycan of vancomycin (Figure 13-**III** and **IV**). The derivatives we are proposing are novel in that they incorporate both natural and unnatural carbohydrate residues that mimic the carbohydrate residues present on natural glycan. The proposed derivatives are inspired by the glycans used by Kahne and coworkers to probe the transglycosylase activity of vancomycin (Figure 13-**I** and **II**) and will be used to determine the specific factors that govern the glycan-glycosyltransferase binding interaction.

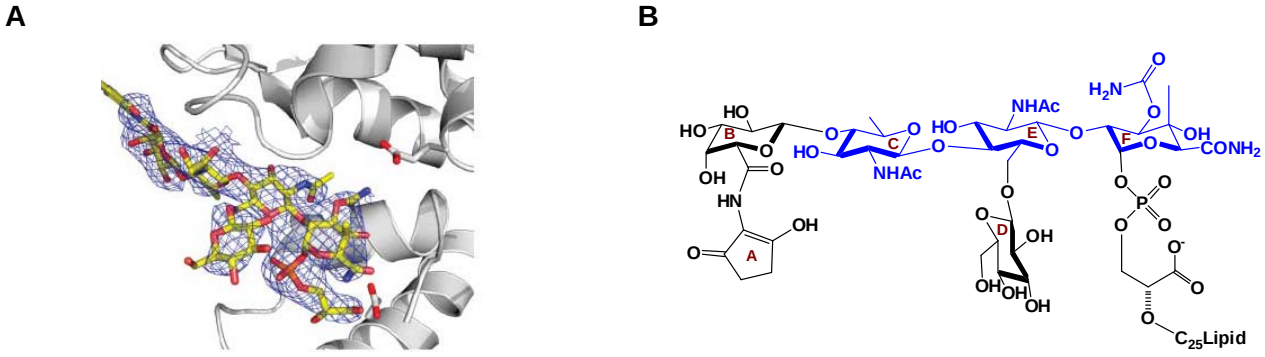
The rationale for the proposed derivatives is based on two parameters that we would like to initially investigate. First, we would like to know how substitution of a natural carbohydrate for an unnatural carbohydrate affects the overall conformation of the glycan. Second, we would like to know whether glycan structures that incorporate unnatural carbohydrates can bind the glycosyltransferases involved in peptidoglycan synthesis.

Figure 13. Vancomycin glycan (**I**), Kahne's chlorobiphenyl glycan derivative (**II**), and two new glycan derivatives: proposed glycan derivative **III** incorporating an unnatural carbohydrate at the vancosamine position and proposed glycan derivative **IV** incorporating an unnatural carbohydrate mimicking the glucose residue of the glycan.



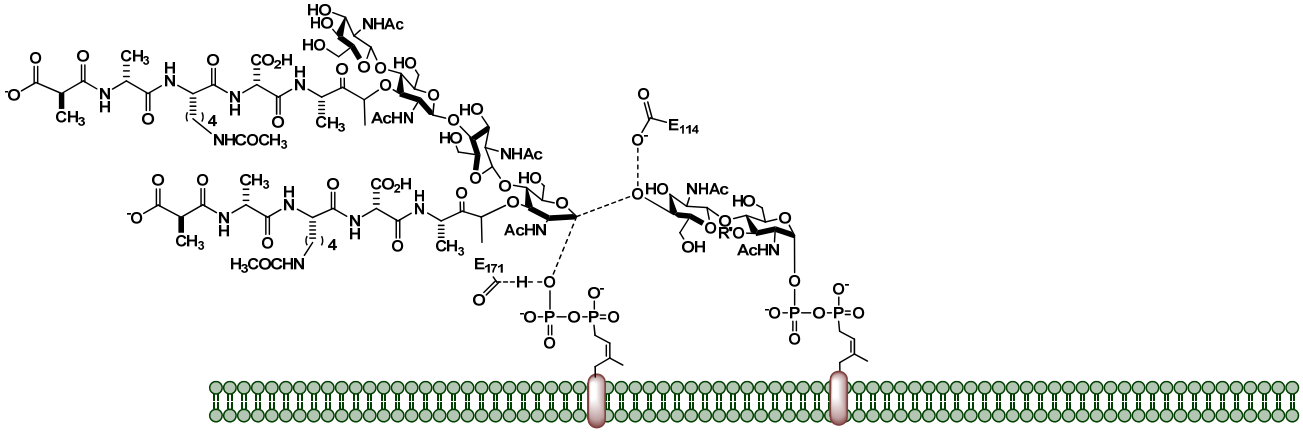
A recent crystal structure of moenomycin-PBP2 glycosyltransferase (from *S. aureus*) co-complex published by Strynadka and coworkers[25] has shed some light on the binding requirements for glycosyltransferases (Figure 14-**A**). Moenomycin (Figure 14-**B**) is a competitive inhibitor of the glycosyltransferases involved in bacterial cell wall biosynthesis. As shown in Figure 14, the C and E rings of moenomycin bind in the same fashion as the NAM-NAG disaccharide of Lipid II binds in the active site of the enzyme. The major difference between Lipid II, which is processed by PBP2, and moenomycin, which inhibits PBP2, is the F ring and the phosphoric acid diester/C25 lipid component. The authors suggested that these two components must be responsible for the inhibitory properties of the antibiotic.

Figure 14. Co-crystal complex of moenomycin and the glycosyltransferase domain of PBP2 **A** and moenomycin **B** (taken and adapted from Strynadka et. al.[25]).



Strynadka and coworkers used the co-crystal structure of the moenomycin-PBP2 glycosyltransferase complex to propose a mechanism for Lipid II polymerization by penicillin binding protein 2 (PBP2). In their report, they suggest that the glycosyltransferase domain of PBP2 binds directly to the disaccharide of Lipid II (or a higher order lipid chain such as Lipid IV). The conformation of the N-acetyl muramyl residue of the disaccharide adopts a half chair conformation upon binding PBP2, and several key glutamine residues play a role in the glycosylation reaction which forms long immature peptidoglycan precursors (Figure 15).

Figure 15. Mechanism for the polymerization of Lipid II (adapted from Strynadka et. al.[25]).



Current research suggests that substituted glycan derivatives directly bind the glycosyltransferases involved in peptidoglycan synthesis. While the exact nature of the binding event is not well understood, there appear to be three different hypotheses that have emerged through the research that has been conducted to date. These hypotheses are:

1. The hydrophobic substituent of the glycan interacts in a competitive manner by binding directly within the active site of the enzyme. This type of binding would restrict the access of peptidoglycan precursors to the glycosyltransferases involved in peptidoglycan synthesis.
2. The hydrophobic substituent of the glycan interacts in a noncompetitive manner by binding to the surface of the glycosyltransferases. This changes the conformation of the active site of the enzyme and indirectly prohibits binding to peptidoglycan precursors.
3. The hydrophobic substituent embeds itself directly into the cell wall, creating an obstruction that blocks the approach of the glycosyltransferases involved in bacterial cell wall biosynthesis.

It has not been suggested that the glycan itself binds directly to glycosyltranferase, and in fact the natural glycan has been shown to be inactive against vancomycin resistant bacteria.[8a] This is surprising, considering that glycosyltransferases such as PBP2 readily accommodate moenomycin which contains carbohydrate residues that bear some resemblance in structure to the carbohydrate residues of the vancomycin glycan.

While there have been no formal suggestions as to why the natural glycan of vancomycin is unable to bind the glycosyltransferases involved in peptidoglycan synthesis, one theory is that this may be because the energy required to distort the vancosamine sugar into the half chair conformation required for binding to glycosyltransferases is too high. The first proposed derivative (Figure 13-**III**) will be used to test this hypothesis by exploring how the substitution of an unnatural sugar at the vancosamine position affects the overall conformation and biological activity of the glycan. The proposed derivative meets the space filling and polar requirements for binding glycosyltransferases, and we believe that the extra carbon in the ring of the unnatural vancosamine sugar will provide greater flexibility in the ring of the sugar that will allow the residue to more readily access the half chair conformation required for binding. The specific question we will address with glycan derivative **III** is: *"Can glycan derivatives incorporating an unnatural carbohydrate at the vancosamine sugar adopt a suitable conformation that will allow them to bind to the glycosyltransferases responsible for peptidoglycan biosynthesis?"*

Kahne and coworkers have shown that the substitution of the C6 carbon of the glucose residue of the vancomycin glycan does not produce a derivative that binds directly to the glycosyltransferases involved in bacterial cell wall biosynthesis.[8b] There is some evidence, however, that a change in the conformation of the glucose residue, and therefore a change in the conformation of the glycan, may provide the vancosamine sugar with greater rotational flexibility making it easier for the glycan to access a conformation suitable to

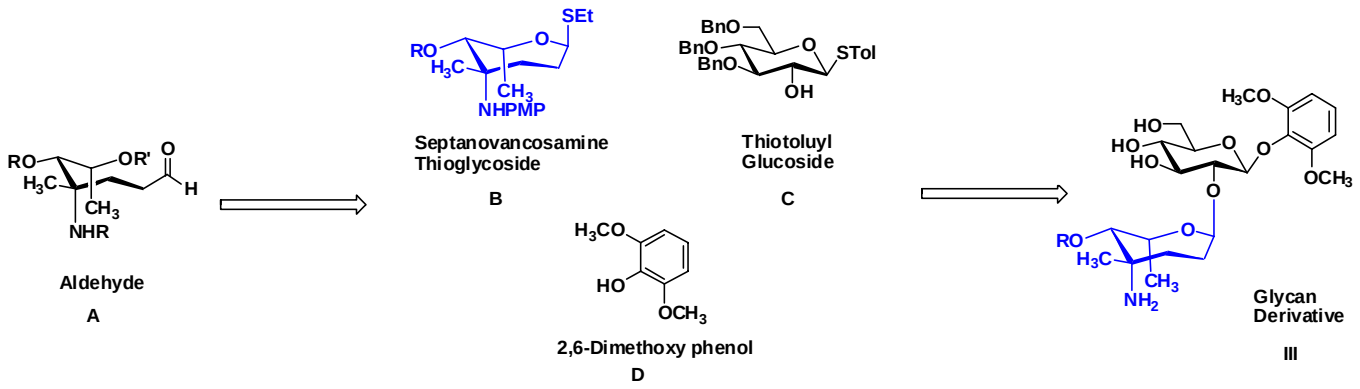
binding glycosyltransferases. The second derivative (Figure 13-**IV**) will address this hypothesis by exploring how the substitution of the glucose residue with an unnatural sugar affects the overall all conformation and biological activity the glycan. The specific question we will address with glycan derivative III is: *"Can glycan derivatives incorporating an unnatural carbohydrate at the glucose residue of the glycan provide a suitable conformation that will allow them to bind to the glycosyltransferases responsible for peptidoglycan biosynthesis?"*

In the sections below we outline the synthesis of the proposed derivatives. We plan to evaluate the activity of these derivatives in the presence of PBP2, a membrane bound glycosyltransferase from *S. aureus*, using isothermal titration calorimetry (ITC) and saturation transfer difference nuclear magnetic resonance spectroscopy (STD-NMR). ITC will be used to determine whether or not the proposed derivatives bind to PBP2. Derivatives that bind PBP2 will then be evaluated using STD-NMR to determine the nature of the binding event. Finally, we will use computational chemistry to aid in our understanding of the results obtained in these studies.

5.2 Preparation of a Glycan Containing a Septanose Residue at the Vancosamine Position

The retrosynthetic analysis of the glycan **III** is shown in Scheme 1 below. Thioacetal cyclization of aldehyde **A** provides access to septanovancosamine thioglycoside **B**. One pot glycosylation of septanovancosamine thioglycoside **B** with thiophenylglucoside **C** and 2,6-dimethoxyphenol **D** provides access to glycan derivative **III**.

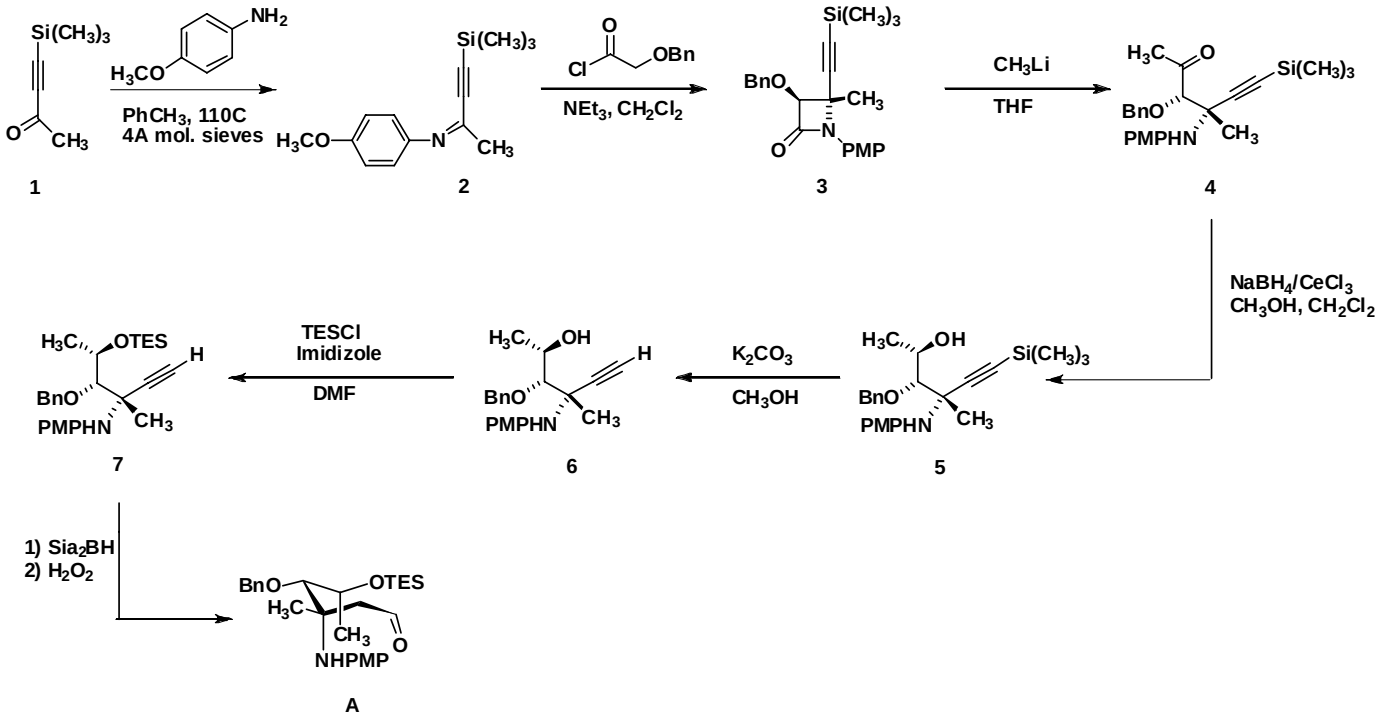
Scheme I. Retrosynthetic analysis of vancomycin glycan derivative **III**.



A. Preparation of aldehyde A

The preparation of aldehyde **A** is shown in Scheme 2. The synthesis of aldehyde A is based on a procedure developed by McDonald and coworkers for the preparation of vancosamine glycals from 4-trimethylsilyl-3-butyn-2-one.[26] Condensation of 4-trimethysilyl-3-butyn-2-one and p-anisidine produces imine **2**. In the next step, imine **2** and benzyloxyacetyl chloride are combined in the presence of triethylamine to produce the corresponding beta-lactam **3**. Beta lactam **3** is then opened using methyl lithium to produce methyl ketone **4**. Reduction of methyl ketone **4** using sodium borohydride in the presence of cerium chloride (heptahydrate) gives alkynol **5** which, when deprotected using potassium carbonate in the presence of methanol, gives the desialylated alkynol **6**. The alcohol of alkynol **6** is then protected using chlorotriethylsilane to give **7**, which undergoes hydroboration oxidation upon reaction with disiamylborane to give aldehyde **A**.

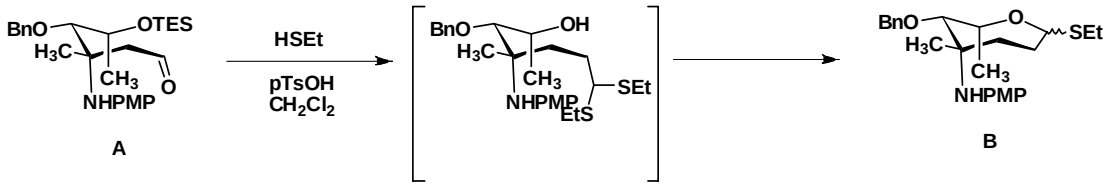
Scheme 2. Preparation of aldehyde **A** from 4-trimethylsilyl-3-butyn-2-one.



B. Preparation of septanovancosamine thioglycoside B

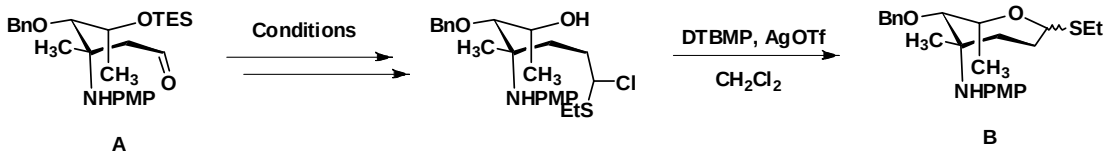
Septanovancosamine thioglycoside **B** can be prepared directly from aldehyde **A** by reacting the aldehyde with ethanethiol in the presence of a catalytic amount of para-toluenesulfonic acid as shown in Scheme 3. While there is no formal precedence for this reaction on a similar system, the same reaction using hydroxyacetals has been described for the synthesis of septanose carbohydrates.[27]

Scheme 3. Preparation of septanovancosamine thioglycoside **B** from aldehyde **A**.



In the event that the above synthesis is met with difficulty, an alternative method described by Hindsgaul and coworkers[28] can be used to access the septanovancosamine thioglycoside **B** as shown below in Scheme 4.

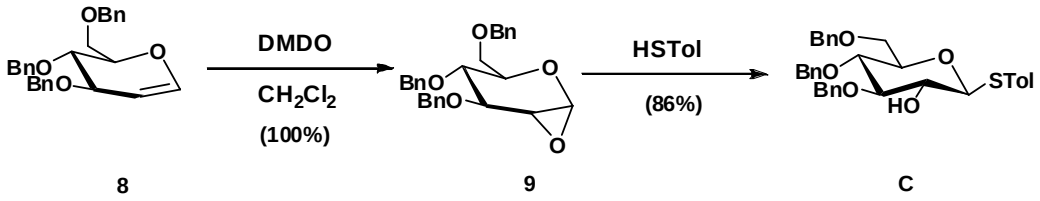
Scheme 4. Alternative preparation of septanovancosamine thioglycoside **B**.



C. Preparation of thiotouly glucoside C

Epoxidation of readily available tri-*O*-benzyl-D-glucal **8** by in situ generation of dimethyl dioxirane (DMDO)[29] provides the corresponding epoxide **9,** which is readily converted to thiotoluyl glucoside **C** using 4-methyl-benzenethiol as shown in Scheme 5.

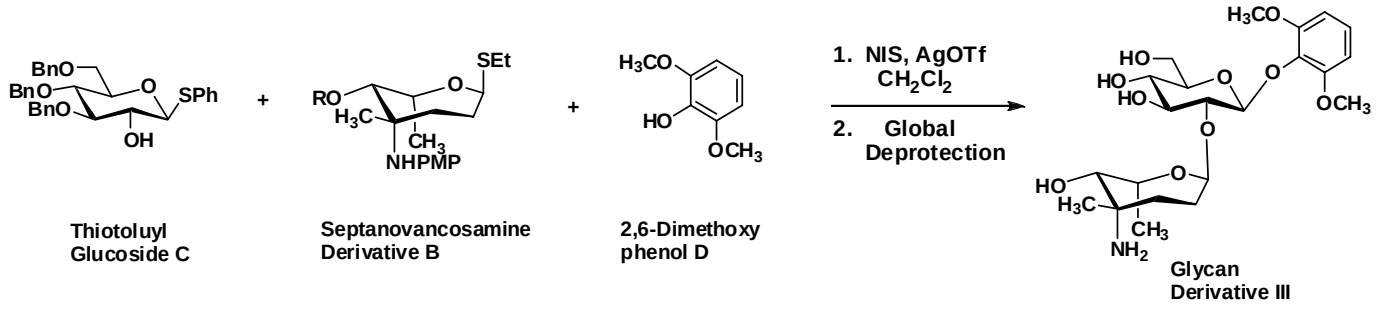
Scheme 5. Preparation of thiotoluyl glucoside **C** from tri-O-benzyl-D-glucal.



D. Preparation of glycan III

Recently, Fu and coworkers[30] have developed a one-pot method for the synthesis of glycans. We plan to employ this method for the synthesis of septanovancosamine glycan derivative **III**. Activation of septanovancosamine derivative **B** using *N*-iodosuccinamide in the presence of silver triflate, followed by addition of thiotoluyl glucoside **C** and 2,6-dimethoxyphenol **D** gives the protected version of glycan **III** (not shown). Global deprotection provides glycan derivative **III**.

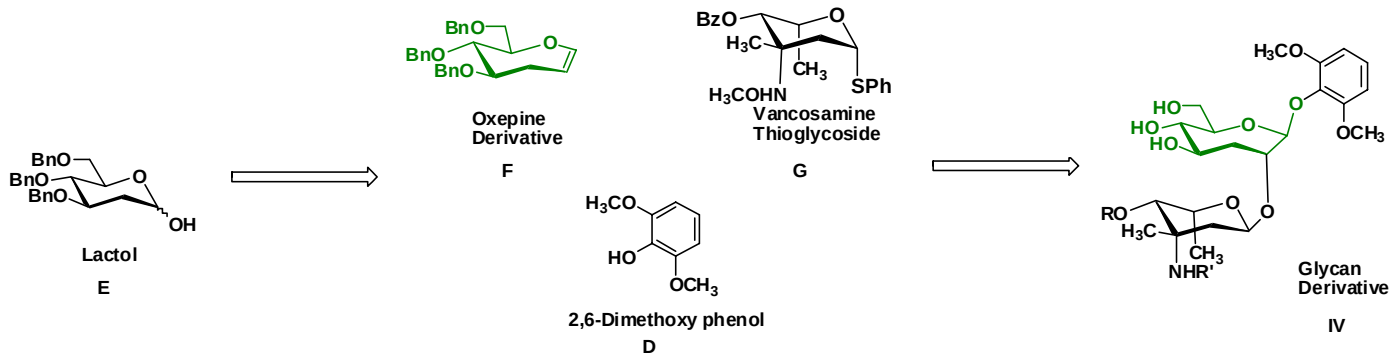
Scheme 6. Preparation of glycan terminating in a septanose residue.



5.3 Preparation of a Glycan Containing a Glucose-Based Septanose Residue

The retrosynthetic analysis of the glycan containing a glucose-based septanose residue is shown in Scheme 7 below. Homologation of 3,4,6 tri-*O*-benzyl-2-deoxy-D-gluco lactol **A** gives oxepine **B**. Reaction of oxepine **B** with 2,6-dimethyoxyphenol **D**, followed by reaction with vancosamine donor **C** and global deprotection provides access to glycan derivative **IV**.

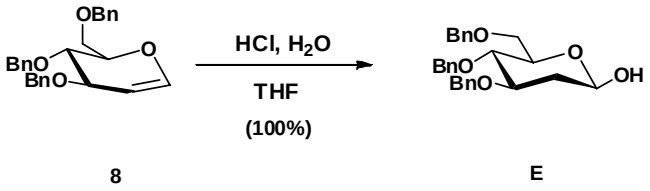
Scheme 7. Retrosynthetic analysis of vancomycin glycan derivative **IV**.



A. Preparation of lactol E

The preparation of lactol **E** is shown in Scheme 8. Hydrolysis of tri-*O*-benzyl-D-glucal with hydrochloric acid in THF provides lactol **E**.

Scheme 8. Preparation of lactol **E** from tri-O-benzyl-D-glucal.

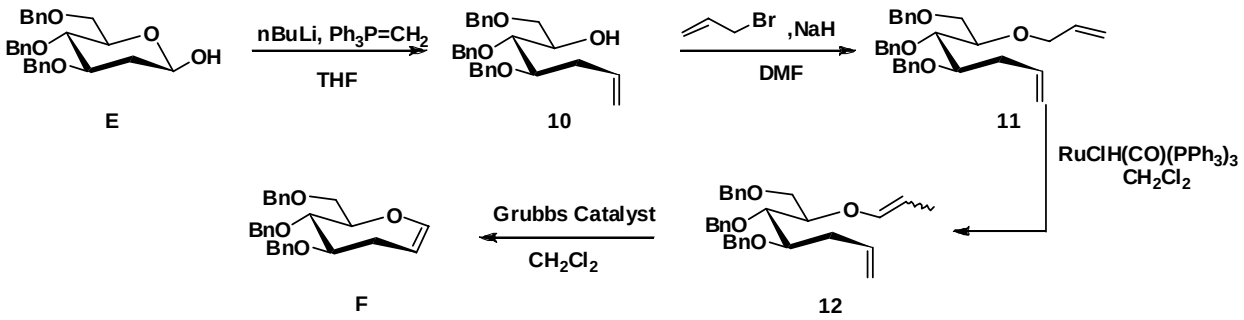


8 **E**

B. Preparation of oxepine F

Oxepine **F** can be prepared from lactol **E** via the four step sequence shown below in Scheme 9. Wittig olefination of lactol **E** to give alcohol **10**, followed by allylation of the free alcohol provides diene **11**. Ruthenium catalyzed migration of the allyl bond generates propenyl ether **12**.[31] Ring closing metathesis of propenyl ether **12** using Grubbs catalyst gives the corresponding oxepine **F**.

Scheme 9. Preparation of oxepine **F** from lactol **E.**

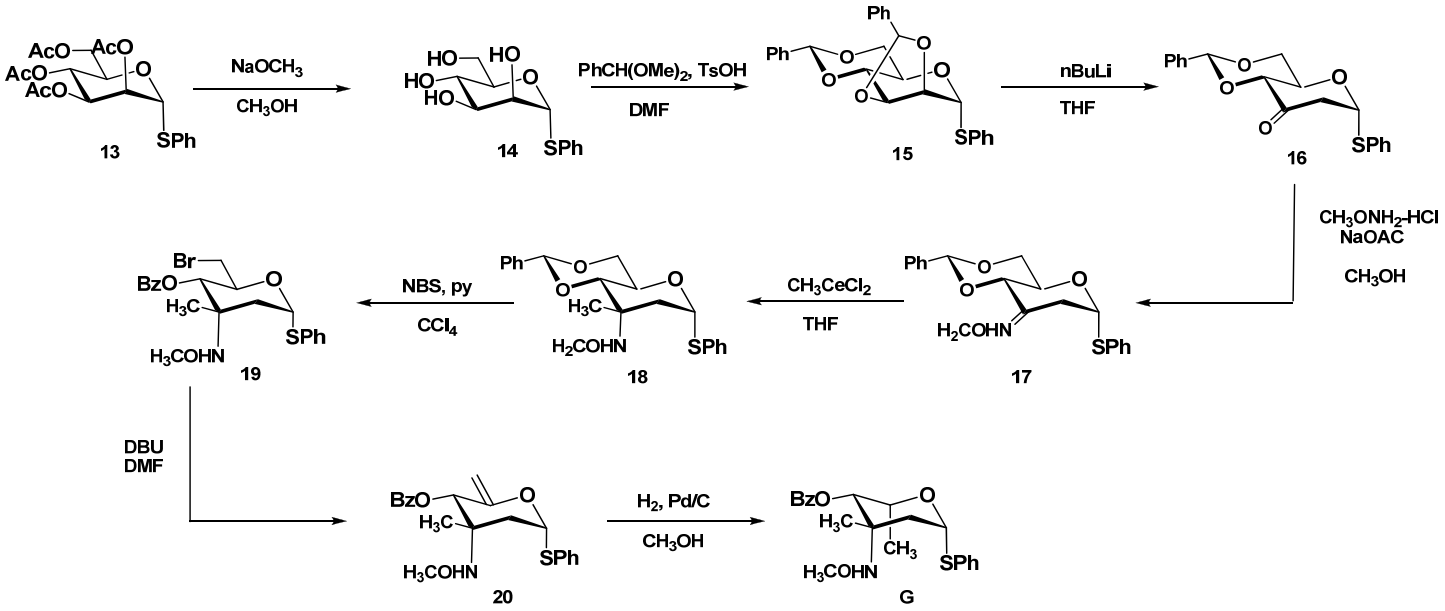


E **10** **11** **12** **F**

C. Preparation of vancosamine thioglycoside derivative G

Preparation of vancosamine derivative **G** is inspired by work published by Hsu and coworkers[32] and is shown in Scheme 10 below. The synthesis of begins with the deacetylation of commercially available 2,3,4,6-tetra-*O*-acetyl-D-thiophenyl-mannopyranoside **13** using sodium methoxide in methanol to give **14**. Reaction of **14** with benzaldehyde dimethylacetal in the presence of para-toluenesulfonic acid gives dibenzylidene deriviatve **15**. Production of the 2-deoxy-thioglycoside **16** using *n*butyllithium, followed the addition of methoxyamine hydrochloride in the presence of sodium acetate and methanol provides oximine **17**. Methylation of oximine **17** using methyl cerium chloride to give **18**, followed by bromination using *N*-bromosuccinamide in carbon tetrachloride gives the corresponding 6-bromo thioglycoside **19**. Base catalyzed elimination of **19** to give alkene **20**, followed by hydrogenolysis provides the target vancosamine thioglycoside derivative **G**.

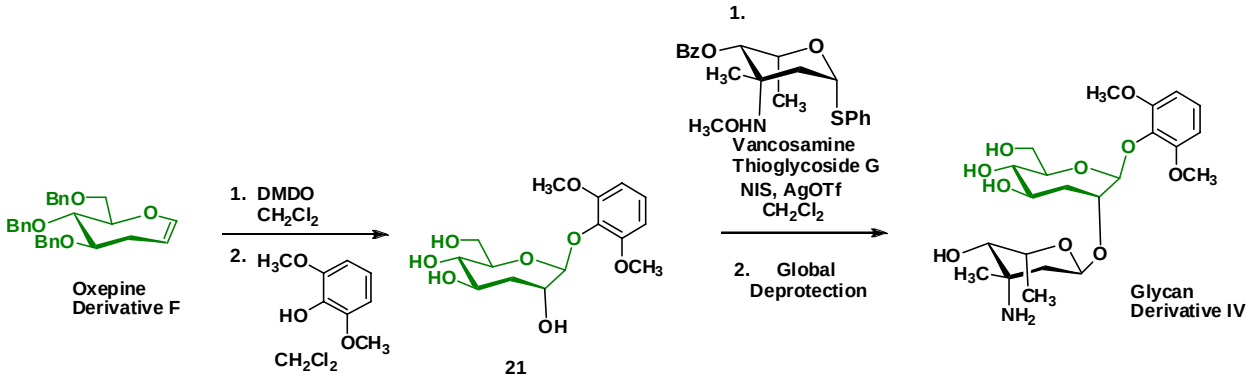
Scheme 10. Preparation of vancosamine thioglycoside **G** from diacetone mannose.



D. Preparation of glycan IV

The synthesis of glycan begins with the coupling to oxepine derivative **F** to 2,6-dimethoxyphenol as shown in Scheme 11. In situ DMDO epoxidation, followed by addition of 2,6-dimethoxyphenol gives **21**. Activation of vancosamine derivative **G** using *N*-iodosuccinamide followed by addition of **21** and global deprotection provides the corresponding glycan **IV**.

Scheme 11. Preparation of glycan terminating in a septanose residue.



5.4 Characterization of Glycans III and IV Using Nuclear Magnetic Resonance, Mass Spectroscopy, and Molecular Modeling Experiments

Conformations of mono- and oligosaccharides play a key role in both the biological functions and applications of these molecules. Like peptides, nucleotides, and their respective biopolymers, monosaccharides and oligosaccharides can adopt three-dimensional conformations that may be relatively fixed or flexible in nature. Elucidation of the three-dimensional conformations of carbohydrates and carbohydrate analogs is imperative to understanding the chemistry of these systems. Therefore, a considerable amount of time and effort has been devoted to being able to accurately assign and further predict the shape of these molecules.

Currently, our understanding of the conformation of complex carbohydrates remains primitive by comparison with the structures of proteins and nucleic acids. Although this is the case, there is no obvious reason why success using NMR spectroscopy and molecular modeling cannot be duplicated for this challenging problem.

A. Nuclear magnetic resonance and mass spectroscopy studies

The intermediate and final products prepared in this study will be analyzed by NMR spectroscopy using the College's Bruker 500MHz NMR Spectrometer. Proton and carbon NMR spectra will be collected and, when needed, two-dimensional spectra will also be obtained. In the event that it becomes necessary to obtain higher field spectra, we currently have access to the 800MHz Bruker NMR spectrometer at Rensselaer Polytechnical Institute.

In order to corroborate the data obtained through NMR experiments, high resolution mass spectra will also be obtained. Since the College does not currently own an instrument capable of obtaining these spectra, samples will be sent to the analytical laboratory at the University of Illinois-Urbana Champaign for analysis. The College currently has an account with the University of Illinois and samples can be run for a nominal fee.

B. Molecular modeling experiments

Students in my research group will apply a host of computational methods in order to help rationalize the glycan-PBP2 binding interactions observed in this study. Computational studies will be conducted using Macro Model, Gaussian, and deMon-NMR software packages. Recent research on septanoses by Peczuh and Haddad[23] has revealed that these molecules tend to adopt predictable low energy twist chair conformations. We plan to use the computational protocols established by Peczuh and Haddad as a first pass to analyze the glycan' prepared in this study.

Macro Model will be used to conduct an initial pseudo Monte Carlo search for each of the glycans prepared in this study (Figure 13-**III** and **IV**), along with the natural vancomycin glycan (Figure 13) and the chlorobiphenyl glycan derivatives prepared by Kahne and coworkers (Figure 13-**I** and **II**). The structures generated from the initial Monte Carlo search will then be optimized with the AMBER force field using the dielectric constant of water. The dielectric constant of water is chosen to reflect the natural environment of the glycans in vivo.

Gaussian will be used to further optimize structures found within a designated range above the global minimum for each of the glycans in Figure 13. The number of structures optimized for each glycan will depend on the initial structures generated from the Monte Carlo search. Structures within the designated range will be optimized using the Minnesota Gaussian Solvation Model (MN-GSM) at the SM5.42/HF/6-31G* level using water as a solvent.

NMR coupling constants will be determined from the SM5.42/HF/6-31G* minimized structures using the deMon-NMR software package. The Perdew and Wang exchange and Perdew correlation function (IGLO III basis set) will be used for these studies. The data generated through these studies will be compared to the data obtained through NMR experiments to determine whether the protocols chosen agree with the data obtained through experiment. NMR data will be generated for the glycans prepared in this study. NMR data for the natural vancomycin glycan and chlorobiphenyl derivatives prepared by Kahne and coworkers have already been published and is available free of charge on the web.

Because there have been no reports of energy minimized structures incorporating both natural and unnatural carbohydrates, we are unable to predict how well the modeling experiments proposed here will work for our systems, and we expect that some force field development will probably be required to obtain computational and experimental data that agree. While I have several years experience with molecular modeling experiments, I have almost no experience developing force fields. Karl Kirschner, a visiting fellow at the Max Planck Institute in Germany and a former faculty member at Hamilton College, has agreed to assist me and my students with this aspect of the project. Karl has over ten years of experience developing modeling programs that can be used to understand complex carbohydrate systems. We are planning to collaborate on the design and development of parameters that can be used to reliably predict the structures of complex disaccharides such as the glycans proposed in this study.

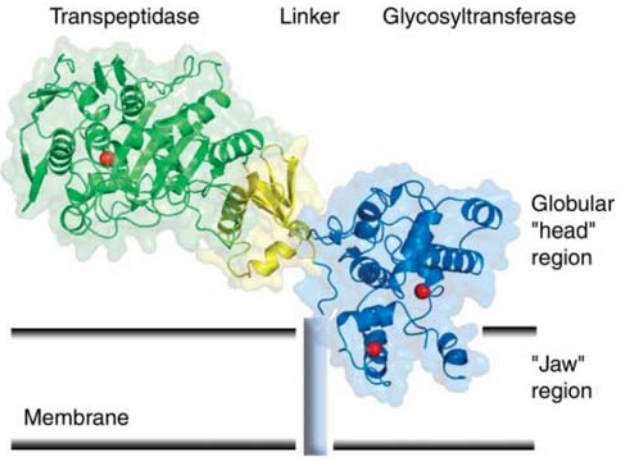
5.5 Biological Investigations Using Penicillin Binding Protein 2

A. Preparation of penicillin binding protein 2 (PBP2)

Penicillin binding protein 2 (PBP2) from *S. aureus* (shown in Figure 16) is chosen as a model protein for this study due to the extensive characterization of this protein and the fact that *S. aureus* is a key target for vancomycin.[16,25] PBP2 is a class A high molecular mass penicillin binding protein that catalyzes the conversion of Lipid II into mature peptidoglycan. PBP2 is composed of two catalytic units held together by a linker. The first catalytic unit encountered by Lipid II is known as the GT domain, or glycosyltransferase domain. This domain, highlighted in blue, is primarily α-helical in nature and is composed of a globular head region that is distal to the cell membrane and a jaw region that is believed to be partially embedded in the cell

membrane. The GT domain is responsible for the biosynthesis of higher order lipid precursors in which Lipid II units are successively linked by glycosylation. Higher order lipid precursors are then cross-linked to form mature peptidoglycan by the transpeptidase unit of PBP2, highlighted in green, which is also primarily α-helical in nature. Interestingly, the GT domain is structurally homologous to catalytic domain of bacteriophage λ lysozyme which hydrolyzes 1-4-β-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues of peptidoglycan.[25]

Figure 16. Penicillin binding protein 1b (PBP1b) from *S. aureus* (taken from Strynadka et. al.[25]). The glycosyltranferase (blue) is responsible for the synthesis of higher order lipid precursors from Lipid II. The transpeptidase (green) cross links successive lipid II units on higher order lipid precursors to form mature peptidoglycan precursors. The two catalytic units are held together by a linker (yellow).



My students and I will prepare histidine tagged multimodular PBP2 as described by Walker and coworkers.[33] Briefly, primers for the gene encoding E. coli PBP2 will be purchased from Invitrogen and the gene will be amplified by PCR using cDNA constructs. The gene will then be subcloned into the pET21b(+) vector and expressed in BL21(DE) *E. coli.* Both the vector and the *E. coli* strain are commercially available from Novagen. Bacteria cultures will be grown at 37C to an optical density of 0.6 at 600nm and protein expression will be induced by adding isopropyl-β-D-thiogalactoside. Samples will be extracted as described and purified using a Ni^{2+} column.

Since I have little experience with DNA amplification, cloning, and protein expression, I have enlisted the help of two individuals who have agreed to assist me and my students with this portion of the project. Professor of Biology and parasitologist Ashleigh Smythe has generously agreed to serve as a consultant to me and my students and will assist our research group with the production of the PBP2 gene. Professor of Chemistry, Didem Vardar-Ulu at Wellesley College, has also agreed to serve as a consultant and will assist me and my students with the cloning, protein expression, and purification stages of this part of the experiment.

B. Measuring PBP2 binding using isothermal titration calorimetry (ITC)

ITC is a thermodynamic technique that allows the study of the interactions of two species.[34] When two species interact, heat is either generated or absorbed and by measuring the interaction heats, binding constants (K_a), reaction stoichiometry (n), and thermodynamic parameters including enthalpy (ΔH) and entropy (ΔS) can be accurately determined. ITC has become an invaluable tool for understanding the processes that govern many biological interactions. Specifically, ITC has been used to study protein interactions with other proteins and biomolecules such as lipids and carbohydrates,[35] enzyme interactions with coenzymes, inhibitors, substrates, and pharmaceutical compounds,[36] antibody studies and antigen-antibody interactions.[37]

In an ITC experiment, the macromolecular solution, for example a protein, is placed into the sample cell. The reference cell contains the buffer solution used in the experiment (minus the macromolecule) and represents a control. Prior to the injection of the titrant (which includes the ligand to be associated) a baseline signal is generated using the reference cell. The time-dependent input of power required to maintain equal temperatures in the sample and reference cell upon addition of the ligand is measured. Upon titration heat is either taken up or evolved depending on whether the macromolecule-ligand association is endothermic or exothermic. If the reaction is exothermic in nature, the temperature in the sample cell will increase. If the reaction is endothermic in nature, the temperature in the sample cell will decrease. The feedback circuit will

compensate, increasing or decreasing power to the sample cell to maintain the temperature. The heat absorbed or evolved during a calorimetric titration is proportional to the fraction of bound ligand. As the ligand concentration increases, the macromolecule becomes saturated and less heat is evolved or absorbed on further addition of titrant.

My students and I will use the College's Microcal ITC to collect data for PBP2 in the presence of the derivatives prepared in this study. Since there is currently no protocol for PBP2 binding experiments using ITC, we plan to adapt the parameters that have been well established for lysozyme[38] to characterize PBP-glycan binding. Parameters for lysozyme were chosen as a starting point since recent research has shown that the lysozyme is structurally homologous to the glycosyltransferase domain of PBP2 that we are interested in studying.[25] The results will be used to determine which derivatives are more effective at binding PBP2 and whether or not any trends exist between the molecules evaluated in this study.

C. Mapping of PBP2 binding using saturation transfer difference nuclear magnetic resonance spectroscopy (STD-NMR)

In recent years STD-NMR has become an extremely useful tool for mapping protein-ligand interactions.[39] This technique is advantageous because it allows the binding component to be directly identified even when mixtures of compounds are present. It also allows for mapping of the binding interaction and is highly sensitive using as little as 1 nmol of protein.[40]

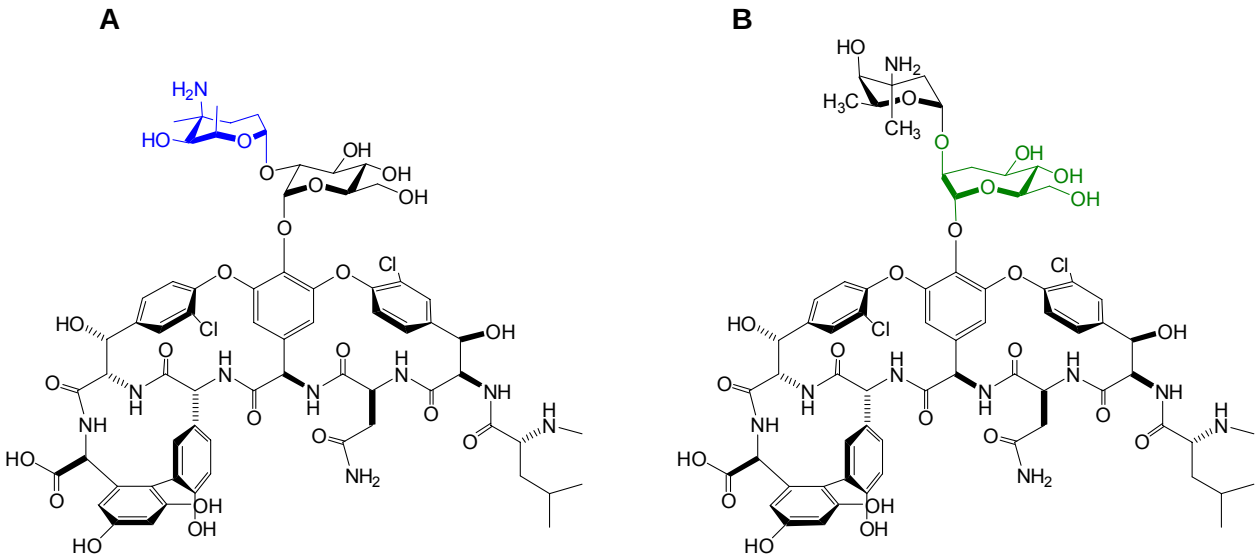
STD works by first irradiating a protein in the presence of a ligand (in excess concentration) at a frequency where no ligand signals resonate. This is known as the on resonance frequency and leads to a selective and efficient saturation of the entire protein by spin diffusion. Saturation is then transferred to the binding parts of the ligand by intermolecular saturation transfer. Protons in close contact with the protein receive the highest degree of saturation while protons with minimal or no contact receive little saturation. In essence, the degree of saturation of the individual protons reflects the proximity of these protons to the protein surface. The frequency is then set to a value different from either of the resonance frequencies of the protein or ligand. This is known as the off resonance frequency. Subtraction of the two spectra gives a spectrum representative of signals resulting from saturation transfer.

An STD-NMR protocol for the binding of moenomycin, a known inhibitor, to PBP1b has recently been established.[41] My students and I will use this protocol as the starting point to develop an STD NMR assay that will be used to corroborate the ITC data and to collect more specific information about the nature of the interaction between PBP2 and the glycans prepared in this study. STD-NMR data will be collected for PBP2 in the presence of the glycan derivatives prepared in order to determine which atoms play a role in binding to PBP2. In addition, competitive STD-NMR experiments will be collected for the glycan derivatives in the presence of moenomycin, a competitive inhibitor, in order to determine the nature of the binding event (competitive or noncompetitive).

5.6 Future Directions

Once the biological activity of the glycans is established, vancomycin derivatives incorporating the new glycans will be prepared. Examples of molecules that will be prepared are shown in Figure 17 below.

Figure 17. Vancomycin derivatives incorporating glycan III **A** and IV **B**.



While there are methods available that will allow us to couple the glycan to the aglycon scaffolding, one of the experiments we would like to attempt is to see if we can harness the power of the enzymes involved in synthesizing vancomycin to incorporate the glycans prepared in this study directly on to the aglycon scaffolding. Kahne and coworkers have recently shown that several of the enzymes involved in the synthesis of vancomycin are promiscuous and will bind and assist in the glycosylation of unnatural sugars.[42]

We would also like to determine the role that these glycans play in maintaining the conformational integrity of the aglycon. Mierke and coworkers conducted modeling studies using vancomycin and found that the orientation of the sugar substituent introduces a certain degree of mobility into the rigid framework of the cyclic peptide that allows it to adopt a suitable conformation for binding peptidoglycan precursors.[6] Studies performed with the glycans prepared in this study would provide more insight into this process.

Finally, we would like to determine the role of these derivatives in vivo. Ultimately we would like to determine if vancomycin derivatives with glycans that incorporate unnatural carbohydrates without long hydrophobic substituent's can inhibit bacterial cell wall biosynthesis by binding directly to glycosyltransferases. This would help solve the current debate in the literature about the role of the hydrophobic substituent in combating vancomycin-resistant strains of bacteria.

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[4] This figure was generated using the glycosyltransferase-inhibitor overlay by published on the web by Strynadka et. al. in "Bacterial Walls Come Tumbling Down." HHMI Press Release PR-HHMI-07-3, published on the web March 09, 2007.

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PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

● New ❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

❑ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ❍ No ❍

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ❍ No ❍

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

❍Yes ●No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance, where applicable, provide an explanation and attach below.

Explanation:

Attachments

CertificationExplanation_attDataGroup0

File Name	Mime Type

NOTIFICATION OF SCIENTIFIC REVIEW ACTION

Release Date: 11/05/2008

Snyder, Nicole
Hamilton College
198 College Hill Rd.
Clinton, NY 13323

Our Reference: 1 R15 AI082525-01 SBCA

The scientific merit review of your application is complete. As part of the initial review, reviewers were asked to provide written evaluations of each application and to identify those with the highest scientific merit. These are, customarily, applications that rank in the top half of applications under review. Only these applications are discussed at the meeting and assigned priority scores. Unscored applications are routinely neither considered at a second level by a national advisory council or board nor considered for funding.

Your application did not receive a score. Although it was not discussed at the meeting, it did receive full written reviews. It is important to note that the unscoring is not a rejection of your application and does not prevent future consideration of a resubmission.

All applicants are strongly advised to read the written critiques carefully to identify project strengths and weaknesses and to consult with the program official listed below to discuss options and to obtain advice. Your summary statement may be found in the Commons (https://commons.era.nih.gov/commons/).

PROGRAM CONTACT:
Zuoyu Xu
301-402-0643
xuzuoyu@mail.nih.gov

If you choose to resubmit, it is important to respond specifically to comments in the summary statement, as outlined in the instructions in the PHS 398 application kit (cf. http://grants1.nih.gov/grants/funding/phs398/phs398.html or http://grant1.nih.gov/grants/funding/424/index.htm).

Enclosure

cc: Business or institutional official of applicant organization

SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT:
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Release Date: 11/05/2008

Application Number: 1 R15 AI082525-01

Principal Investigator

SNYDER, NICOLE

Applicant Organization: HAMILTON COLLEGE

Review Group: SBCA
Synthetic and Biological Chemistry A Study Section

Meeting Date: 10/15/2008
Council: JAN 2009
Requested Start: 05/01/2009

RFA/PA: PA06-042
PCC: M30C BR
Dual PCC: P257MF
Dual IC(s): GM

Project Title: NIH-AREA: Understanding the Role of the Vancomycin Glycan in Binding Glycosyltran

SRG Action: **

Human Subjects: 10-No human subjects involved

Animal Subjects: 10-No live vertebrate animals involved for competing appl.

Project Year	Direct Costs Requested
1	150,000
TOTAL	150,000

**NOTE TO APPLICANT: As part of the initial scientific merit review process, reviewers were asked to identify those applications with the highest scientific merit, generally the top half of applications that they customarily review. At the study section meeting, those applications were discussed and assigned a priority score. All other applications, including this application, did not receive a score. Provided is a compilation of reviewers' comments prepared prior to the meeting, without significant modification or editing by NIH staff.

NEW INVESTIGATOR

1R15AI082525-01 Snyder, Nicole

NEW INVESTIGATOR

DESCRIPTION (provided by applicant): Vancomycin is a glycopeptide antibiotic used in the clinical setting for the treatment of methicillin- resistant Staphylococci and Enterococci. Vancomycin is composed of two bioactive components, a cyclic peptide component (aglycon) and a functionalized peripheral carbohydrate (glycan), that work together to inhibit the biosynthesis of peptidoglycan, a major component of the cell wall of gram-positive bacteria. Over the past twenty years, several vancomycin-resistant strains of bacteria have been detected. This has led researchers to search for new and more potent derivatives of vancomycin. Recent attempts aimed at reversing vancomycin resistance have focused on modifying the glycan component of vancomycin. The glycan is believed to play an important role in inhibiting bacterial cell wall biosynthesis by binding directly to the glycosyltransferases that convert peptidoglycan precursors into mature peptidoglycan, although the exact nature of this event is not well understood. In this proposal we present the design and development of two novel glycan derivatives of vancomycin that incorporate a combination of natural and unnatural carbohydrates. The proposed derivatives will be evaluated for biological activity against penicillin binding protein 2 (PBP2), a membrane bound glycosyltransferase isolated from S. aureus, using biophysical (isothermal titration calorimetry), spectroscopic (saturation transfer difference NMR spectroscopy), and computational techniques. The data collected from the studies outlined in this proposal will be used to develop a more specific understanding of the factors that influence the glycan-glycosyltransferase binding interaction. This information will ultimately be used to design more potent derivatives of vancomycin that can be used to combat resistant strains of gram-positive bacteria.

PUBLIC HEALTH RELEVANCE: Vancomycin is a glycopeptide antibiotic used in the clinical setting to treat methicillin resistant strains of Staphylococcus and Enterococcus. This proposal highlights the design, development, and biological evaluation of two novel derivatives of the vancomycin glycan. The proposed derivatives will be used to develop a better understanding of the factors that influence the glycan-glycosyltransferase binding interaction responsible for inhibiting bacterial cell wall biosynthesis. The data collected from the experiments outlined in this proposal will ultimately be used to design more potent derivatives of vancomycin that can be used to combat resistant strains of gram positive bacteria.

CRITIQUE 1:

Significance: Vancomycin-resistant bacteria are a serious health threat. The development of new, more potent derivatives of vancomycin that are effective against vancomycin-resistant strains of bacteria would be highly significant. The approach here is an exploration of the affect of changing the carbohydrate portion of vancomycin rather than the peptide portion (which is more common).

Approach: The PI proposes to synthesize two glycan analogs, both of which contain 7-membered ring sugars in place of the natural six-membered ring glycans (III and IV, page 35). The stated reason for choosing septanoses is that the proposed derivatives meet the space-filling and polar requirements for binding glycosyltransferases. The PI postulates that the extra carbon in the ring will serve to provide greater flexibility in the ring, allowing the residue to more readily access the half chair conformation that is required for binding to the glycosyltransferases. Kahne's analogs I and II serve as the inspiration for these compounds, although they are quite different.

While the seven-membered rings are an interesting idea, the syntheses of III and IV are lengthy, especially for undergraduates. The PI should re-evaluate her analog choice and substitute compounds that will serve analogously in the studies without requiring such intensive synthesis efforts. The PI does not have enough evidence that these will be good compounds to merit the performance of such a lengthy synthesis. The PI should also more thoroughly justify how the results in these studies, where

the peptide portion of vancomycin is entirely omitted, will be applicable for the full vancomycin derivative if use of that compound as a new antibiotic is the ultimate goal.

Penicillin binding protein 2 (PBP2) will be used for binding studies. This protein will be obtained from E. coli and purified. ITC and STD-NMR (saturation transfer difference) experiments are proposed. Both these characterization methods would be excellent training for undergraduates.

Innovation: The innovation of substituting a 7-membered ring for one of the residues in the sugar portion of vancomycin is high. However, more justification for why a septanose is likely to be an active compound should be provided, as the level of innovation is reduced without this information.

Investigators: The investigator is a new PI with an excellent publication record from her PhD at Connecticut (Peczuh). She started at Hamilton in 2007 after two years as a visiting professor at Wellesley College.

Environment: Hamilton is clearly an excellent undergraduate institution with excellent instrumentation and facilities.

Overall Evaluation: This is an interesting, well-written proposal from a new investigator. The basic idea of studying modified glycans to address the issue of vancomycin resistant bacteria is excellent. The idea should be tested first with simpler compounds, however. There is not enough evidence at this time to indicate that the seven membered rings will make outstanding analogs and are worth the lengthy synthetic expenditure that is required.

CRITIQUE 2:

Significance: Vancomycin is a glycopeptide antibiotic that has been effectively used for the treatment of infection by gram positive bacteria, including penicillin-resistant Staphylococcus and Enterococci strains. Nevertheless, the emergence of vancomycin resistant bacterial strains in recent years has urged the development of vancomycin analogs or other new types of antibiotics to combat the drug-resistant strains. This application proposes to make vancomycin glycan analogs that may possess activities in blocking the function of the bacterial transglycosylase essential for bacterial peptidoglycan biosynthesis.

Approach: This is a new R15 application with a focus on structural modification on vancomycin glycan structure to reverse its antibiotic activity against vancomycin resistant bacterial strains. Vancomycin is a glycopeptide that consists of a disaccharide moiety and a cyclic peptide moiety. The PI proposes to replace each of the monosaccharide components with a rare, unnatural 7-membered ring septanose structure. The PI hypothesizes that replacement with a conformationally more flexible sugar unit at the vancomycin glycan may enhance the binding of the derivative to the bacterial glycosyltransferase (the transglycosylase), thus leading to enhanced antibacterial activity against vancomycin resistant strains. While the new structures themselves look interesting, the hypothesis lacks structural and biological foundation. First, it was previously reported that the natural disaccharide moiety in vancomycin did not bind directly to transglycosylase (Science, 1999, 284, 507); second, for those glycan modified derivatives that showed antibacterial activities against vancomycin resistant strains, as reported by Nagarajan, Kahne, and co-workers, the introduction of a hydrophobic substituent on the sugar moiety was essential for the activity (J. Antibiot, 1993, 46, 1181; Science, 1999, 284, 507). These studies suggested that the modified carbohydrate derivatives exhibited their antibacterial activities by inhibiting the key transglycosylation steps in peptidoglycan biosynthesis. Possible mechanism of action includes interaction of the hydrophobic residue with bacterial membrane and proteins involved in the transglycosylation step. But no data so far support the hypothesis that the enzyme was the direct target; third, there was no clue that conformational change in the sugar moiety played any role in this process; and finally, the oligosaccharide recognition mode in the complex of moenomycin and the transglycosylase domain of PBP2 (reported by Strynadka's group, Science, 2007, 315, 1402) might not

serve as a basis to consider that the glycan in vancomycin binds to the transglycosylase. In fact, moenomycin can be viewed as a lipid II substrate mimic, but vancomycin exhibits its antibacterial activity in a different mechanism. Overall, the disaccharide moiety in vancomycin does not seem to mimic the glycan in moenomycin and/or peptidoglycan.

To make the designed carbohydrate analogs, compounds III and IV in Figure 13, the PI proposed a one-pot condensation of three components (B, C, D) to form the disaccharide derivative III (Scheme 1 and Scheme 6). This is risky and problematic. The PI did not discuss any possible outcome of the stereochemistry. In the two glycosylation steps, the stereo-selectivity of the glycosidic bond needs to pay particular attention, as no neighboring group assistance or other anomeric control strategy was applied. The PI should have realized this difficulty. A classic, step-wise coupling reactions should be the first choice. For the synthesis of building blocks A and B, racemic intermediates (e.g., 3-7) would be generated. It was not clear whether the PI would use the racemic compounds for the final synthesis. For the preparation of compound IV, the PI proposes to make three intermediates D, F, and G. The synthesis of intermediate F shown in Scheme 9 by Grubbs' RCM was a nice design. For the synthesis of intermediate G (Scheme 10), it was not clear whether the conversion of 17 to 18 would give a clear steric control. This also applies to the conversion of 20 to G. The PI should elaborate on these transformations. For the final assembly (Scheme 11), the stereochemistry should be discussed with a backup plan.

Once the target compounds are synthesized, the PI proposes to perform NMR conformational studies and molecular modeling. This aim seems straightforward. For the biological aspects, the PI proposes 1) to overproduce penicillin binding protein 2 (PBP2), 2) to measure PBP2 binding with the synthetic derivative by ITC, and 3) to map PBP2's binding by STD-NMR. Surprisingly, the PI did not clearly propose to test the antibacterial activity of the synthetic compounds first. The above proposed binding experiments seem to be merit only if the synthetic compounds exhibit antibacterial activity. In fact, even if some of the synthetic compounds show sort of antibacterial activity, it is still a big question whether the target of the compounds is PBP2. The overall research design and structure of the proposal need to be dramatically revised. It is highly speculative to consider PBP2 as the target for the modified glycans in Kahne's inhibitor. Instead, it would be more attractive if the PI focuses on modifying Kahne's inhibitor (must have a lipid moiety on!) and tries to study the relationships between structural modification and anti-bacterial activity. The work on stepwise synthesis of the inhibitors and analysis of the stereo-chemistry would be ideal for training undergraduates, but not the proposed one-pot synthesis.

Innovation: The incorporation of an unnatural seven-membered ring septanose structure in vancomycin glycan is an interesting idea. But the hypothesis that the sugar conformations somehow affect the binding of vancomycin to the glycosyltransferase (the transglycosylase) is only a speculation. In fact, we don't even know whether the vancomycin glycan actually interacts with the transglycosylase or not (Existing experimental data suggested that the enzyme itself was not the target of Kahne's inhibitor, although the inhibitor was implicated to interfere with the transglycosylation steps).

Investigators: Dr. Snyder received an appropriate training in carbohydrate chemistry. She did a nice work on the synthesis and conformational studies of unnatural, ring-expanded monosaccharide derivatives. She is qualified to direct the synthetic work proposed here.

Environment: Hamilton College has the basic facility to support the proposed study.

Overall Evaluation: This application proposes to make vancomycin glycan analogs that may show improved anti-bacterial activities. Hence the proposal addresses an important biomedical problem. However, the research design and approaches toward the goal are weak. The major concern is that the design compounds and the their potential bacterial target (the enzyme) may have no direct connection. The incorporation of an unnatural seven-membered ring septanose structure in the vancomycin glycan is an interesting idea, but the hypothesis that the conformations of vancomycin glycan somehow affect the binding of vancomycin to the glycosyltransferase (the transglycosylase) is just speculative, as the

existing experimental data suggest otherwise. Kahne and co-workers have clearly demonstrated that the hydrophobic modification on the sugar moiety was the key for the compounds of this class to gain the new anti-bacterial activities (the mechanism is still elusive). Unfortunately, this key element did not reflect in the PI's design. The synthetic part of the proposal also requires more sophisticated design and analysis of the stereochemistry of transformations such as glycosylation.

CRITIQUE 3:

Significance: Due to the emergence of vancomycin resistant bacterial strains there is an urgent need for the development of new vancomycin analogs. This proposal focuses on the carbohydrate portion of vancomycin by proposing to make six member ring analogues of the known disaccharide. Thus, the proposal addresses an important biomedical problem and plans to make novel structures not address by the studies of Kahne.

Approach: In this new application, the PI plans to build upon chemistry she helped to develop as a graduate student. During her Ph.D. she synthesized and studies several unnatural, ring-expanded monosaccharide. While I am sure the proposed to septanose disaccharide analogues will lead to interesting SAR-studies, the proposed synthesis and choice of specific targets was not compelling. As the PI most certainly knows from her own experience as a Ph.D. student, the glycosylation of the pyranones does not easily translate to the glycosylation chemistry of the septanose. Thus, some effort to further develop this chemistry to the septanoses would be required before a detail medicinal chemistry study can be undertaken.

Investigators: Dr. Snyder received an appropriate training in carbohydrate chemistry. She is qualified to direct the synthetic work proposed here.

Environment: Hamilton College has the basic facility to support the proposed study.

Overall Evaluation: This is a very adventurous proposal that has the potential to add to the SAR-studies associated with finding new antibiotics. Unfortunately, the proposal suffers from a lack of detail in terms of synthetic details and biological studies. A revised application would benefit greatly from a more detail review of the work from the Kahne and a focus on the issues associated with septanose glycosylation chemistry. That is to say, a proposal that convincingly described how several septanonse analogues of the Kahne disaccharides were to be made and how they were going to be tested would be well received by the committee.

Budget: The budget is reasonable.

MEETING ROSTER

Synthetic and Biological Chemistry A Study Section
Biological Chemistry and Macromolecular Biophysics Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
SBCA
October 15, 2008 - October 16, 2008

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

Alexander von Humboldt
Stiftung/Foundation

Antrag auf ein Forschungsstipendium in Deutschland für Bewerberinnen und Bewerber*) aus dem Ausland

Application for a Research Fellowship in Germany for Applicants from Abroad

Bitte füllen Sie das Formular maschinenschriftlich aus.
Please complete the form in typescript.

Name | name	Nicole Leigh Snyder

Antrag auf ein | Application for a

- ☐ Humboldt-Forschungsstipendium für Postdoktoranden[1]
  Humboldt Fellowship for Postdoctoral Researchers
- ☒ Humboldt-Forschungsstipendium für erfahrene Wissenschaftler[2]
  Humboldt Fellowship for Experienced Researchers
- ☐ Georg Forster-Forschungsstipendium für Postdoktoranden[1]
  Georg Forster Fellowship for Postdoctoral Researchers
- ☐ Georg Forster-Forschungsstipendium für erfahrene Wissenschaftler[2]
  Georg Forster Fellowship for Experienced Researchers

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Tick **one** programme only!

Bitte vor dem Ausfüllen unbedingt die Programminformation und **beigefügte Hinweise für eine vollständige Bewerbung** beachten (Seite 12 - 14)! | It is important to read the Programme Information and attached **Guidelines for completing the application** before filling in the form (pages 12 - 14)!

[1] Als Postdoktoranden gelten überdurchschnittlich qualifizierte Wissenschaftler, die am Anfang ihrer wissenschaftlichen Laufbahn stehen und ihre Promotion vor nicht mehr als vier Jahren abgeschlossen haben. | The term postdoctoral refers to highly-qualified researchers at the beginning of their academic careers who completed their doctorates less than four years ago.

[2] Als erfahrene Wissenschaftler gelten überdurchschnittlich qualifizierte Wissenschaftler mit einem klaren eigenständigen Profil, die ihre Promotion vor nicht mehr als zwölf Jahren abgeschlossen haben. | Experienced researchers are highly-qualified academics with a clearly defined academic profile who completed their doctorates less than twelve years ago.

*) Im Folgenden wird aus Gründen der sprachlichen Vereinfachung nur die männliche Form verwendet. Es sind jedoch stets Personen männlichen und weiblichen Geschlechts gleichermaßen gemeint.

1. Persönliche Angaben zum Bewerber | Applicant's personal details

Nachname surname	**Snyder**
Vorname(n) first name(s) / given name(s)	**Nicole**
Früherer Nachname former surname	**Snyder**
Geburtsdatum (TT.MM.JJJJ) date of birth (dd.mm.yyyy)	**13.07.1978**
Geschlecht gender	☐ männlich / male ☒ weiblich / female
Staatsangehörigkeit nationality	**United States of America**
Gegenwärtige berufl. Stellung present professional position	**Assistant Professor of Chemistry**
Höchster akademischer Grad highest academic degree	**Ph.D.**

2. Welche Adresse soll für die Korrespondenz benutzt werden? | Indicate to which address correspondence should be sent.

☒ Institutsadresse / address of the institute

☐ Privatadresse / private address

3. Institution, an der Sie tätig sind | Institution at which you are currently working

Universität / Institution university / institution	**Hamilton College**		
Institut department / institute	**Department of Chemistry**		
Straße, Postfach street, P.O. Box	**198 College Hill Road**		
Postleitzahl postal code	**13323**	Ort city / town	**Clinton**
Land country	**U.S.A.**	Telefonnummer phone number	**1-315-859-4742**
E-Mail-Adresse e-mail address	**nsnyder@hamilton.edu**		

4. Privatanschrift – nur falls Kontaktadresse | Private address – only if contact address

Straße, Postfach street, P.O. Box			
Postleitzahl postal code		Ort city / town	
Land country		Telefonnummer phone number	
E-Mail-Adresse e-mail address			

5. Letzte Institution im Ausland, falls Sie sich bereits in Deutschland aufhalten | Last institution abroad if you are already working in Germany

Universität / Institution university / institution			
Institut department / institute			
Straße, Postfach street, P.O. Box			
Postleitzahl postal code		Ort city / town	
Land country			

6. Gastgeber und Gastinstitut | Host and host institute

Titel / Nachname title / surname	**Seeberger**
Vorname(n) first name(s)	**Peter**
Universität / Institution university / institution	**Max-Plank-Institute of Colloids and Interfaces**
Institut department / institute	**Department of Biomolecular Systems**
Straße, Postfach street, P.O. Box	**Am Muhlenberg 1**
Postleitzahl postal code	**14476**
Ort city / town	**Potsdam-Golm**
Land country	**Germany**
Telefonnummer phone number	**+49(0)30-838-54004**
Fax fax	
E-Mail-Adresse e-mail address	**peter.seeberger@mpikg.mpg.de**

7. Zweiter Gastgeber und Gastinstitut, falls Sie Ihr Forschungsprojekt in Zusammenarbeit mit einem zweiten Gastgeber durchführen möchten | Second host and host institute if you intend to carry out your research project in cooperation with a second host

Titel / Nachname title / surname	
Vorname(n) first name(s)	
Universität / Institution university / institution	
Institut department / institute	
Straße, Postfach street, P.O. Box	
Postleitzahl postal code	
Ort city / town	
Land country	
Telefonnummer phone number	
Fax fax	
E-Mail-Adresse e-mail address	

7a. Geplanter Zeitraum und geplante Anzahl der Monate für den Aufenthalt am zweiten Institut, falls die Arbeiten an zwei Instituten durchgeführt werden sollen | Projected period and number of months to be spent at the second host institute if the research project is to be carried out at two host institutes

Von (TT.MM.JJJJ) from (dd.mm.yyyy)	**06.01.2010**
Bis (TT.MM.JJJJ) to (dd.mm.yyyy)	**31.07.2011**
Anzahl der Monate number of months	**14**

8. Hochschulausbildung / -abschlüsse (Ph.D., C.Sc., etc.; bitte landesübliche Bezeichnung angeben) | University education / degrees (please give names of degrees as customary in your country)

von (MM.JJJJ) from (mm.yyyy)	08.2000	bis (MM.JJJJ) to (mm.yyyy)	12.2005
Name der Hochschule name of institution	University of Connecticut		
Ort city	Storrs, Connecticut	Land country	U.S.A
Hauptstudien-Prüfungsfach major subject studied / examined	Chemistry	Abschluss degree	Ph.D.

von (MM.JJJJ) from (mm.yyyy)	08.1996	bis (MM.JJJJ) to (mm.yyyy)	05.2000
Name der Hochschule name of institution	Westminster College		
Ort city	New Wilmington, Pennsylvania	Land country	U.S.A.
Hauptstudien-Prüfungsfach major subject studied / examined	Chemistry	Abschluss degree	B.S.

von (MM.JJJJ) from (mm.yyyy)	08.1996	bis (MM.JJJJ) to (mm.yyyy)	05.2000
Name der Hochschule name of institution	Westminster College		
Ort city	New Wilmington, Pennsylvania	Land country	U.S.A.
Hauptstudien-Prüfungsfach major subject studied / examined	Biology	Abschluss degree	B.S.

von (MM.JJJJ) from (mm.yyyy)		bis (MM.JJJJ) to (mm.yyyy)	
Name der Hochschule name of institution			
Ort city		Land country	
Hauptstudien-Prüfungsfach major subject studied / examined		Abschluss degree	

9. Bereiten Sie sich gegenwärtig auf ein Examen vor (z. B. Ph.D.)? | Are you currently preparing for an examination or academic degree (e. g. Ph.D.)?

Erwartetes Datum (MM.JJJJ) expected date (mm.yyyy)		Name der Hochschule name of institution	
Ort city		Examen degree	
Hauptstudien-Prüfungsfach major subject studied / examined			

10. Akademischer / Beruflicher Werdegang inklusive Studien- und Forschungsaufenthalte im Ausland | Academic / Professional record including study and research stays abroad

von (MM.JJJJ) from (mm.yyyy)	07.2007	bis (MM.JJJJ) to (mm.yyyy)	Current
Stellung position	Assistant Professor of Chemistry		
Arbeitgeber employer	Hamilton College		
Ort city	Clinton, New York	Land country	U.S.A.

von (MM.JJJJ) from (mm.yyyy)	08.2005	bis (MM.JJJJ) to (mm.yyyy)	06.2007
Stellung position	Visiting Assistant Professor of Chemistry		
Arbeitgeber employer	Wellesley College		
Ort city	Wellesley, Massachusettes	Land country	U.S.A.

von (MM.JJJJ) from (mm.yyyy)	08.2000	bis (MM.JJJJ) to (mm.yyyy)	12.2005
Stellung position	Graduate Student		
Arbeitgeber employer	University of Connecticut		
Ort city	Storrs, Connecticut	Land country	U.S.A.

von (MM.JJJJ) from (mm.yyyy)	08.1996	bis (MM.JJJJ) to (mm.yyyy)	05.2000
Stellung position	Undergraduate Student		
Arbeitgeber employer	Westminster College		
Ort city	New Wilmington, Pennsylvania	Land country	U.S.A.

von (MM.JJJJ) from (mm.yyyy)		bis (MM.JJJJ) to (mm.yyyy)	
Stellung position			
Arbeitgeber employer			
Ort city		Land country	

von (MM.JJJJ) from (mm.yyyy)		bis (MM.JJJJ) to (mm.yyyy)	
Stellung position			
Arbeitgeber employer			
Ort city		Land country	

10a. Bitte hier Ihre **derzeitige Stellung** angeben | Please state **your current position**

von (MM.JJJJ) from (mm.yyyy)	07.2007	bis (MM.JJJJ) to (mm.yyyy)	Current
Stellung position	Assistant Professor		
Arbeitgeber employer	Hamilton College		
Ort city	Clinton, New York	Land country	U.S.A.

11. Welche Bedeutung hat der beantragte **Forschungsaufenthalt in Deutschland** für Ihre **weitere Karriere? |** What will be the impact of your **research stay in Germany** on your **future career**?

This fellowship will provide me with the opportunity to learn complex oligosaccharide synthesis, purification and characterization, as well as carbohydrate-based vaccine development from the premier research group in this field. I will use the skills I learn to expand my independent research program in carbohydrate chemistry at Hamilton College.

11a. Weitere berufliche Pläne oder Karriereziele nach Ihrem Forschungsaufenthalt **| Future professional plans** or career goals following your stay

Weitere Pläne future plans	Professor of Chemistry; Hamilton College; Clinton, NY
Land country	U.S.A.

12. Haben Sie Ihre **wissenschaftliche Laufbahn** nach der Promotion **unterbrochen** (z.B. Erziehungszeiten)? **| Did you interrupt your academic career** after your Ph.D. (e.g. due to parental leave)?

Im Falle einer Unterbrechung tragen Sie im nachfolgenden Textfeld bitte den Zeitraum (von TT.MM.JJJJ bis TT.MM.JJJJ) und den Grund für diese ein.
In case of an interruption please fill in the period (from dd.mm.yyyy to dd.mm.yyyy) and the reason of it.

Bei Erziehungszeiten Geburtsdaten der Kinder (TT.MM.JJJJ) **|** In case of parental leave children's dates of birth (dd.mm.yyyy)

Wissenschaftl. Profil und Forschungsprojekt | Academic profile and research project

13. Forschungsgebiet | Field of research

Wiss. Fachgebiet research area	Organic Chemistry	Fachgebietscode[3] code of research area	2F04
ggf. 2. wiss. Fachgebiet 2nd research area if applicable	Pharmaceutical Chemistry	Fachgebietscode[3] code of research area	2F0902

14. Angaben zur **Dissertation** und zum **Abschluss der Promotion** | Details of **doctoral thesis** and **doctoral degree**

Thema der Doktorarbeit topic of thesis *(for Ph.D., C.Sc.,etc.)*	New Perspectives on the Synthesis and Function of Septanose Carbohydrates		
Datum der Promotion (TT.MM.JJ) date of doc. degree (dd.mm.yy)	18.12.05	ggf. Bewertung der Promotion grade obtained if applicable	

15. **Beantragte Förderzeit** für den Aufenthalt am Gastinstitut | **Fellowship period** applied for at the host institute

Postdoktoranden | Postdoctoral researchers

von (MM.JJJJ) from (mm.yyyy)		bis (MM.JJJJ) to (mm.yyyy)		Anzahl der Monate | number of months	

Erfahrene Wissenschaftler | Experienced researchers

Zeitraum 1 | period 1		Zeitraum 2 | period 2		Zeitraum 3 | period 3		Anzahl der Monate | number of months
von (MM.JJJJ) from (mm.yyyy)	06.2010	von (MM.JJJJ) from (mm.yyyy)		von (MM.JJJJ) from (mm.yyyy)		
bis (MM.JJJJ) to (mm.yyyy)	08.2011	bis (MM.JJJJ) to (mm.yyyy)		bis (MM.JJJJ) to (mm.yyyy)		14

16. Angaben zum **Forschungsprojekt** am Gastinstitut | Details of **research project** at the host institute

Arbeitstitel Ihres Forschungsvorhabens | Working title of proposed research project

The Synthesis and Biological Evaluation of A2G2F: A Novel Glioblastoma Multiforme (GBM)-Associated N-Linked Glycan with Potential Therapeutic Applications

Schlüsselbegriffe[4] (bis zu fünf) zur Definition Ihres speziellen Forschungsthemas am Gastinstitut | Keywords[4] (up to five) to define your special research topic at the host institute

Schlüsselwörter keywords	synthesis, carbohydrate, vaccine, glioblastoma, tumor

Kurze inhaltliche Zusammenfassung (max. 20 Zeilen) des geplanten Forschungsvorhabens (ausführliche Darstellung bitte separat beifügen) | brief summary (20 lines max.) of proposed research project (please give detailed description separately)

Glioblastoma multiforme (GBM) is the most aggressive type of primary brain tumor in humans and accounts for 52% of all primary brain tumor cases. The median survival time from the time of diagnosis is just over one year, with only one in every twenty patients surviving for more than three years. The most common methods for the treatment of GBM are palliative and include surgery, radiation therapy, and chemotherapy. More recently, a number of tumor-pulsed dendritic cell vaccines have been developed for the treatment of patients with GBM. While these vaccines have improved the prognosis for many patients with GBM, by nature they remain patient specific and are highly constrained by the time required to obtain peptide antigen from individual tumor cell lines. Therefore, a more effective and tumor specific, rather than patient specific therapeutic is needed to treat GBM. The research in this proposal will address this issue by focusing on the synthesis, characterization and evaluation of a conjugate vaccine based on the expression of A2G2F, an N-linked glycan found in high concentrations on the surface of GBM tumors. The proposed vaccine will be used to further an understanding of the role of A2G2F in GMB growth and development, and the knowledge obtained through the experiments outlined in this proposal will serve as a foundation for the rational design of new carbohydrate-based therapeutics for the treatment of GBM.

[3] Fachgebietscode entsprechend Fachgebietskatalog der Humboldt-Stiftung (s. Website) | Code of research area according to the Humboldt Foundation's research area index (see website)

[4] Die Schlüsselbegriffe zu Ihrem Forschungsthema dienen der Bestimmung fachlich geeigneter unabhängiger Gutachter. Bitte berücksichtigen Sie dies bei der Begriffsauswahl. | The key words on your research topic help to identify suitable specialist reviewers. Please take this into consideration when choosing them.

Fortsetzung | Continuation

17. Nur für Bewerber im **Georg Forster-Forschungsstipendienprogramm** | Only to be answered by applicants in the **Georg Forster Fellowship Programme**

Inwieweit sind die erwarteten Ergebnisse des beantragten Forschungsaufenthalts sowie die zu erlernenden Methoden und Techniken für die weitere Entwicklung Ihres Herkunftslandes relevant? | To what extent are the results of the research stay and the methods and techniques to be acquired expected to be relevant for the further development of your country?

Angaben zu Sprachkenntnissen des Bewerbers | Applicant's language proficiency

18. Sprachkenntnisse | Knowledge of languages

Schätzen Sie Ihre Lese-, Schreib-, Hör- und Sprechfähigkeit in folgenden Sprachen ein. | Assess your proficiency in reading, writing, listening comprehension and speaking in the following languages.

1 - herausragend | excellent, 2 - gut | good, 3 - mittelmäßig | fair, 4 - ausreichend | sufficient, 5 - ungenügend | insufficient

		Lesen reading	Schreiben writing	Verstehen listening	Sprechen speaking
Englisch | English		**1**	**1**	**1**	**1**
Deutsch | German		**4**	**4**	**4**	**4**
Andere | others	**Spanish**	**4**	**4**	**4**	**4**

19. Besuchte **Deutschkurse** | **German language courses** attended

Zeitraum (MM.JJ-MM.JJ) Period (mm.yy-mm.yy)	Name der Sprachschule name of language school	Ort place/city
08.97-05.98	Westminster College	New Wilmington, Pennsylvania
02.00-05.00	Westminster College	New Wilmington, Pennsylvania

Haben Sie eine Deutschprüfung abgelegt? Welche, wann, wo? | Have you taken a German language examination? If so, which, when and where?

As a graduate student at the University of Connecticut, I took and passed a technical German proficiency exam in May of the year 2003.

20. Sprachstipendium | Grant for an **intensive German language course**

Wünschen Sie **vor** Antritt des Forschungsstipendiums ein Stipendium für einen deutschen Intensiv-Sprachkurs? Die Teilnahme an den Sprachkursen wird empfohlen. Der Intensiv-Sprachkurs in Deutschland liegt unmittelbar vor dem Forschungsaufenthalt, der Beginn der Forschung am Gastinstitut verschiebt sich also um den Zeitraum, der für den Sprachkurs vorgesehen ist. Die Zeit des Sprachkurses wird nicht zur Stipendiendauer gerechnet. | Are you interested in a special grant for an intensive German language course before starting the research fellowship? Participation in such courses is recommended. Intensive language courses in Germany take place immediately prior to the research stay; this means that the commencement of the research work at the host institutes is postponed by the time envisaged for language studies. The time required for the language course is not included in the duration of the fellowship period.

☐ Ja (2 Monate) yes (2 months) ☐ Ja (4 Monate) yes (4 months) ☒ Nein no

Wenn möglich beginnend am (TT.MM.JJJJ)
If possible starting on (dd.mm.yyyy)

Zusätzliche Angaben zur Bewerbung | Additional general information

21. Haben Sie sich schon einmal um ein Forschungsstipendium der Alexander von Humboldt-Stiftung **beworben**? | **Have you previously applied for a Research Fellowship** from the Alexander von Humboldt Foundation?

☒ Nein / no ☐ Ja / yes Jahr / year _____

22. Bewerben Sie sich **zurzeit** auch bei einer **anderen Institution** um ein **Stipendium**? | Are you **applying** for a **fellowship** to **any other institution**?

☒ Nein / no ☐ Ja / yes

Wenn ja, bei welcher Institution? / If so, to which institution? .....

Bitte informieren Sie uns umgehend, falls die Entscheidung über Ihre Parallelbewerbung bei einer anderen Institution getroffen wird. Teilen Sie uns auch mit, wenn Sie sich nach Einreichung Ihrer Bewerbung bei der Humboldt-Stiftung noch bei einer anderen Institution um ein Stipendium bewerben. | Please inform us immediately if a decision is made on your application to any other institution. Please also inform us if you apply for a fellowship from any other institution after having submitted your application to the Humboldt Foundation.

23. Wie sind Sie auf das Forschungsstipendienprogramm der Alexander von Humboldt-Stiftung **aufmerksam geworden?** Mehrfachnennungen sind möglich. | **How did you find out about** the Alexander von Humboldt Foundation's **Fellowship Programmes**? Multiple answers are possible

☒ Gastgeber / host

☐ Betreuer der Doktorarbeit / doctoral supervisor

☒ Humboldtianer / Humboldt fellow

☒ Weitere Fachkollegen / colleagues

☐ Internetrecherche / internet search

☐ Artikel, Anzeige in Zeitschriften, Newslettern (in welcher Zeitschrift?) / articles, adverts in magazines, newsletters (in which?) .....

☐ Informationsvortrag, Beratung (durch welche Stelle?) / information talks, advisory service (given by?) .....

☐ Sonstige / other _____

24. Einverständniserklärung | Declaration of consent

Ich erkläre hiermit, dass alle Angaben richtig und vollständig sind, und dass ich gesundheitlich in der Lage bin, das geplante Forschungsvorhaben in Deutschland durchzuführen.

Ich bin damit einverstanden, dass meine persönlichen Daten gespeichert und im Falle der Stipendienverleihung Name, Vorname, akademischer Titel, Nationalität, Ort und Name der Institution, an der ich tätig bin, Ort und Name der Gastinstitution in Deutschland sowie die geplanten Förderzeiten, Auswahldatum, Fachgebiet und Schlüsselbegriffe zur Definition meines speziellen Forschungsthemas in Deutschland in Listen durch die Humboldt-Stiftung veröffentlicht werden, die auch der Bekanntgabe der persönlichen Daten an amtliche Stellen und Institutionen der Wissenschaftsförderung und -kooperation dienen.

I hereby declare that the above statements are correct and complete and that I am of sound health and not physically handicapped in a way that would prevent me from carrying out the envisaged research project in Germany.

The Humboldt Foundation has my permission to electronically store my personal data which are required for my research fellowship application and the fellowship stay. If a research fellowship is awarded, the following information may be included in lists published by the Humboldt-Foundation and passed on to German official authorities and institutions involved in scientific promotion and cooperation: name, first name, academic degree, nationality, place and name of the institution at which I am currently working, place and name of the host institution and the planned research period, decision date, field of research and key words to define my special field of research during my stay in Germany.

Datum / date .....

Unterschrift / signature .....

25. Name und Adresse des **Betreuers der Doktorarbeit** | Name and address of **doctoral supervisor** or **director of post-graduate studies**

Titel / Nachname title / surname	**Peczuh**		
Vorname(n) first name(s)	**Mark**		
Universität / Institution university / institution	**University of Connecticut**		
Ort city / town	**Storrs, Connecticut**	Land country	**U.S.A.**
E-Mail-Adresse e-mail address	**mark.peczuh@uconn.edu**		

26. Weitere **Referenzgutachter** | Additional **referees**

Titel / Nachname title / surname	**Rosenstein**		
Vorname(n) first name(s)	**Ian**		
Universität / Institution university / institution	**Hamilton College**		
Ort city / town	**Clinton, New York**	Land country	**U.S.A.**
E-Mail-Adresse e-mail address	**irosenst@hamilton.edu**		

Titel / Nachname title / surname	**Nolen**		
Vorname(n) first name(s)	**Ernest**		
Universität / Institution university / institution	**Colgate University**		
Ort city / town	**Hamilton, New York**	Land country	**U.S.A.**
E-Mail-Adresse e-mail address	**enolen@colgate.edu**		

Titel / Nachname title / surname	**Lowary**		
Vorname(n) first name(s)	**Todd**		
Universität / Institution university / institution	**University of Alberta**		
Ort city / town	**Edmonton, Alberta**	Land country	**Canada**
E-Mail-Adresse e-mail address	**todd.lowary@ualberta.edu**		

Titel / Nachname title / surname	**Bundle**		
Vorname(n) first name(s)	**David**		
Universität / Institution university / institution	**University of Alberta**		
Ort city / town	**Edmonton, Alberta**	Land country	**Canada**
E-Mail-Adresse e-mail address	**dave.bundle@ualberta.edu**		

Zusätzliche persönliche Angaben für den Fall einer Stipendienbewilligung | Additional personal information in case the application is approved

27. Weitere persönliche Angaben zum Bewerber | Applicant´s further personal details

Im Folgenden bitten wir Sie um weitere persönliche Angaben. Diese ermöglichen der Humboldt-Stiftung im Falle einer positiven Entscheidung, Ihnen die Verleihungsunterlagen so rasch wie möglich zuzustellen. Sie dienen außerdem dazu, die Voraussetzungen für Entscheidungen über Anträge für Krankenversicherungsbeihilfe, Familienzulage und die Finanzierung von Deutschkursen zu schaffen und die erforderlichen Haushaltsmittel frühzeitig zu planen. Zusätzlich wären wir Ihnen dankbar, wenn Sie uns bereits jetzt ein **Passfoto** zur Verfügung stellen würden, das wir für Ihre Humboldt-Ausweiskarte benötigen. Im Falle einer Ablehnung Ihres Stipendienantrages werden nachfolgende Daten nicht elektronisch erfasst. **Die Daten sind für die Auswahlentscheidung nicht relevant und werden dem zuständigen Ausschuss nicht vorgelegt.** | Below we would like to ask for some further personal details. In case of a positive decision, they will enable the Humboldt Foundation to send you the award documents as soon as possible. In addition, they will be used to create the necessary conditions to take decisions on applications for subsidy towards health insurance, family allowances and grants for German language courses and to budget the necessary financial means in advance. In addition, we would be grateful if you sent us already now a **passport photograph** which we need for your Humboldt identity card. If you are not awarded a Humboldt Research Fellowship, the following data will not be stored electronically. **These data is not relevant to the Selection Committee's decision and is not presented to the Committee.**

Geburtsland des Bewerbers applicant's country of birth	**U.S.A.**
Geburtsort des Bewerbers applicant's place of birth	**New Castle, Pennsylvania**
Familienstand marital status	**Married**
Nachname des Ehepartners surname of marital partner	**Lee**
Vorname(n) des Ehepartners first name(s) of marital partner	**Christopher**

Geburtsdaten von Kindern - nur falls nicht unter Nr. 12 angegeben (TT.MM.JJJJ) | Children's dates of birth - only if not completed under No. 12 (dd.mm.yyyy)

Research Summary: The Synthesis and Biological Evaluation of A2G2F: A Novel Glioblastoma Multiforme (GBM)-Associated *N*-Linked Glycan with Potential Therapeutic Applications

1.0 Introduction

Glioblastoma multiforme (GBM) is the most aggressive type of primary brain tumor in humans and accounts for 52% of all primary brain tumor cases. The median survival time from the time of diagnosis is just over one year, with only one in every twenty patients surviving for more than three years. The most common methods for the treatment of GBM are palliative and include surgery, radiation therapy, and chemotherapy. More recently, a number of tumor-pulsed dendritic cell vaccines have been developed for the treatment of patients with GBM. While these vaccines have improved the prognosis for many patients with GBM, by nature they remain patient specific and are highly constrained by the time required to obtain peptide antigen from individual tumor cell lines. Therefore, a more effective and tumor specific, rather than patient specific therapeutic is needed to treat GBM. The research in this proposal will address this issue by focusing on the synthesis, characterization and evaluation of a conjugate vaccine based on the expression of A2G2F, an *N*-linked glycan found in high concentrations on the surface of GBM tumors. The proposed vaccine will be used to further an understanding of the role of A2G2F in GMB growth and development, and the knowledge obtained through the experiments outlined in this proposal will serve as a foundation for the rational design of new carbohydrate-based therapeutics for the treatment of GBM. The uniting goals of the research project are:

1. To synthesize A2G2F using an automated synthetic approach.
2. To conjugate the *N*-linked glycan to keyhole limpet hemocyanin (KLH), a carrier protein.
3. To assess the ability of the conjugate vaccine prepared in this study to elicit an immune response.

The overall questions we are trying to address are: "What is the role of A2G2F in GBM growth and development?" and furthermore, "Can a conjugate vaccine based on the *N*-linked glycan A2G2F be used to induce an immune response against GMB?"

2.0 Background and Significance

2.1 Glioblastoma Multiforme Pathology and Immunology

Gliomas are aggressive primary brain tumors that account for the majority of all brain tumor cases.[1] There are four grades of glioblastoma: pilocytic astrocytoma (grade I), astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma multiforme or GBM (grade IV). GBM is the most aggressive form of glioblastoma and is characterized by the presence of pseudopalisading necrosis and hyperplasic blood vessels.[2] Patients with GBM often exhibit seizures, although other symptoms such as difficulties in speech, vision problems, and decreased sensation will often occur earlier but generally go unnoticed.[3] Patients afflicted with GBM usually die within two years of diagnosis.[4]

Over the past twenty years, two subsets of GBM associated antigens have been identified:[5] (i) those whose expression is restricted to transformed glial tissue in the brain, and (ii) those that are expressed in both tumor and normal tissues in the brain. GBM associated antigens are generally recognized by cytotoxic T-lymphocytes (CTL's), white blood cells that are responsible for eliminating antigen from the host's system. CTL's bind to host cells presenting tumor antigen bound to Class I MHC molecules on the host cells surface. Through a series of chemical reactions, CTL's release perforin and granulysin which activates the caspase cascade ultimately leading to apoptosis.

Several MHC class I protein and glycoprotein antigens, including tyrosine related protein 2 (TRP-2),[6] absent in melanoma 2 (AIM-2),[7] human epidermal growth factor receptor 2 (HER-2),[8] glycoprotein 100 (gp100),[8] melanoma antigen family 1 (MAGE-1),[8] interleukin 13 receptor α-2 (IL13Rα2),[6] sex determining region Y-box 2 (SOX2),[9] and epidermal growth factor receptor vIII (EGFRvIII)[6] have been identified on GBM tumors. Of these, only MAGE-1, SOX2 and EGFRvIII are specific to GBM tissue in the brain. In addition, a number of *N*-linked glycans, including A2G2F,[10] A3G3,[10] and $H_7N_3F_1$-690 (structural isomer 5)[11] have been identified as potential antigens. These *N*-linked glycans are generally found in higher concentrations on the surface of GBM tumor cells in comparison to normal

brain tissue. Because of the nature of the above described protein and carbohydrate antigens, they have become important targets for the development of therapeutics to prevent and treat GBM.

2.2 Current Treatments for Glioblastoma Multiforme

Multidisciplinary multimodal treatment approaches involving combinations of palliative and symptomatic therapy are the most common forms of treatment for GBM today. Surgery, guided by 5-aminolevulinic acid is the first stage of treatment for patients with GBM and can be used to remove over 98% of the tumor.[12] Whole brain and three dimensional conformal radiation therapies have also been used to reduce tumor size.[13] Chemotherapy is also used, often in conjunction with surgery and radiation. Temozolomide is the most common chemotherapeutic agent used due to limited systemic toxicity.[14]

Recently, immunotherapeutic procedures involving dendritic cell vaccines have been established. Dendritic cells pulsed with tumor antigens, specifically autologous antigen derived peptides or antigens present in autologous tumor cell lysates, have been shown to increase the median survival time of the patient. For example, Yu[15] and coworkers demonstrated that autologous tumor specific peptide-pulsed dendritic cells could be used to enhance cytotoxic T-lymphocyte responses resulting in the targeting and destruction of tumor cells. Yamanaka[16] and Yu[17] also showed that autologous tumor lysate consisting of undefined tumor-associated antigens could also be used to generate a similar immune response without requiring the time needed to extract and purify tumor specific peptides. More recently, Wu and coworkers[18] described a vaccine based on dendritic cells raised against autologous glioma cell lysate EGFRvIII which has been effective for the treatment of GBM through phase II clinical trials.

While dendritic cell vaccines have improved the prognosis for many patients with GBM there are several problems with this mode of therapy. First, these vaccinations are patient specific and are constrained by the time required to obtain antigen specific peptides. While using nonspecific antigens from the tumor lysates of GBM patients has decreased the overall time that it takes to prepare these vaccines, using nonspecific antigen also increases the risk of developing an uncontrolled autoimmune response. In the case of the EGFRvIII vaccine, while this vaccine has been highly effective, tumor cells obtained from patients with recurrent GBM no longer expressed EGFRvIII, rendering the vaccine useless for the continuous treatment of GBM. Finally, for all of these treatments, the personalized nature of these vaccines makes them relatively expensive and impractical for mainstream therapy. Therefore, a more effective and tumor specific, rather than patient specific vaccine is needed to treat GBM.

3.0 Design and Synthesis of a Carbohydrate-Based Conjugate Vaccine Targeted at GBM

In this study we propose to synthesize and evaluate a carbohydrate-based vaccine based on A2G2F, an α-1,6-fucosylated biantennary *N*-glycan structure present in increased levels in glioblastoma multiforme tumor and cell lines. The conjugate vaccine prepared in this study will be the first carbohydrate-based vaccine targeted at GBM, and will be used to further an understanding of role A2G2F plays in GMB growth and development with the goal of designing new carbohydrate-based therapeutics for the treatment of GBM.

In the first four months of the study period, we propose to synthesize A2G2F using an automated solid-phase synthetic approach. The glycan prepared in this part of the study will be subsequently conjugated to keyhole limpet hemocyanin (KLH) through known chemistry and formulated for vaccination. The specific question we are trying to address in this phase of the study is: ***Can the A2G2F glycan be synthesized via a fully automated, solid-phase synthetic approach?***

3.1 Design and Rationale

Carbohydrates have become important antigenic targets for vaccine development over the past thirty years.[19] In particular, *N*-linked glycans, which cover the outermost surface of cells and play key roles in cellular communication and cell to cell interaction, have become the rational choice for the design of glycoconjugate vaccines targeted at cancer. However, two key factors have limited development in this area. The first is the ability to structurally identify tumor specific *N*-linked glycans,

which are often complex and difficult to isolate. The second limitation is fast and reliable methods for the preparation of these glycans for incorporation into therapeutics. This proposal addresses the second issue using a glycan, A2G2F, for which the structure has already been established.

In 2005, Yamanaka and coworkers[10] identified A2G2F (Figure 1), a biantennary bigalactosylated fucosylated structure which was shown to be expressed in increased levels on glioblastoma multiforme tissue. A2G2F was found to be as high as 2.9% (content ratio) on GBM tumor cells in comparison to less than 0.1% for normal brain tissue, and was the only biantennary structure found at such proportionate levels on GBM tumor cells, suggesting that A2G2F may serve as a novel molecular marker and target for the treatment of GBM.

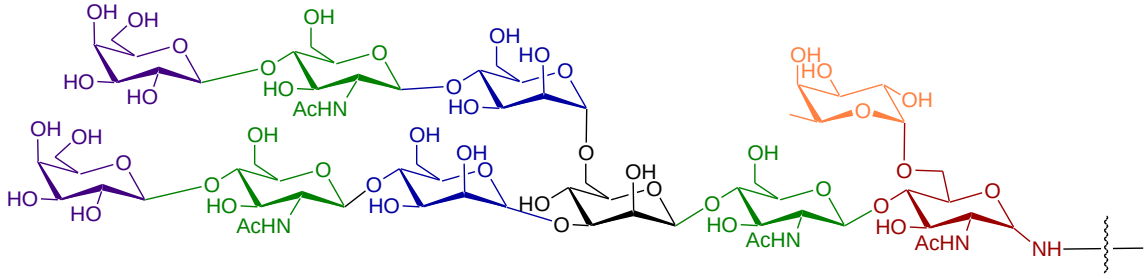


Figure 1. A2G2F—N-linked glycan found in high concentrations on the surface of GBM.

Interestingly, A2G2F is analogous in structure to the glycan component of prostate specific antigen (PSA) with the only significant difference being the α-1,6-fucosylation of the reducing *N*-acetlyglucosamine residue, suggesting that fucosylation may play a unique role in GMB growth and development. The overexpression of A2G2F has also recently been implicated in pulmonary metastasis,[20] further suggest that while A2G2F may serve as a specific marker for GMB tumors in the brain, the expression of A2G2F and structures analogous to A2G2F may play a more important role in general cancer development. Therefore, an expeditious and efficient route for the preparation of these glycan structures, such as the one outlined in this proposal, may prove useful in the study of other types of tumors.

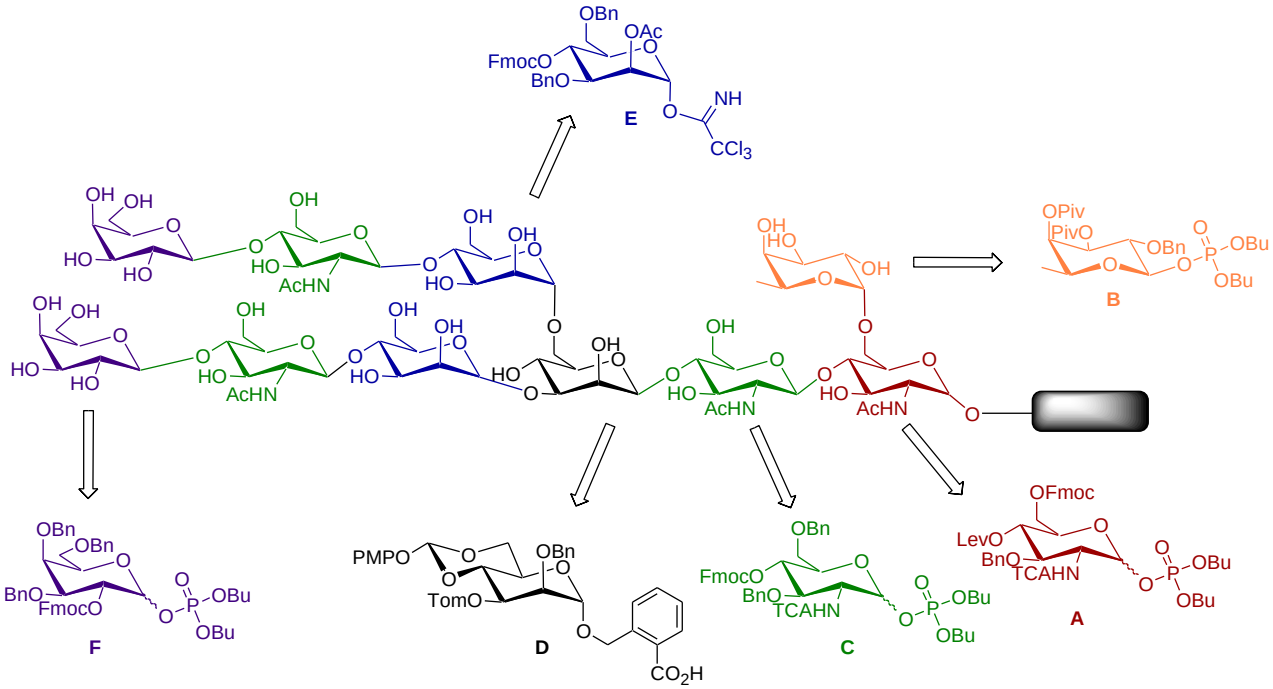
3.2 Automated Oligosaccharide Synthesis of A2G2F

The design of rapid and high yielding methods for the total synthesis of complex glycans has been a major limitation in the field of carbohydrate chemistry. Recently, Seeberger and coworkers have made extensive strides towards an automated solid-phase synthetic approach for the preparation of complex oligosaccharides. [21] For example, Seeberger and coworkers have recently demonstrated the synthesis of the prostate cancer antigen globo H hexasaccharide using an automated solid-phase oligosaccharide approach.[22] The synthesis of this complex glycan was accomplished in just over one day, a significant improvement over previously reported traditional syntheses.

Automated solid-phase oligosaccharide synthesis uses an automated synthesizer to make glycosidic linkages in a similar manner to the way peptide synthesizers are used to make peptide linkages. In general, selectively functionalized monosaccharide building blocks (donors) are added sequentially to a growing oligosaccharide chain (acceptor) linked to a solid support. Each glycosidic linkage is made by activating the appropriately functionalized monosaccharide building block in the presence of the acceptor to couple to the two molecules. After glycosylation, the growing oligosaccharide is washed, the appropriate protecting groups are removed to expose the next functional group for coupling, and the process is repeated until the desired oligosaccharide has been synthesized. The oligosaccharide is then removed from the solid support and further functionalized for use.

The synthesis of A2G2F has not been accomplished to date; however the glycan component of PSA and several glycan-PSA variants have been synthesized using traditional chemical approaches.[23] The synthesis proposed below is the first example of a fully automated synthetic approach to an α-1,6-fucosylated biantennary *N*-glycan and is only one of a handful of examples of highly branched glycans which have been synthesized using an automated oligosaccharide approach.[21]

A retrosynthesis for A2G2F is shown below in Scheme 1. Ideally, we will be able to use six monosaccharide building blocks (**A** through **F**) to access the glycan. These building blocks are accessible through commercially available materials and can be synthesized in no more than three to four steps per monosaccharide. The major challenges with this synthesis will focus on determining the reaction conditions required to make the various glycosidic linkages selectively and in high yield. While the *trans* coupling required to generate the linkages between most of the monosaccharides illustrated below has been established in other systems, the *cis* linkage required to install residue **D** remains a specific challenge for this particular system. Detailed synthetic steps required to prepare A2G2F are outlined in Schemes 1 through 4 below.



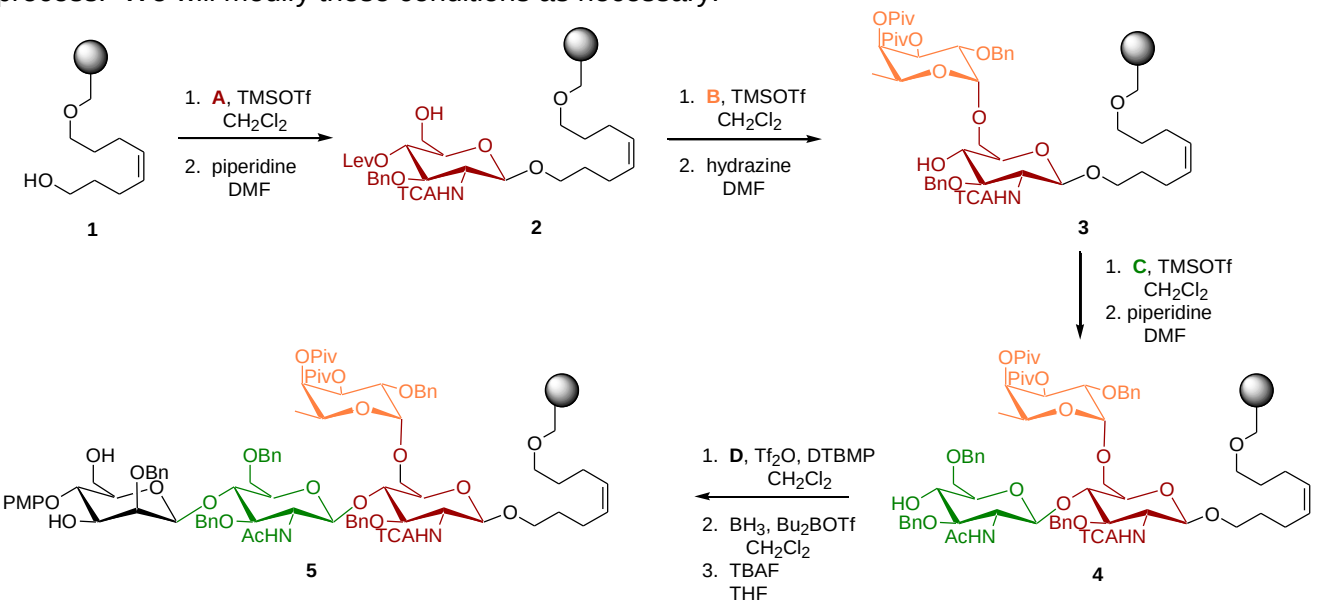
Scheme 1. Retrosynthetic analysis of A2G2F

The synthesis of A2G2F will begin with the preparation of core **5** as shown in Scheme 2. It is important to note that core **5**, minus the α-1,6-linked fucose residue, was previously synthesized by Seeberger and coworkers[24] using an automated approach. However, the revised synthesis described below uses methodology that has been developed since the original synthesis was published, and allows for the incorporation of the fucose residue.

Coupling of *N*-acetyl glucosamine donor **A** to a chloromethylated polystyrene resin using an octenediol linker **1** as described by Seeberger and coworkers is accomplished using trimethylsilyl trifluoromethanesulfonate (TMSOTf).[24] Removal of the 9-fluorenylmethoxycarbonyl (F-moc) protection group by the addition of piperidine gives acceptor **2**. Glycosylation of donor **B** using acceptor **2** in the presence of TMSOTf, followed by the removal of the levulinoyl (Lev) protection group using hydrazine gives acceptor **3.** Reaction of donor **C** and acceptor **3** in the presence of TMSOTf, followed by the removal of the Fmoc protection group gives acceptor **4.**

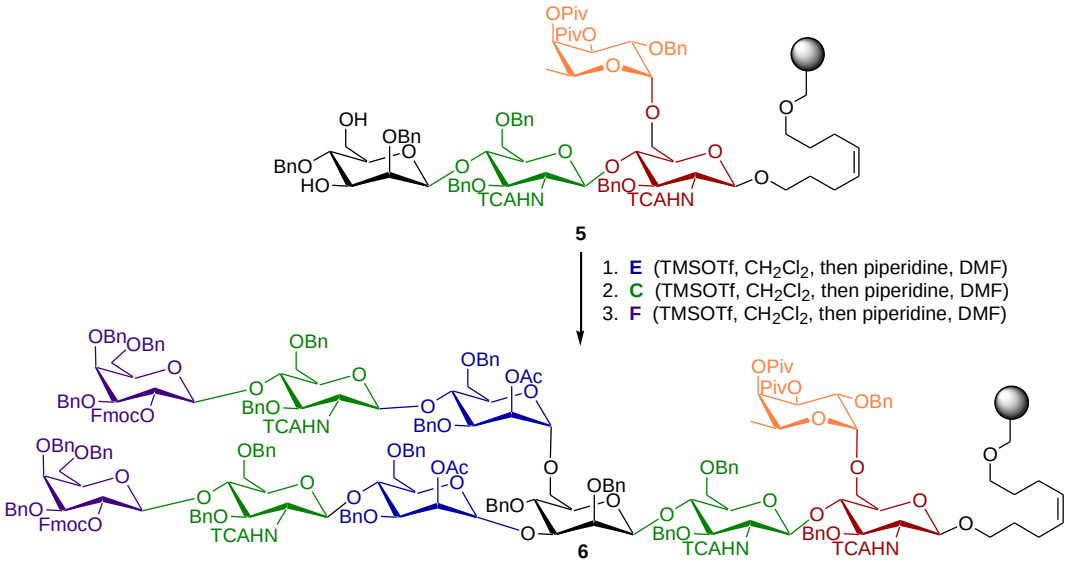
The final reaction to form the core of A2G2F involves reaction monosaccharide **D** with acceptor **4** in the presence of TMSOTf and di-*t*-butyl-4-methylpyridine (DTBMP), followed by the selective opening of the para-methoxyphenyl (PMP) acetal and removal of the [(triisopropylsilyl)oxy]methyl (Tom) protection group to give **5.** Monosaccharide **D** is a readily accessible derivative of the benzylidene protected monosaccharide recently reported by Seeberger and coworkers for the automated incorporation of β-mannosidic linkages into complex oligosaccharides.[25] While the deprotection of the Tom group using tetrabutylammonium floride (TBAF) has been established for automated oligosaccharide synthesis, the screening for a rapid and facile approach for the selective ring opening of the PMP acetal to give the free 6-OH for the next glycosylation reaction must be

conducted to determine the appropriate reaction conditions. We plan to begin with conditions established by Wei and coworkers[26] for the deprotection of thioglycosides using borane and dibutylboryl triflate since the reaction conditions required are mild and compatible with the automated process. We will modify these conditions as necessary.



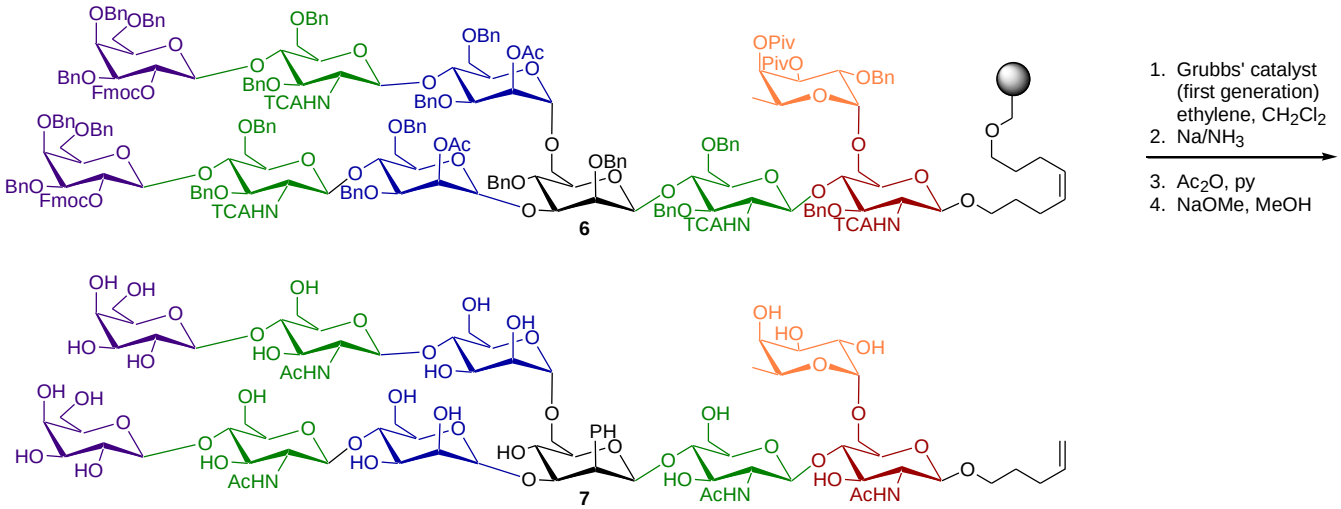
Scheme 2. Synthesis of the protected A2G2F glycan core.

The final A2G2F glycan is constructed using a parallel approach as shown in Scheme 3. Molecule **5** is reacted with donor **E** and TMSOTf, followed by the removal of the Fmoc protecting groups using piperidine to provide for the subsequent acceptor (not shown) which is then coupled to donor **C** and then donor **F** using the same conditions to give protected glycan **6**.



Scheme 3. Final stages of the protected A2G2F glycan.

The final steps in the synthesis of A2G2F are shown in Scheme 4 and include the removal of the solid support using Grubbs first generation catalyst,[27] followed by deprotection of the protecting groups using Birch conditions, peracetylation with acetic anhydride, and deprotection of the O-acetates to give the free glycan **7**.



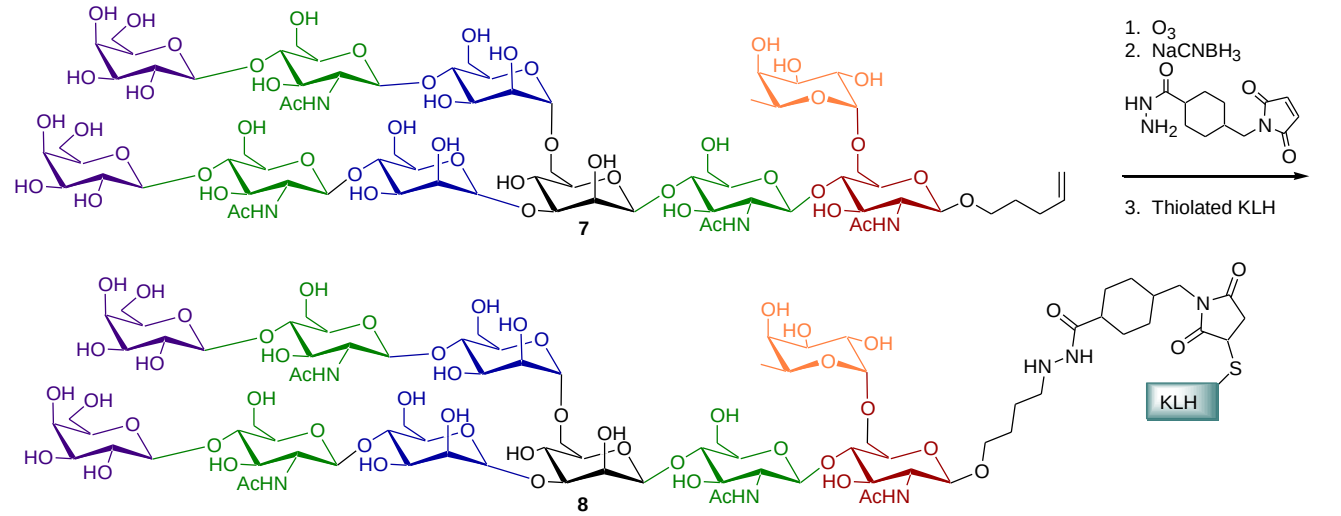
Scheme 4. Preparation of glycan **9** as a precursor to conjugation for vaccine development.

Reaction progress will be monitored by UV-Visible spectroscopy. Purification of the final glycan (**7**) will be accomplished by semipreparative HPLC using the methodology described by Seeberger and coworkers for the automated synthesis of the core pentasaccharide as a starting point.[24]

3.3 Conjugate Vaccine Development

Once the A2G2F oligosaccharide has been synthesized and purified, the next goal will be to conjugate the glycan to keyhole limpet hemocyanin (KLH). KLH, a highly potent T-cell dependent immunostimulatory protein and adjuvant, was chosen for this study due to the successful use of KLH as carrier protein for other GBM vaccines under development. For example, EGFRvIII-peptide vaccinations PEP-3-KLH/CDX-110 use KLH as a carrier protein and adjuvant.[28]

KLH conjugation is based on procedures previously established by Danishefsky and cowokers for PSA glycan conjugation to KLH.[29] Ozonolysis of the n-pentenyl group of **7** gives the corresponding aldehyde (not shown). Condensation of the aldehyde with 4-(4-*N*-maleimidomethyl)cyclohexane-1-carboxyl hydrazide (MMCCH) in the presence of sodium cyanoborohydride ($NaCNBH_3$) provides for the activated glycan which is then reacted with thiolated KLH to provide the target vaccine **8**.



Scheme 5. Preparation of the target vaccine.

4.0 Biological Studies of a Carbohydrate-Based Vaccine Targeted at Glioblastoma Multiforme

The second half of this proposal focuses on the biological evaluation of the A2G2F-KLH conjugate that will be prepared in the first half of this study. More specifically, we plan to determine whether the A2G2F-KLH conjugate vaccine can elicit an immune response. If an immune response is generated, we will determine the nature of the response and whether the response is effective enough prevent the development of GBM and/or treat patients afflicted with GBM. The question we are addressing in this phase of the proposal is: ***"Can a conjugate vaccine based on A2G2F be used to elicit an immune response against GB?"***

In order to study the carbohydrate-based conjugate vaccine prepared above, we plan to use the mouse glioma model recently developed by Verma and coworkers[30] at the Salk Institute. This model was chosen for its ability to produce tumors that exhibit the hallmarks of human gliomas including high density lesions, intratumoral hemorrhage, necrosis, nuclear pleomorphism, and high mitotic activity while mimicing the randomly occurring mutations that often occur with GBM.

In addition to determining whether the A2G2F-KLH conjugate vaccine can elicit an immune response, there are two major concerns that need to be addressed in these studies. The first concern is the "immunologically privileged" status of the central nervous system. While considerable research has been conducted to establish the ability of whole cell, protein, and peptide-based vaccines to mount an immune response against intracranial glioma cells, to the best of our knowledge there has been no research published on the ability of carbohydrate-based vaccines to elicit an immune response against these cells. Therefore, it is unknown whether the vaccine will need to be directly injected intracerebrally, or whether a simpler subcutaneous injection will be able to generate a favorable immune response. In order to address this issue, we have outlined studies below in which the vaccine is administered both intracerebrally and subcutaneously in the mice used in this study. These studies should allow us to determine which method is more effective at stimulating an immune response. ELISA and antibody-mediated cytotoxicity assays will be used to measure the nature of the immune response as a function of time.

The second concern is autoimmunity, especially since A2G2F has been found in high levels in human lung tissue. Recently, Okada and coworkers showed that CTLs raised *in vitro* against synthetic IL13Rα2, a protein found in high levels in glioma samples, but also in normal brain and testicular tissue, were able to specifically kill IL13Rα2 glioma cell lines without any observed autoimmunity.[31] Therefore, we are hopeful that we will be able to elicit a beneficial immune response specific to glioma cells that express the A2G2F glycan with similar results. In an effort to address this issue, we will clinically monitor the mice used in this study.

4.1 Murine Glioma Model

As previously noted, the mouse glioma model developed by Verma and coworkers[30] will be used for the immunological studies outlined below. This model has been used to successfully induce glioblastoma multiforme-like tumors in adult immunocompetent mice using Cre-l*oxP*-controlled lentiviral vectors expressing oncogenes. Briefly, GFAP-Cre transgenic mice will be crossed with *Tp53*$^{-/-}$ mice to produce the GFAP-Cre*Tp53*$^{+/-}$ strain required for these studies. Both murine strains are available commercially from The Jackson Laboratories. Tomo H-RasV12 and Tomo AKT lentiviral vectors will be prepared as described by Verma and coworkers.[32] Vectors will be mixed in a 1:1 ratio and will be stereotaxically injected (0.8uL) into the hippocampus of mice ages 8-16 weeks old depending on the type of study.

4.2 Survival Studies

The survival studies outlined below are modeled based on work by Lillehel and coworkers.[33] The goal of these studies is to determine the long range survival rates for mice that have been immunized with the A2G2F glycan conjugate prior to and after transduction with the Tomo H-RasV12/AKT lentiviral vectors. The assays for these studies are briefly outlined below.

Prophylactic Assay. Six separate groups of eight week old GFAP-Cre*Tp53*$^{+/-}$ mice will be immunized either intracerebrally or subcutaneously in the hind leg using (i) KLH in phosphate buffer saline (PBS) and (ii) the A2G2F glycan in PBS as a control and (iii) the A2G2F-KLH conjugate vaccine in PBS. One week after immunization, mice will be challenged by sterotaxic injection of the Tomo H-RasV12 and Tomo AKT lentiviral vectors into the hippocampus as described by Verma and coworkers.[30] Long term survival rates will be determined for mice in this group (see statistical analysis section) and will be compared to those animals in the therapeutic group.

Therapeutic Assay. Four separate groups of eight week old GFAP-Cre*Tp53*$^{+/-}$ mice will be challenged with the Tomo H-RasV12/AKT lentiviral vectors by the method described above for the mice in the prophylactic assay. Two sets of mice will be immunized with the A2G2F-KLH conjugate vaccine on the same day as the tumor challenge, one set intracerebrally and the other subcutaneously in the hind leg. The other two sets of mice will be immunized with the A2G2F-KLH conjugate vaccine seven days after the tumor challenge. Long term survival rates will be determined for mice in this group (see statistical analysis section) and will be compared to those animals in the prophylactic group.

Statistical Analysis. Survival curves will be estimated for each group using the Kaplan-Meier method[34] and will be compared using the Cox proportional hazards regression model.[35] Long term survival will be defined as greater than double the median survival time of KLH vaccinated mice.[28b] Statistical significance will be determined at the 0.05 level.

4.3 Serological Assays

An ELISA assay will be used to monitor antibody titers as a function of time in an effort to monitor the immune response prior to and after both lentiviral vector challenges and vaccinations. Serum samples will be collected from mice in both studies at regular intervals prior to and after challenge and immunization. Briefly, 96-well flat bottom plates will be pre-coated with approximately 50ng of A2G2F glycan. The amount of glycan was chosen based on Danishefky's work with PSA fragments which are analogous in structure to the A2G2F glycan.[23] Mouse serum antibody bound to A2G2F will be detected as described by Sampson and coworkers[28b] using secondary biotinylated goat antibodies specific form mouse IgG, IgG1 or IgG2a antibodies (available from Amersham Life Science) and observed with streptavidin-alkaline phosphatase and p-nitrophenyl phosphate at 405nm. If time permits, it would also be interesting to compare the specificity of the antibodies produced for A2G2F to the non-fucosylated glycan. This glycan would be readily accessible using the automated method previously described by Seeberger and cowokers.[24]

4.4 Cytotoxicity Assays

Cytotoxicity assays, based on work by Danishefsky and coworkers[29] on globo H hexasaccharide, will be used to determine the ability of the A2G2F-KLH conjugate vaccine to stimulate both antibody dependent cell mediated cytotoxicity. Briefly, Sera will be taken from each set of mice at regular intervals before and after immunization. Sera samples will be diluted two to four fold from their maximum antibody titer as determined by ELISA, and incubated with human U-87MG glioma cells (commercially available from ATCC). After incubation, cells will be washed, treated with biotinylated goat antibodies specific form mouse IgG, IgG1 or IgG2a antibodies and analyzed by flow cytometry for mean percent positive cells.

5.0 Timeline

The following timeline is based on fourteen months of support to begin in June 01, 2010 and end on July 31, 2011.

June 01, 2010 through September 30, 2010: Design and Synthesis of the A2G2F-KLH Conjugate Vaccine (Section 3.0)--Based on the technology available in the Seeberger laboratory and the protocols already in place for the design and synthesis of the monosaccharides needed for this study, we believe it will take approximately four months to synthesize the quantity of material

needed for the immunological studies outlined in the second half of this proposal. A conjugation expert, already in place at the MPI, will be able to assist with the KLH conjugation studies which may take up to an additional month. We plan to publish the novel synthesis of the A2G2F-KLH conjugate vaccine outlined in this proposal as a communication upon full characterization of the vaccine.

June 01, 2010 through July 21, 2011: Immunological Studies (Section 4.0)—The immunological studies outlined in this proposal will take almost one year to complete. First, several months will be required to produce the GFAP-Cre*Tp53*$^{+/-}$ mice. Once the mice are eight weeks old, experimentation can begin. Approximately four to five months are needed for tumor generation after transduction. Upon transduction, it is expected that the survival studies will require between six to ten months to complete based on the life span of the GFAP-Cre*Tp53*$^{+/-}$ strain. Therefore, we will begin coordinating with animal facilities personnel immediately upon my arrival at the MPI to ensure that mouse models are ready as the vaccine becomes available in order to generate data in a timely fashion. The results obtained from the studies outlined in the second half of this proposal will be the first immunological studies on a carbohydrate-based conjugate vaccine targeted at glioblastoma multiforme. We hope to publish the results of these studies as a full paper upon completion of the outlined experiments and any others deemed necessary as part of our immunological investigations.

6.0 Future Studies

A2G2F is only one of several glycans found on the surface of glioblastoma cells. As previously mentioned glycans A3G3,[10] and $H_7N_3F_1$-690 (structural isomer 5)11 have also been identified as potential antigens. The work in this proposal will set the stage for a more comprehensive study of the roles that these glycans play in tumor development. Ultimately, in collaboration with the Seeberger group, we would like to be able to study each glycan individually with the long term goal of developing multivalent, heterogeneous carbohydrate-based conjugate vaccines incorporating glycan structures that have been shown to stimulate an immune response against GBM.

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Professional Experience:

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-Principal investigator of a research team focusing on the design and synthesis of natural and unnatural carbohydrate systems that can be used to probe a number of key macromolecule-carbohydrate interactions.
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-Principal investigator of a research group that primarily focused on the production of a vancomycin derivative incorporating an unnatural carbohydrate at the vancosamine position.
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-Developed synthetic routes towards the production of carbohydrate-based oxepines (ring expanded glycals) for use as precursors in the synthesis of septanose monosaccharides.
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Awarded in Pittsburgh, Pennsylvania in May 2000

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Publications:

1. Peczuh, M.W.; Snyder, N.L. "Septanals: Ring Expanded Glycals for the Synthesis of Septanose Carbohydrates." *Tetrahedron Letters* **2003**, 44, 4057-4061.

2. Peczuh, M.W.; Snyder, N.L.; Fyvie, W.S. "Synthesis, Crystal Structure, and Reactivity of a D-xylose Based Oxepine." *Carbohydrate Research* **2004***, 339(6),* 1163-1171.

3. DeMatteo, M. P.; Snyder, N.L.; Morton, M.; Baldisseri, D.; Haddad, C.M.; Peczuh, M.W. "Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl α-D-Glycero-D-idoseptanoside and Methyl β-D-Glycero-D-guloseptanoside." *Journal of Organic Chemistry* **2005**, *70*, 24-38*.*

4. Castro, S.; Duff, M.; Snyder, N.L.; Morton, M.; Kumar, C.V.; Peczuh, M.W. "Recognition of Ring Expanded Carbohydrates by Concanavalin A: The Effect of Anomeric Configuration on Binding." *Organic Biomolecular Chemistry* **2005**, *3,* 3869-3872*.*

5. Snyder, N.L.; Peczuh, M.W. Haines, H.M. "Recent Developments in the Synthesis of Oxepines." *Tetrahedron* **2006***, 62,* 9301-9320.

6. Castro, S.; Cherney, E. C.; Snyder, N. L.; Peczuh, M. W. "Synthesis of Substituted Septanosyl-1,2,3-triazoles." *Carbohydrate Research* **2007**, *342(10)*, 1366-1372*.*

7. Markad, S.D; Xia, S.; Snyder, N.L.; Hadad, C. M.; Peczuh, M. W. "Stereoselectivity in the Epoxidation of Carbohydrate Based Oxepines." *Journal of Organic Chemistry* **2008***, 73(16)*, 6341-6354.

8. Ruppel, J. V.; Gauthier, T. J.; Perman, J.A.; Snyder, N.L.; Zhang, X.P. "Asymmetric Cobalt-Catalyzed Cyclopropanation with Succinimidyl Diazoacetate: General Synthesis of Optically Active Cyclopropyl Carboxamides." *Organic Letters* **2009**, *11,* 2273-2276.

9. Ruppel, J. V.; Fields, K. B.; Snyder, N. L.; Zhang, X. P. "Metalloporphyrin-Catalyzed Asymmetric Atom/Group Transfer Reactions." In *The Porphyrin Science Handbook*; Kadish, K.; Smith, K. M.; Guillard, R. Eds. (Invited Chapter—*Submitted*)

10. Fields, K. B.; Ruppel, J. V.; Snyder, N. L.; Zhang, X. P. "Porphyrin Functionalizaton via Palladium-Catalyzed Carbon–Heteroatom Cross-Coupling Reactions." In *The Porphyrin Science Handbook*; Kadish, K.; Smith, K.; Guillard, R. Eds. (Invited Chapter—*Submitted*)

The following above-mentioned papers have evolved from my doctoral thesis: 1, 2, 3, 4, 6, 7

The following above-mentioned papers have been submitted for review: 3, 7, 8

Service

GRE Review 2009

Hosted by Professors Snyder, Wile and Van Wynsberghe

All review sessions will be held from 7:00pm until 9:00pm on Sunday evenings in G042.

Review Date	Topic(s)	Host
September 13, 2009	General Chemistry Review	Snyder
September 20, 2009	Organic Chemistry	Snyder
September 27, 2009	Bioorganic Chemistry/Biochemistry	Snyder
October 04, 2009	Inorganic Chemistry	Wile
October 11, 2009	Physical Chemistry	Van Wynsberghe
October 18, 2009	Analytical Chemistry	Wile/Snyder
October 25, 2009	GRE Test	Snyder

*GRE Test will be graded and returned by Monday, October 26, 2009 so that you can use the last few weeks to review weak areas.

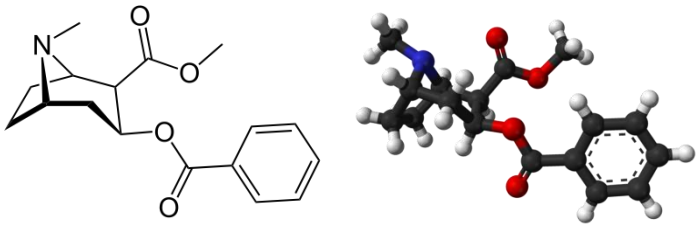
GCMS Analysis of Cocaine on Money

Science Saturdays

December 06, 2008

Background and Significance:

The lifetime of U.S. bill ranges from two to five years.[1] Recently, the analysis U.S. currency indicated that 79% of the money in general circulation is contaminated with at least 0.1 µg, and more than half with at least 1.0 µg of cocaine.[2] Currency analyzed from Canada[3] and the U.K[4] show similar statistics. Some scientists attribute the levels of cocaine on U.S. currency to direct contact with drugs, while others believe the contamination is due to indirect transfer, such as from contaminated bank counting machines.[5]



In this laboratory experiment we will use the scientific method to qualitatively determine whether currency in general circulation is contaminated with cocaine. First we will develop a working hypothesis, after which we will perform an experiment to extract the cocaine and will analyze the cocaine using a technique called gas-chromatography-mass spectroscopy or GCMS. Finally, we will analyze and interpret the data collected from the GCMS to draw conclusions confirm or reject our initial hypothesis.

Using the Scientific Method to Determine the Incidence of Cocaine on US Currency:

There are seven major steps to the scientific method. We will use these steps to test whether or not 79% of the currency in general circulation is contaminated with cocaine.

Step 1: Define the question

Step 2: Gather information and resources

Step 3: Form a hypothesis

Step 4: Perform an experiment and collect data (see procedure below).

Step 5: Analyze and interpret the data collected

Step 6: Publish the results

Step 7: Retest

Procedure:

The following procedure will be used to extract cocaine from circulating US currency:

1. Fold a $1, $5, $10, or $20 ten to twelve times to form an accordion and insert the currency into a 25mL vial.
2. Add 5mL of 0.1M HCl (be careful with the acid and wear gloves!), close and shake the vial for five minutes.
3. Add 2mL of 0.5M NH_3, (try not to breathe the ammonia solution) close the vial, and shake the vial for one minute.
4. Pass the mixture of solutions using a syringe through a C-18 SPE cartridge.
5. Fill the now empty syringe with 2mL of distilled water and pass the water through the C-18 SPE cartridge.
6. Fill the now empty syringe with 2mL of CH_2Cl_2 and pass the CH_2Cl_2 through the SPE cartridge into a clean GCMS vial.
7. Label your vial and provide your instructor with the labeled vial for analysis.

Discussion Questions:

1. What is the purpose of adding hydrochloric acid (HCl) to the currency?

2. What is the purpose of adding ammonia (NH_3) before the extraction?

3. What is the purpose of passing water through the C-18 SPE cartridge?

4. What is the purpose of passing CH_2Cl_2 through the C-18 SPE cartridge?

5. What can you conclude from your GCMS data?

6. Based on the class data, which bills contain more cocaine?

7. In what ways could you improve your experiment?

References:

1 U.S. Bureau of Engraving and Printing, “Money Facts” http://www.bep.treas.gov
2 Oyler, J., Darwin, W. D., Cone, E. J. *J. Anal. Toxicol.,* **1996**, *20*, 213.
3 Hudson, J. C. *J. Can. Soc. Forensic Sci.,* **1989**, *22*, 203.
4 Sleeman, R., Burton, F., Carter, J., Roberts, D. *Analyst* **1999**, *124*, 103.
5 Sleeman, R., Burton, F., Carter, J., Roberts, D, Hulmston, P. *Anal. Chem.*, **2000**, *72(11)*, 397A

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