New Synthetic Applications for Ring-Closing Metathesis and Cross-Metathesis Employing Well-Defined Ruthenium Alkylidenes

Thesis by

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For my family

"If all you got to live for is what you left behind,
Get yourself a powder charge and seal that silver mine.
Lost my boots in transit, baby, pile of smokin' leather,
I nailed a retread to my feet and prayed for better weather."

-The Grateful Dead

Acknowledgments

My first acknowledgement has to go to Professor Bob Grubbs. To be direct, coming to Caltech and joining Bob's group has been one of the best moves of my life, and the past 5 years have been tremendous. While I was told initially, "you can't go wrong joining Bob's group...", I was a little wary during those first few months here, where everything I had been taught as an undergrad at Oberlin seemed trivialized, and I wondered what I had got myself into. The complete scientific freedom and lack of structure was daunting, to say the least, but over time, I began to feel more confident in myself as a scientist, and I really began to appreciate the whole "hands-off" environment. Now, I would not have wanted it any other way. In view of other lab managing strategies, I think Bob has it down, and I really feel that I have been able to flourish as a chemist, and as a person, here in his laboratories. Through the bad times and the good, I feel Bob has always been supportive, and I thank him whole-heartedly for that. Being involved in a research project pretty much out of the confines of what was normally pursued in the Grubbs' group was especially a challenge at times, but Bob always pointed me in the right direction when I was getting off course, and had the amazing way of seeing long term goals when I was caught in the thick of minutia. Thanks for your insight, and sense of humor.

Next, I would like to thank the other members of my committee-Harry Gray, Barbara Imperiali, and Jackie Barton. I appreciate the support you have given me over the past 5 years. Harry deserves many thanks for recruiting me to come to Caltech when I was an undergrad at Oberlin: you were right, Harry, Caltech is a special place, and I made the right choice coming here. I would also like to thank Barbara specifically for helpful discussions about my project and about future paths. Having two women chemists on my Ph.D. committee has been especially inspirational to me, and I thank you for your enthusiasm and optimism for the future.

While I could list off many past Grubbs' group members to thank, Scott Miller deserves to be recognized first. When I came to Caltech, I really thought I was going to do polymer chemistry with Bob. After a few sessions talking to Scott, though, the next thing I knew I was wrists deep in peptide couplings and was trying to break new ground doing metathesis in the context of peptides. I can't thank Scott enough for persuading me to work with him on this project, a project which has kept me excited up to this very day. Scott's hard work in lab, scientific rigor, and amazing knowledge of chemistry really impressed me and shaped me during my "formative" years. When I was overwhelmed, he helped me keep everything in perspective, and was always there for a good pep talk coupled with a strong cup of coffee. His support, even from afar, has meant a tremendous amount to me. Thanks Scott, for all your help, in and out of lab.

Next, I want to thank all of the great post-docs (or equivalents) Bob has had in the group during my time here, especially, Cassandra Frasier, Bernhard Mohr, Geoff Coates, Osamu Fujimura, and Peter Schwab. The hard work and creativity of this bunch was a real inspiration to me when I first showed up on the scene. Cassandra and Bernie deserve special thanks for taking me "under their wings" when I was just starting out in lab. Their calmness and even-keeled approach to lab work kept me sane during some stressed out points, and they were always able to show me that there is more out there in the world other than chemistry, Cassandra has my utmost respect for really getting her career started at Virginia, and I look forward to having her as a mentor when I get my career started, wherever or whatever that might be. I also thank Cassandra for being a much needed woman friend when our numbers were low around lab.

As for other women friends, I want to send some thank yous to Mary Gin, Amy Giardello, and Melanie Sanford for being a great bunch to hang out with, either at lunch on Wednesdays, or elsewhere. Coming from an undergrad experience where feminism was the norm, and women were about 51% of the population, being in Southern California at a male-dominated technical institute was a big change. I have come to grips

with it now, but having a good safety net of women who feel the same way has meant a lot to me. Thanks for your openness, kindness, and humor.

I have to single out Dan O'Leary at this point to say "thank you." Dan came to the group on a sabbatical last year and started up work on my bench. Little did I know, that we would start working together on the cross-metathesis project, a project that has been a blast and luckily has kept us in contact even after he stopped work here. It was great to work along side someone so jazzed about chemistry. His enthusiasm was truly infectious, and we had to keep each other in check when the project started spiraling out of control. I also want to thank him for sharing his NMR expertise. Dan has an amazing way of getting people "connected," and I really want to thank him for his continued support and insight.

The Grubbs' group seems to have always been a hotbed of characters, and I've had the privilege of hanging out with some really good people. Whether on the group camping trip, on the softball field, or at a seedy bar, there have been some awesome times. Eric Dias, Bobby Maughon, Bill Zuercher, Marcus Weck, Tom Kirkland, Todd Younkin, and Melanie Sanford deserve thanks for being a great bunch of people to hang out with. Delwin (big D) Elder deserves special thanks for his friendship, and always having a big toothy smile on his face. Adam Matzger should be thanked for all of his help with computing issues, but beyond that, for being a great guy to hang out with in general. His sarcasm is unparalleled, and I have really appreciated his dry wit, especially while writing this thesis. Newcomer Arnab Chatterjee deserves special mention for keeping me company during some late nights while I wrote this thesis. His humor kept me going at some low points, and I want to thank him for keeping me laughing instead of crying.

Last but definitely not least, I want to thank my boyfriend Dave Lynn. While I never would have thought we would be together when we were sharing a bench 4 years ago, that must have been dramatic foreshadowing. Dave Lynn's an awesome man, and I

feel privileged that we are together now. His friendship, support, and respect mean more to me than I can say, and I owe a lot to him for making my experience here at Caltech fantastic. I also owe him for critically (and I mean critically) reading this entire thesis cover to cover. Thanks for everything, Dave.

Finally, I want to thank my parents for their encouragement from day one. Thanks to you two, and thanks to my brother, Martin, for always believing in me.

Abstract

The development of well-defined ruthenium alkylidene $(PCy_3)_2Cl_2Ru{=}CHPh$ (**1**) has revolutionized the application of the olefin metathesis reaction in organic synthesis. This thesis describes: (1) the application of ring-closing metathesis (RCM) to the synthesis of constrained cyclic peptides, (2) the conformational analyses of these peptide architectures, and (3) the development of new selective cross-metathesis (CM) methodology employing alkylidene **1**.

Chapter 2 describes the initial application of RCM to the synthesis of cyclic amino acids and peptide β-turns. Introduction of olefin functionality into peptidic structures proved straightforward, and treatment with ruthenium alkylidene **1** generated cyclic amino acids and dipeptides in excellent yields. Tetrapeptide diene analogs of naturally-occurring disulfide β-turns proved to be robust substrates for RCM, and the macrocyclic olefin products could be prepared either in solution or on solid support.

Chapter 3 describes the application of RCM to the synthesis of macrocyclic hexapeptide β-sheets. The acyclic hexapeptide diene frameworks were modeled after hexapeptide disulfides reported to adopt β-sheet conformations. Treatment with ruthenium alkylidene **1**, however, afforded only low yields of the desired 20-membered macrocycles: conformational analyses suggested that the hexapeptides adopted a helical rather than β-sheet conformation in the apolar solvents in which RCM was performed. These peptide systems were redesigned in Chapter 4 so that macrocyclization was favored when the peptides adopted a helical conformation. A series of macrocyclic heptapeptides were prepared in high yield *via* RCM which were shown to adopt 3_{10}-helical conformations both in solution and in the solid state.

Chapter 5 describes new CM methodology involving the coupling of terminal olefins with symmetrically disubstituted olefins to generate heterodimeric cross-products in excellent yields and with high *trans* selectivity. CM was employed in an initial self-

metathesis step to synthesize a variety of disubstituted olefins with diverse functionality, which were then further processed in the CM with terminal olefins. Finally, a new CM application was introduced involving the metathesis of acrolein acetal derivatives with terminal olefins to generate α,β-unsaturated aldehydes.

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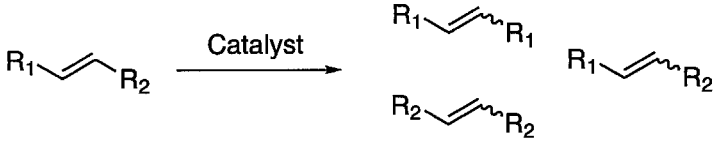
Chapter 1

General Introduction to Olefin Metathesis: Efficient Methodology for the Synthesis of Peptidomimetics

Part I: Introduction to Olefin Metathesis

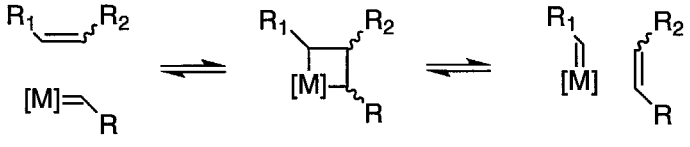
The olefin metathesis reaction is a transition metal alkylidene catalyzed transformation in which the vicinal substituents on two olefins are effectively interchanged. For example, in Eq 1 a *trans*-disubstituted olefin is converted into a statistical mixture of olefin products.[1] In the first step of the now accepted olefin metathesis mechanism proposed by Chauvin (Eq 2),[2] a formal [2+2] cycloaddition occurs between a metal alkylidene and an olefin substrate to generate a metallacyclobutane intermediate. Productive retrocycloaddition affords a new metal alkylidene and the olefin metathesis product. These fundamental steps are frequently reversible, and the reaction is under thermodynamic control.

Olefin Metathesis:



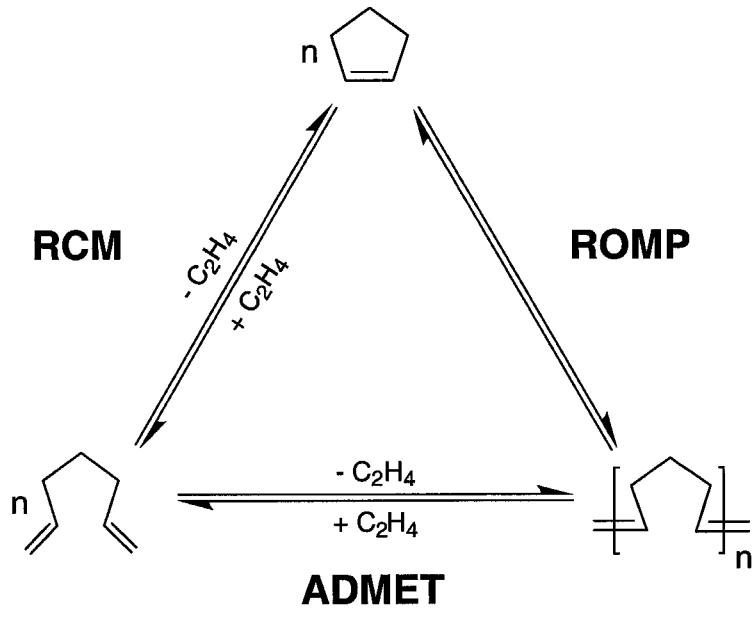
(Eq 1)

Accepted Mechanism:



(Eq 2)

The diverse reaction pathways accessible *via* olefin metathesis are a principle reason for the considerable research effort in this area over the past 20 years. The reaction depicted in Eq 1, the acyclic cross metathesis of alkenes (CM), is one of the simplest metathesis processes, and its utility is greatly enhanced when both product and *cis/trans* selectivity can be achieved (as discussed in more detail below). In addition to this type of basic cross-metathesis reaction, three other classes of transformations which can be performed *via* olefin metathesis are shown in Scheme 1.

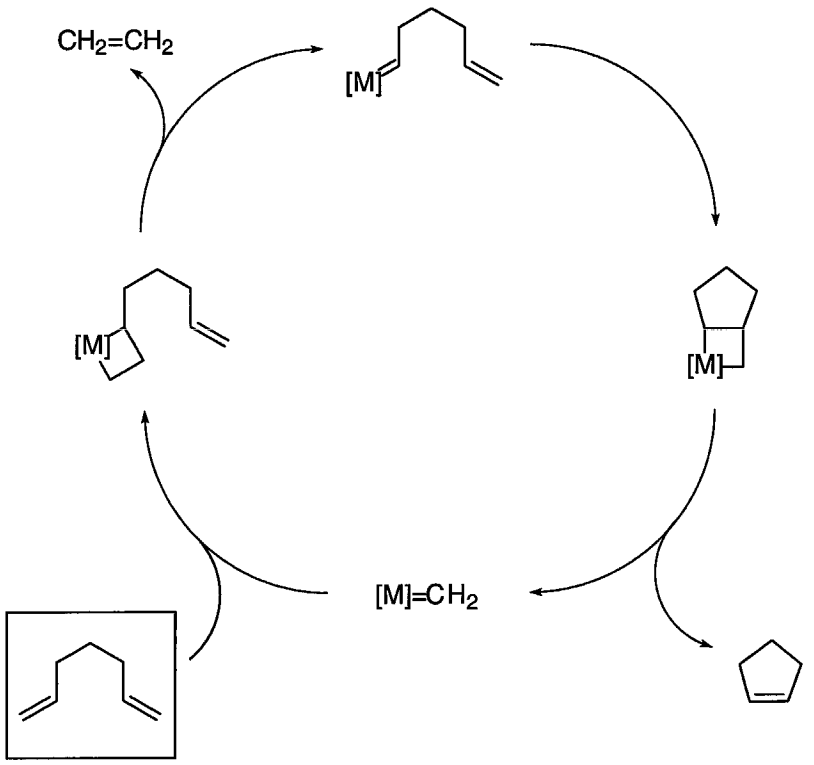


Scheme 1. Olefin metathesis reaction pathways.

Ring-opening metathesis polymerization (ROMP) is the oldest of the three reactions shown in Scheme 1.[3] Typically, a strained cycloalkene can be polymerized to yield a corresponding polyolefin. Highly-strained substrates, such as norbornene, are easily polymerized using a variety of single and multicomponent catalyst systems. Because polymerizations are entropically disfavored, it is the enthalpic contribution provided by the release of ring strain in the monomer that supplies the driving force for this reaction. Accordingly, olefins with less ring strain are more difficult to polymerize; for example, only the more active catalysts polymerize cyclooctenes and cyclopentenes. ROMP has been successfully used to synthesize a variety of telechelic,[4] electroluminescent,[5] and liquid-crystalline[6] polymers in a controlled manner, often with narrow molecular weight distributions.

In comparison to ROMP, the ring-closing metathesis (RCM) of α,ω-dienes to produce cyclic alkenes (Scheme 1) is a relatively new reaction.[7] The thermodynamic profile of RCM is exactly the opposite of ROMP; the formation of cyclic alkenes is enthalpically disfavored, while the production of ethylene provides an entropic driving

force for the reaction. The mechanism of RCM is shown in more detail in Scheme 2. In addition to simple RCM to form five-, six-, seven-, and eight-membered rings,[8] macrocyclic,[9] and solid-supported compounds[10] have also been successfully synthesized. RCM is increasingly being utilized as a key step in the synthesis of natural products and natural product analogs.[11]



Scheme 2. Mechanism of RCM.

Acyclic diene metathesis polymerization (ADMET, Scheme 1) is the least common of these three metathesis reactions.[12] While the thermodynamics of ADMET are similar to RCM, the enthalpic change is approximately zero. ADMET generally produces a variety of cyclic and macrocyclic olefins, in addition to the desired polymers, resulting in a complex mixture of products. Therefore, ROMP is generally favored over ADMET for controlled polymer synthesis.

In addition to these four elementary metathesis transformations, seemingly endless combinations and variations of these reactions have been investigated. For

instance, alkynes can be polymerized to yield poly(acetylenes) with more active catalysts.[13] Acyclic triynes have also been converted to benzene derivatives by a novel fourfold yneyne metathesis cascade comparable to acetylene cyclotrimerization.[14] Incorporation of unsaturation into α,ω-dienes in the form of either alkynes or cycloalkenes has been used to synthesize a wide variety of bicyclic compounds *via* tandem intramolecular eneyne or enene metathesis.[15] The latter transformation, a combination of ring-opening and ring-closing metathesis, has been used to synthesize polymers containing cyclopentene units from commercially available 1,2-poly(butadiene).[16] The ring-opening cross-metathesis (ROM) of cyclic alkenes in the presence of terminal olefins has also been used to synthesize several small organic and silicon-containing molecules.[17,18] Intermolecular eneyne cross-metathesis of alkenes with terminal alkynes has been shown to afford high yield yields of disubstituted diene products.[19] Finally, yneyne cross-metathesis has been reported as an exceptional methodology for the homo- and heterodimerization of terminal and internal alkynes.[20]

Olefin Metathesis Catalysts

Historically, olefin metathesis catalysts have been ill-defined mixtures of transition metal salts (e.g. WCl_6, $MoCl_5$, $ReCl_5$) with main group organometallic compounds (e.g. $RAlCl_2$, SnR_4).[1,21] The inclusion of small amounts of oxygen-containing compounds (ROH, ROR, O_2) in these mixtures was shown to lead to an increase in activity, and the catalyst mixtures were generally active at room temperature. Catalysts based upon late transition metals such as ruthenium and osmium (e.g. $[Ru(H_2O)_6](tos)_2]$) were found to be less active than salts of earlier transition metals.[22] However, in comparison to the earlier systems, these salts were more tolerant of polar and protic functional groups such as alcohols and water.[23] Although these "classical" catalyst systems were easily generated, they were poorly defined with respect to the actual

catalytic species. Therefore, their activities could not be well controlled, complicating their use as initiators in polymerizations and in organic synthesis.

In contrast to the variety of olefin metathesis-based transformations that have been studied (as discussed above), a relatively small number of well-defined, single component transition-metal catalysts have been developed. Shown in Figure 1 are representative well-defined olefin metathesis catalysts based upon metal alkylidene and metallacyclobutane frameworks. In general, bimolecular decomposition of non-heteroatom substituted metal carbenes to form olefinic coupling products is very facile. As a result, sterically bulky ligands have commonly been employed to protect the metal alkylidenes. In addition, bulky substituents on the carbene moiety itself are required in some cases.

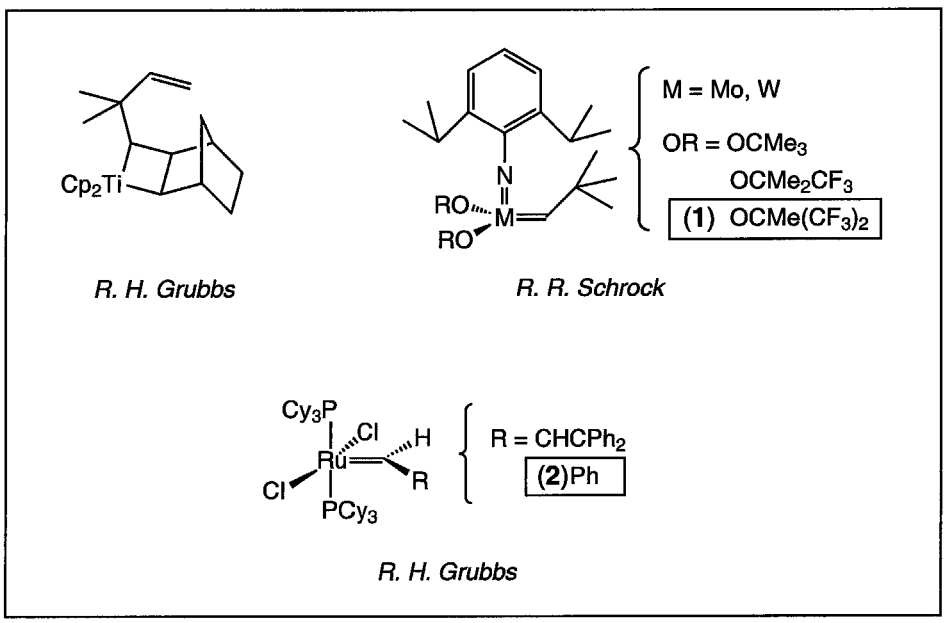


Figure 1. Single-component transition metal catalysts for olefin metathesis. The highlighted alkylidenes (Mo (**1**) and Ru (**2**)) have seen the most application since their disclosure.

The titanacyclobutane complex (Figure 1) was the first example of an isolated metallacyclobutane intermediate in olefin metathesis, providing direct evidence for the Chauvin mechanism (Eq 2). Cleavage of the titanacyclobutane occurs to form a titanium alkylidene, which subsequently reacts with other olefins to form new

titanacyclobutanes.[24] However, the reactivity of titanium carbenes in olefination reactions with carbonyls including esters, ketones, and aldehydes has severely limited the pool of substrates that can be employed.

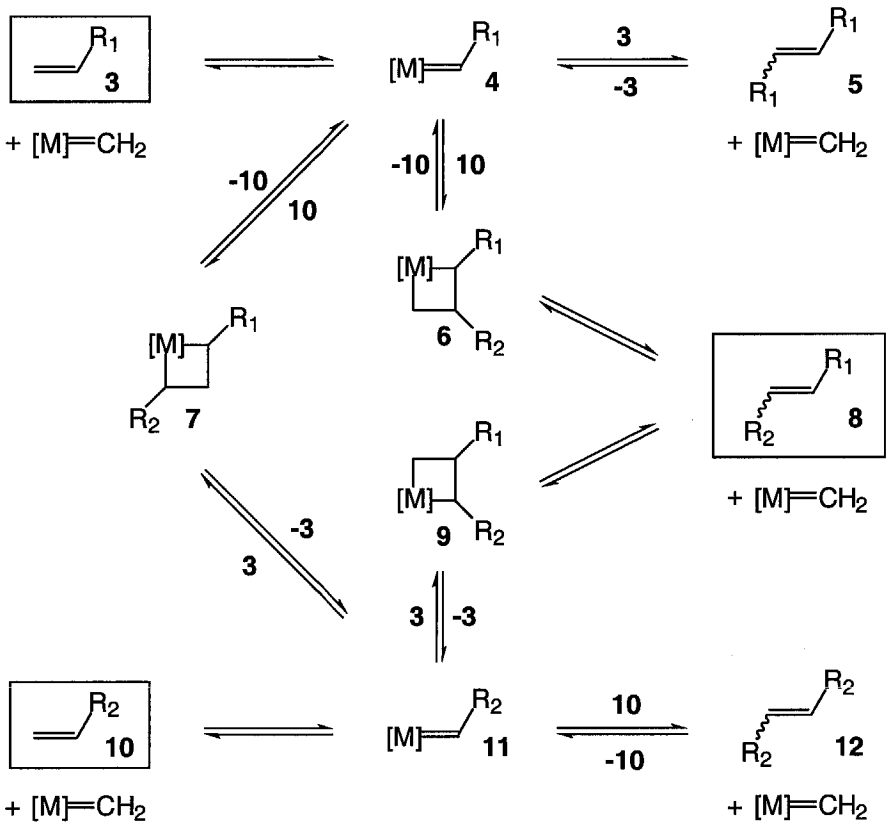
The molybdenum and tungsten catalysts developed by Schrock *et al.* (Figure 1) have been commonly used for olefin metathesis.[25,26] The commercial availability of the molybdenum alkylidene **1** makes it especially convenient for routine use, as the syntheses of both it and the tungsten analog are fairly complicated. Both catalysts exhibit remarkably high activities, and can even be used to polymerize alkynes or cyclooctatetraenes to make poly(acetylenes). In addition, molybdenum alkylidene **1** has been found to be particularly useful in RCM to produce tri- and tetrasubstituted olefins. Recently, highly active asymmetric variants of molybdenum alkylidene **1** have been developed for the use in catalytic kinetic resolutions and enantioselective desymmetrizations through RCM.[27] However, in conjunction with their high activities, this catalyst family as a whole shows limited stability, decomposing slowly at room temperature under inert atmosphere, such that rigorous exclusion of oxygen and water is necessary when these catalysts are used. Their stabilities toward functional groups are also limited; for example, molybdenum alkylidene **1** will olefinate aldehydes, and the tungsten analog both ketones and aldehydes. Thus, although highly active, these catalysts have several practical limitations for routine use in polymer and organic synthesis.

Recently, the use of ruthenium (II) alkylidenes (Figure 1) for olefin metathesis has been developed by Grubbs *et al.*[28] These catalysts are readily available through several inexpensive and straightforward preparations, including reaction of the appropriate ruthenium precursor with cyclopropenes,[28b] diazo compounds,[28c,d] *gem*-dihalo compounds,[29] and propargyl and vinyl chlorides.[30] Generally, these compounds are stable to air in the solid state, and react with oxygen only slowly in solution to produce the corresponding aldehydes. Their stabilities toward multiple functional groups—including

aldehydes, alcohols, amides, and carboxylic acids—while retaining high metathesis activity has been demonstrated.[31] Furthermore, their stability toward water eliminates the need for rigorous drying of solvents and reagents. The advent of these easily accessible, functional group tolerant, and active catalysts has dramatically increased the volume of current research effort directed toward the application of the olefin metathesis reaction in organic and polymer synthesis.

Olefin Cross-Metathesis

While the volume of work in the area of RCM, ROMP, ADMET, and combinations thereof dramatically overshadow that reported for olefin cross-metathesis (CM) to date, the development of the molybdenum and ruthenium alkylidenes **1** and **2** (Figure 1) has enabled some of the first studies directed toward selective CM. Issues of poor product and *trans/cis* selectively plagued early CM efforts employing "classical" catalysts.[32] A majority of these early reports involved the synthesis of insect pheromone natural products: these compounds are frequently isolated from natural sources as a specific ratio of *cis* and *trans* isomers, so CM proved to be a moderately effective route toward these product mixtures.[33] However, for application to synthetic organic chemistry in general, some degree of control over *trans/cis* and product selectivity is essential.



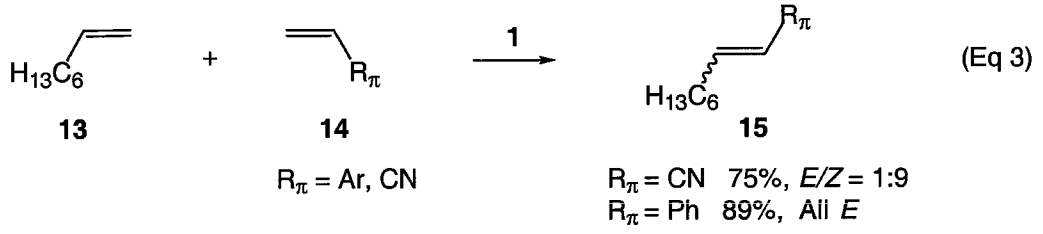
Scheme 3. General reaction scheme of terminal olefin cross-metathesis. Ethylene, the stoichiometric cross-metathesis by-product of retrocycloaddition, has been omitted for clarity.

The simplified CM reaction between two terminal olefins (**3** and **10**) catalyzed by a metal methylidene is depicted in Scheme 3. Generally, this reaction proceeds to yield three unique products: one heterodimeric product (**8**) and two homodimeric products (**5** and **12**), each as a mixture of olefin isomers. The methylidene complex can react with terminal olefins **3** and **10** to form new alkylidene complexes **4** and **11**. Subsequent cycloaddition with the starting material olefins **3** and **10** leads to the formation of metallacyclobutanes **6**, **7**, and **9**. Only the retrocycloaddition reaction of the mixed metallacyclobutanes **6** and **9** generates the desired heterodimeric cross-product **8**. The other reaction pathways are unproductive, or lead to homodimers **5** and **10**.

The composition of dimeric products (**5**, **8**, and **12**) depends on the ratio of the concentrations of alkylidenes **4** and **11**, as well as their specific reactivities toward the

starting material terminal alkenes **3** and **10** (Scheme 3). Recently, there have been a number of reports of CM employing catalysts **1** and **2** where the reactivities of the two olefin reactants has been modified such that productive heterodimerization has been favored over unproductive homodimerization. These cases are discussed briefly below.

Crowe *et al.* have shown that small, alkyl-substituted olefins (**13**) undergo chemo- and stereoselective cross-metathesis with π-substituted terminal olefins (**14**) such as styrene and acrylonitrile in the presence of molybdenum alkylidene **1** (Eq 3).[34]



13 **14** **15** (Eq 3)

R_π = Ar, CN

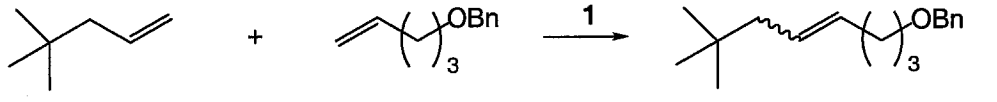
R_π = CN 75%, *E/Z* = 1:9
R_π = Ph 89%, All *E*

Crowe argued that the extended π-system of olefin **14** served as a good "alkylidene donor," favoring formation of a new metal alkylidene (**16**). In contrast, the alkyl-substituted olefin (**13**) was more nucleophilic and preferred to react with this new metal alkylidene (**16**) to generate a metallacyclobutane intermediate (**17**). This could then fragment to form a methylidene and the observed cross-metathesis products (**15**) in good yields. Further evidence in support of this theory was obtained in the highly selective cross-metathesis of allyltrimethylsilane with π-substituted olefins, which was attributed to the pronounced nucleophilic character of the silane (due to the β effect of silicon).[35] Interestingly, in the case of acrylonitrile (Eq 3), a 9:1 mixture of *cis* and *trans* olefin isomers (**15**) were isolated, while the product **15** was purely *trans* in the case of styrene. A clear explanation for this stereoselectivity was not reported. However, in the case of acrylonitrile, it was proposed that the selectivity is kinetically controlled (because the less stable product is formed preferentially), and is probably related to the small size and/or the electron-withdrawing properties of the cyano substituent.[34b] Unfortunately, this model is not transferable to any other π-substituted olefins.

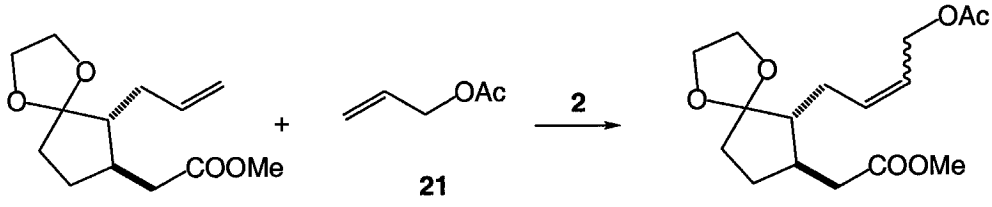
16 $R_\pi = \pi$-donating **17** R_{nuc}= nucleophilic

Figure 2. Electronic contributions towards CM.

Steric factors have also been shown to direct selective CM (Eq 4 and Eq 5). For example, Blechert *et al.* have shown that employing either alkylidene **1** or **2**, metathesis of sterically hindered olefins (**18** and **19**) in the presence of excess smaller terminal olefins (**20** and **21**) results in selective CM for the heterodimeric products (**22** and **23**).[36] The products **22** and **23** were isolated as the predominately *trans* mixtures of olefin isomers. However, no systematic study was carried out to determine the extent to which steric factors direct the product selectivity and/or stereoselectivity of CM.



18 **20** **22** 70% *E*:*Z* = 5:1 (Eq 4)



19 **21** **23** 73% *E*:*Z* = 2:1 (Eq 5)

Blechert *et al.* have also studied the selective CM of allyltrimethylsilane with terminal alkenes, and have observed exceptionally high selectivities in these reactions.[36] In contrast to Crowe,[35] however, they believe that β effect of silicon is not the only factor involved in governing the chemoselectivity of the reaction. For example, Blechert observed that 4,4-dimethylpentene (**18**), a direct carbon analog of allyltrimethylsilane, showed comparable reactivity and selectivity in CM although it lacks a putative β effect.

Furthermore, Blechert observed that employing either alkylidene **1** or **2**, selective CM could be carried out between terminal olefins with double bonds of comparable electron densities.[36]

To date, the work of Blechert and Crowe represents the most comprehensive studies of terminal olefin CM with well-defined olefin metathesis catalysts **1** and **2**. Overall, no general model for the selectivity of CM can be derived form this work. The reduced tendency of sterically hindered and conjugated olefins to undergo self-metathesis, along with the possible precomplexation of the metal alkylidene complex by polar heteroatom substitutents,[36] offers a viable starting point for increasing the understanding of the observed selectivities. However, because the desired heterodimeric cross-product can be generated by various reaction pathways, the suppression of the self-metathesis of one reaction partner alone does not allow for the proposal that a selective mode of action is operative. On the other hand, selective CM is often observed between olefins which completely homodimerize in the absence of another reaction component. Clearly, the current knowledge of the factors governing CM selectivity remains insufficient.

Part II: Introduction to Peptidomimetics

Peptides and proteins play an essential role in the control and modulation of virtually all biological processes.[37] For example, they regulate biological functions by behaving as enzymes, inhibitors, hormones, and receptors. The enormous growth in the fields of molecular biology, peptide synthesis, structure elucidation (nuclear magnetic resonance, X-ray crystallography), and molecular modeling has considerably increased the understanding of the intimate relationship between peptide and protein structure and their ultimate biological function. The dramatic growth in these fields, along with recent progress in the synthesis and evaluation of vast combinatorial peptide libraries has

focused attention on small peptides as important lead structures for the development of potential therapeutic agents.[38]

However, problems of metabolic instability severely restrict the use of many naturally-occurring peptides themselves as therapeutic candidates. These pharmaco-kinetic problems underscore the importance of establishing general synthetic strategies for converting peptides into peptidomimetics. A peptidomimetic, or non-peptide, is a compound which generally abandons naturally-occurring peptide primary structure, but retains those essential chemical functionalities and the ability to display them in a characteristic three-dimensional pattern which is complimentary to the peptide receptor.[39] As a result, the effects of the original peptide are either inhibited (antagonist, inhibitor), or duplicated (agonist).

Rational Design of Peptidomimetics

As opposed to many combinatorial approaches, much thought must be put into the rational design of peptidomimetics in order to produce effective medicinal candidates. Chemical synthesis is the sole tool which enables the availability of peptidomimetics. The complete arsenal of synthetic organic chemistry, therefore, can compliment modern peptide chemistry to expand the structural variation accessible in the laboratory.

Conformationally restricted peptides and amino acids assume a prominent role in current peptidomimetic design.[39] Numerous approaches to the design and synthesis of rigidified peptidomimetics have been reported, including side chain and amide linkage modification. A common theme among many of the constrained peptidomimetic design strategies applied to date has been the introduction of some form of cyclic sub-structure into a peptide-based framework.[40] This cyclic moiety is most often introduced through installation of a covalent or non-covalent bridge between different parts of the molecule. The bridging can either occur within a single amino acid residue, or may involve several amino acid residues. Frequently, this cyclization results in an increased affinity of the

peptide for a particular biological receptor, with simultaneously reduced sensitivity to cellular peptidases. Furthermore, rigidified peptide mimetics can also serve as tools in basic biostructural research; for example, in the construction of model systems that probe the origins of folding preferences.[41]

Induction and Stabilization of Protein Secondary Structures via Cyclization

The structures of most proteins appear to be made up of segments of secondary structure which intricately pack to generate complex architectures.[37] These key structural elements include β-turns, β-sheets, and α-helices, all which have been shown to be important recognition sites for protein-ligand, protein-protein, and protein-DNA interactions, among others. A considerable amount of effort has been directed toward introducing cyclic moieties into small peptides exhibiting these key secondary structural motifs in order to effectively lock the peptides into their bioactive conformations. Notably, significant progress has been made toward stabilizing synthetic β-turns,[39,42] β-sheets,[43] and helical peptides[44] through the incorporation of covalent or non-covalent linkages between constituent amino acid side-chains (Figure 3). Examples include salt bridges,[45] lactams,[44] disulfide bridges,[46] hydrophobic interactions,[47] and metal ligation between natural[48] and unnatural amino acids.[49] Strategic modulation of the linkage element and the peptide primary structure has allowed for the synthesis of an extensive pool of peptidomimetics exhibiting specific protein secondary structural motifs, for use as model compounds to probe the intricacies of protein conformation and folding, and also as potent therapeutics. Finally, these cyclic structures have been incorporated into larger protein architectures with the hope of nucleating specific protein secondary and tertiary structures.[41]

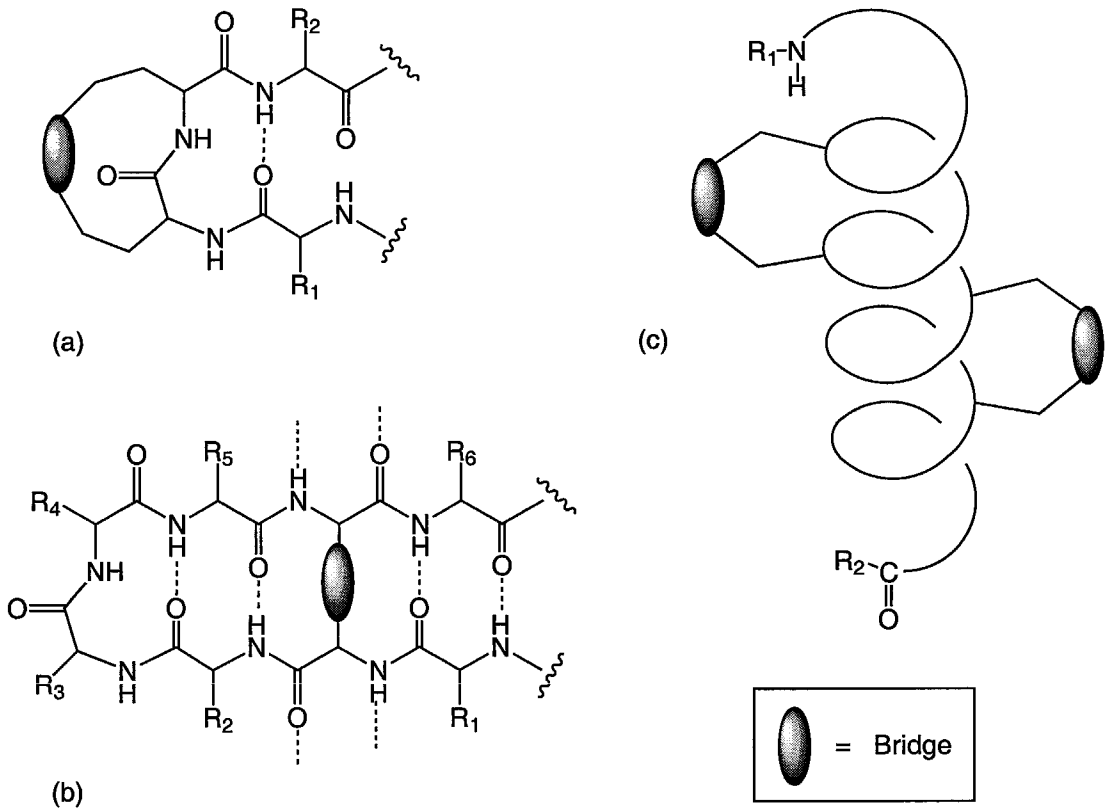


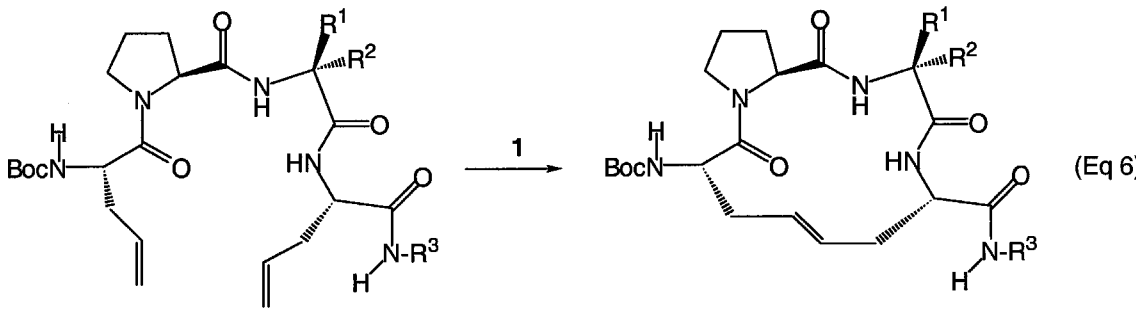
Figure 3. Introduction of side chain bridges in peptides exhibiting (a) β-turn, (b) β-sheet, and (c) helical secondary structural motifs.

The synthesis of cyclic peptidomimetics of such great diversity involves utilizing synthetic methods not commonly employed in traditional peptide chemistry, which is limited to those methods which do not react with amides and other functionalities associated with amino acid side chains. Recently, we have reported the syntheses of conformationally restricted peptides and peptide architectures employing olefin metathesis.[50] This powerful C-C bond formation strategy is now being utilized with some frequency to successfully synthesize cyclic peptidomimetics, due to the compatibility of new transition metal alkylidene **2** with the polar functional groups regularly found in biomolecules. Examples of all of the cyclic secondary structure mimetics shown schematically in Figure 3 wherein the side chain bridge(s) were introduced *via* RCM have been synthesized to date.

Part III: Thesis Research

The advent of well-defined alkylidenes **1** and **2** has revolutionized the application of the olefin metathesis reaction. The majority of this thesis describes the application of olefin metathesis to the synthesis of constrained cyclic peptides. Specifically, ring-closing metathesis (RCM) has been employed to cyclize small dienic peptides which exhibit unique protein secondary structural motifs. The introduction of olefinic functionality into peptide structures was straightforward, and the RCM transformation employing ruthenium alkylidene **2** proceeded smoothly to afford high yields of cyclic products.

Chapter 2 describes the initial application of RCM to the synthesis of cyclic amino acids and β-turn peptides. Cyclic amino acids and dipeptides with ring sizes of six to eight atoms could be generated in good yield upon treatment of acyclic substrates with ruthenium alkylidene **2** in organic solvents. In attempts to generate macrocyclic peptides *via* RCM, we employed a class of naturally-occurring tetrapeptide disulfide β-turns as an initial structural template. We envisioned replacing the disulfide bond with a carbon-carbon double bond by RCM to generate a 14-membered macrocycle. Replacing cysteines with allylglycines proved an effective method for strategic introduction of terminal olefin functionality into peptides. The RCM of dienic tetrapeptide analogs of disulfide β-turns proved to be a general procedure for the synthesis of tetrapeptide macrocycles (Eq 6). Furthermore, this RCM methodology could be applied to dienic peptides either in solution or bound to solid support.



In Chapter 3, the synthesis of cyclic β-sheet model peptides *via* RCM is described. The acyclic dienic precursor peptides were based upon hexapeptide disulfides previously reported to adopt β-sheet conformations (Figure 4). Systematic replacement of the two cysteine residues in these peptides generated the acyclic diene analogs, in direct analogy to the preparation of the β-turn systems introduced in Chapter 2. The β-sheet conformations of the hexapeptides were believed to preorganize them for RCM. However, treatment with ruthenium alkylidene **2** afforded only low yields of the desired 20-membered macrocycles, either in solution or on solid support. Potential explanations for these low yields are presented, the favored explanation being that the hexapeptides adopted a helical rather than β-sheet conformation in the apolar solvents in which RCM was performed, and that this conformation was unfavorable for macrocyclization.

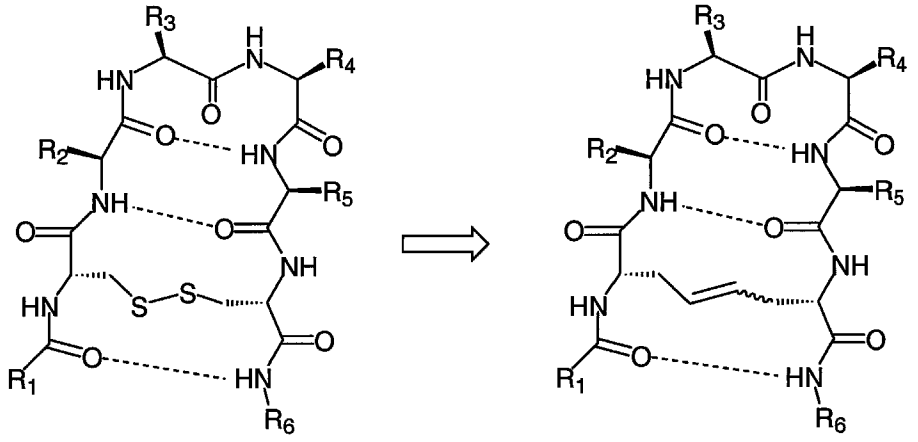
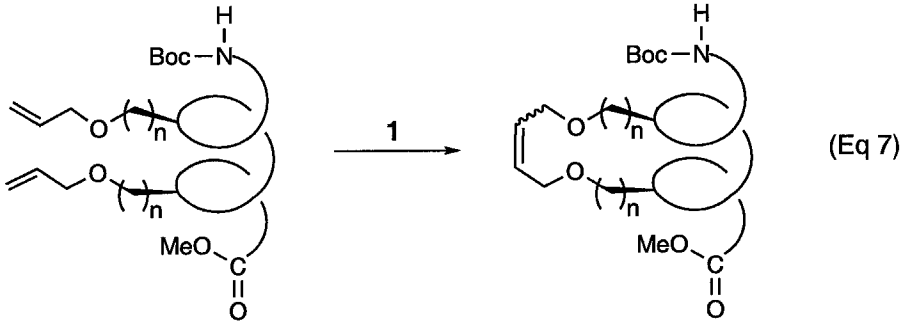


Figure 4. A generic β-sheet hexapeptide disulfide and the corresponding C=C analog.

The observed tendency of the hexapeptides described in Chapter 3 to adopt helical conformations is applied in Chapter 4, where the efficient synthesis of macrocyclic peptides adopting helical conformations by RCM is described. Hydrophobic heptapeptide analogs were prepared based upon a previously reported model peptide shown to adopt an α-helical conformation both in apolar organic solvents and in the solid state. In the acyclic dienic precursors, olefinic functionality was installed by the strategic incorporation of serine and homoserine (*O*-allyl) ethers in the place of the two alanine

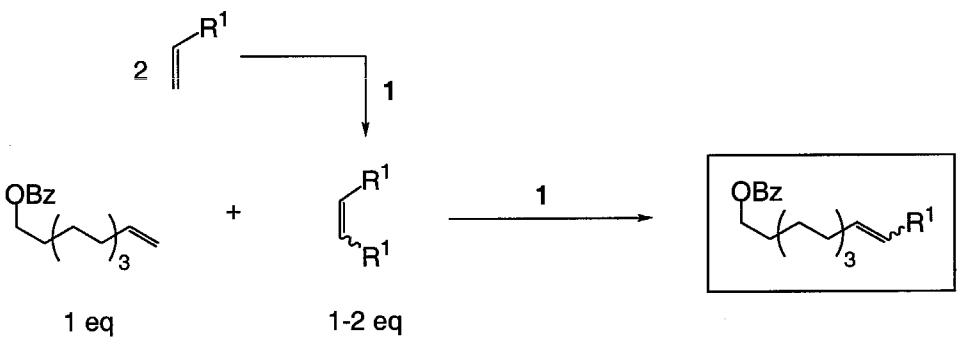
residues in the model system (Eq 7). Specifically, the olefinic residues were positioned in the peptide sequence so that their side chains extended out of one face of the peptide helix, and therefore would be well-positioned for RCM macrocyclization if the diene analogs adopted a helical conformation similar to that of the model system. Indeed, treatment of the acyclic peptide dienes with ruthenium alkylidene **2** in apolar solvents generated the desired macrocyclic peptides in high yields. Detailed conformational analyses of both the acyclic and cyclic heptapeptides in solution strongly suggested that the acyclic dienes are preorganized in a 3_{10}-helical conformation for RCM, and that this conformation was maintained in the macrocyclic peptide products. An X-ray crystal structure of one of the macrocyclic heptapeptides confirmed that the peptide also adopted a 3_{10}-helical conformation in the solid state. Finally, it is believed that the macrocyclization of hydrophobic peptide helices is uniquely suited to RCM in organic solvents, because helical conformations are frequently favored in apolar media.



(Eq 7)

The remaining portion of this thesis focuses upon olefin cross-metathesis (CM). As previously discussed in this chapter, methods for chemo- and regioselective CM remain scarce, primarily because a clear rationale for the factors dictating selectivity in CM has not been disseminated. Described in Chapter 5 is selective CM methodology involving the reaction of terminal olefins with symmetrically disubstituted olefins to generate heterodimeric cross-products in excellent yields (Eq 8). An excess of the disubstituted olefin was required for optimal yields, and the mixtures of olefin

stereoisomers isolated were always predominantly *trans*. The *trans/cis* ratio could be increased dramatically by either increasing the relative steric bulk of the substituents on the disubstituted olefin, or by incorporating steric bulk in the allylic position of the terminal olefin component. Systematic comparison of selected disubstituted olefins with their monosubstituted counterparts revealed the disubstituted olefins to be more reactive for CM. A plausible explanation for this heightened reactivity is presented. While this method proved highly effective employing disubstituted olefins with allylic heteroatoms, the yields of the desired cross-products were reduced as this functionality was placed further away from the olefin.

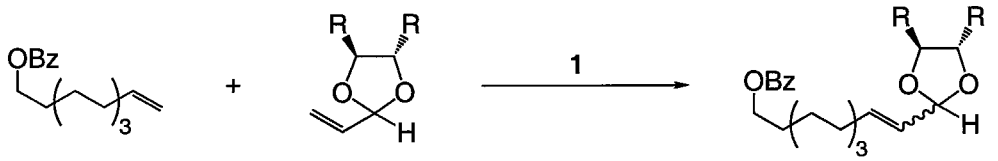


(Eq 8)

Due to the limited availability of symmetrical disubstituted olefins, we employed CM in an initial self-metathesis step to synthesize a variety of disubstituted olefins with diverse functionality (Eq 8). A variety of novel amino acid, peptide, and carbohydrate containing homodimers were synthesized utilizing some of the peptide alkene precursors previously prepared in Chapters 2-4. The majority of these homodimers were processed further in the CM with terminal olefins to generate a structurally-diverse pool of heterodimeric products in good yields,

Finally, at the end of Chapter 5, a new CM application is introduced involving the metathesis of acrolein acetal derivatives with terminal olefins to generate α,β-unsaturated aldehydes (Eq 9). Acrolein acetals proved to be exceptionally robust substrates for CM, and depending on the structures of the acetal, generated predominantly *trans* cross-

products. Preliminary investigations of the CM of chiral acetal derivatives for use in potential kinetic resolutions *via* CM are also addressed.



1 eq 1-2 eq (Eq 9)

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Chapter 2

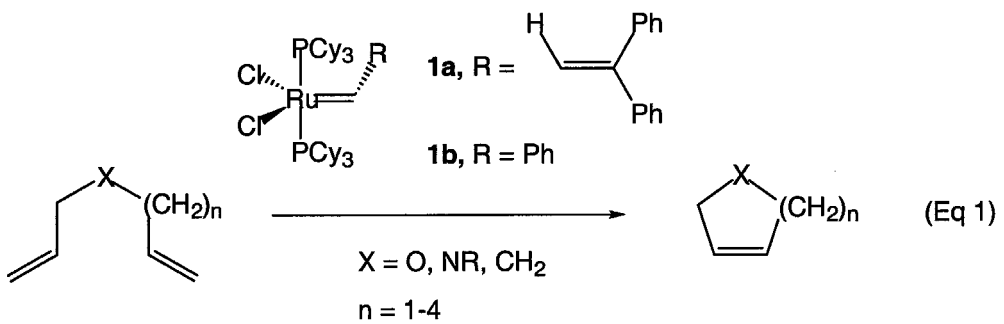
Application of Ring-Closing Metathesis to the Synthesis of Constrained Cyclic Amino Acids and Peptides[†]

Abstract

Ruthenium complexes **1a** and **1b** have been applied to the ring-closing metathesis (RCM) reactions of a number of dienic peptide substrates. The substrate scope includes rings of 6 to 20 members. In addressing macrocyclic peptides, a class of tetrapeptide disulfides inspired the synthesis of the carbon-carbon bond analogs. Replacement of cysteine residues with allylglycines resulted in the acyclic precursors, which were subjected to RCM to afford the corresponding macrocycles. In addition, several macrocycles were prepared which were not based upon disulfide bridge-containing species found in nature. The method was found to function on dienic peptides which were either dissolved in organic solvents or bound to solid supports.

Introduction

As described in Chapter 1, ruthenium complexes **1a** and **1b** have been found to efficiently catalyze ring-closing metathesis (RCM) reactions to form five, six, seven and eight-membered carbocycles and heterocycles (Eq 1).[1,2,3] Metathesis-based strategies for carbocycle and heterocycle synthesis, based on alkylidenes of ruthenium and molybdenum, are now being applied with regularity in natural products synthesis.[4] The extraordinary functional group tolerance of ruthenium-based catalysts (**1**) has also enabled the recent synthesis of cyclic amino acids and peptides, containing multiple heteroatoms, acidic protons, and hydrogen bonds.[5,6] In addition, Ghadiri *et al.* have recently demonstrated that these structural features can be engineered into cyclic β-sheet systems which can efficiently dimerize and undergo template driven inter- and intramolecular olefin metathesis.[5a,n] In this chapter, our findings concerning the scope of RCM in the synthesis of cyclic amino acids and peptides will be described.



(Eq 1)

Given the prominent role of conformationally restricted amino acids and peptides in the design of peptidomimetics (discussed in detail in Chapter 1), we sought to apply the functional group tolerant catalysts (**1**) to the synthesis of this class of molecules.[7] Many approaches to the synthesis of a vast array of these compounds have been described. Amide linkage modification, side chain modification, and *de novo* synthesis of particular structural motifs are among the strategies employed to date. Cyclic substructures are likewise frequently incorporated, as these motifs serve to restrict the

conformational space of the molecules. Such modifications often result in increased affinity for a given biological receptor, with simultaneously diminished sensitivity to cellular peptidases. Because RCM based on catalysts **1a** and **1b** was emerging as a powerful method for carbocycle and heterocycle synthesis in the presence of a wide range of functional groups, we endeavored to apply this strategy to the synthesis of peptide-based structures.

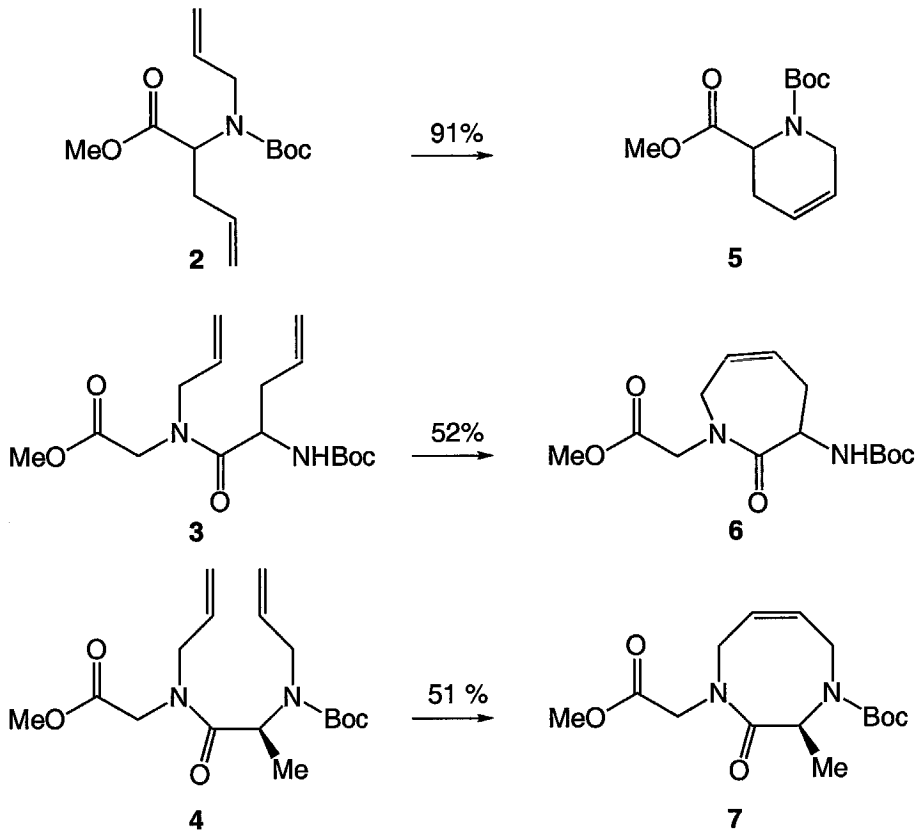
Results and Discussion

Cyclic Amino Acids

Our initial objective was the synthesis of simple amino acid derivatives containing various ring sizes, which might subsequently be introduced into peptides. Depending on the desired ring size, our approach required the introduction of alkene functionality at either C_α, or at the amide nitrogen of the amino acid. Alkene incorporation at C_α is facilitated by the commercial availability of (+/-)-, (+)- and (-)-allylglycine. In addition, highly efficient methodology for the asymmetric synthesis of this unnatural amino acid is also available.[8] Introduction of alkene functionality at the amide nitrogens has also been addressed. In particular, Seebach *et al.* have shown that peptides can be converted to the poly(*N*-allylamides) upon exposure to allyl bromide in the presence of the P4-phosphazene base.[9] Synthesis of the RCM precursors **2-4** then proceeded in a straightforward fashion employing conventional peptide coupling and derivatization techniques.[10]

The results of the RCM experiments are shown in Scheme 1.[11] Treatment of modified amino acid **2** with catalyst **1a** under standard RCM reaction conditions (5 mol % **1a**, 0.2 M in $CHCl_3$, 25 °C) furnished the dehydro-pipicolinate **5** in good yield (91%) within 1h. In contrast, substrates **3** and **4** required more vigorous conditions for ring closure, and the isolated yields for the corresponding cyclizations were reduced. Nevertheless, subjection of acyclic dienes **3** and **4** to the above RCM conditions afforded

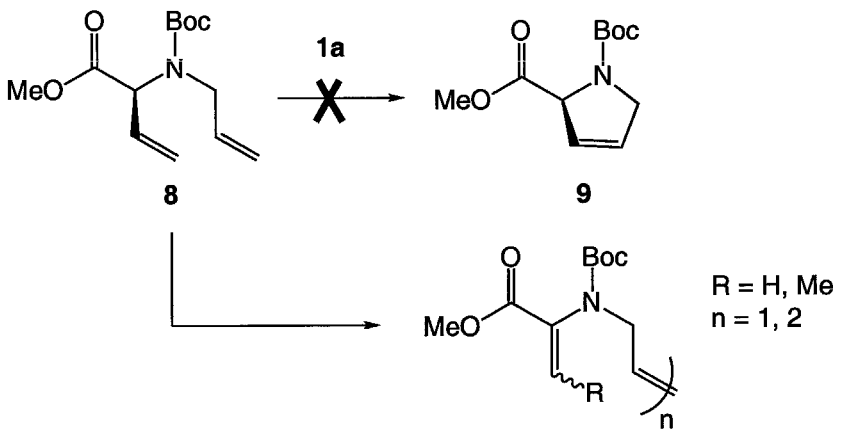
the seven-membered ring **6** in 52% yield and the eight-membered ring **7** in 51% yield, respectively. The latter two transformations appear to be limited by the ring strain inherent to the cyclic product, which requires the reactions be run at high dilution so as to minimize competing intermolecular oligomerization processes.[12]



Scheme 1. Synthesis of cyclic amino acids *via* RCM employing catalyst **1a**.

A notable limitation of the RCM strategy employing **1** for the synthesis of cyclic amino acids is illustrated by our unsuccessful attempt to prepare dehydroproline (**9**) by this method (Scheme 2). Initially, the vinyl glycine derivative **8** was projected to undergo facile, rapid RCM with **1a** to afford the 5-membered ring in analogy to the other 5-membered rings we had studied.[13] However, upon exposure to the ruthenium-based catalyst **1a**, only starting material and acyclic α,β-unsaturated esters were recovered.

Although no rigorous mechanistic studies were carried out, we attributed this undesired reactivity to the labile acidic C_α-H associated with the vinyl glycinate structure (**8**). Indeed, the corresponding N-Boc vinyl glycinol derivative has been recently reported to undergo the RCM reaction in 95% yield.[14] Furthermore, substitution of the N-Boc protecting group of **8** with an N-trityl group renders **8** a reactive substrate for RCM, giving the N-trityl derivative of **9** in 70% yield.[15] The fact that a bulkier N-protecting group is prerequisite for ring-closure suggests that coordination of the amine to the ruthenium complex **1b** could be another reason for the poor reactivity of diene **8**. The synthesis of the dehydroproline derivative **9** was not pursued further at this point.

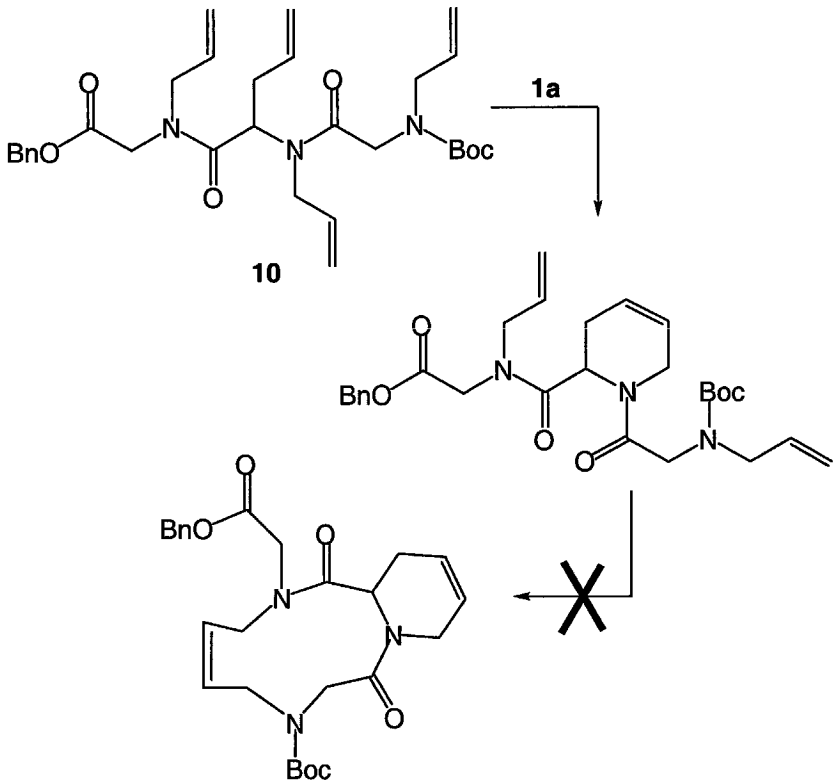


Scheme 2. Attempted synthesis of dehydroproline derivative **9** *via* RCM employing catalyst **1a**.

Macrocyclic Peptides

At this stage, we turned our attention to the synthesis of macrocyclic peptide structures. Our initial approach to this problem was based on the incorporation of alkene functionality at the amide nitrogens (Scheme 3). Synthesis of *N,N,N*-(triallyl)peptide **10** was accomplished by conventional peptide coupling chemistry,[10] followed by allylation with the P4-phosphazene base.[9] Our initial projection was that the substrate, upon treatment with the metathesis catalyst, would undergo a bicyclization reaction wherein a rapid 6-membered ring closure would be followed by a macrocyclization. However,

treatment of **10** with **1a** resulted in only monocyclization, and no macrocyclic compounds were obtained.[16] While the reasons for this lack of macrocyclization were not definitively established, we attributed the low reactivity of the structure to either the ring strain inherent to the bicyclic product, or kinetic problems associated with its formation. As a result, we sought an alternative approach to macrocyclic peptide formation.[17]



Scheme 3. Treatment of *N,N,N*-(triallyl)peptide **10** with catalyst **1a** yields only a monocyclic product.

Dicarba-Analogs of Naturally-Occurring, Macrocyclic Disulfides

In order to contend with the potential thermodynamic and kinetic problems associated with peptide macrocyclizations, we turned our attention to examples of macrocyclic peptides which were found in Nature. While examples of such peptides are numerous, we were attracted to a class of disulfide-stabilized tetrapeptides found in a number of redox active proteins (Figure 1).[18] Importantly, each member of this class contains a disulfide bridge which locks the tetrapeptide into a β-turn type structure.[19]

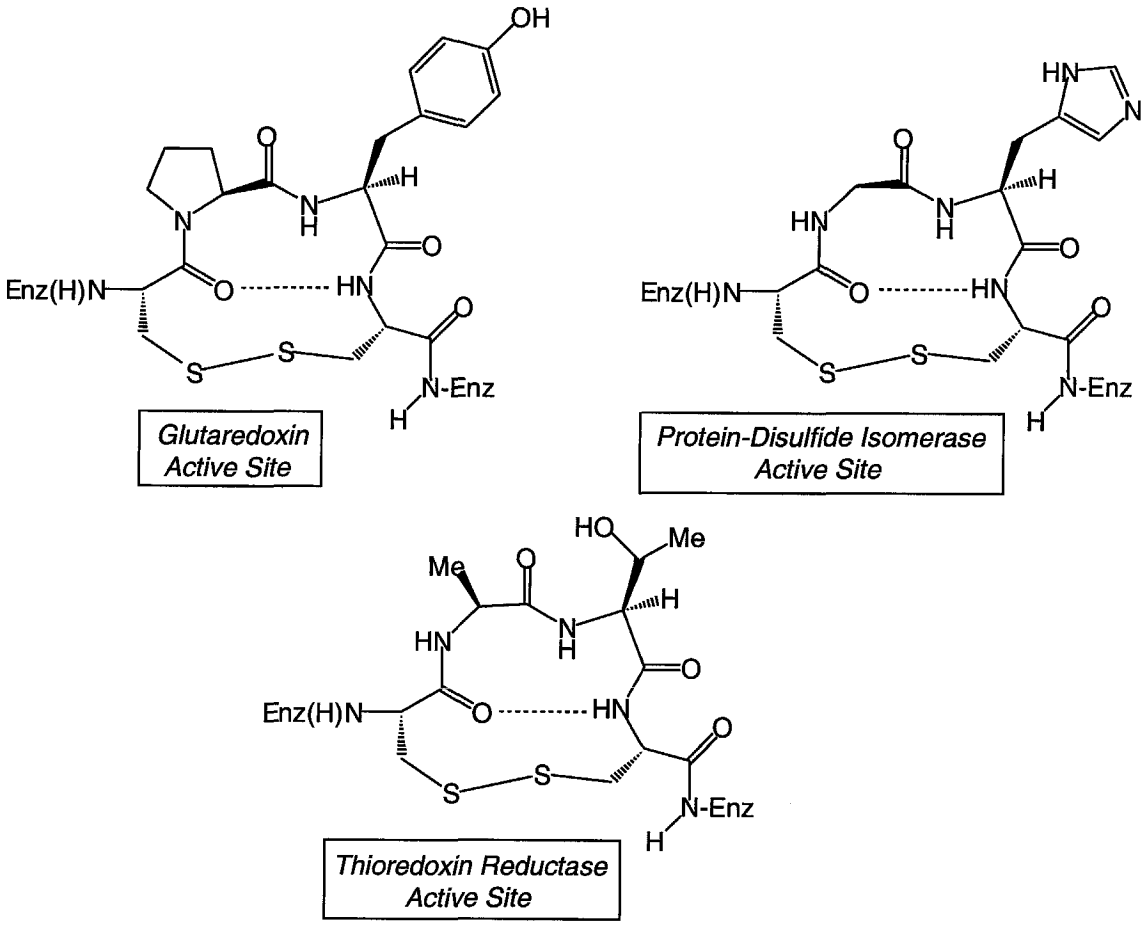
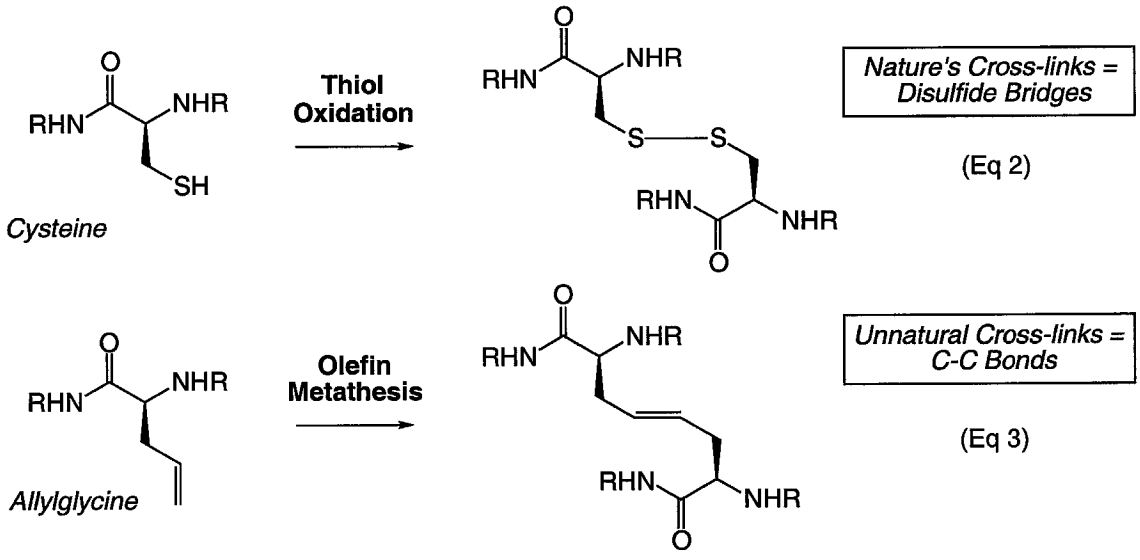


Figure 1. Naturally occurring disulfide-stabilized β-turns.

Our objective was to replace the disulfide bridges in these known systems with carbon-carbon bonds. So-called "dicarba" peptides of this nature have themselves been prepared previously in peptidomimetic research.[20] Replacement of the disulfide bridge in biologically active compounds can provide drugs which have increased metabolic stabilities, as the covalent cross-links are inert to conditions which can reduce disulfide-based cross-links.[21] Our approach was to design a laboratory analogy for the oxidation of cysteines to disulfide bridges (Eq 2). Replacement of cysteines with allylglycines in acyclic peptides sets up RCM instead of thiol oxidation (Eq 3). In the presence of a metathesis catalyst, a carbon-carbon bond is then formed in place of the disulfide bridge.



Balaram *et al.* have reported the disulfide-stabilized β-turn **11** as an analog of the redox active β-turns above, and this substrate stimulated us to attempt to prepare the analogous tetrapeptide olefin (Figure 2).[22] Significantly, Balaram has shown that both **11** and the corresponding acyclic (bis)benzyl thioether **12** possess the illustrated transannular 4→1 hydrogen bond (a diagnostic of peptide β-turns) in $CDCl_3$ solvent, and we believed that this feature might help dispose the acyclic diene analog toward a conformation suitable for macrocyclization.[23]

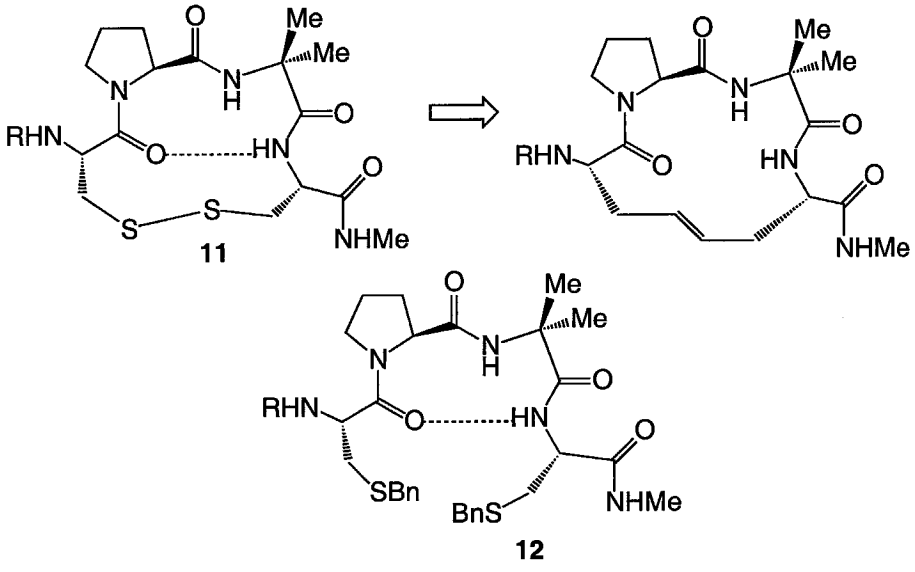
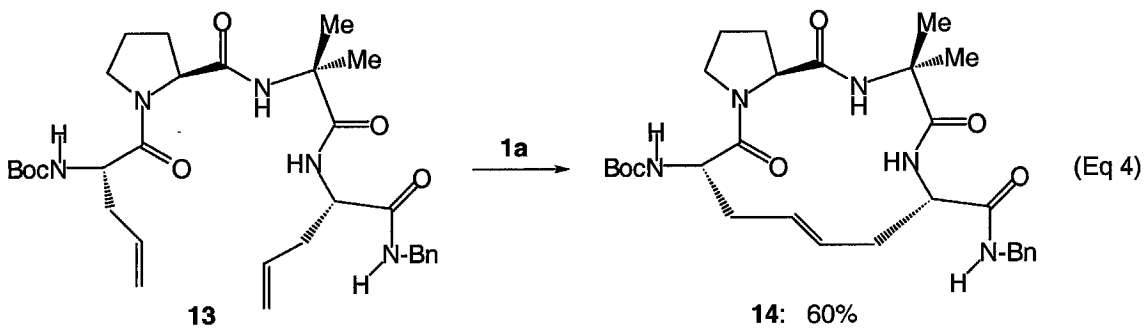


Figure 2. Balaram's disulfide-stabilized peptide β-turn **11** and the C=C analog.

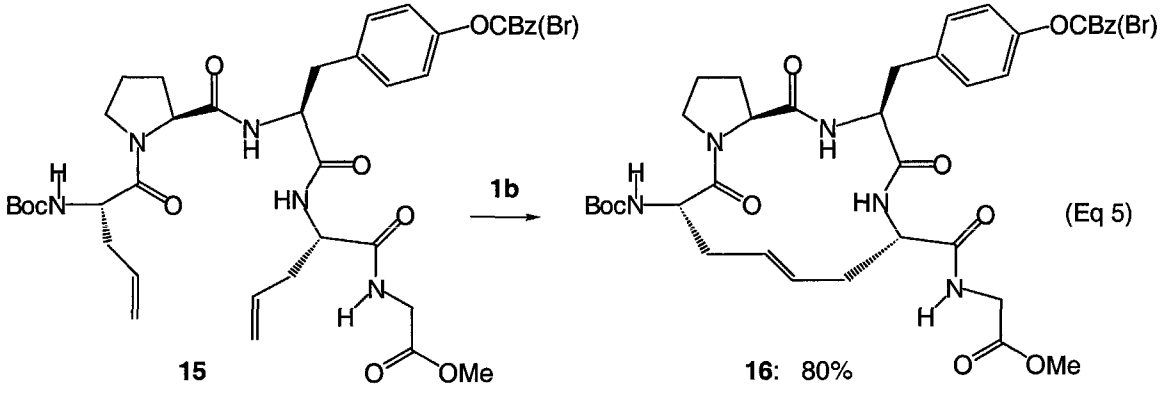
Since disulfide bridges possess different dihedral angle requirements relative to olefins,[24] it was not obvious at the outset of our study whether (*S*)-allyl glycine would be the optimal stereoisomer for replacement of the L-cysteine. Therefore, in our initial study of this system, we prepared a statistical mixture of the four stereoisomers of acyclic dienes **13** employing (+/-)-allylglycine. When this mixture of the four diastereomers was treated with catalyst **1a** (20 mol %, 0.002M in CH_2Cl_2, 40 °C), a single macrocycle **14** diastereomer was obtained. The majority of the reaction mixture was composed of unreacted dienes. When diastereomerically pure (*S,S,S*)-**13** was subjected to the reaction conditions (*S,S,S*)-**14** was obtained in 60% yield, and the product was identical to that obtained from the analogous experiment on the mixture (Eq 4). From this point onwards, we exclusively employed (*S*)-allyl glycine in the synthesis of all dienic peptides.[25]



(Eq 4)

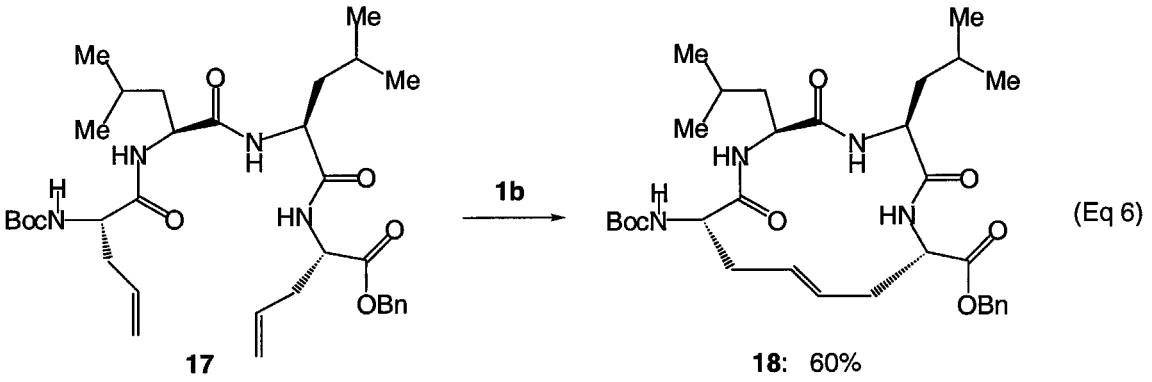
In order to explore the scope of the process, and in order to assay the role of preorganization in facilitating ring closure, we prepared additional tetrapeptides in which we systematically removed the conformationally constrained amino acids proline and α-aminoisobutyric acid (Aib).[26] The Pro-Aib sequence is known to dramatically restrict the conformational space of peptides, and was found to impose a type III (3_{10} helical) β-turn conformation[27] on Balaram's cyclic disulfide **11** in $CDCl_3$ solution.[28] Therefore, we examined substrates in which these amino acids were replaced with less rigidifying residues.

Replacement of the Aib residue with protected tyrosine resulted in the formation of peptide **15** (Eq 5). The Pro-Tyr dipeptide sequence is that which spans the cysteine residues in the glutaredoxin active site (Figure 1). The Cys-Pro-Tyr-Cys cyclic disulfide had been previously studied by Balaram *et al.* and was found to adopt a stable type I β-turn[19] conformation in $CDCl_3$ solution.[29] Compound **15** therefore represented an attempt to synthesize a carbon-carbon bond stabilized β-turn mimic of the active site of glutaredoxin.



Exposure of **15** to ruthenium alkylidene **1b** under standard macrocyclization conditions (0.004 M, CH_2Cl_2, 40 °C) resulted in clean formation of the macrocycle **16** in 80% yield (Eq 5).[30] This experiment demonstrated that two consecutive conformationally constrained amino acids were not required for the synthesis of tetrapeptide macrocycles by RCM.

Replacement of both the proline and the Aib residues with leucines resulted in peptide **17** (Eq 6), which was devoid of conformationally restricted amino acids in the bridging positions. To our knowledge, there has been no conformational analysis performed on the tetrapeptide disulfide analog. Once again, exposure to alkylidene **1b** resulted in very efficient macrocyclization to afford cyclic tetrapeptide **18** (Eq 6). These results established that there is no rigorous requirement for the Pro-Aib sequence as the bridging residues for the synthesis of tetrapeptide macrocycles by RCM.



(Eq 6)

Conformational Analyses of Cyclic Dicarba-Tetrapeptides

In order to ascertain the extent to which the macrocyclic products containing carbon-carbon double bonds resemble their disulfide bridge analogs, an investigation of the solution conformations cyclic tetrapeptides was undertaken. As indicated above, Balaram's Cys-Pro-Aib-Cys system **11** was found by a series of IR and NMR experiments to possess an intramolecular, transannular H-bond between the two Cys residues. The corresponding data on the carbon-carbon bond analogs of these systems is discussed below.

Table 1 lists the NH region of the IR spectra of the cyclic peptides **14**, **16**, and **18**. In each case, the characteristic bands for both non hydrogen-bonded NH groups (>3400 cm^{-1}), and intramolecular C=O···H-N hydrogen bonds (<3400 cm^{-1}) appear.[31] Although it is difficult to definitively establish from the IR data alone which of the amide N-H groups is involved in intramolecular hydrogen bonding, these data are consistent with the possibility of transannular intramolecular H-bonding in the cyclic structures.

Table 1. IR data for cyclic tetrapeptides (2 mM in CH_2Cl_2, 25 °C)

peptide	**14**	**16**	**18**
Non H-bonded NH	3424 cm^{-1}	3426 cm^{-1}	3419 cm^{-1}
H-bonded NH	3324 cm^{-1}	3354 cm^{-1}	3339 cm^{-1}

To further delineate the H-bonding network in the cyclic tetrapeptides, we turned to ^{1}H NMR spectroscopy (Figures 3 and 4). Compounds **14** and **18** were studied to identify the presence of any transannular 4→1 hydrogen bonding, which is diagnostic of β-turn type conformations.[19] The participation of specific NH groups in hydrogen bonds was established by examining: (1) the temperature dependence of the NH chemical shifts ($-\Delta\delta/\Delta T$) in $(CD_3)_2SO$, and (2) the solvent dependence of the chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures.[24] In both of these studies, if an amide NH chemical shift is changing considerably more slowly than the other NHs, this is believed to indicate that the amide NH is less affected by solvent/temperature changes due to its involvement in an intramolecular hydrogen bond. Proton assignments of the olefinic and amide regions of the ^{1}H NMR spectra of **14** and **18** are shown on the illustrated plots, and were established employing conventional decoupling techniques (Figures 3a and 4a).

For cyclic peptide **14**, an intramolecular transannular H-bond was identified by the above analysis (Figure 3). The temperature dependence ($-\Delta\delta/\Delta T$) of the key allylglycine-NH was found to be 0.003 ppm/K. In contrast, the ($-\Delta\delta/\Delta T$) for all the other amide resonances are > 0.007 ppm/K, reflecting full solvent exposure. Likewise, in the solvent titration measurements, the allylglycine-NH shows very minor changes in chemical shift with increasing $(CD_3)_2SO$ concentration, while the other amide resonances shift steadily downfield. These results are very similar to the observations of Balaram *et al.* for the tetrapeptide disulfide **11**, where the illustrated Cys(1)-C=O···HN-Cys(4) was established by these methods.[22] Therefore, we concluded that a transannular 4→1 H-bond existed in $CDCl_3$ solution between the allylglycine(1) and (4) of **14**.

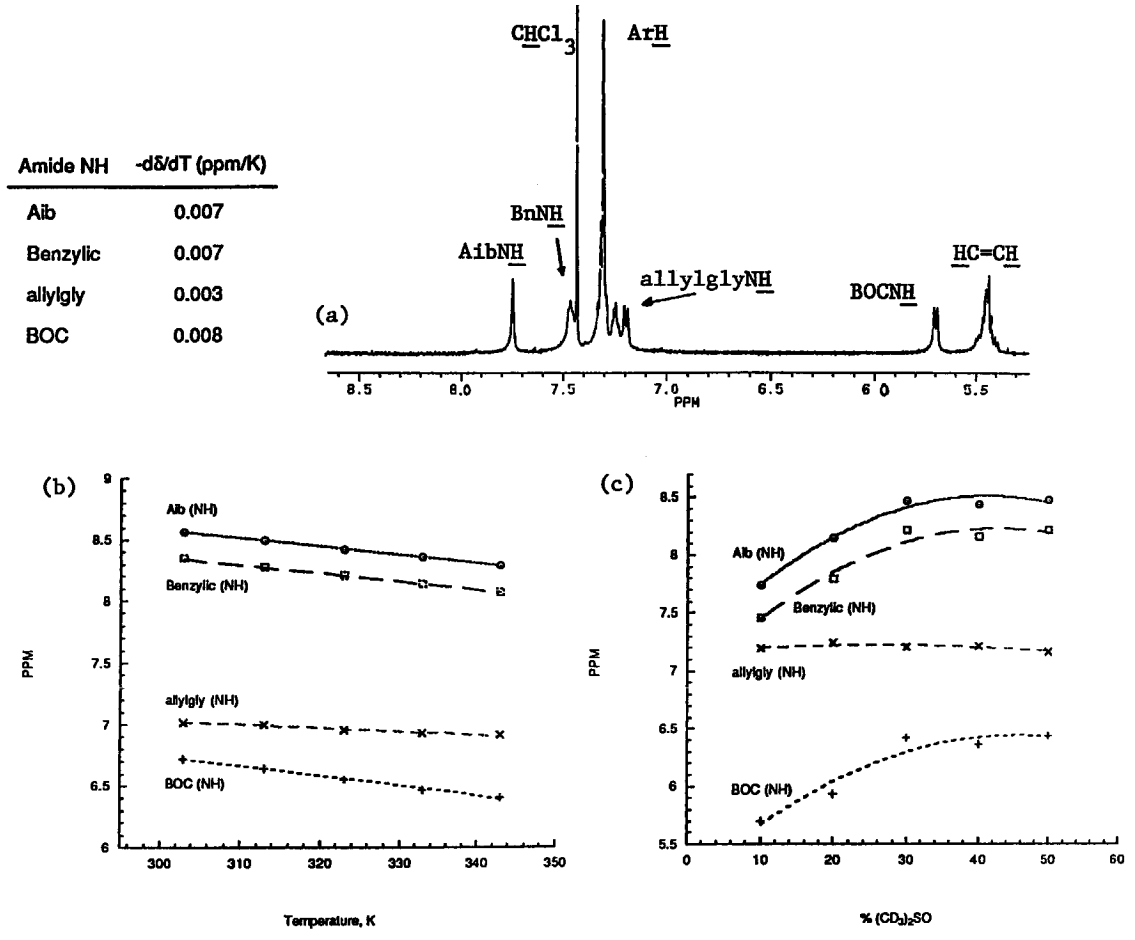


Figure 3. (a) Amide-NH and olefin region of the 500 MHz ^{1}H NMR spectrum of **14** in 10% $(CD_3)_2SO/CDCl_3$. (b) Temperature dependence of chemical shift for amide protons in $(CD_3)_2SO$ solution ($-\Delta\delta/\Delta T$). (c) Dependence of chemical shift for amide protons in $CDCl_3$ solution with increasing $(CD_3)_2SO$ concentration.

Employing the same NMR techniques as for **14**, an analogous transannular H-bond was identified in cyclic peptide **18** (Figure 4). The temperature dependence ($-\Delta\delta/\Delta T$) of the key allylglycine-NH was found to be ~0.00 ppm/K, or otherwise non-existent. Interestingly, the ($-\Delta\delta/\Delta T$) for the Boc-NH resonance was > 0.007 ppm/K, reflecting full solvent exposure, while the two leucine amide NH resonances exhibited intermediate temperature dependencies, denoting moderate solvent exposure.[32] Similarly, in the solvent titration measurements, the allylglycine-NH showed very minor changes in chemical shift with increasing $(CD_3)_2SO$ concentration, while the Boc-NH shifted steadily downfield and the two leucine NHs shifted at a more moderate rate. These

results suggested that cyclic tetrapeptide **18** assumed a "pseudo" β-turn conformation, where the diagnostic transannular 4→1 allylglycine H-bond was present along with weak 3→1 γ-turn-type H-bonding between both of the leucines NHs and the carbonyls of allylglycines (1) and (4). Such simultaneous 4→1 and 3→1 H-bonding patterns have been reported previously for small β-turn model peptides.[19,33] While no further conformational analysis was pursued at this point to better establish the structure of **18**, the heightened flexibility of the peptide backbone of **18** relative to **14** could allow it to undergo subtle conformational changes in solution which permit multiple simultaneous H-bonding patterns.

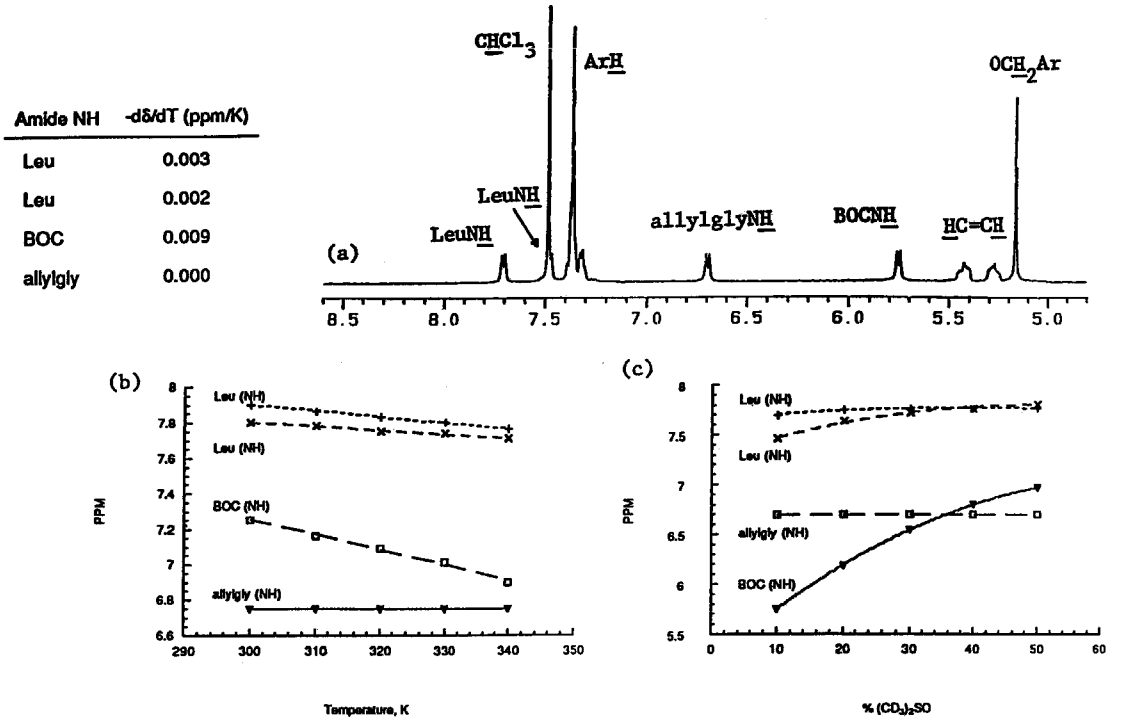
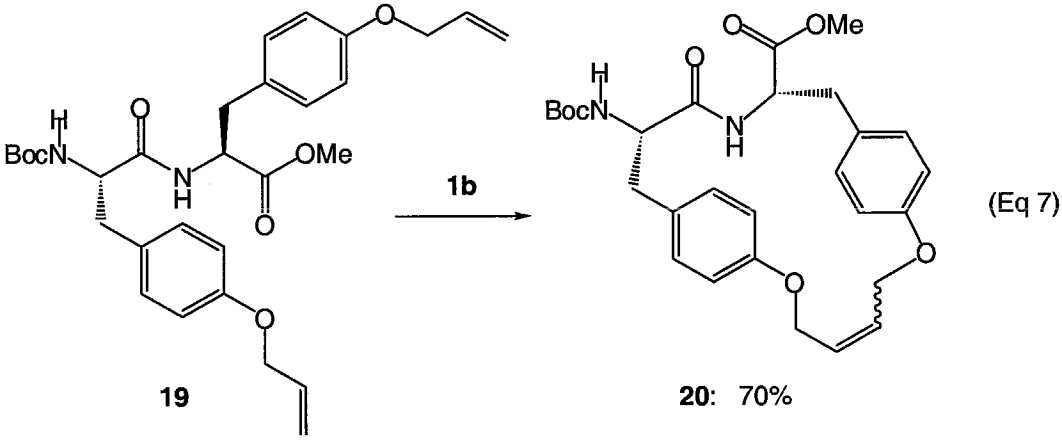


Figure 4. (a) Amide-NH and olefin region of the 500 MHz ^{1}H NMR spectrum of **18** in 10% $(CD_3)_2SO/CDCl_3$. (b) Temperature dependence of chemical shift for amide protons in $(CD_3)_2SO$ solution $(-\Delta\delta/\Delta T)$. (c) Dependence of chemical shift for amide protons in $CDCl_3$ solution with increasing $(CD_3)_2SO$ concentration.

Additional Examples

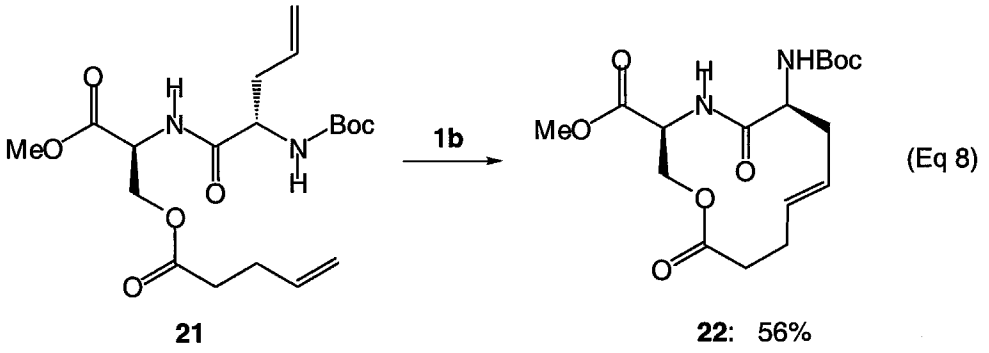
In an effort to understand the generality of the method for introducing carbon-carbon bond cross-links into peptide systems, we explored several examples which are

not based on the tetrapeptide framework. In these cases, we also studied examples wherein alkene functionality is not solely based on allylglycine. For example, the bis(*O*-allyl)-tyrosine dimer **19** undergoes efficient RCM to produce the 20-membered ring macrocycle **20** in 70% yield (Eq 7).[34] It is interesting to note that acyclic peptide **19** has no apparent structural preorganization by intramolecular hydrogen bonding.[23] Nevertheless, at high dilution (0.002 M) it undergoes successful RCM to yield macrocyclic peptide **20**.



(Eq 7)

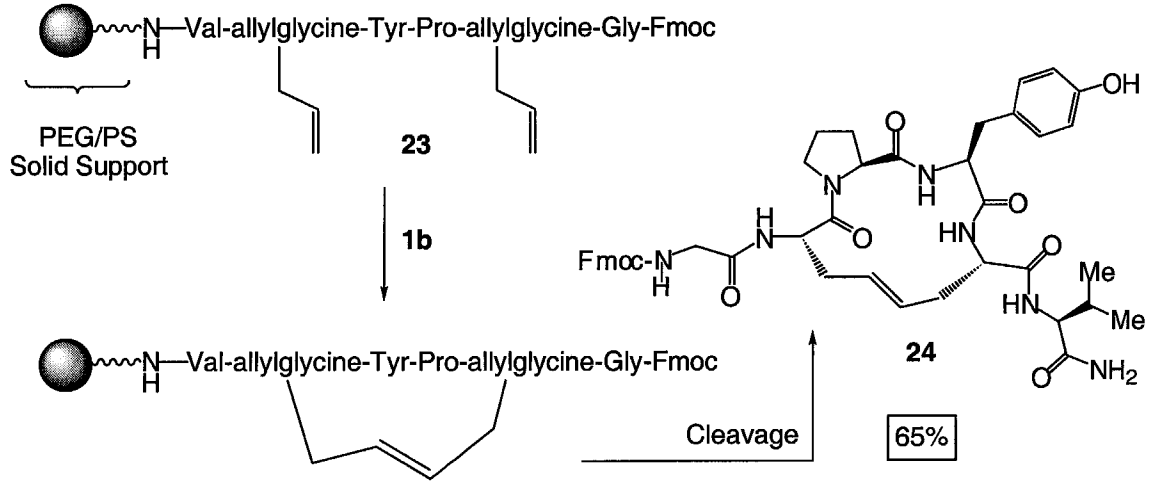
Likewise the Ser-allylglycine dipeptide derivative **21** can be converted to macrocycle **22** by RCM in 56% yield (Eq 8). Interestingly, this system required extremely high dilution conditions to minimize dimer formation. When this cyclization was performed at conventional cyclization concentration (0.002 M), dimeric products were observed. However, further dilution to the somewhat extreme level of 0.0005 M resulted in the exclusion of dimer. Detailed discussion of intermolecular olefin cross-metathesis and its application to the controlled synthesis of dimeric peptides will be discussed in Chapter 5. Compounds **18** and **20** represent two of the three macrocyclic dipeptides[6i] reported to date synthesized *via* RCM which are *devoid* of any apparent conformational bias.[35]



21 **22**: 56%

Preliminary Application of RCM to Solid Support

Our initial efforts to study the RCM of alkene-containing peptides wherein we incorporated > 5 amino acid residues were thwarted by the low solubility of the substrates in the organic solvents where catalysts **1** display their highest activities. As a result, we began to examine the feasibility of performing the ring-closing reactions on solid support-bound peptides.[36,37] To demonstrate the compatibility of the catalyst with solid phase techniques, we revisited one of the tetrapeptide macrocycles that was found to undergo efficient RCM in solution (Scheme 4). We resynthesized a solid support-bound analog of tetrapeptide **15** using conventional solid phase peptide synthesis techniques on PEG/PS resin (**23**).[10,38,39]



Scheme 4. Synthesis of cyclic hexapeptide **24** *via* RCM on solid support.

To effect RCM, the polymer beads were first swelled in CH_2Cl_2, the solvent of choice for maximum activity of **1**. Catalyst **1b** was then introduced and the mixture was heated to 40 °C for 22 h, in analogy to the conditions for the solution phase RCM reaction. Upon cleavage from the resin, HPLC analysis revealed the presence of two peaks in a 2:1 ratio, the minor peak corresponding to the acyclic peptide (Figure 5a). Analysis by LRMS (FAB) confirmed the identity of the two peaks as the starting material, and the ring-closed product **24** (Figure 5b). This experiment demonstrates that catalysts **1** react efficiently with solid support-bound substrates.[40,41] Application of this solid support methodology to the synthesis of larger, conformationally rigidified peptides will be addressed in Chapter 3.

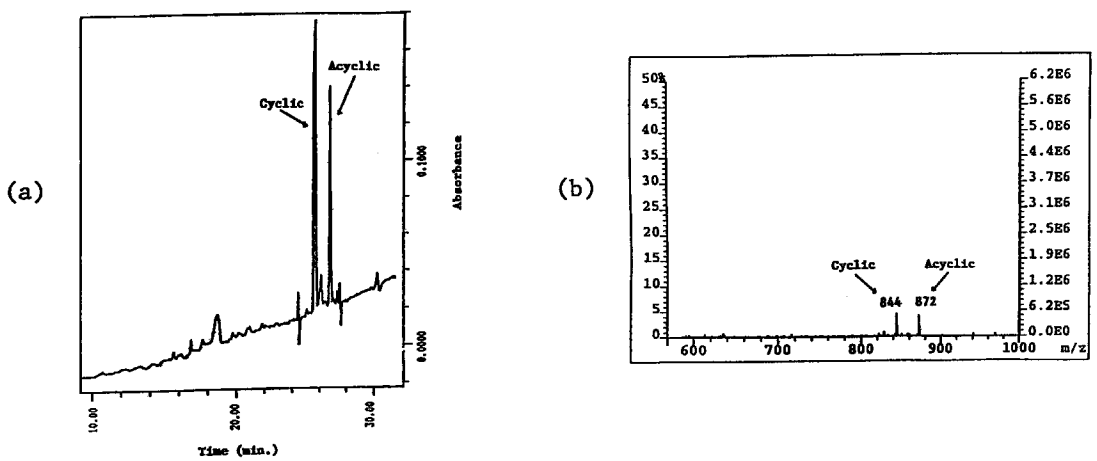


Figure 5. (a) HPLC trace of mixture of acyclic and cyclic glutaredoxin analogs (**23** and **24**) synthesized on solid support. (b) Low resolution FABMS of mixture of acyclic and cyclic glutaredoxin analogs (**23** and **24**).

Summary and Conclusions

Ruthenium alkylidenes **1a** and **1b** have been shown to demonstrate high metathesis activity for the RCM of amino acids and peptides containing olefins. A range of substrates was examined and ring sizes ranging from six to twenty were generated in good to excellent yields. The method was found to be particularly well-suited for the

synthesis of tetrapeptide, carbon-carbon double bond mimics of naturally-occurring tetrapeptide disulfides. Solution conformational analysis of two of the cyclic tetrapeptides (**14** and **18**) revealed the presence of intramolecular 4→1 H-bonding which was analogous to that which had been reported for the corresponding disulfide-bridged β-turn system (**11**). In addition, RCM employing ruthenium alkylidene **1b** was demonstrated for the first time to be compatible with substrates bound to solid supports. These experiments illustrate a very straightforward synthetic strategy for the synthesis of conformationally rigidified cyclic peptides which contain non-native carbon-carbon bond cross-links. Application of RCM to the synthesis of more complex cyclic peptides exhibiting β-sheet and helical secondary structural motifs support will be discussed in Chapters 3 and 4.

Acknowledgements

This research was generously supported by grants from the NIH and the NSF. Prof. Scott J. Miller is acknowledged for his tremendous intellectual and technical contributions to this work, along with his inexhaustible enthusiasm for this project as a whole. In addition, Professor Andrew G. Myers and Dr. James Gleason are gratefully acknowledged for providing samples of optically pure (*S*)- and (*R*)-allylglycine, as well as for helpful discussions. Professor Barbara Imperiali, Dr. Mary Struthers, Dr. Ranabir Sinha Roy, and Dr. Eldon E. Baird are acknowledged for assistance with solid phase peptide synthesis and many helpful discussions.

Experimental Section

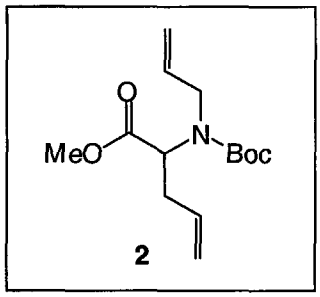
General Experimental Considerations. NMR spectra were recorded on a General Electric QE-300 or Bruker AM-500 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet

(t), quartet (q), and multiplet (m). Major rotamer peaks are reported in spectra that are not fully coalesced. The olefin configurations for **14**, **16**, **18**, and **22** were assigned by sequential irradiation of the C_β-protons and analysis of the resulting patterns. The notation d(m) refers to the value of the primary J value extracted from this analysis. Infrared spectra were obtained on a Perkin-Elmer 1600 Series FT-IR. Optical rotations were recorded on a Jasco DIP-181 digital polarimeter at 589 nm and are reported as $[\alpha]_D$ (concentration in grams/100 mL solvent). Low and high resolution mass spectra were provided by either the Chemistry and Biology Mass Spectrometry Facility (Caltech) or the Southern California Mass Spectrometry Facility (University of California, Riverside).

Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Flash column chromatography was performed using Silica Gel 60 (230-400 mesh) from EM Science.[42] Commercially available reagents and starting materials were purchased from Aldrich, Sigma, Applied Biosystems, Peptides International, and PerSeptive Biosystems, and used as delivered unless noted otherwise. Catalysts **1a** and **1b** were prepared according to the published procedures.[3]

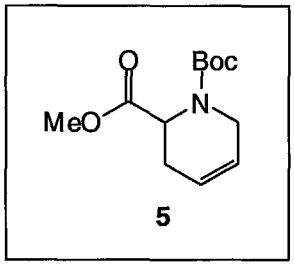
Peptide Synthesis. Peptides **13**, **15**, and **17** were synthesized by conventional solution phase synthesis methods using a racemization free fragment condensation strategy. Couplings were mediated by *N,N*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT).[10] The Boc group was used to protect the N-terminus, and the C-terminus was protected as a methyl ester. Deprotections were performed using 1:1 trifluoroacetic acid/CH_2Cl_2 and saponification, respectively. All intermediates were characterized by ^1H NMR and TLC, and if necessary purified by column chromatography on silica gel.

All ring-closing metathesis (RCM) reactions were carried out under an argon atmosphere with dry, degassed solvents under anhydrous conditions.[43]



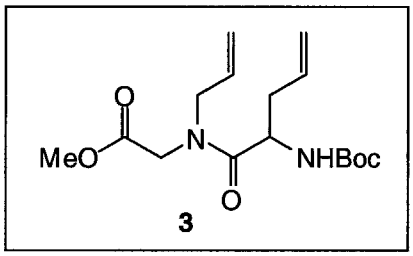
2

***N*-Allyl-*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-DL-norvaline, methyl ester (2).** To a 0 °C solution of (+/-)-*N*-Boc-allylglycine methyl ester (1.4 g, 6.1 mmol) in 30 mL of DMF was added allyl bromide (581 μL, 6.7 mmol) followed by sodium hydride (160 mg, 6.7 mmol). Gas evolution was observed, and the reaction mixture assumed a pale yellow color. The solution was stirred for 1.5 h at 0 °C and 30 min at 25 °C before it was quenched by addition of 20 mL of dilute aqueous NH_4Cl. The product was extracted with three 20 mL portions of Et_2O, dried over $MgSO_4$ and purified by flash chromatography (1.5 cm x 12 cm of silica gel, 20% EtOAc/hexanes) to afford 900 mg (55%) of **2** as a colorless oil. ^1H NMR ($CDCl_3$, 500 MHz, not coalesced): δ 5.77-5.60 (2H, br m), 5.10-4.95 (4H, br m), 4.5-3.6 (3H, br m), 3.65 (3H, s), 2.80-2.65 (1H, br m), 2.60-2.45 (1H, br m); ^{13}C NMR ($CDCl_3$, 125 MHz, not coalesced): δ 171.7, 155.4, 154.6, 135.0, 134.5, 134.4, 134.2, 120.4, 117.6, 117.3, 116.1, 80.5, 80.4, 59.0, 58.2, 51.9, 50.5, 49.0, 34.7, 33,8, 28.2; IR (neat, cm^{-1}): 3079, 2978, 1745, 1697, 1643, 1453; TLC R_f = 0.50 (30% EtOAc/hexane); HRMS (EI) calcd for $C_{14}H_{24}N_1O_4$ $[M+H]^+$ 270.1705, found 270.1698.



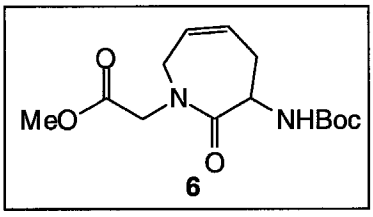
5

1-*tert*-Butyl 2-methyl (±)-3,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (5). To a 25 °C solution of acyclic diene **2** (170 mg, 0.636 mmol) in 7 mL of C_6H_6 was added ruthenium catalyst **1a** (29 mg, 0.032 mmol, 5 mol %). The orange-brown solution was stirred at this temperature for 2 h before it was concentrated and applied directly to a silica gel column. Chromatography (1.5 cm x 12 cm silica gel; solvent gradient: 5% EtOAc/hexane to 20% EtOAc/hexane) afforded 139 mg (91%) of **5** as a clear oil. ^{1}H NMR ($CDCl_3$, 300 MHz, not coalesced): δ 5.77-5.65 (1H,br m), 5.01-4.80 (1H, br m), 4.20-3.70 (2H, br m), 3.71, 3.70 (3H, 2 x s), 2.65-2.50 (2H, br m), 1.49, 1.46 (9H, 2 x s); ^{13}C NMR ($CDCl_3$, 75 MHz, not coalesced): δ 172.3, 155.9, 124.5, 124.2, 122.4, 122.0, 80.4, 52.4, 52.3, 51.0, 42.3, 41.6, 28.4, 26.7, 26.6; IR (neat, cm^{-1}): 2976, 1746, 1694, 1454, 1403; TLC R_f = 0.40 (30% EtOAc/hexane); HRMS (EI) calcd for $C_{12}H_{18}N_1O_4$ $[M-H]^+$ 240.1236, found 240.1236.



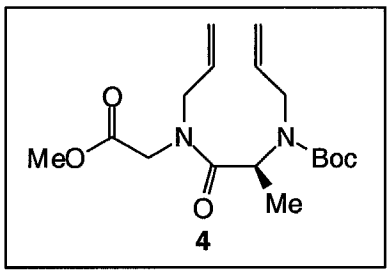
3

***N*-Allyl-*N*-[*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-DL-norvalyl]glycine, methyl ester (3).** To a solution of *N*-allyl-*N*-Boc-Gly-methyl ester (1.17 g, 5.10 mmol) in 20 mL of CH_2Cl_2 was added 10 mL of TFA. The mixture was stirred for 1 h before it was concentrated and redissolved in 25 mL of CH_2Cl_2. The solution was washed with 100 mL of saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated. The unpurified amine was then dissolved in 15 mL CH_2Cl_2 and treated with (+/-)-*N*-Boc-allylglycine (400 mg, 1.86 mmol), DCC (383 mg, 1.86 mmol) and DMAP (25 mg, 0.121 mmol). The mixture was stirred for 1h as a white precipitate formed. The mixture was filtered, concentrated and chromatographed (1.5 cm x 10 cm silica gel, 20% EtOAc/hexanes to 50% EtOAc/hexane) to afford 566 mg (93%) of **3** as a clear oil ^{1}H NMR ($CDCl_3$, 500 MHz, not coalesced): δ 5.81-5.70 (2H, m), 5.29-5.04 (5H, m), 4.65 and 4.40 (1H, q, J = 8.2 Hz), 4.21 (1H, d, J = 7.2 Hz), 4.05-3.80 (3H, m), 3.82 (1H, d, J = 7.2 Hz), 3.71 and 3.68 (3H, 2 x s), 2.50-2.40 (1H, m), 2.34-2.30 (1H, m), 1.38 and 1.37 (9H, 2 x s); ^{13}C NMR ($CDCl_3$, 125 MHz, not coalesced): δ 172.3, 172.1, 169.5, 169.3, 155.2, 155.0, 132.9, 132.6, 132.2, 118.5, 118.4, 118.1, 79.6, 79.5, 52.3, 52.0, 51.2, 49.9, 49.7, 49.4, 48.2, 46.9, 37.6, 37.3, 28.2, 28.1; IR (neat, cm^{-1}): 3320, 2979, 1747, 1713, 1650, 1514, 1454; TLC R_f = 0.40 (30% EtOAc/hexane); HRMS (EI) calcd for $C_{16}H_{27}N_2O_5$ $[M+H]^+$ 327.1920, found 327.1914.



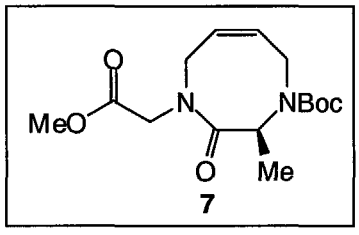
6

Methyl (±)-3-[(*tert*-butoxycarbonyl)amino]-2,3,4,7-tetrahydro-2-oxo-1*H*-azepine-1-acetate (6). To a 50 °C solution of acyclic diene **3** (160 mg, 0.491 mmol) in 60 mL of $CHCl_3$ was added ruthenium catalyst **1a** (29 mg, 0.032 mmol, 5 mol %). The orange-brown solution was stirred at this temperature for 4 h before it was concentrated and applied directly to a silica gel column. Chromatography (1.5 cm x 12 cm silica gel; 25% EtOAc/hexane) afforded 76 mg (52%) of **6** as a clear oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.76-5.68 (2H, m), 4.91 (1H, m), 4.52 (1H, br d, J = 17 Hz), 4.35 (1H, d, J =17.4 Hz), 4.03 (1H, d, J = 17.4 Hz), 3.70 (3H, s), 3.70 (1H, m), 3.32 (1H, dd, J = 17.6, 7.2 Hz), 2.63 (1H, dd, J =18.1, 4.1 Hz), 2.23 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.9, 169.4, 154.9, 129.9, 123.6, 79.5, 52.2, 50.1, 50.0, 47.3, 33.2, 28.3; IR (neat, cm^{-1}) 3272, 2973, 1759, 1715, 1650, 1538, 1487, 1454; TLC R_f = 0.15 (30% EtOAc/hexane); HRMS (EI) calcd for $C_{14}H_{23}N_2O_5$ $[M+H]^+$ 299.1607, found 299.1603.



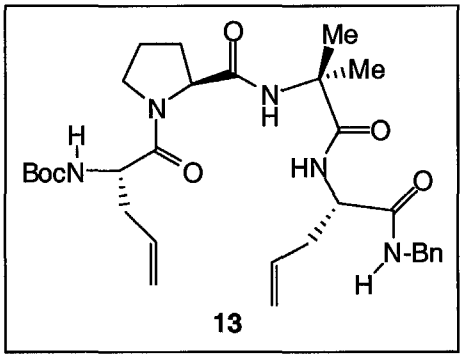
4

***N*-Allyl-*N*-[*N*-allyl-*N*-(*tert*-butoxycarbonyl)-L-alanyl]glycine, methyl ester (4).** To a -78 °C solution of *N*-Boc-Ala-Gly-methyl ester (180 mg, 0.66 mmol) in 5 mL of THF was added allyl bromide (125 mL, 1.45 mmol) followed by phosphazene-P4 base (968 μL, 1.38 mmol). The mixture was stirred at -78 °C for 1 h and then warmed to 25 °C for 30 min. The reaction mixture was concentrated and purified by chromatography (1.5 cm x 12 cm silica gel, 25% EtOAc/hexane) to afford 44 mg (20%) of **4** as a colorless oil. ^{1}H NMR (toluene-d_8, 300 MHz, 80 °C - not fully coalesced): δ 5.8 (1H, br m), 5.65 (1H, br m), 5.30-4.80 (5H, m), 4.20-3.70 (6H, m), 3.40 (3H, s), 1.40 (9H, s), 1.28 (3H, d, J = 6.8 Hz); ^{13}C NMR (toluene-d_8, 125 MHz, 80 °C, not fully coalesced): δ 172.0, 169.7, 155.8, 136.9, 134.1, 117.3, 115.7, 80.1, 51.4, 47.6, 46.5, 28.6, 16.4; IR (neat, cm^{-1}): 2980, 1754, 1660, 1450; TLC R_f = 0.55 (50% EtOAc/hexane); HRMS (EI) calcd for $C_{17}H_{29}N_2O_5$ $[M+H]^+$ 341.2076, found 341.2064.



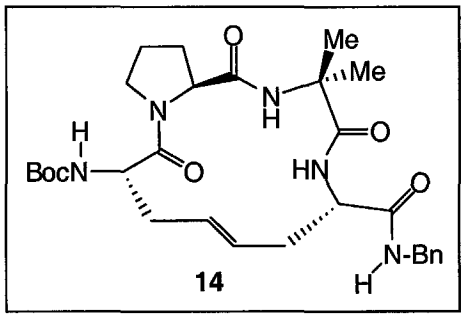
7

Methyl (3*S*,6*Z*)-4-(*tert*-butoxycarbonyl)-3,4,5,8-tetrahydro-3-methyl-2-oxo-1,4-diazocine-1(2*H*)-acetate (7). To a 50 °C solution of acyclic diene **4** (17 mg, 0.050 mmol) in 5 mL of C_6H_6 was added ruthenium catalyst **1a** (7 mg, 0.008 mmol, 16 mol %) as a solution in 5 mL of C_6H_6. The orange-brown solution was stirred at this temperature for 24 h before it was concentrated and applied directly to a silica gel column. Chromatography (1.5 cm x 12 cm silica gel; 25% EtOAc/hexane) afforded 8 mg (51%) of **7** as a clear oil. ^{1}H NMR (toluene-d_8, 300 MHz, 80 °C - not fully coalesced): δ 5.60 (1H, m), 5.37 (1H, m), 5.00 (1H, br m), 4.40-3.00 (6H, br m), 3.35 (3H, s), 1.40 (3H, br d, J = 7 Hz), 1.38 (9H, s); ^{13}C NMR (C_6D_6, 125 MHz, 70 °C - not fully coalesced): δ 171.2, 169.3, 154.3, 134.4 (br), 125.2 (br), 79.9, 51.2, 49.9, 45.4, 43.2 (br), 30.0. 28.4, 17.1; IR (neat, cm^{-1}): 2926, 1755, 1693, 1659, 1436, 1393; TLC R_f = 0.30 (50% EtOAc/hexane); HRMS (EI) calcd for $C_{15}H_{24}N_2O_5$ $[M]^+$ 312.1685, found 312.1678.



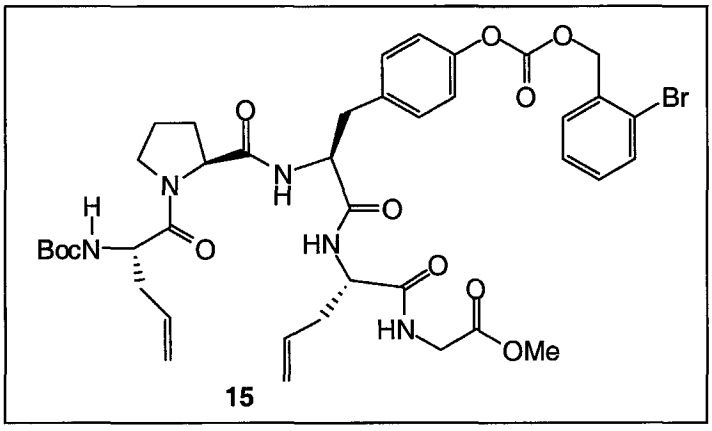
13

***N*-Benzyl-*N*^{2}-[*N*-[1-[*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-L-norvalyl]-L-prolyl]-2-methylalanyl]-4,5-didehydro-L-norvalinamide (13).** Tetrapeptide **13** was prepared according to the standard solution protocol described in the general experimental above. ^{1}H NMR (CD_3CN, 500 MHz): δ 7.74 (1H, br t), 7.29 (5H, m), 7.22 (1H, m), 7.0 (1H, br s), 5.83-5.78 (2H, m), 5.55 (1H, br d), 5.18-5.05 (4H, m), 4.42-4.21 (5H, m), 3.99 (1H, br t), 3.76 (1H, m), 3.60 (1H, m), 2.75 (1H, m), 2.5-1.1.7 (8H, m), 1.40 (12H, s), 1.35 (3H, s); ^{13}C NMR (CD_3CN, 125 MHz): δ 175.5, 174.0, 173.5, 172.6, 157.0, 140.8, 136.6, 135.0, 129.7, 128.7, 128.1, 119.3, 118.1, 80.5, 63.6, 58.3, 54.8, 53.8, 49.0, 43.9, 36.9, 36.6, 30.0, 29.0, 27.6, 26.3, 24.8; IR (CH_2Cl_2, cm^{-1}): 3425, 3339, 2982, 2934, 1668, 1498, 1439; TLC R_f = 0.45 (100% EtOAc); $[\alpha]_D$ = -53.0 (c = 0.7, CH_2Cl_2); HRMS (FAB) calcd for $C_{31}H_{46}N_5O_6$ $[M+H]^+$ 584.3448, found 584.3471.



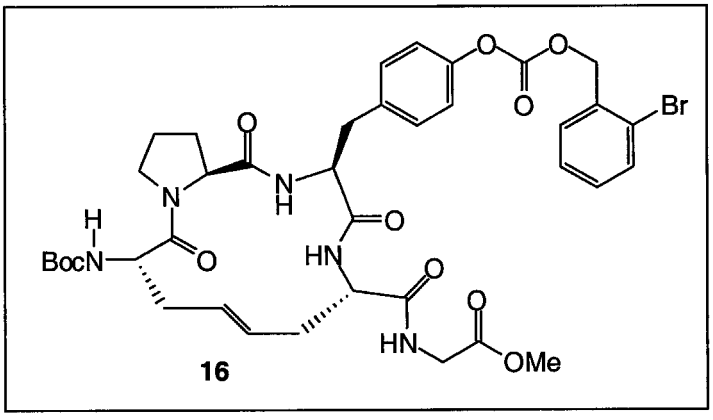
14

***tert*-Butyl-(6*S*,8*E*,11*S*,16a*S*)-6-(benzylcarbamoyl)-1,2,3,4,5,6,7,10,11,12,14,15,16,16a-tetradecahydro-3,3-dimethyl-1,4,12-trioxopyrrolo[1,2-*a*]-[1,4,7]-triazacyclotetradecine-11-carbamate (14).** To a solution of acyclic diene **13** (210 mg, 0.360 mmol) in 80 mL of CH_2Cl_2 was added *via* cannula a solution of ruthenium catalyst **1a** (67 mg, 0.072 mmol) predissolved in 20 mL CH_2Cl_2. The orange-brown solution was heated to 40 °C and stirred at this temperature for 20 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (1.5 cm x 15 cm silica gel; solvent gradient: 50% EtOAc/hexane to 100% EtOAc) afforded 120 mg (60%) of **14** as an off-white powder. Macrocycle **14** can be recrystallized by dissolving the powder in CH_2Cl_2 and layering the solution with hexanes. White needles and prisms result. However, upon removal of solvent the amorphous white powder is reobtained. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ 7.33-7.21 (5H, m), 7.01 (1H, br d, J = 7.2 Hz), 6.91 (1H, br t), 6.53 (1H, br s), 5.58 (1H, d, J = 7.8Hz), 5.51-5.42 (1H, d(m), J = 15 Hz), 5.39-5.30 (1H, d(m), J = 15 Hz), 4.65-4.59 (2H, m), 4.50 (1H, q, J = 6 Hz), 4.29 (1H, dd, J = 15, 5.0 Hz), 4.20 (1H, t, J = 7.2 Hz), 3.69 (1H, m), 3.58 (1H, m), 2.6-1.88 (8H, m), 1.50 (3H, s), 1.41 (9H, s), 1.35 (3H, s); ^{13}C NMR (CD_2Cl_2, 125 MHz): δ 175.4, 172.5, 172.1, 172.0, 155.0, 139.9, 131.0, 129.6, 128.9, 128.5, 128.3, 80.5, 62.2, 61.9, 58.4, 53.4, 48.7, 44.2, 34.9, 34.4, 29.3, 29.2, 26.8, 23.9; IR (CH_2Cl_2, cm^{-1}): 3424, 3324, 3054, 2985, 1691, 1632, 1498, 1444; TLC R_f = 0.20 (100% EtOAc); $[\alpha]_D$ = +63.8 (c = 0.73, CH_2Cl_2); HRMS (FAB) calcd for $C_{29}H_{42}N_5O_6$ $[M+H]^+$ 556.3135, found 556.3145.



15

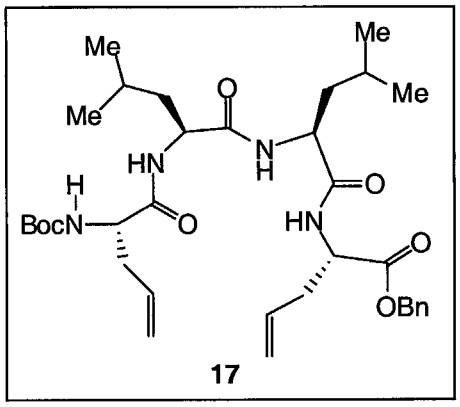
***N*-[*N*-[*O*-[[(*o*-Bromobenzyl)oxy]carbonyl]-*N*-[1-[*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-L-norvalyl]-L-prolyl]-L-tyrosyl]-4,5-didehydro-L-norvalyl]-glycine, methyl ester (15).** Pentapeptide **15** was prepared according to the standard solution protocol described in the general experimental above.[44] ^{1}H NMR ($(CD_3)_2SO$, 500 MHz): δ 8.34 (1H, br t, *J* = 5 Hz), 7.94 (1H, d, *J* = 8 Hz), 7.89 (1H, d, *J* = 8 Hz), 7.71 (1H, d, *J* = 8 Hz), 7.57 (1H, d, *J* = 8 Hz), 7.46 (1H, t, *J* = 8 Hz), 7.36 (1H, t, *J* = 8 Hz), 7.29 (3H, amide NH obscured, apparent d, *J* = 8 Hz), 7.13 (2H, d, *J* = 7 Hz), 6.91 (1H, d, *J* = 8 Hz), 5.83-5.70 (2H, m), 5.32 (2H, s), 5.12- 5.01 (4H, m), 4.48 (1H, br q, *J* = 9 Hz), 4.40-4.30 (2H, br m), 4.21 (1H, br q, *J* = 7 Hz), 3.91-3.80 (2H, m), 3.63 (3H, s), 3.60-3.51 (2H, m), 3.06 (1H, dd, *J* = 14, 4 Hz), 2.87 (1H, dd, *J* = 14, 9 Hz), 2.40 (1H, m), 2.33 (2H, m), 2.22 (1H, m), 1.95 (1H, m), 1.82-1.71 (3H, br m), 1.36 (9H, s); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C - not fully coalesced): δ 172.1, 172.0, 171.4, 170.9, 170.3, 155.6, 153.3, 150.2, 134.8, 134.4, 133.3, 127.7, 123.5, 120.0, 118.8, 118.5, 79.8, 69.6, 63.0, 60.7, 55.9, 52.9, 52.2, 47.6, 41.3, 36.9, 36.5, 28.7, 28.4, 25.2; IR (CH_2Cl_2, cm^{-1}): 3415, 3333, 2923, 2851, 1759, 1672, 1605, 1503, 1441, 1364; TLC R_f = 0.27 (83% EtOAc/hexane); $[\alpha]_D$ = -38.0 (c = 1.1, CH_2Cl_2); HRMS (FAB) calcd for $C_{40}H_{51}N_5O_{11}Br$ $[M+H]^+$ 856.2768, found 856.2794.



16

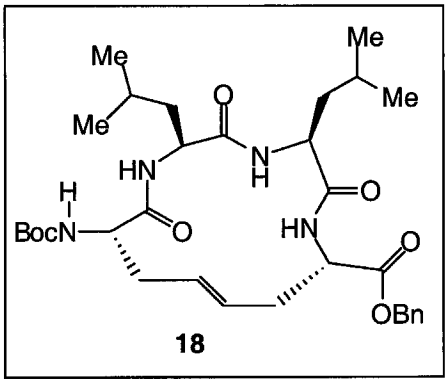
***tert*-Butyl-(3*S*,6*S*,8*E*,11*S*,16a*S*)-3-[*p*-[[[(*o*-bromobenzyl)oxy]carbonyl]-oxy]benzyl]-1,2,3,4,5,6,7,10,11,12,14,15,16,16a-tetradecahydro-6-[[(methoxycarbonyl)-methyl]-carbamoyl]-1,4,12-trioxypyrrolo[1,2-*a*][1,4,7]-triazacyclotetra-decine-11-carbamate (16).** To a solution of acyclic diene **15** (200 mg, 0.234 mmol) in 53 mL of CH_2Cl_2 was added via syringe a solution of ruthenium catalyst **1b** (58 mg, 0.072 mmol) predissolved in 10 mL CH_2Cl_2. The purple solution was heated to 45 °C, and turned orange-brown in color over a 30 min period. The solution was stirred at 45 °C for 23 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (3 cm x 15 cm silica gel; solvent gradient: 80% EtOAc/hexane to 100% EtOAc) afforded 155 mg (80%) of **16** as an off-white powder. ^{1}H NMR ($(CD_3)_2SO$, 500 MHz): δ 8.22 (1H, br t, J = 6 Hz), 7.88 (1H, br d, J = 9 Hz), 7.71 (1H, d, J = 8 Hz), 7.57 (1H, d, J = 7 Hz), 7.46 (1H, t, J = 7 Hz), 7.36 (2H, t, J = 7 Hz), 7.22 (2H, d, J = 8 Hz), 7.13 (3H, amide NH obscured, apparent d, J = 8 Hz), 6.69 (1H, d, J = 9 Hz), 5.46 (1H, d(m), J = 15 Hz), 5.36 (1H, d(m), J = 15 Hz), 5.31 (2H, s), 4.58 (2H, m), 4.47 (1H, m), 3.97 (1H, m), 3.85 (2H, m), 3.68 (1H, m), 3.64 (3H, s), 3.36 (2H, m), 2.80 (1H, m), 2.46 (2H, m), 2.11 (2H, m), 1.84 (1H, m), 1.71 (2H, m), 1.37 (9H, s), 1.23 (1H, m); ^{13}C NMR ($(CD_3)_2SO$, 75 MHz): δ 171.8, 170.8, 170.2, 170.0, 168.9, 155.0, 152.7, 149.1, 136.5, 134.0, 132.7, 130.7, 130.6, 130.3, 130.1, 128.0, 127.4, 122.9, 120.5, 78.2, 69.2, 60.8, 53.1, 51.7, 51.2, 50.7, 46.5, 35.3, 34.8, 28.5, 28.0, 24.4; IR (CH_2Cl_2, cm^{-1}): 3426,

3354, 2954, 2923, 2851, 1759, 1687, 1621, 1508, 1446, 1369, 1164; TLC R_f = 0.21 (83% EtOAc/hexane); $[\alpha]_D$ = -29.0 (c = 1.1, CH_2Cl_2); HRMS (FAB) calcd for $C_{38}H_{47}N_5O_{11}Br$ $[M+H]^+$ 828.2443, found 828.2455.



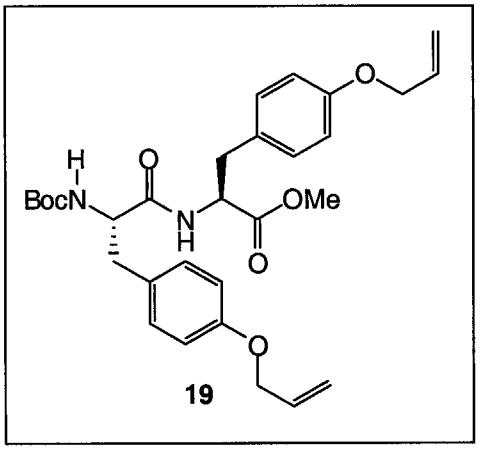
17

***N*[*N*-[*N*-(tert-Butoxycarbonyl)-4,5-didehydro-L-norvalyl]-L-leucyl]-L-leucyl]-4,5-didehydro-L-norvaline, benzyl ester (17).** Tetrapeptide **17** was prepared according to the standard solution protocol described in the general experimental above. ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C - not fully coalesced): δ 7.33 (5H, m), 6.91 (1H, apparent s), 6.73 (1H, apparent s), 6.64 (1H, d, J = 7 Hz), 5.74-5.63 (2H, m), 5.19-5.02 (6H, m), 4.66 (1H, q, J = 7 Hz), 4.49 (1H, q, J = 9 Hz), 4.39 (1H, q, J = 8 Hz), 4.11 (1H, m), 2.61-2.38 (4H, m), 1.73-1.46 (6H, m), 1.43 (9H, s), 0.96-0.86 (12H, m); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C - not fully coalesced): d 171.9, 171.8, 171.4, 135.7, 133.1, 132.6, 128.8, 128.6, 128.5, 80.7, 67.2, 52.3, 51.9, 41.3, 41.1, 36.8, 36.5, 34.2, 29.9, 28.5, 25.9, 25.1, 25.0, 24.9, 23.2, 23.0, 22.2, 22.1; IR (CH_2Cl_2, cm^{-1}): 3414, 3339, 2961, 2929, 1740, 1694, 1505, 1456, 1365, 1169; TLC R_f = 0.41 (80% CH_2Cl_2/EtOAc); $[\alpha]_D$ = -29.5 (c = 1.2, CH_2Cl_2); HRMS (FAB) calcd for $C_{34}H_{53}N_4O_7$ $[M+H]^+$ 629.3914, found 629.3914.



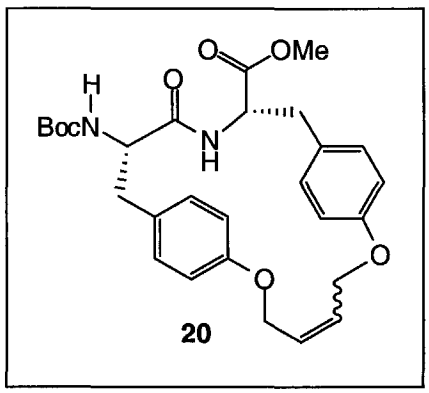
18

***tert*-Butyl (3*S*,6*S*,9*S*,11*E*,14*S*)-14-[(benzyloxy)carbonyl]-3,6-diisobutyl-2,5,8-trioxo-1,4,7-triazacyclotetradec-11-ene-9-carbamate (18).** To a solution of acyclic diene **17** (285 mg, 0.453 mmol) in 100 mL of CH_2Cl_2 was added *via* syringe a solution of ruthenium catalyst **1b** (112 mg, 0.136 mmol) predissolved in 22 mL CH_2Cl_2. The purple solution was heated to 45 °C, and turned orange-brown in color over 20 min. The solution was stirred at 45 °C for 21 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (3 cm x 15 cm silica gel; eluent: 80% CH_2Cl_2/EtOAc) afforded 163 mg (60%) of **18** as an off-white powder. ^{1}H NMR ($(CD_3)_2SO$, 500 MHz): δ 7.90 (1H, d, J = 9), 7.80 (1H, d, J = 8), 7.37 (5H, m), 7.24 (1H, d, J = 8 Hz), 5.46 (1H, d(m), J = 15 Hz), 5.16 (1H, d(m), J = 15 Hz), 5.13 (2H, s), 4.48 (1H, m), 4.21 (2H, m), 4.03 (1H, m), 2.45-2.12 (4H, br m), 1.66-1.45 (6H, m), 1.38 (9H, s), 0.87 (6H, apparent d, J = 6 Hz), 0.81 (6H, apparent t, J = 6 Hz); ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 171.9, 170.8, 170.6, 130.3, 128.1, 127.7, 127.6, 127.5, 78.7, 66.0, 53.4, 52.8, 50.6, 50.0, 40.2, 39.7, 34.6, 33.7, 24.4, 24.2, 24.1, 22.5, 22.2, 20.4, 20.3; IR (CH_2Cl_2, cm^{-1}): 3419, 3339, 2958, 2929, 1740, 1671, 1602, 1515, 1365, 1158; TLC R_f = 0.24 (80% CH_2Cl_2/EtOAc); $[\alpha]_D$ = -114.0 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{32}H_{49}N_4O_7$ $[M+H]^+$ 601.3601, found 601.3610.



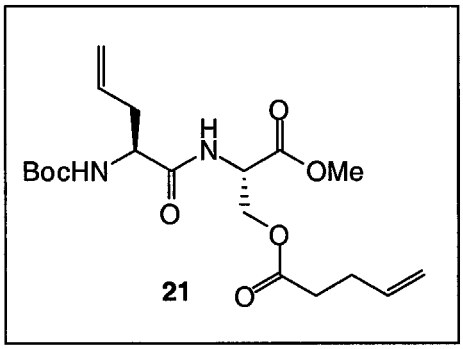
19

***O*-Allyl-*N*-[*O*-allyl-*N*-(*tert*-butoxycarbonyl)-L-tyrosyl]-L-tyrosine, methyl ester (19).** To a solution of *N*-Boc-dityrosine methyl ester (3.26 g, 7.10 mmol) in 30 mL of acetone was added allyl bromide (1.71 mL, 19.8 mmol) and finely powdered K_2CO_3 (2.94 g, 21.3 mmol). The reaction mixture was stirred for 48 h at 25 °C before being filtered through a celite pad. Purification of the residue by chromatography (3 cm x 12 cm silica gel, solvent gradient: 20% EtOAc/hexane to 50% EtOAc/hexane) afforded **19** as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.06 (2H, d, J = 8.4 Hz), 6.87 (2H, d, J = 8.4 Hz), 6.79 (2H, d, J = 8.4 Hz), 6.75 (2H, d, J = 8.4 Hz), 6.38 (1H, br d, J = 7.5 Hz), 6.00 (2H, m), 5.35 (2H, dd, J = 17.1, 0.6 Hz), 5.23 (2H, br d, J = 9.3 Hz), 4.92 (1H, br s), 4.71 (1H, br d, J = 6.2 Hz), 4.46 (4H, br t, J = 4.1 Hz), 4.29 (1H, br s), 3.63 (3H, s), 2.95 (4H, m), 1.38 (9H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.3, 170.7, 157.6 (br d), 155.1, 133.2, 130.2, 130.1, 128.6, 127.8, 117.3, 117.2, 114.8, 114.7, 79.9, 68.7, 68.6, 55.8, 53.3, 51.9, 37.3, 37.0, 28.1; IR (CH_2Cl_2, cm^{-1}): 3420, 2981, 2932, 1742, 1713, 1681, 1610, 1510, 1361; TLC R_f = 0.50 (50% EtOAc/hexane); $[\alpha]_D$ = +20.7 (c = 2.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{30}H_{39}N_2O_7$ $[M+H]^+$ 539.2757, found 539.2766.



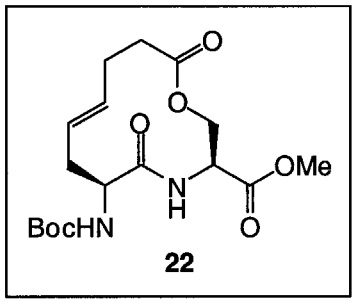
20

***tert*-Butyl-(13*S*,16*S*)-13-(methoxycarbonyl)-15-oxo-2,7-dioxa-14-azatricyclo-[16.2.2.-$2^{8,11}$]tetracosa-4,8,10,18,20,21,23-heptaene-16-carbamate (20)**. To a 50 °C solution of acyclic diene **19** (130 mg, 0.241 mmol) in 100 mL of CH_2Cl_2 was added ruthenium catalyst **1b** (29 mg, 0.072 mmol). Within 5 min, the purple solution became orange-brown and the solution was stirred for an additional 2.5 h, when TLC analysis showed full disappearance of starting material. Triethylamine (1 mL) was added to the solution to deactivate any remaining active catalyst. The solution was then concentrated to afford an oily brown mixture. Purification by chromatography (3 cm x 12 cm silica gel, 50% EtOAc/hexane) afforded 83 mg (68%) of **21** as a white powder. The olefin configuration was not assigned. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.04 (2H, br s), 6.77 (2H, br s), 6.68 (4H, t, J = 8.5 Hz), 5.90 (1H, br m), 5.83 (2H, s), 5.25 (1H, br s), 4.85 (1H, br s), 4.66 (4H, m), 4.39 (1H, br t), 3.70 (3H, s; -minor rotamer at 3.77), 3.34 (1H, br d, J = 12.6 H), 2.98 (2H, br s), 2.63 (1H, dd, J = 14.0, 8.9 Hz), 1.49 (9H, s); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C - not fully coalesced): δ 171.2, 171.1, 156.8, 156.6, 155.5, 130.5, 130.2, 130.0, 129.7, 129.5, 129.2, 127.4, 115.6, 115.4, 115.3, 80.9, 67.0, 66.7, 64.7, 64.1, 55.6, 52.7, 52.1, 37.4, 36.2, 28.3; IR (CH_2Cl_2, cm^{-1}): 3683, 3411, 2933, 1744, 1713, 1676, 1611, 1511, 1484; TLC R_f = 0.45 (50% EtOAc/hexane); $[\alpha]_D$ = +46.4 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{28}H_{35}N_2O_7$ $[M+H]^+$ 511.2444, found 511.2437.



***N*-[*N*-(*tert*-Butoxycarbonyl)-4,5-didehydro-L-norvalyl]-*O*-4-pentenoyl-L-serine, methyl ester (21).** *N*-Boc serine methyl ester (1.20 g, 5.47 mmol) was dissolved in 75 mL CH_2Cl_2 and treated with 4-pentenoic acid (559 μL, 5.47 mmol), DCC (1.13 g, 5.47 mmol), and DMAP (100 mg, 0.82 mmol). A white precipitate formed immediately, and the solution was stirred for 12 h. The mixture was filtered, washed with 50 mL of a 10% citric acid solution, followed by 50 mL saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$, and concentrated to afford 1.65 g of the crude esterified product as a pale yellow oil with some crystalline domains. To the crude product (1.65 g, theoretical 5.47 mmol) dissolved in 15 mL of CH_2Cl_2 was added an excess of TFA (7.67 mL, 99.6 mmol). The solution was allowed to stir at room temperature for 2.5 h after which the solution was concentrated to an orange oil and dried under high vacuum. The oil was then taken up in 100 mL of CH_2Cl_2 and treated with triethylamine (915 μL, 6.56 mmol) at room temperature. After stirring for 15 min, *N*-Boc-allylglycine (1.18 g, 5.47 mmol), HOBT (1.11 g, 8.21 mmol), and DCC (1.13 g, 5.47 mmol) were added to the solution. A white precipitate formed immediately, and the solution was allowed to stir at room temperature for 9 h. The mixture was then filtered, washed with 75 mL of a 10% citric acid solution, and subsequently washed with 75 mL saturated $NaHCO_3$ solution. The product was dried over $MgSO_4$, and concentrated to yield a yellow oil. Purification by column chromatography (4 cm x 15 cm silica gel, solvent gradient 25% EtOAc/hexane to 50% EtOAc/hexane) afforded 1.19 g (55%) of **21** as a clear oil. ^1H NMR ($CDCl_3$, 500 MHz): δ 6.92 (1H, br d, J = 7 Hz), 5.81-5.68 (2H, m), 5.15-4.97 (5H, m), 4.80-4.78 (1H,

m), 4.42 (1H, dd, J = 11, 4 Hz), 4.36 (1H, dd, J = 11, 3 Hz), 4.17 (1H, m), 3.73 (3H, s), 2.54-2.43 (2H, m), 2.38 (2H, m), 2.32 (2H, m), 1.41 (9H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.6, 171.6, 169.7, 155.6, 136.6, 133.1, 119.2, 115.8, 80.4, 63.8, 52.9, 52.0, 36.8, 34.1, 33.7, 28.8, 28.4; IR (CH_2Cl_2, cm^{-1}): 3680, 3427, 2981, 1746, 1716, 1685, 1494, 1438; TLC R_f = 0.21 (75% hexane/EtOAc); $[\alpha]_D$ = +13.1 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{19}H_{31}N_2O_7$ $[M+H]^+$ 399.2131, found 399.2140.



22

***tert*-Butyl-(3*S*,6*S*,8*E*)-3-(methoxycarbonyl)-5,12-dioxo-1-oxa-4-azacyclododec-8-ene-6-carbamate (22).** To a solution of acyclic diene **21** (50 mg, 0.125 mmol) in 230 mL of CH_2Cl_2 was added via syringe a solution of ruthenium catalyst **1b** (30 mg, 0.036 mmol) predissolved in 20 mL CH_2Cl_2. The purple solution was heated to 45 °C, and turned orange-brown in color over 20 min. The solution was stirred at 45 °C for 20 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (3 cm x 10 cm silica gel; eluent: (50% EtOAc/hexane) afforded 28 mg (56%) of **22** as an off-white powder. ^{1}H NMR ($(CD_3)_2SO$, 500 MHz, 60°C - not fully coalesced): δ 7.47 (1H, br d, J = 8 Hz), 6.03 (1H, apparent s), 5.48 (1H, d(m), J = 15 Hz), 5.32 (1H, d(m), J = 15 Hz), 4.72 (1H, m), 4.52 (1H, apparent t, J = 11 Hz), 4.16 (1H, dd, J = 11, 4 Hz), 4.07 (1H, m), 3.64 (3H, s), 2.44-2.18 (6H, m), 1.41, 1.40 (9H, 2 x s); ^{13}C NMR ($(CD_3)_2CO$, 75 MHz, 25 °C - not fully coalesced): δ 172.2, 170.5, 169.5, 155.0, 131.7, 126.0, 78.5, 61.0, 55.0, 52.3, 51.9, 50.1, 35.0, 34.0 ; IR (CH_2Cl_2, cm^{-1}): 3691, 3422, 2929, 1736, 1720, 1683, 1517, 1485; TLC R_f = 0.30 (50% EtOAc/hexane); $[\alpha]_D$ = +41.1 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{17}H_{27}N_4O_7$ $[M+H]^+$ 371.1818, found 371.1812.

Solid phase synthesis of hexapeptide (23). Peptide **23** was prepared by manual solid-phase peptide synthesis.[10,45] Fmoc-Pal-PEG-PS resin (substitution 0.20 mmol/g) was used to afford C-terminal primary amides. N^{α}-fluorenylmethyloxycarbonyl (Fmoc) protection was employed for all amino acids in the solid-phase synthesis, with the tyrosine phenol protected as a *tert*-butylester. Each amino acid was coupled sequentially to the peptide chain grown from the C-terminal amino acid using *N,N*-diisopropylcarbodiimide/ 1-hydroxybenzotriazole. A complete coupling in each step was monitored by a quantitative Ninhydrin test.[46] Unreacted N-termini were acetylated employing an acetic anhydride/HOBT/diisopropylethylamine capping protocol. Fmoc groups were cleaved with 20% piperidine in dimethylformamide (DMF). The peptides were deprotected and cleaved form the resin by treatment with a solution of trifluoroacetic acid(TFA)/anisole/thioanisole (90:5:5) for 2 h.

Solid phase RCM protocol. To a suspension of solid-support bound peptide **23** (300 mg resin, 0.06 mmol theoretical bound peptide) in 22 mL CH_2Cl_2 was added *via* syringe a solution of ruthenium catalyst **1b** (25 mg, 0.030 mmol) predissolved in 5 mL CH_2Cl_2. The solution turned from pink to orange-brown over 3 h. The suspension was heated to 40 °C and gently stirred for 22 h. The beads were then filtered, rinsed with CH_2Cl_2, DMF, and MeOH, respectively, and dried under high vacuum. To 270 mg dried resin was added 3 mL of a solution of TFA:anisole:thioanisole (90:5:5). The suspension was shaken gently at room temperature for 2h, after which the beads were filtered and rinsed with a 0.5 mL of TFA. The filtrate was reduced in volume to ~0.5 mL to yield a brown oil. Trituration with 2:1 ether/hexane afforded the crude peptide mixture as an off-white solid. The solid was dissolved in deionized H_2O, and freeze-dried to afford a cream powder which was a mixture of 65% **24** and 35% **23** (as determined by HPLC, LRMS (FAB), and ^{1}H NMR analyses).

References and Notes

† The majority of this chapter was previously reported in a separate publication. See: Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606-9614.

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(11) Compounds were identified on the basis of their ^{1}H NMR, ^{13}C NMR, IR, and HRMS characteristics (see Experimental section). The cases involving *C*-allylglycine in Schemes 1 and 2 were performed on the racemic mixtures.

(12) This issue is addressed in more detail in reference 2g.

(13) Vinyl glycine derivative **8** was prepared by derivatization of *N*-allyl-*N*-Boc-methionine methyl ester in analogy to the procedure of Rapoport. See: (a) Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817-4820. (b) Meffre, P.; Voquang, L.; Voquang, Y.; Le Goffic, F. *Synth. Commun.* **1989**, *19*, 3457-3468.

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(16) A monocyclized product was identified by HRMS (FAB) [M-28 mu]. No ions corresponding to the bicyclization product could be detected [M-56 mu]. The monocyclized product was formed at approximately the same rate as the 6-membered cyclic product **5**, and no other product was formed after elongated reaction time. The formation of cyclic product **5** was previously found to be fast and effectively irreversible. Therefore, we believe that treatment of substrate **10** under the analogous reaction conditions leads to the 6-membered monocyclization product as opposed to the 11-membered monocyclization product.

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(25) Interestingly, when the (*i+1*) and (*i+2*) residue stereocenters are inverted in the case of Boc-allylglycine-D-Pro-D-Ala-allylglycine-$N(Me)_2$, no ring closure to form the cyclic peptide is observed upon treatment with catalyst **1b**. This, coupled with the evidence that the stereocenters at the (*i*) and (*i+3*) residues must be of (*S*)-configuration for the successful formation of macrocycle **14**, strongly suggests that ring-closure is favored for homochiral tetrapeptide systems. (This D-Pro-D-Ala tetrapeptide derivative was initially synthesized in hopes that it would assume a minimal β-hairpin conformation, and this secondary structure could be stabilized by C-C double bond formation. For similar acyclic tetrapeptides with inverted (*i+1*) and (*i+2*) stereocenters which have been shown to assume β-hairpin conformations, see: Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975-6985.)

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(31) For interpretation of amide N-H IR stretches in CH_2Cl_2 solutions, see: (a) Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280-3281. (b) Liang, G.-B.; Desper, J. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 925-938. (c) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164-1173. (d) Rao, C. P.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *Biochemistry* **1980**, *19*, 425-431.

(32) The two leucine residues of cyclic peptide **18** could not be differentiated by standard ^{1}H NMR techniques.

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(34) The olefin geometry of cyclic peptide **22** could not be assigned by standard ^{1}H NMR decoupling techniques.

(35) For a macrocyclic tripeptide synthesized *via* RCM devoid of any apparent conformational constraint, see 6o. For examples of macrocyclic peptides prepared *via* RCM which were designed to be conformationally biased toward cyclization, see references 6a, f, and p.

(36) For literature on other cyclization reactions performed on solid support-bound substrates, see: (a) Hiroshige, M.; Hauske, J. R.; Zhou, P. *J. Am. Chem. Soc.* **1995**, *117*, 11590-11591. (b) Andreu, D.; Albericio, F.; Sole, N. A.; Munson, M. C.; Ferrer, M.; Barany, G. *Methods Mol. Biol. (Totowa, NJ)* **1994**, *35* (Peptide Synthesis Protocols), 91. (c) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4708-4712. (d) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580-11581. (e) Zhao, Z.; Felix, A. M. *Pept. Res.* **1994**, *7*, 218-223. (f) Albericia, F.; Hammer R. P.; Garciaechevaerria, C.; Mollins, M. A.; Chang, J. L.; Munson, M. C.; Pons, M.; Giralt, E.; Barany, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 402-413. (g) Apsimon, J. W.; Dixit, D. M. *Can. J. Chem.* **1982**, *60*, 368-370.

(37) For a recent review of transition metal mediated reactions in solid phase organic synthesis and combinatorial chemistry, see: Andres, C. J.; Whitehouse, D. L.; Deshpande, M. S. *Curr. Opin. Chem. Biol.* **1998**, *2*, 353-362.

(38) The additional N-terminal Gly and C-terminal Val residues introduced into hexapeptide **23** are the native residues flanking the β-turn of the glutaredoxin active site. The C-terminal Val was added initially to act as a one residue tether between the dienic tetrapeptide portion of interest and the resin.

(39) PEG-PS resin, or TentaGel resin, is composed of an insoluble polyethylene glycol-polystyrene copolymer (ca. 70% PEG) cross-linked with 2% divinyl benzene. Despite its higher cost, the better solvation of PEG-PS in a wide range of protic and aprotic solvents, and its observed reaction rate enhancement has made PEG-PS resin a popular choice for solid phase chemistry in recent years. See: Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113-129.

(40) For examples of RCM reactions performed on solid support reported after the disclosure of this work, see: (a) reference 5e. (b) Schuster, M.; Pernerstorfer, J.;

Blechert, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1979-1980. (c) van Maarseveen, J. H.; den Hartog, J. A. J.; Engelen, V.; Finner, E.; Visser, G.; Kruse, C. G. *Tetrahedron Lett.* **1996**, *37*, 8249-8252. (d) Piscopio, A. D.; Miller, J. E.; Koch, K. *Tetrahedron Lett.* **1997**, *38*, 7143-7146. (e) Piscopio, A. D.; Miller, J. E.; Koch, K. *Tetrahedron Lett.* **1998**, *39*, 2667-2670. (f) Nicolaou, K. C.; Winssinger, N.; Vourloumis, D.; Ohshima, T.; Kim, S.; Pfefferkorn, J.; Xu, J.- Y.; Li, T. *J. Am. Chem. Soc.* **1998**, *120*, 10814-10826. (g) Pernerstorfer, J.; Schuster, M.; Blechert, S. *Synthesis* **1999**, 138-144.

(41) For application of RCM as a cyclization/cleavage protocol to cleave cyclic β-turn tetrapeptides analogous to **24** from solid support, see reference 6e.

(42) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925

(43) CH_2Cl_2 was purified by passage through a solvent column composed of activated alumina (A-2) and supported copper redox catalyst (Q-5 reactant). See: Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

(44) A minor impurity remains in compound **15**, even after repeated column chromatography and recrystallization. Peptide **15** is greater than 95% pure by ^1H NMR. This impurity is not present in the cyclized product **16**.

(45) Merrified, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 1476-1482.

(46) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147-157.

Chapter 3

Application of Ring-Closing Metathesis to the Synthesis of Constrained Cyclic Peptide β-Sheets

Abstract

Ruthenium complex **1** had been applied to the synthesis of cyclic hexapeptide β-sheets *via* RCM. Two acyclic hexapeptide diene analogs (**3** and **10**) were prepared based upon two previously studied peptide disulfides (**2** and **9**) shown to adopt β-sheet conformations. The cysteine residues in these systems were replaced with allylglycine residues, and it was believed that the conformational disposition of the hexapeptide frameworks toward β-sheet conformations would preorganize the acyclic diene analogs **3** and **10** for facile macrocyclization *via* RCM. Treatment with alkylidene **1** in CH_2Cl_2 solutions, however, generated only low yields of the desired 20-membered macrocyclic products. The low yields were believed to be due to acyclic dienes **3** and **10** adopting *helical* conformations in organic solvents, as opposed to β-sheet conformations. In a helical conformation, the two allylglycine units in hexapeptides **3** and **10** are situated on opposing faces of the helix, and this orientation was believed to disfavor RCM. Through comparison with a related acyclic hexapeptide system (**5**), acyclic peptide dienes **3** and **10** were believed to assume the desired β-sheet conformations in polar media (MeOH, H_2O). The insolubility of alkylidene **1** in polar media precluded performing RCM in these solvents. Attempts at circumventing this solubility issue are presented, including performing the macrocyclization reactions on solid-support bound peptides, and attempting RCM in aqueous media with a water soluble, ruthenium alkylidene.

Introduction

After the synthesis and conformational analysis of the 14-membered cyclic β-turn analogs described in Chapter 2, we turned our attention to the synthesis of larger macrocyclic peptides which exhibit β-sheet conformations by RCM.[1] Cyclic, nonhydrolyzable β-sheet peptides may be useful to peptide and medicinal chemists because compact β-sheets have been shown to play key roles in protein-protein[2] and protein-RNA[3] recognition. As shown in Chapter 2, the replacement of cysteine residues in peptide sequences with allylglycine units generated highly active acyclic precursors for RCM, and we chose to employ this strategy again in the synthesis of macrocyclic β-sheet hexapeptides (Figure 1). We became interested in a pair of well-characterized hexapeptide peptide disulfides known to adopt β-sheet/β-hairpin conformations.[4] Simple replacement of the cysteines in the (*i*) and (*i*+5) positions with (*S*)-allylglycines residues generated the desired acyclic diene precursors. The designed hexapeptides could then be envisioned as "extended" β-turn structures analogous to those described in Chapter 2, with simply two additional residues in the primary sequence and potentially three intramolecular transannular H-bonds instead of one.[4]

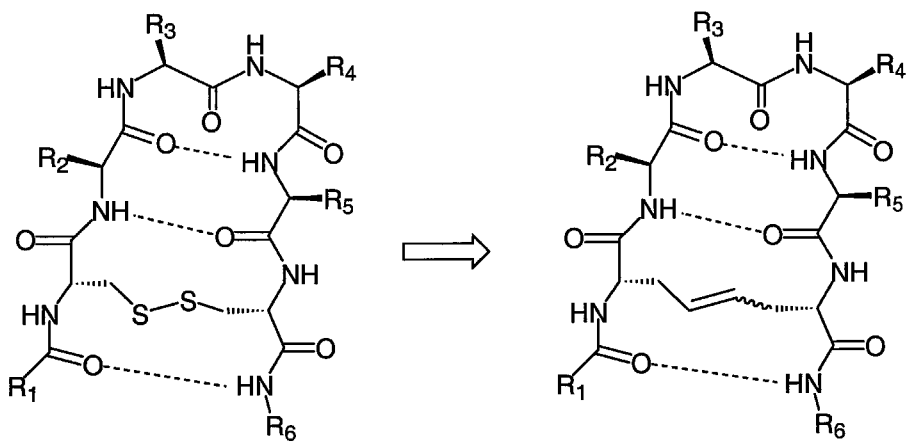


Figure 1. A generic β-sheet hexapeptide disulfide and the corresponding C=C analog.

We predicted that our acyclic hexapeptide diene analogs would be able to assume this extended H-bonding network (maintaining from one to three H-bonds), and thus be

predisposed towards RCM upon treatment with ruthenium carbene $(PCy_3)_2Cl_2Ru{=}CHPh$ (**1**)[5] to yield the 20-membered macrocyclic products. The synthesis of two hexapeptide β-sheet systems and their macrocyclizations *via* RCM are described below.

Results and Discussion

Synthesis of a Cyclic C=C-Stabilized β-Sheet via RCM

The first system we investigated in the synthesis of macrocyclic β-sheets by RCM was based upon a disulfide-stabilized hexapeptide β-sheet (**2**) reported by Balaram *et al.* (Figure 2).[6] Hexapeptide **2** was composed entirely of hydrophobic residues, including one conformationally restricted α-aminoisobutyric acid (Aib) unit, and was completely soluble in organic solvents (i.e. CH_2Cl_2, $CHCl_3$, C_6H_6). Conformational analyses of cyclic peptide **2** indicated that it adopted an antiparallel β-sheet conformation both in the solid state and in $CDCl_3$ solution, nucleated by the β-bend at the Aib-Ala sequence and stabilized by three intramolecular hydrogen bonds of the type: Boc C=O···HN-methylamide, Leu C=O···HN Val, and Val C=O···HN Leu.

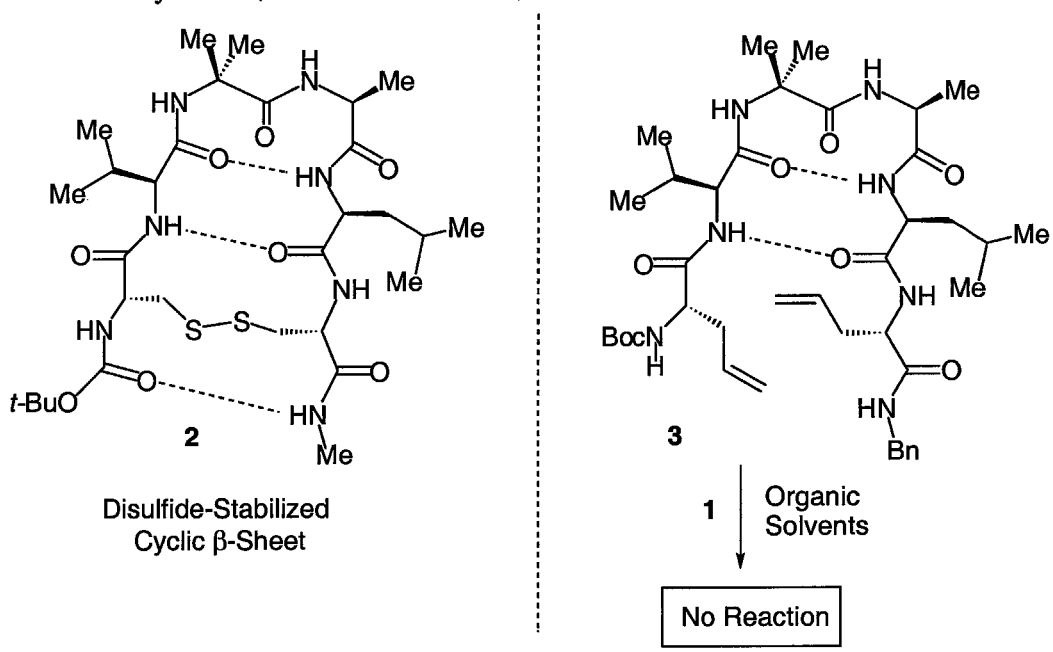


Figure 2. Balaram's cyclic hexapeptide β-sheet **2** and an acyclic diene analog **3.**

We prepared acyclic diene analog **3** by standard solution-phase peptide synthesis, incorporating two allylglycine units in place of the two cysteines (Figure 2).[7,8] However, treatment of **3** with catalyst **1** employing standard RCM macrocyclization conditions (20 mol % **1**, 0.005 M in $CHCl_3$, 40 °C, 24 h) generated no detectable product.

The lack of reactivity exhibited by acyclic diene **3** was rationalized through comparison to conformational studies performed on the acyclic bis-benzyl thioether precursor (**5**) to disulfide **2** (Figure 3). These conformational analyses were reported by Balaram *et al.* some time after the disclosure of the structure of disulfide **2**, and were found to be in unique contrast to the analogous studies performed on **2**.[9]

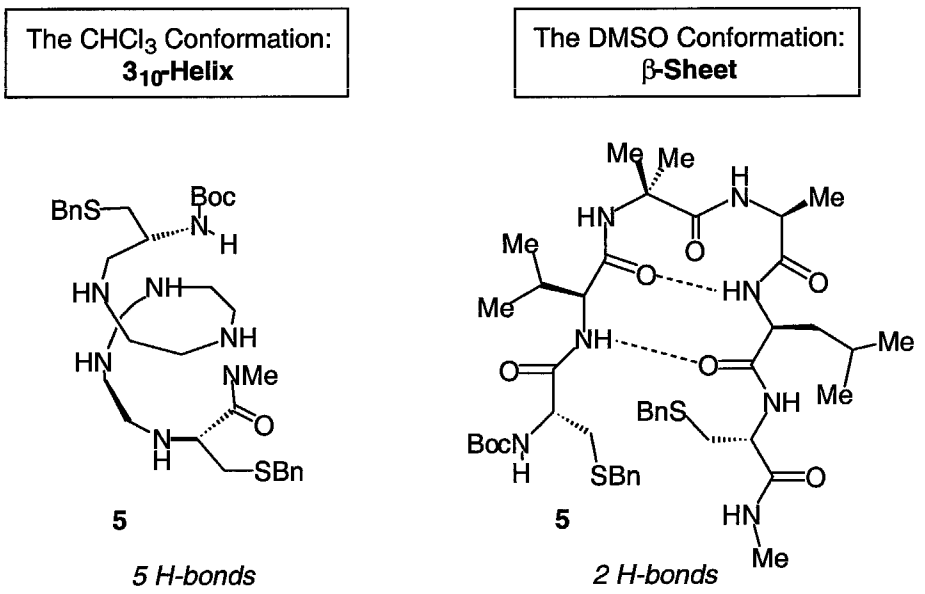


Figure 3. Conformational analysis of Balaram's bis-benzyl acyclic precursor **5** in apolar and polar media.

Specifically, the conformation of acyclic bis-benzyl thioether **5** was shown to be highly dependent on solvent (Figure 3). In contrast to disulfide **2**, its acyclic precursor **5** was found (by standard ^1H NMR techniques) to assume a 3_{10}-helix conformation in $CDCl_3$ solution, stabilized by five intramolecular H-bonds involving residues 3-6 and the terminal methylamide. This result was not entirely surprising, because the presence of a single Aib residue in small hydrophobic peptide sequences has been shown to promote

helical folding in apolar solvents.[10,11] Studies of acyclic peptide **5** in solvent mixtures established a smooth conformational transition on going from $CDCl_3$ to $(CD_3)_2SO$, where the peptide helix gradually unwound and assumed a more extended structure approximating a β-sheet conformation with two intramolecular transannular H-bonds. The more polar solvent was believed to disrupt the five intramolecular H-bonds of the 3_{10}-helix favored in apolar environments, allowing acyclic peptide **5** to adopt the less ordered, more flexible β-sheet conformation.[12]

Therefore, in analogy to acyclic bis-benzyl thioether **5**, we believed that acyclic diene **3** was also conformationally labile. While no ^1H NMR spectroscopic investigations of the solution structure of **3** were completed, we felt confident that its primary structure homology with **5** would confer it with similar conformational behavior in apolar and polar media. We believed that the conformational bias we had designed into peptide diene **3** (i.e. β-sheet) was being negated when we attempted RCM in CH_2Cl_2 or $CHCl_3$ solutions: diene **3** was most likely adopting a 3_{10}-helical conformation which maximizes the amount of intramolecular H-bonding within the structure, places the two critical olefinic side chains on directly opposing faces of the peptide helix, and thus disfavors macrocyclization (Figure 4). Indeed, qualitative IR analysis of **3** in CH_2Cl_2 solution (1.0 mM, 25 °C) showed a very strong amide N-H stretching band at 3319 cm^{-1} relative to the N-H band at 3427 cm^{-1}, suggesting that the majority of the amide NHs in **3** are involved in intramolecular H-bonding.[13] Furthermore, far UV circular dichroism (CD) spectra of **3** in trifluoroethanol (TFE), a solvent known to be strongly helix promoting comparable to $CHCl_3$,[14] also indicated that the peptide backbone of **3** was adopting a right-handed helical conformation. A CD spectrum of **3** in TFE (1.0 mM, 25 °C) is shown in Figure 5: the two negative bands at approximately 203-205 (π-π^*) and 218-222 (n-π^*) are transitions characteristic of largely helical conformations in short peptides.[15] Specifically, the n-π^* absorption is considerably weaker than that at π-π^*, which corroborates well with CD spectra for known 3_{10}-helical peptides.[16]

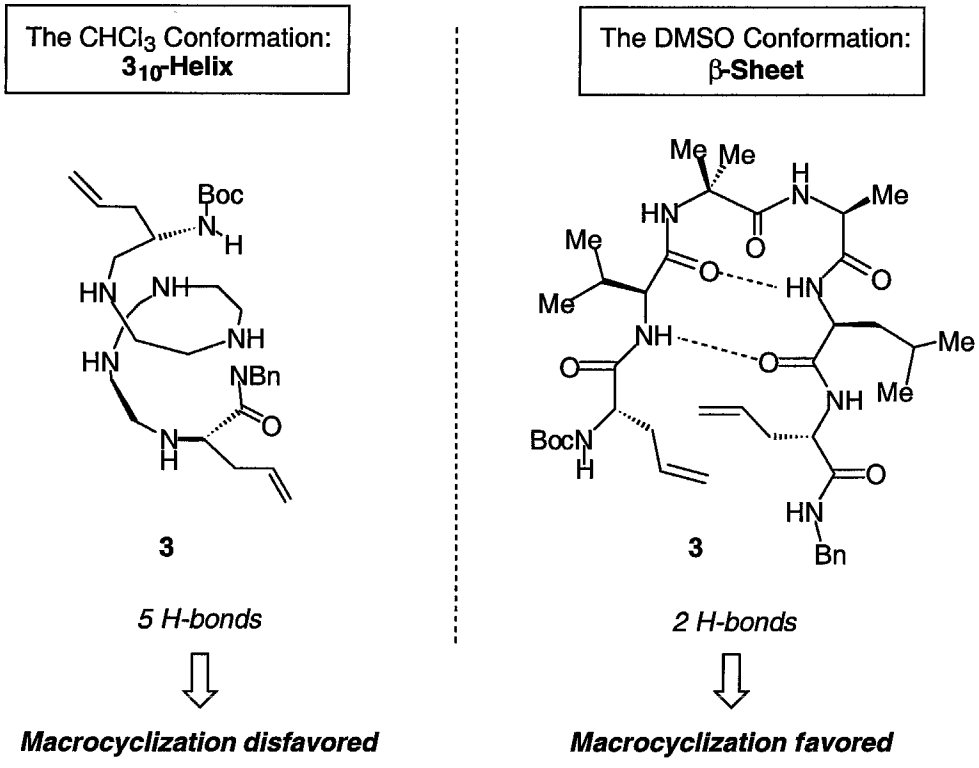


Figure 4. Conformational rationale for the lack of reactivity toward RCM exhibited by acyclic hexapeptide diene **3**.

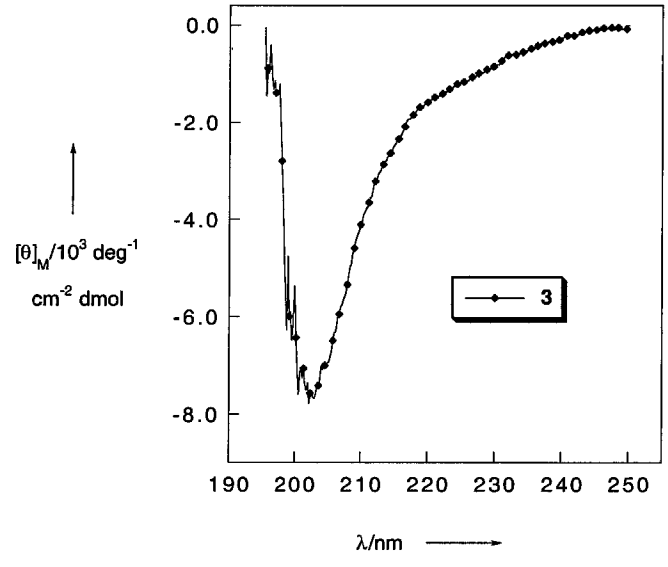
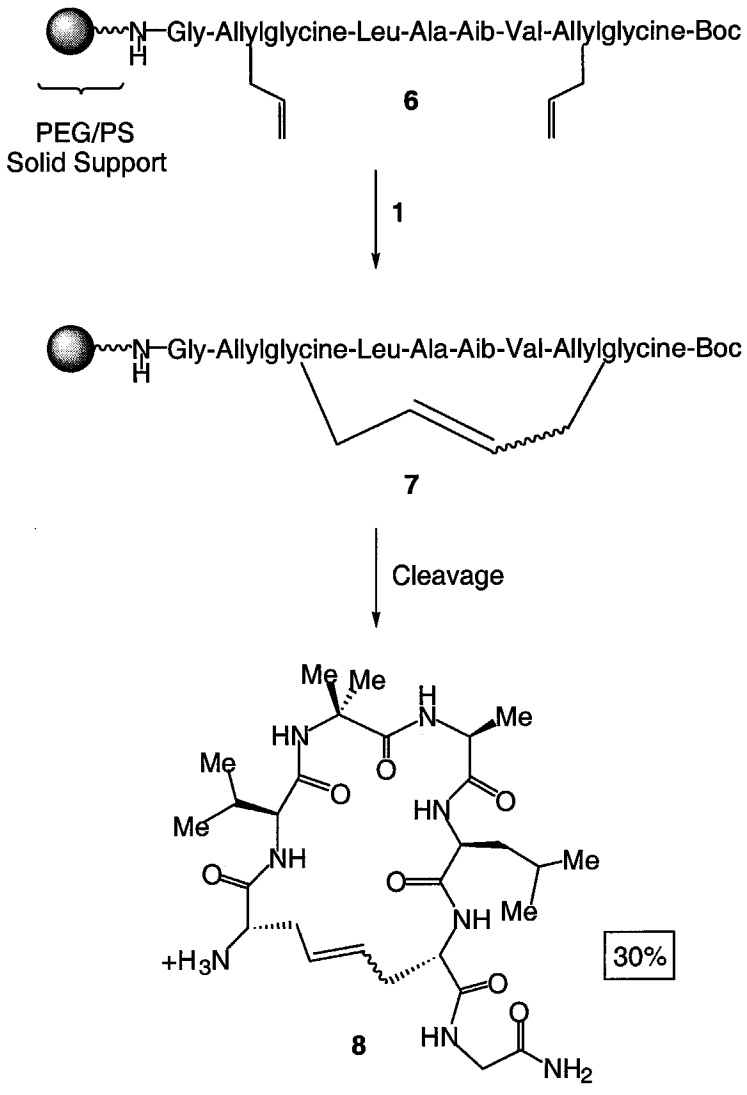


Figure 5. CD spectrum (250-195 nm) of acyclic hexapeptide **3** (1 mM) in TFE at 25 °C. Total molar ellipticity values are given.

Thus, in analogy to bis-benzyl thioether **5**, we believed that acyclic diene **3** was adopting the desired β-sheet conformation in polar solvents ($(CD_3)_2SO$, H_2O), with the two allylglycine units in close proximity to each other due to the antiparallel packing of the peptide about the Aib-Ala β-bend (shown schematically in Figure 4). The insolubility and inactivity of metathesis catalyst **1** in such polar media precluded attempts at RCM in these solvents. Unfortunately, attempts at circumventing these conformational issues by conducting the RCM of **3** in miscible apolar/polar solvent mixtures (MeOH or dioxane in CH_2Cl_2, 1:10 to 1:1) did not yield any macrocyclic product. The lowered stability and solubility of catalyst **1** in these solvent systems could also be reason for the lack of any observable RCM product.

In order to attempt RCM in a solvent environment both compatible with catalyst **1** and with acyclic diene **3** adopting the desired β-sheet conformation, we next attempted the RCM of the solid support bound analog of **3** (**6**) (Scheme 1).[7,17] As described in Chapter 2, we had previously confirmed the compatibility of catalyst **1** with solid support bound substrates in apolar media. We believed at the outset that the polar nature of the TentaGel polymeric resin (70% polyethylene glycol: 30% polystyrene)[18] could generate a more polar environment for the bound substrate (**6**), and that this could predispose **6** to adopt the desired β-sheet conformation. Indeed, much of the success reported in the use of TentaGel type resin in peptide synthesis has been attributed to the better solvation of the growing peptide in the swollen polar polymeric matrix; the polarity of the polymeric matrix has been compared to that of dimethylformamide (DMF) even when the beads are suspended in $CHCl_3$ solutions.[18] Treatment of solid support bound diene **6** with alkylidene **1** under standard solution phase RCM conditions (20 mol % **1**, 0.005 M in CH_2Cl_2, 40 °C, 24 h), and subsequent cleavage from the resin afforded a 30% yield of the desired macrocycle **8** (Scheme 1).[19] While the yield of 20-membered macrocycle **8** was low, we were encouraged that we could actually generate the macrocycle *via* RCM. Furthermore, employing solid support to alter the effective polarity of solvent appeared to

be an excellent approach to manipulating polar dienic structures which have either low solubility or undesired conformational preferences in apolar media.



Scheme 1. Solid phase RCM of macrocyclic peptide **8**.

Unfortunately, systematically altering the concentration, catalyst loading, and reaction temperature did not act to increase the yield of **8**. Furthermore, due to the inability to completely purify macrocycle **8** away from the acyclic starting material **6**, and the low yield of **8** overall, detailed conformational analysis of macrocycle **8** was not attempted. Instead, we turned our attention to the synthesis of another dicarba-analog of

a naturally-occurring β-sheet disulfide, with the hope of generating a dienic system more predisposed toward macrocyclization

Synthesis of Cyclic Oxytocin Analogs via RCM

A naturally occurring macrocyclic peptide of particular interest is the neurohypophyseal nonapeptide hormone oxytocin (**9**). Shown in Figure 6, oxytocin (**9**) consists of a 20-membered ring and an acyclic tripeptide tail. The cyclic portion of the peptide includes a disulfide bridge between the (*i*) and (*i*+5) residues. The conformation of oxytocin (**9**) consists of two β-turns: one, a β-sheet turn, in the cyclic moiety involving the sequence Tyr(2)-Ile-Gln-Asn(5), and a second involving the C-terminal sequence Cys(6)-Pro-Leu-Gly(9).[20] Oxytocin (**9**) elicits smooth muscle contraction, causing milk ejection and uterine contractions in mammals, and is employed routinely to induce labor in humans. In the elucidation of the hormonal activity of oxytocin, it was discovered that the peptide was only active when in its oxidized, cyclized form. Furthermore, the redox activity of the disulfide bridge was not implicated in the activity of the hormone.[21]

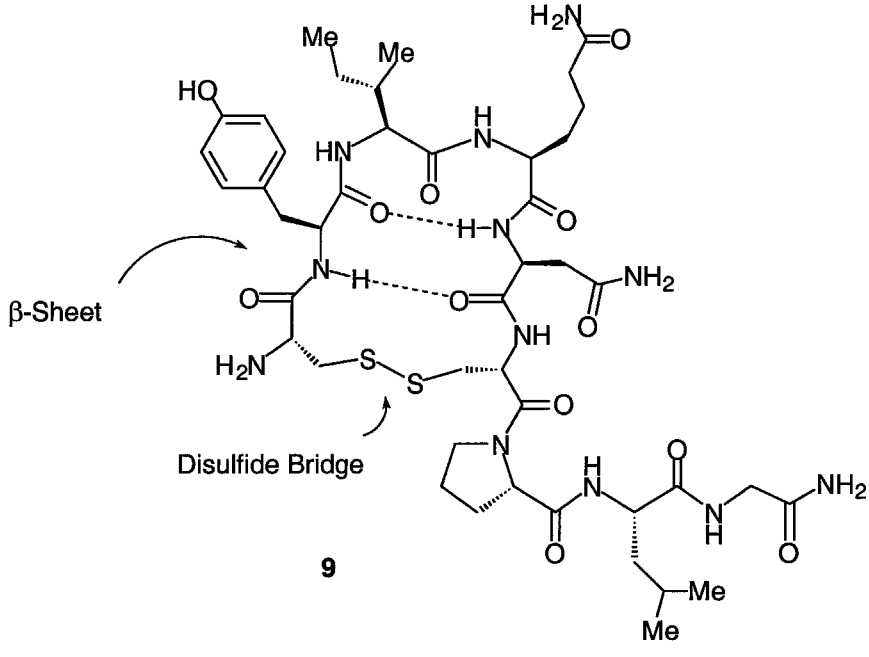
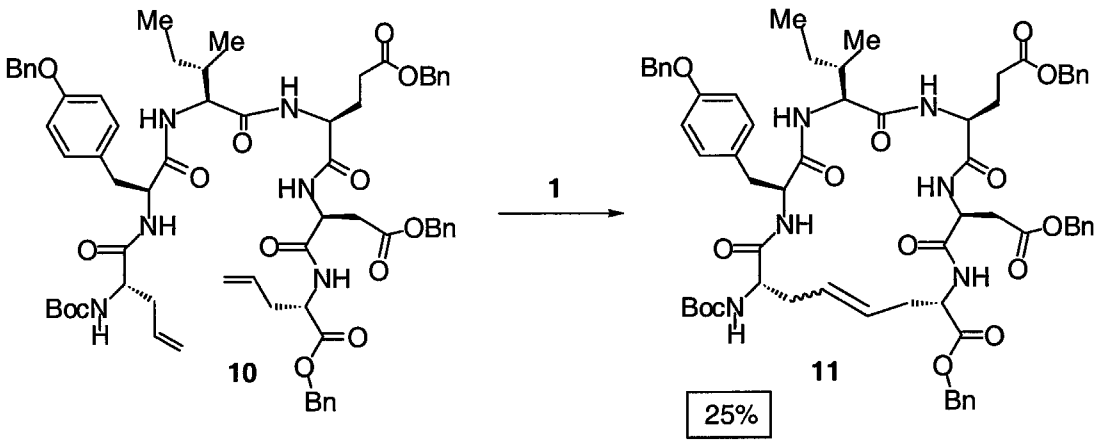


Figure 6. The nonapeptide hormone oxytocin (**9**) with structural features identified.

Before these structure/activity studies on oxytocin (**9**) were carried out, it was believed that disulfide bridges found in peptides and proteins were always involved in redox processes, and did not play a purely structural role. In oxytocin (**9**), it became evident that disulfide bridges behave frequently as covalent cross-links in proteins, forming once the protein has folded into its native conformation, and acting to "lock" the protein in this conformation. The Tyr-Ile-Gln-Asn β-turn in oxytocin **9**, essential to its biological activity, is therefore believed to be "locked" in place by the disulfide bridge in the context of a cyclic β-sheet.[22]

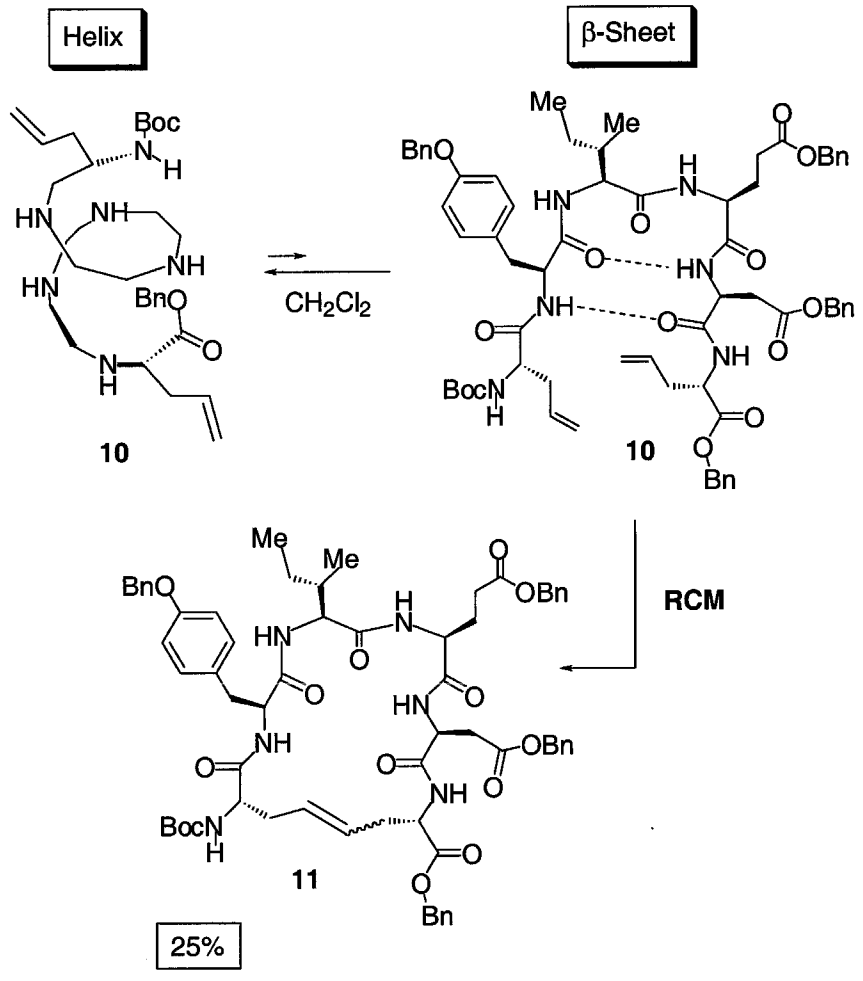
An oxytocin analog wherein the cyclic substructure essential for its hormone activity is not readily cleavable could potentially be *more* potent than the natural hormone. Much effort has been directed toward this goal, and numerous analogs of oxytocin (**9**) have been reported where the disulfide bridge has been replaced by an alternative bridging moiety.[23] One restriction in the design of these analogs is that a ring size of 20 members must be maintained: analogs with larger or smaller rings have been found to be virtually devoid of biological activity.[24] Our strategy of replacing cysteines with allylglycines was then compatible for designing a dicarba-analog of oxytocin (**9**) because the cyclized product would maintain the requisite 20-membered ring.

In order to prepare an olefin analog of oxytocin (**9**), acyclic hexapeptide **10** was synthesized in solution as a model compound.[7] Due to the limitation of working in apolar organic solvents (see above), peptide side chain functionality was protected with hydrophobic protecting groups to heighten the solubility of the hexapeptide **10** in organic media.[25] Also, the C-terminal tripeptide tail of oxytocin (**9**), not essential for its activity or part of the cyclic β-sheet sub-structure, was not included in acyclic analog **10** so as to further aid its solubility in organics. Upon exposure of **10** to standard solution phase RCM conditions (20 mol % **1**, 0.005 M in CH_2Cl_2, 40 °C, 24 h), macrocyclic peptide **11** was isolated in 25% yield (Scheme 2).[26] Unfortunately, all attempts to optimize this reaction did not lead to an improved yield of macrocycle **11**.



Scheme 2. Synthesis of a dicarba-analog **11** of the oxytocin disulfide fragment by RCM.

In rationalizing the low yield of macrocycle **11** *via* RCM (Scheme 2), we again turned to the Balaram's conformational analysis of acyclic bis-benzyl thioether **5**,[9] as we had in the analysis of acyclic hexapeptide diene **3** (see above). We hypothesized that hydrophobic peptide **10** could also favor a helical conformation in apolar media, as opposed to the desired more, extended β-sheet conformation (Scheme 3). Thus, *over the lifetime of catalyst* **1** in CH_2Cl_2 at elevated temperature (ca. 24 h), the 25% yield of macrocyclic product **11** could potentially be related to the conformational equilibrium between the helical and β-sheet conformations accessed by acyclic peptide **10**, where the former conformation was favored over the latter. However, we can not disregard the possibility of acyclic peptide **10** adopting some other conformation other than helical which disfavors macrocyclization. Indeed, oxytocin (**9**) and very closely related analogs have been found to be exceedingly conformationally flexible in solution and in the solid state.[27] Unfortunately, as we had observed with hexapeptide **3**, treatment of **10** with catalyst **1** in miscible polar/apolar solvent mixtures (MeOH or dioxane in CH_2Cl_2, 1:10 to 1:1) in attempt to perturb the conformational equilibrium shown in Scheme 3 did not yield any macrocyclic product.



Scheme 3. The conformational equilibrium of **10** in CH_2Cl_2 precludes facile macrocyclization *via* RCM to yield **11**.

While we were confident that the low yield of **11** was dependent on **10** assuming a unfavorable conformation for RCM in CH_2Cl_2, no ^1H NMR solution phase conformational analyses of **10** or **11** were undertaken to confirm the above speculations. However, qualitative IR data for **10** and **11** in CH_2Cl_2 solutions (1.0 mM, 25 °C) showed strong amide NH stretching bands at 3325 and 3347 cm^{-1} relative to those at 3421 and 3420 cm^{-1}, respectively, suggesting that **10** and **11**, like acyclic diene **3**, adopted conformations in CH_2Cl_2 solution which maximized the number of intramolecular C=O···H-N H-bonds.[Error! Bookmark not defined.] Interestingly, the intensity of the intramolecularly H-bonded NH stretch for cyclic peptide **11** was weaker relative to that

of **10**, indicating that the conformation of the cyclized peptide **11** was less-structured than its acyclic precursor (**10**) in CH_2Cl_2 solution.

In view of the success we had achieved earlier in the synthesis of macrocyclic peptide **8** on solid support (see above), we next attempted the synthesis of a dicarba-analog of oxytocin (**9**) *via* RCM on solid support. Due to the ease of solid phase peptide synthesis,[7] we prepared the acyclic nonapeptide dicarba-analog (**12**) of oxytocin (**9**) on solid support, only differing from native oxytocin (**9**) in that the two cysteine residues were replaced with allylglycines (Figure 7). Unfortunately, treatment of **12** with catalyst **1** under our standard heterogeneous RCM conditions yielded no macrocyclic product. All attempts to optimize these reaction conditions did not afford any macrocyclic product. We speculate that the unprotected primary amide functionality of **12** could potentially coordinate to catalyst **1** and therefore shut down RCM.

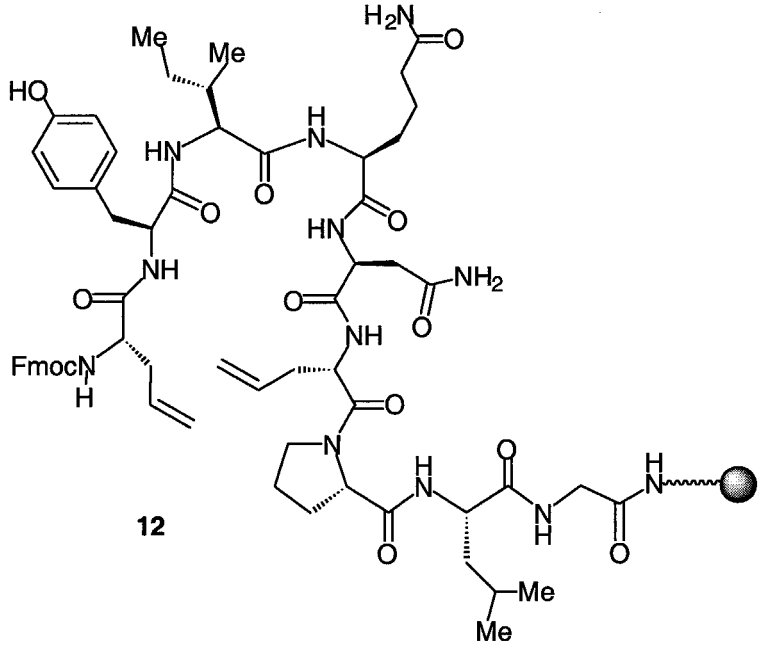


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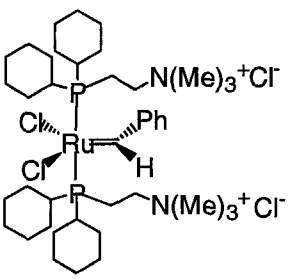
Figure 7. Solid support-bound nonapeptide dicarba-analog (**12**) of oxytocin (**9**).

Application of Water Soluble Alkylidenes to the Synthesis of Cyclic Peptide β-Sheets

During the course of the design and synthesis of the hexapeptides described herein, a family of new water soluble, ruthenium alkylidenes were discovered which were active for olefin metathesis in protic media.[28] Due to the postulated conformational bias of hexapeptide dienes **3** and **10** toward β-sheet conformations in polar media (as discussed above), we believed that the application of water soluble alkylidenes such as **13** towards the RCM of **3** and **10** could be an effective route for macrocyclization. However, treatment of both **3** and **10** with catalyst **13** (30 to 100 mol %) in MeOH-d_4 (25 °C to 45 °C, 24 h) afforded no detectable macrocyclic product by ^{1}H NMR. We speculate that the lower activity of catalyst **13** toward the RCM of α,ω-type dienes relative to catalyst **1** could be partially responsible for these failed cyclizations. Alkylidene **13** has been recently shown to be much more reactive in the RCM of acyclic dienes containing one internal olefin.[29] The design of peptide dienes structurally related to **3** and **10** which contain one internal olefin substituent is the topic of ongoing research in our laboratory.[30]



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Summary and Conclusions

Ruthenium complex **1** has been applied to the synthesis of cyclic hexapeptide β-sheets *via* RCM. Two acyclic hexapeptide diene analogs (**3** and **10**) were prepared based upon two previously studied peptide disulfides known to adopt β-sheet conformations (**2** and **9**). The cysteine residues in the latter systems (**2** and **9**) were replaced with

allylglycine residues, and it was believed that the conformational disposition of the hexapeptide frameworks toward β-sheet conformations would preorganize the acyclic diene analogs **3** and **10** for facile macrocyclization *via* RCM. Treatment with catalyst **1** in CH_2Cl_2 solutions, however, generated none or only low yields of the desired 20-membered macrocyclic products. The low yields were believed to be due to acyclic dienes **3** and **10** adopting predominantly *helical* conformations in organic solvents, as opposed to the desired β-sheet conformations. In a helical conformation, the two allylglycine units in the acyclic hexapeptides **3** and **10** are situated on opposing faces of the helix, and this orientation was believed to disfavor RCM. Through comparison with a related acyclic hexapeptide system (**5**), the acyclic peptide dienes **3** and **10** were believed to predominantly assume the desired β-sheet conformations in polar media (MeOH, H_2O). The insolubility of catalyst **1** in polar media precluded performing RCM in these solvents. However, in the case of acyclic hexapeptide **3**, performing the macrocyclization reaction on a solid support bound dienic precursor (**6**) afforded a 30% yield of the desired macrocyclic peptide **8**. In contrast, an improved macrocyclization yield was not observed when a nonapeptide dicarba-analog (**12**) of oxytocin (**9**) was prepared on solid support and treated with catalyst **1**.

While the generation of cyclic β-sheet peptides macrocycles *via* RCM remained a goal, the conformational analysis of the systems described herein sparked our interest in the design of macrocyclic peptide helices *via* RCM. Indeed, while it was not the desired result at the outset, we believed we had been successful in the design of dienic peptides which readily adopt helical conformations in organic media, the solvents in which catalyst **1** exhibits its highest activity (Figure 4 and Scheme 3). We believed we could redesign these systems so that macrocyclization was favorable, thereby potentially trapping the peptides in a helical conformation by C-C bond formation. Specifically, we believed two details of the peptide structures would have to be manipulated: (1) the olefinic residues would have to be moved in the primary peptide sequence so that their

side chains were directed out of the same face of the helix, and (2) the length of the olefinic side chains would have to be increased (Figure 8). Details of our implementation of these two design strategies in the synthesis of macrocyclic heptapeptide helices *via* RCM are given in Chapter 4.

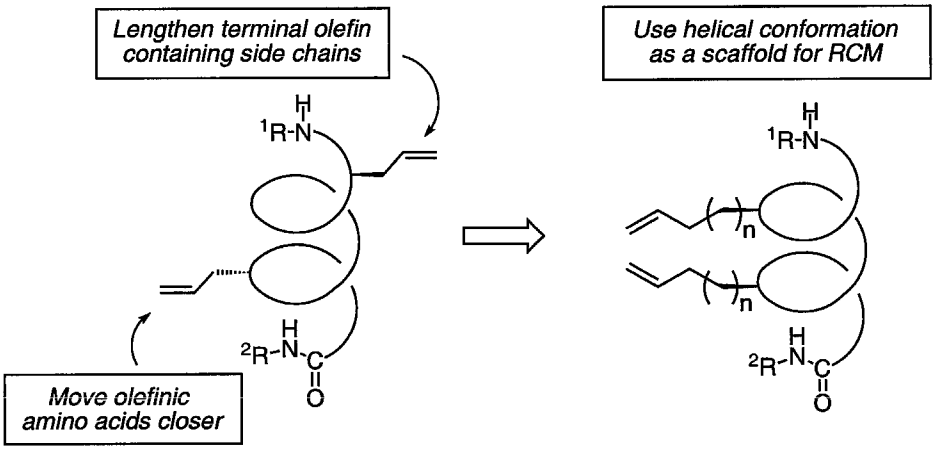


Figure 8. Rational design of acyclic peptide dienes preorganized in a helical conformation for macrocyclization *via* RCM.

Acknowledgements

This research was generously supported by grants from the NIH and the NSF. Prof. Scott J. Miller is acknowledged for his tremendous intellectual and technical contributions to this work, along with his inexhaustible enthusiasm for this project as a whole. Glenn M. Samnis is acknowledged for his assistance in the synthesis of the oxytocin analogs described herein. Professor Andrew G. Myers and Dr. James Gleason are gratefully acknowledged for providing samples of optically pure (*S*)- and (*R*)-allylglycine, as well as for helpful discussions.

Experimental Section

General Experimental Considerations. NMR spectra were recorded on a Bruker AM-500 spectrometer. Chemical shifts are reported in parts per million (ppm)

downfield from tetramethylsilane (TMS) with reference to internal solvent. Multiplicities are abbreviated as follows: singlet (s), doublet (d), doublet-of-doublet (dd), triplet (t), quartet (q), and multiplet (m). All NMR spectra were collected at room temperature unless otherwise noted. The reported ^{13}C NMR data include all peaks observed and no peak assignments were made. Infrared spectra were obtained on a Perkin-Elmer 1600 Series FT-IR. Optical rotations were recorded on a Jasco DIP-1010 digital polarimeter at 589 nm and are reported as $[\alpha]_D$ (concentration in grams/100 mL solvent). Low and high resolution mass spectra were provided by either the Chemistry and Biology Mass Spectrometry Facility (Caltech) or the UCLA Mass Spectrometry Facility (University of California, Los Angeles).

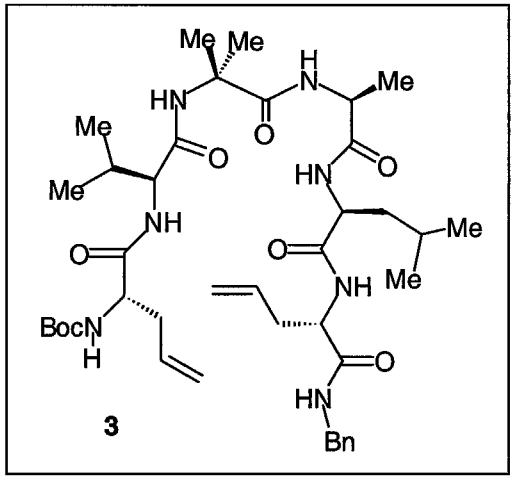
Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Flash column chromatography was performed using Silica Gel 60 (230-400 mesh) from EM Science.[31] Commercially available reagents and starting materials were purchased from Aldrich, Sigma, Applied Biosystems, and ChiroChem chemical companies, and used as delivered unless noted otherwise. Catalyst **1**[5] and **13**[28] was prepared according to the published procedures. All ring-closing metathesis (RCM) reactions were carried out under an argon atmosphere with dry, degassed solvents under anhydrous conditions.[32]

Solution phase peptide synthesis. Peptides **3** and **10** were synthesized by conventional solution phase synthesis methods employing a racemization free fragment condensation strategy. Couplings were mediated by *N,N*-dicyclohexylcarbodiimide (DCC)/1-hydroxy-benzotriazole (HOBT).[7] The Boc group was used to protect the N-terminus, and the C-terminus was protected as a methyl ester. Deprotections were performed using 1:1 trifluoroacetic acid/CH_2Cl_2 and saponification, respectively. All intermediates were characterized by ^1H NMR and TLC, and if necessary purified by column chromatography on silica gel.

Solid phase peptide synthesis. Peptides **6**, **12**, and **14** were prepared by manual

solid phase peptide synthesis.[7,33] Fmoc-Pal-PEG-PS resin (substitution 0.20 mmol/g) was used to afford C-terminal primary amides. In the synthesis of hexapeptide **6**, Boc-allylglycine-Val-Aib-Ala-Leu-OH was prepared initially in solution employing *N*-Boc chemistry[7] and coupled to allylglycine-Gly-functionalized resin in the last step. Otherwise, each amino acid was coupled sequentially to the peptide chain grown from the C-terminal amino acid using *N,N*-diisopropylcarbodiimide/ 1-hydroxybenzotriazole. N^{α}-fluorenylmethyloxycarbonyl (Fmoc) protection was employed for all amino acids in the solid-phase synthesis. A complete coupling in each step was monitored by a quantitative Ninhydrin test.[34] Unreacted N-termini were acetylated employing an acetic anhydride/HOBT/diisopropylethylamine capping protocol. Fmoc groups were cleaved with 20% piperidine in dimethylformamide (DMF). The peptides were deprotected and cleaved form the resin by treatment with a solution of trifluoroacetic acid (TFA)/anisole/thioanisole (90:5:5) for 2 h.

Circular Dichroism Studies. CD spectra of peptide **3** were recorded on a Jasco J-600 spectropolarimater equipped with a JFC data processor (J-600 series spectropolarimater system software, Ver. 2.00) using 1 mm pathlength cuvettes. The scan speed was 5 nm/min, and spectra were averaged over 4 scans. Spectral baselines were obtained under analogous conditions as that for the samples. All spectra are baseline subtracted, converted to a uniform scale of molar ellipticity, and replotted. The temperature was maintained at 25 °C and the sample concentration was 1.0 mM.



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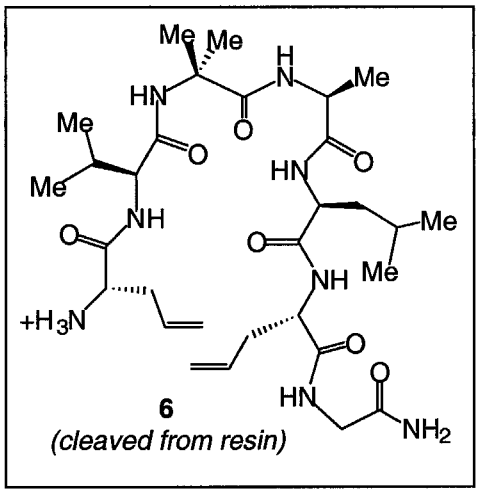
Acyclic peptide 3. Hexapeptide **3** was prepared according to the methods described in the general experimental section above. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.70 (1H, s), 7.65 (1H, d, J = 6.0 Hz), 7.58 (1H, t, J = 5.9 Hz), 7.34 (1H, masked d, J = 8.8 Hz), 7.31 (3H, app d, J = 7.6 Hz), 7.24 (2H, t, J = 7.4 Hz), 7.16 (1H, t, J = 7.2 Hz), 6.79 (1H, d, J = 3.9 Hz), 5.81-5.72 (2H, br m), 5.59 (1H, app s), 5.22 (2H, app d, J = 12.6 Hz), 5.10 (1H, app d, J = 17.1 Hz), 5.00 (1H, app d, J = 20.4 Hz), 4.57 (2H, m), 4.31 (1H, dd, J = 5.1 Hz, 15.0 Hz), 4.17 (1H, m), 3.93 (1H, m), 3.81 (1H, app br s), 3.75 (1H, m), 2.89 (1H, m), 2.62 (1H, m), 2.48 (1H, m), 2.38 (1H, m), 2.20 (1H, m), 1.86-1.21 (2H, br m), 1.48 (9H, masked s), 1.46 (3H, s), 1.43 (3H, s), 1.41 (3H, s), 1.07 (1H, br m), 0.98-0.93 (9H, br m), 0.87 (3H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 194.4, 176.4, 175.1, 173.9, 173.7, 171.9, 171.6, 157.1, 138.8, 134.7, 132.5, 128.3, 127.8, 126.8, 120.2, 117.3, 81.8, 61.0, 57.0, 56.2, 54.1, 53.4, 52.0, 49.1, 43.4, 39.6, 35.9, 35.5, 34.1, 29.1, 28.4, 27.3, 25.8, 25.2, 25.16, 23.6, 23.4, 21.1, 19.2, 18.1, 16.8. IR (CH_2Cl_2, cm^{-1}): 3427, 3319, 2958, 2935, 2955, 1663, 1531, 1484, 1221, 1161; TLC R_f = 0.60 (50% EtOAc/Hexane); HRMS (FAB) calcd for $C_{40}H_{63}N_7O_8$ $[M+H]^+$ 770.4816, found 770.4807.



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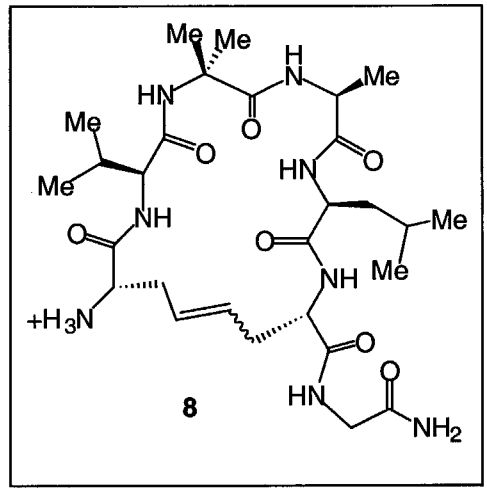
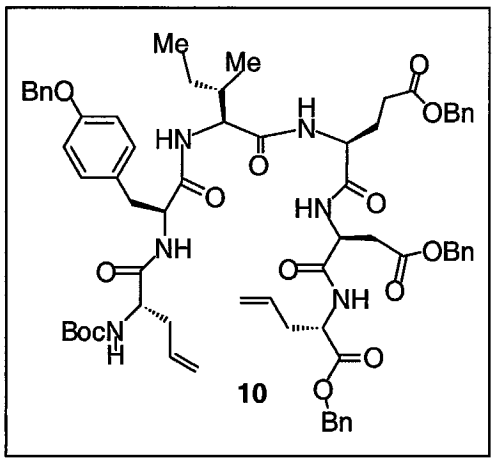
(cleaved from resin)



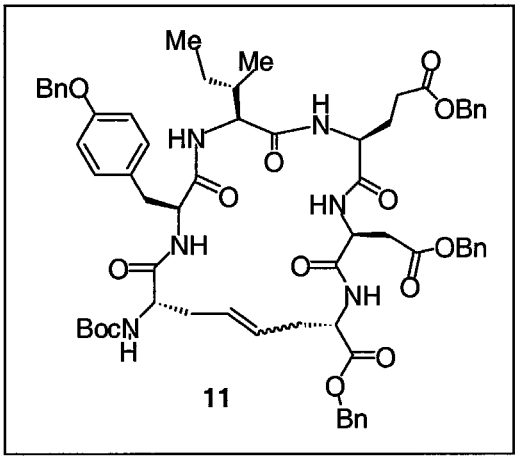
8

Cyclic peptide 8. To a suspension of solid support bound peptide **6** (600 mg resin, 0.12 mmol theoretical bound peptide) in 30 mL CH_2Cl_2 was added *via* syringe a solution of ruthenium catalyst **1** (20 mg, 0.024 mmol, 20 mol %) predissolved in 2 mL CH_2Cl_2. The solution turned from pink to orange-brown over 3 h. The suspension was heated to 40 °C and gently stirred for 24 h. The beads were then filtered, rinsed with CH_2Cl_2, DMF, and MeOH, respectively, and dried under high vacuum. To 580 mg dried resin was added 5 mL of a solution of TFA:anisole:thioanisole (90:5:5). The suspension was shaken gently at room temperature for 2h, after which the beads were filtered and rinsed with a 0.5 mL of TFA. The filtrate was reduced in volume to ~0.5 mL to yield a brown oil. Trituration with 2:1 ether/hexane afforded the crude peptide mixture as an off-white solid. The solid was dissolved in deionized H_2O, and freeze-dried to afford a cream powder which was a mixture of 30% **8** and 70% **6** (as determined by LRMS (FAB) and ^{1}H NMR analyses). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 5.57-5.31 (2H, br m, internal cyclic olefin resonances for **8**), integrated versus peaks at: δ 5.78-5.67 (2H, br m, internal olefin resonances for **6**), 5.18-4.97 (4H, terminal olefin resonances for **6**). The olefin configuration of **8** was not assigned. LRMS (FAB) calcd for **8** $C_{28}H_{49}N_8O_7^+$ $[M]^+$ 609.4, found 609.2. LRMS (FAB) calcd for **6** $C_{30}H_{53}N_8O_7^+$ $[M]^+$ 637.4, found 637.2.



10

Acyclic peptide 10. Hexapeptide **10** was prepared according to the standard solution phase protocol described in the general experimental above. ^{1}H NMR ($CDCl_3$, 500 MHz, 325 K): δ 7.41-7.26 (21 H, br m), 7.18 (2H, t, J = 7.5 Hz), 7.05 (2H, d, J = 8.3 Hz), 6.90 (2H, d, J = 8.4 Hz), 6.83 (1H, br s), 6.59 (1H, d, J = 4.6 Hz), 5.75 (1H, m), 5.51 (1H, m), 5.18-5.02 (12H, br m), 4.95 (1H, m), 4.77 (1H, app s), 4.63 (1H, m), 4.44 (1H, m), 4.41 (1H, m), 4.18 (1H, m), 3.91 (1H, m), 3.10 (1H, m), 3.02 (2H, d, J = 6.1 Hz), 2.95 (1H, m), 2.64-2.38 (4H, br m), 2.26 (2H, m), 2.10 (1H, m), 1.41-1.08 (4H, br m), 1.40 (9H, s), 0.86 (6H, m). (The small peaks at 4.11, 3.50, 1.93, 1.71, and 1.61 ppm in the ^{1}H NMR spectrum are due to a trace amount of dicyclohexylurea contaminant left over from peptide coupling reactions (<5%).) ^{13}C NMR ($CDCl_3$, 125 MHz, 325 K): δ 173.1, 173.0, 172.5, 171.8, 171.4, 171.2, 171.1, 170.3, 158.7, 133.0, 132.6, 130.3, 128.9, 128.8, 128.7, 128.4, 128.36, 128.34, 128.3, 128.27, 128.2, 127.6, 119.9, 118.8, 115.9, 81.6, 70.5, 67.0, 66.7, 66.6, 60.1, 55.7, 55.4, 53.8, 52.7, 50.2, 49.5, 36.4, 36.3, 36.2, 36.0, 34.2, 31.2, 28.4, 26.6, 26.0, 25.2, 25.1, 15.9, 11.7. IR (CH_2Cl_2, cm^{-1}): 3421, 3325, 3054, 2984, 2933, 2855, 1734, 1669, 1521, 1453, 1421, 1214, 1168, 1017; TLC R_f = 0.74 (30% EtOAc/CH_2Cl_2); LRMS (FAB) calcd for $C_{67}H_{80}N_6O_{14}$ $[M+H]^+$ 1192.6, found 1193.2.



11

Cyclic peptide 11. To a solution of acyclic diene **10** (140 mg, 0.12 mmol) in 22 mL of CH_2Cl_2 was added *via* syringe a solution of ruthenium catalyst **1** (19.3 mg, 0.023 mmol, 20 mol %) predissolved in 2 mL of CH_2Cl_2. Within 20 min, the purple solution became orange-brown, and the solution was stirred for 24 h at 40 °C. The solution was then concentrated to 0.5 mL and purified directly on a silica gel column (3x12cm, 20% EtOAc/CH_2Cl_2 to 100% EtOAc as eluent) to afford 35 mg (25%) of macrocycle **11** as an off-white powder. Standard NMR techniques (homonuclear decoupling, variable temperature ^1H and ^{13}C NMR, COSY) did not allow for the discrimination between **11** being isolated as a mixture of olefin isomers, adopting two or more conformations at 298 K, or a combination of both. ^1H NMR ($CDCl_3$, 500 MHz, major isomer reported): δ 7.63 (1H, br d), 7.41-7.30 (22H, br m), 7.17 (1H, d, J = 4.4 Hz), 7.05 (2H, d, J = 7.8 Hz), 7.00 (1H, br s), 6.89 (2H, d, J = 8.2 Hz), 5.32 (2H, m), 5.18-5.03 (8H, br m), 5.00 (1H, s), 4.57 (1H, m), 4.48 (2H, m), 4.33 (1H, m), 4.18 (1H, m), 3.87 (1H, m), 3.26 (1H, m), 3.12-2.95 (2H, m), 2.55-2.35 (3H, br m), 2.30 (1H, m), 2.20 (3H, m), 2.07 (1H, m), 1.72-1.12 (4H, br m), 1.27 (9H, masked s), 0.89-0.85 (6H, br m). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.9, 173.6, 173.2, 173.0, 171.9, 171.6, 171.4, 171.2, 171.1, 170.9, 170.8, 170.5, 158.6, 158.5, 156.4, 137.0, 136.0, 130.4, 130.3, 128.9, 128.8, 128.77, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.7, 126.3, 115.8, 115.7, 81.8, 70.3, 67.1, 67.0, 66.8, 66.7, 66.6, 60.6, 60.4, 57.2, 56.3, 55.2, 53.5, 52.2, 51.6, 35.9, 35.7, 34.9, 34.5, 33.9, 33.6, 32.1, 30.9, 29.9, 29.6, 29.0,

28.4, 28.3, 27.0, 26.7, 24.8, 22.9, 16.0, 15.9, 14.3, 11.7. IR (CH_2Cl_2, cm^{-1}): 3420, 3347, 3056, 2962, 2926, 2856, 1732, 1698, 1682, 1651, 1558, 1506, 1457, 1418, 1095, 1014; TLC R_f = 0.57 (30% EtOAc/ CH_2Cl_2); LRMS (FAB) calcd for $C_{65}H_{76}N_6O_{14}$ $[M+H]^+$ 1164.5, found 1165.6.

General RCM Procedure in MeOH-d_4 and D_2O. Peptide **3**, **10**, or **14** was placed in a vial and dissolved in MeOH-d_4 or D_2O. Alkylidene **13** was placed in a separate vial and dissolved in MeOH-d_4 or D_2O. The catalyst and substrate solutions were combined, placed in an NMR tube, and the tube was sealed with a rubber septum. The reaction was heated to 45 °C, and monitored by ^1H NMR spectroscopy. Conversion to product was determined *via* the disappearance of internal acyclic (~5.8 ppm) and terminal olefin peaks (~5.1 ppm) and the formation of new, internal cyclic olefin peaks (~5.5 ppm).

References and Notes

(1) For another example of the application of RCM to the synthesis of macrocyclic β-sheet peptides, see: (a) Clark, T. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, *117*, 12364-12365. (b) Clark, T. D.; Kobayashi, K.; Ghadiri, M. R. *Chem. Eur. J.* **1999**, *5*, 782-792.

(2) Strynadka, N. C. J.; Jensen, S. E.; Alzari, P. M.; James, M. N. G. *Nat. Struct. Biol.* **1996**, *3*, 290-297.

(3) Varani, G. *Acc. Chem. Res.* **1997**, *30*, 189-195, and references therein.

(4) A β-hairpin, or two peptide strands connected by a short loop, can be viewed as the smallest increment of antiparallel β-sheet secondary structure. The most common loop contains only two residues, and in this case, the two residues are also the two central residues of a β-turn. While the majority of the hexapeptide sequences discussed throughout this chapter are technically β-hairpins, they will be titled β-sheets for reasons of clarity. See: Gellman, S. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 717-725.

(5) For the preparation and characterization of catalyst **1**, see: (a) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Eng.* **1995**, *34*,

2039-2041. (b) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100-110. (c) Belderrain, T. R.; Grubbs, R. H. *Organometallics* **1997**, *16*, 4001-4003.

(6) (a) Kishore, R.; Raghothama, S.; Balaram, P. *Biopolymers* **1987**, *26*, 873-891. (b) Karle, I.; Kishore, R.; Raghothama, S.; Balaram, P. *J. Am. Chem. Soc.* **1988**, *110*, 1958-1963.

(7) Bodansky, M. *Peptide Chemistry*; Springer-Verlag: New York, 1988, pp. 55-146, and references therein.

(8) Acyclic diene **3** was initially synthesized by Scott J. Miller. The methylamide of disulfide **2** was replaced with a benzylamide in **3** in order to introduce a UV chromophore for aid in synthetic manipulation.

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(10) The ability of disulfide **2** to adopt a β-sheet conformation in apolar media was actually found to be an anomaly, and was rationalized to be due to the conformational constraint imposed on the peptide by the disulfide bridge. We wished to harness this same constraint in the design of dicarba-analog **3**. See reference 9.

(11) For a comprehensive review of the conformational constraint imposed on peptide backbones by the inclusion of one or more Aib residues, see: Karle, I. L. *Biopolymers* **1996**, *40*, 157-180.

(12) Conformational lability similar to that observed for acyclic peptide **5** has also been established for a putative β-sheet model peptide, Boc-$(Val)_2$-Aib-Pro-$(Val)_3$-OMe. In this case, a 3_{10}-helix is observed in crystals and in $CDCl_3$ solution, but a central β-turn with extended arms is favored in $(CD_3)_2SO$. See: Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, H.; Balaram, P. *Biopolymers* **1990**, *29*, 1433-1442.

(13) The characteristic IR bands for non hydrogen-bonded amide NH groups are usually found at ~3440 cm^{-1}, while those for intramolecular C=O···H-N hydrogen bonds appear at ~3340 cm^{-1}. For interpretation of amide N-H IR stretches in CH_2Cl_2 solutions, see: (a) Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280-3281, and references therein.

(14) Goodman, M.; Listowsky, I.; Masuda, Y.; Boardman, F. *Biopolymers* **1963**, *1*, 33-42.

(15) For examples of small helical Aib containing peptides exhibiting similar CD ellipticities in TFE, see: (a) Banerjee, A.; Raghothama, S.; Balaram, P. *J. Chem. Soc., Perkin Trans. II* **1997**, 2087-2094. (b) Karle, I. L.; Banerjee, A.; Bhattachrjya, S.; Balaram, P. *Biopolymers* **1996**, *38*, 515-526.

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(17) The additional Gly residues in solid support bound analog **6** was included because at the outset of our solid phase studies we believed that a one residue spacer between the resin and peptide **6** would afford **6** with heightened conformational mobility; therefore, **6** would not "recognize" it was bound to solid support.

(18) PEG-PS resin, or TentaGel resin, is composed of an insoluble polyethylene glycol-polystyrene copolymer (ca. 70% PEG) cross-linked with 2% divinyl benzene. See: Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113-129.

(19) The yield was determined by ^1H NMR and LRMS analyses of the mixture of **6** and **8** obtained after cleavage from the solid support. The olefin configuration of macrocycle **8** was not assigned.

(20) (a) Urry, D. W.; Walter, R. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 956-958.

(21) (a) Rudinger, J.; Jost, K. *Experientia* **1964**, *20*, 570-571. (b) Keller, O.; Rudinger, J. *Helv. Chim. Acta* **1974**, *57*, 1253-1259.

(22) Some controversy remains if the 20-membered ring of oxytocin actually adopts a true β-sheet conformation. The controversy stems in part from the torsional angles of the β-turn portion not approximating those of that for the average β-sheet. The two intramolecular H-bonds depicted in Figure 5 have been identified, however, and we believed at the outset that these interactions would themselves provide a conformational bias in the macrocyclization of an acyclic diene analog of **9** *via* RCM. See reference 6b, and references cited therein.

(23) For some notable examples of oxytocin analogs devoid of disulfide bridges, see: (a) Kobayashi, A.; Hase, S.; Kiyoi, R.; Sakakibara, S. *Bull. Chem. Soc. Japan* **1969**, *42*, 3491-3495. (b) Hill, P. S.; Smith, D. D.; Slaninova, J.; Hruby, V. J. *J. Am. Chem. Soc.* **1990**, *112*, 3110-3113. (c) Smith, D. D.; Slaninova, J.; Hruby, V. J. *J. Med. Chem.* **1992**, *35*, 1558-1563. (d) Wahl, F.; Mutter, M. *Tetrahedron Lett.* **1996**, *37*, 6861-6864.

(24) (a) Ressler, C.; du Vigneuad, V.; *J. Am. Chem. Soc.* **1957**, *79*, 4511-4515. (b) Lutz, W. B.; Ressler, C.; Nettleton, D. E., Jr.; du Vigneaud, V. *J. Am Chem. Soc.* **1959**, *81*, 167-173. (c) Jarvis, D.; Bodansky, M. du Vigneaud, V. *J. Am Chem. Soc.* **1961**, *83*, 4780-4784.

(25) Gln(4) and Asn(5) were replaced with protected Glu and Asp residues, respectively, due to the ready availability of protected Glu and Asp residues and to aid solubility in organics. The replacement of the Gln and Asn residues with structurally similar residues was not believed at the outset to perturb the structure of acyclic peptide **10** relative to oxytocin **9**. Attempts at preparing the acyclic hexapeptide diene analog that included the native Gln and Asn residues failed due to solubility problems; therefore, no legitimate comparison of the conformational predisposition of **10** relative to a "native" oxytocin analog could be made.

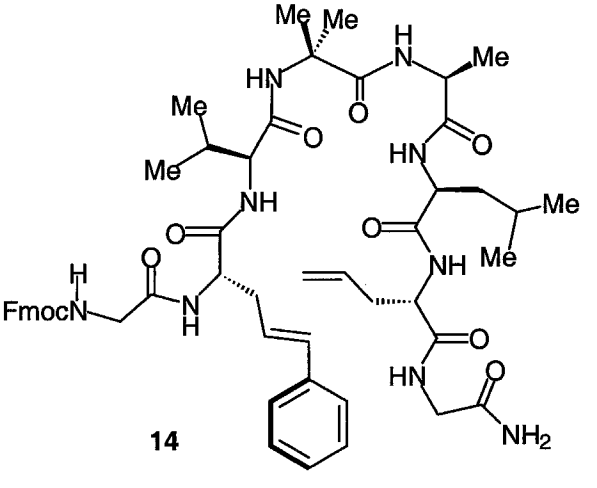
(26) Standard NMR techniques (homonuclear decoupling, variable temperature ^{1}H and ^{13}C NMR, COSY) did not allow for the discrimination between **11** being isolated as a mixture of olefin isomers and/or adopting two or more conformations at 298 K.

(27) Wood, S. P.; Tickle, I. J.; Treharne, A. M.; Pitts, J. E.; Mascarenhas, Y.; Li, J. Y.; Husain, J.; Cooper, S.; Blundell, T. L.; Hruby, V. J.; Buku, A.; Fischman, A. J.; Wyssbrod, H. R. *Science* **1986**, *232*, 633-636, and references therein.

(28) (a) Mohr, B.; Lynn, D. M.; Grubbs, R. H. *Organometallics* **1996**, *15*, 4317-4325. (b) Lynn, D. M.; Mohr, B.; Grubbs, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 1627-1628.

(29) For details of this increased reactivity, see: Kirkland, T. A.; Lynn, D. M.; Grubbs, R. H. *J. Org. Chem.* **1998**, *63*, 9904-9909.

(30) An octapeptide (**14**), structurally similar to hexapeptide **3** yet containing one styrenylalanine and one allylglycine unit, was prepared *via* solid phase peptide synthesis.[7] Octapeptide **14** was cleaved from the resin to yield a fully MeOH and H_2O soluble substrate. Treatment of **14** with alkylidene **13** in MeOH-d_4 and D_2O solutions afforded no detectable macrocyclic product by ^{1}H NMR.



14

(31) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925

(32) CH_2Cl_2 was purified by passage through a solvent column composed of activated alumina (A-2) and supported copper redox catalyst (Q-5 reactant). See: Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

(33) Merrified, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 1476-1482.

(34) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147-157.

Chapter 4

Highly Efficient Synthesis of Covalently Cross-Linked Peptide Helices by Ring-Closing Metathesis[†]

Abstract

Heptapeptides containing residues with terminal olefin derivatized side chains (**3** and **4**) have been treated with ruthenium alkylidene **1** and undergone facile ring-closing olefin metathesis (RCM) to give 21- and 23-membered macrocyclic peptides (**5** and **6**). The primary structure of peptides **3** and **4** was based upon a previously studied heptapeptide (**2**) which was shown to adopt a predominantly 3_{10}-helical conformation in $CDCl_3$ solution, and an α-helical conformation in the solid state. We predicted that replacement of the (*i*) and (*i+4*) Ala residues of **2** with residues containing unbranched side chain terminal olefin groups would place the two olefins in proximity to one another, provided that our dienic analogs also adopted a helical conformation analogous to **2**, and that the derivatized side chains were of appropriate length. Circular dichroism, IR, and solution phase ^1H NMR studies strongly suggested that acyclic precursors **3** and **4** and the fully saturated macrocyclic products **7** and **8** also adopted 3_{10}-helical conformations in apolar organic solvents. Single crystal X-ray diffraction of cyclic peptide **8** showed it to exist as a right-handed 3_{10}-helix up to the fifth residue in the solid state. The relative ease of introducing carbon-carbon bonds into peptide secondary structures by RCM and the predicted metabolic stability of these bonds renders olefin metathesis an exceptional methodology for the synthesis of rigidified peptide architectures. Specifically, we believe the macrocyclization of hydrophobic peptide helices is uniquely suited to RCM in organic solvents because helical conformations are frequently favored in apolar media.

Introduction

Due to the frequency of helical secondary structures in peptides and proteins,[1] considerable effort has been directed toward the design of small molecule helix mimetics and stabilized helix structures. Designed organic template molecules that initiate α-helix formation in peptide sequences have been reported.[2] Short α-helical peptides have also been stabilized by incorporation of naturally occurring capping motifs[3] and by stabilization of the intrinsic helix dipole.[4] Notably, significant progress has been made toward stabilizing synthetic α-helical peptides through the incorporation of covalent or non-covalent linkages between constituent amino acid side chains. Examples include salt bridges,[5] lactams,[6] disulfide bridges,[7] hydrophobic interactions,[8] and metal ligation between natural[9] and unnatural amino acids.[10] In several of these cases, it was found that substantial helix stabilization was achieved when the linkage was placed between the (*i*) and (*i+4*) residues in the peptide backbone. Such a linkage encompasses approximately one turn of the helical peptide backbone, and places the tethered side chains on the same side of the helix.

As addressed in Chapters 1-3, the extraordinary functional group tolerance of olefin metathesis catalyst $(PCy_3)_2Cl_2Ru{=}CHPh$ (**1**)[11] has enabled the synthesis of cyclic amino acids[12] and peptides[13] exhibiting β-turn[14] and β-sheet[15] secondary structure *via* ring-closing olefin metathesis (RCM).[16] This transformation effectively introduces non-native carbon-carbon bond constraints which may afford enhanced biostability in peptides and proteins. This chapter describes a concise synthesis and structural analysis of a series of cyclic helical peptides wherein RCM is used to incorporate a carbon-carbon tether between amino acid side chains. The design principles addressed at the close of Chapter 3 for the synthesis of acyclic peptide dienes predisposed in a helical conformation for RCM in apolar organic solvents will be employed, through strategic derivatization of a known helical peptide scaffold with terminal olefin functionality displayed on one side of the helix.

Selection of a Model Heptapeptide Helix

We chose to study hydrophobic peptide model systems from the outset, partly because the use of apolar sequences permits characterization of conformation in poorly solvating organic solvents, where folding is mainly controlled by intramolecular hydrogen bonding, non-bonded interactions, and electrostatic effects.[17] Furthermore, ruthenium alkylidene **1** exhibits its highest metathesis activity in this media.[18] We became interested in a hydrophobic peptide (**2**) studied by Karle and Balaram *et al.*,[19] whose solubility in organic solvents would be compatible with alkylidene **1**. Heptapeptide **2** contains two repeat units of valine-alanine-leucine (Val-Ala-Leu) separated by one α-aminoisobutyric acid residue (Aib), as shown in Figure 1.

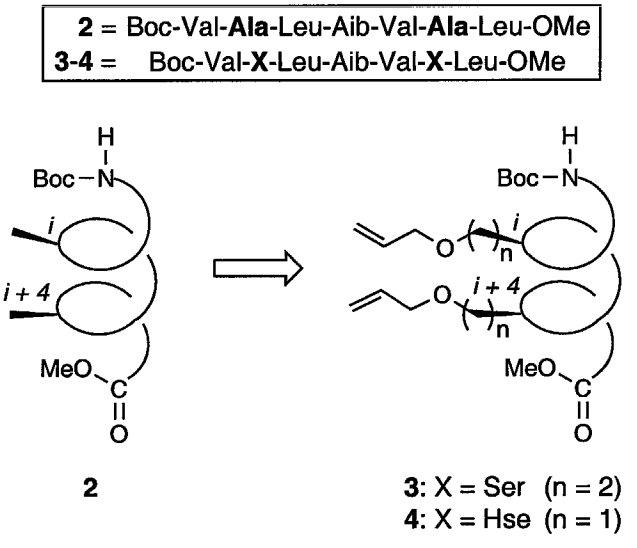


Figure 1. Karle and Balaram's heptapeptide **2**, and two dienic analogs **3** and **4**.

As mentioned in Chapter 3, the Aib residue is well-known to stabilize 3_{10}- and/or α-helical conformations in apolar oligopeptides, and is frequently found in peptides produced by microbial sources.[20] Examples of such Aib-rich peptides include the antibiotics alamethicin, zervamicin, and trichogin A IV, which are purported to adopt helical conformations within lipid bilayer membranes and aggregate therein to form ion channels. Heptapeptide **2** was shown to adopt a predominantly α-helical conformation in

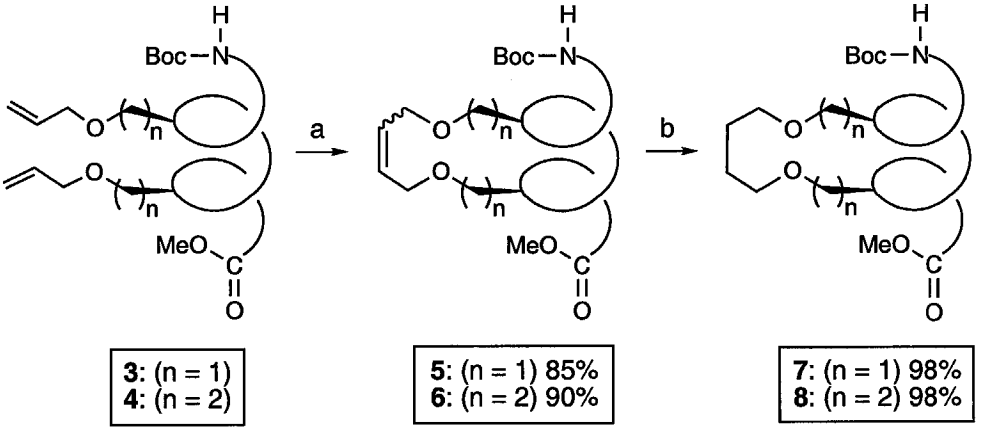
the solid state by X-ray crystallography, and was found to adopt a structurally similar 3_{10}-helical conformation in $CDCl_3$ by solution-phase ^1H NMR analyses.[19] Encouraged by these structural results, we endeavored to use heptapeptide **2** as a scaffold upon which to build acyclic peptide dienes. Furthermore, we were excited about the prospect of eventually applying RCM to the synthesis of constrained analogs of the above peptide antibiotics, in order to probe the requirements of a helical conformation on their ion channel forming activity.

Results and Discussion

Synthesis of Cyclic Heptapeptides via RCM

Through analysis of the X-ray structure of model peptide **2** and molecular modeling, we predicted that replacement of the (*i*) and (*i+4*) Ala residues of **2** with residues containing unbranched side chain terminal olefin groups would place the two olefins in proximity to one another, provided that our dienic analogs also adopted a helical conformation, and that the derivatized side chains were of appropriate length. L-Serine (Ser) and L-homoserine (Hse) *O*-allyl ethers were selected as olefin containing residues due to their ready availability and trivial derivatization as allyl ethers.[21] Acyclic peptide dienes **3** and **4**, shown schematically in Figure 1, were then prepared by standard solution phase peptide chemistry.[22]

Dienes **3** and **4** were each treated with alkylidene **1** (20 mol % **1**, 5 mM in **3** or **4**, $CHCl_3$, 25 °C, 3-4 h) to yield 21- and 23-membered macrocyclic alkenes **5** and **6** in 85% and 90% yields, respectively (Scheme 1).[23] Each macrocycle was isolated as a mixture of olefin isomers (ca. 5:1 *E/Z*).[24] Catalytic hydrogenation (10% Pd-C, 1 atm. H_2, EtOH, 25 °C, 2 h) then afforded the saturated species **7** and **8** in excellent yields. The high yield and relatively fast rate of the RCM transformation suggested that the acyclic dienes were preorganized for ring closure in organic solvents. This prompted closer examination of the structures of acyclic peptides **3** and **4**, along with cyclic products **7** and **8**.



Scheme 1. Synthesis of peptide macrocycles *via* a two-step RCM and hydrogenation procedure. a) 20 mol % **1**, $CHCl_3$, 25 °C; b) cat. 10% Pd-C, 1 atm. H_2, EtOH, 25°C.

IR and CD Studies of the Acyclic and Cyclic Peptides

Table 1 lists the amide NH region of the IR spectra of the heptapeptides **3**, **4**, **7**, and **8** in $CHCl_3$ solutions (1.0 mM, 25 °C). In each case, the characteristic bands for both non hydrogen-bonded NH groups (>3400 cm^{-1}), and intramolecular C=O···H-N hydrogen bonds appear (<3400 cm^{-1}).[25] Notably, the latter bands were considerably stronger than the former, indicating that the majority of the amide NHs in structures **3**, **4**, **7**, and **8** are involved in intramolecular H-bonds in dilute $CHCl_3$ solutions (approximating the RCM reaction conditions). Although it is difficult to definitively establish from the IR data alone which of the amide NH groups are involved in intramolecular hydrogen bonding, these data are consistent with the possibility of extensive transannular intramolecular H-bonding in both the acyclic (**3** and **4**) and cyclic structures (**7** and **8**), suggesting that helical conformations could be adopted in $CHCl_3$.

Table 1. IR amide NH bands for acyclic and cyclic heptapeptides (1.0 mM in $CHCl_3$, 25 °C)

peptide	**3**	**4**	**7**	**8**
Non H-bonded NH	3428 cm^{-1}	3438 cm^{-1}	3431 cm^{-1}	3426 cm^{-1}
H-bonded NH	3323 cm^{-1}	3325 cm^{-1}	3331 cm^{-1}	3325 cm^{-1}

Further qualitative measurements of the conformation of the heptapeptides were obtained by far UV circular dichroism (CD). CD spectra of solutions of peptides **3**, **4**, **7**, and **8** in trifluoroethanol (TFE) at 25 °C are shown in Figure 2. For peptides and proteins, TFE has been shown to be a strongly helix promoting solvent comparable to $CHCl_3$,[26] and thus CD measurements in TFE can be correlated to the conformations accessed by the peptides during the RCM reaction. Two negative bands at approximately 203-205 (π-π^*) and 218-222 (n-π^*) nm are observed for all four compounds, transitions characteristic of largely helical conformations in short peptides. The n-π^* absorptions are considerably weaker than those for π-π^*, a trend that has been observed experimentally for numerous short 3_{10}-helical peptides,[27] and reported recently to be the standard CD pattern for right-handed 3_{10}-helical peptides by Tonolio *et al.*[28]

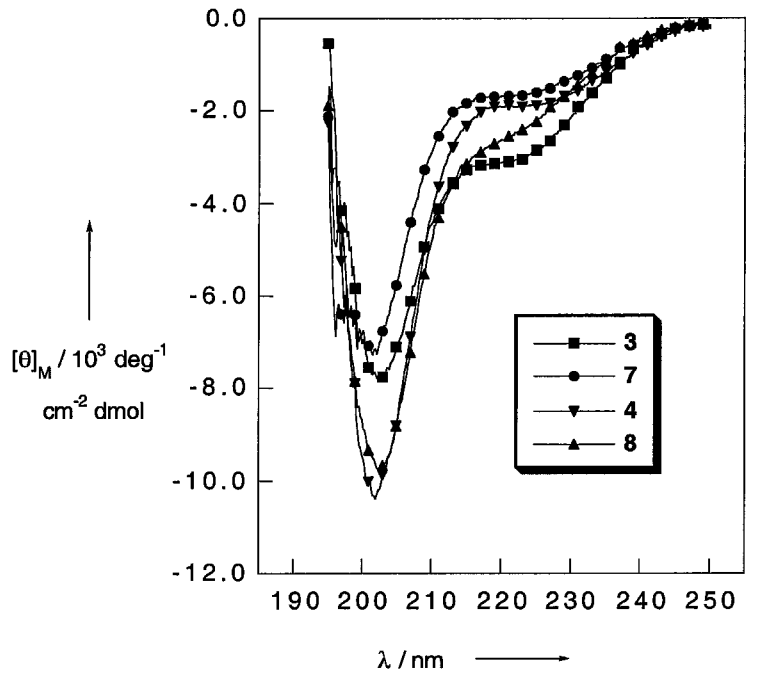


Figure 2. CD spectra (250-195 nm) of peptides **3**, **4**, **7**, and **8** (1 mM) in TFE at 25 °C. Total molar ellipticity values are given.

Importantly, these IR and CD data together suggest that the peptide backbones of acyclic dienes **3** and **4** *are preorganized* in a helical conformation for RCM in organic

solvents. Furthermore, the CD ellipticities for macrocycles **7** and **8** do not appear to differ noticeably from their acyclic precursors **3** and **4**, indicating that significant conformational changes did not take place upon macrocyclization.[29]

NMR Studies of the Acyclic and Cyclic Peptides

We employed standard ^{1}H NMR techniques (previously described in Chapter 2) to further examine the participation of the amide NH groups in **3**, **4**, **7**, and **8** in intramolecular H-bonding. Plots of the amide NH, olefin, and C_α-H portions of the ^{1}H NMR spectra of compounds **3-8** in $CDCl_3$ are shown in Figures 3 and 4. Relevant NMR parameters for the amide resonances of peptides **3**, **4**, **7**, and **8** are summarized in Tables 2 and 3 (residues are numbered sequentially from N- to C-termini). Assignment of the relative spin systems were made employing standard ^{1}H spin decoupling techniques. The Val (1) NHs were distinguished on the basis of the well-established tendency of Boc NH groups to resonate at high field in $CDCl_3$.[30] The Aib (4) NHs were the only sharp singlet NH resonances in the ^{1}H NMR spectra, and therefore could be unambiguously assigned.

The Ser (2) and Hse (2) NH resonances could be assigned roughly through comparison to the chemical shift of Ala (2) in Karle's peptide **2** and other peptides (in $CDCl_3$) with structures similar to those described herein;[31] however, this assignment is by no means definitive. Comparison to **2** and other known peptides did not assist in the assignment of the Leu NH resonances. We turned to analysis of the $J_{HNC\alpha H}$ values in $CDCl_3$ to better establish the identity of these four residues. $J_{HNC\alpha H}$ values in $CDCl_3$ have been observed to be small at the N-termini of helical peptides (~2-5 Hz), and grow larger toward the C-termini (6-8 Hz).[31] Assuming that the peptides could adopt helical conformations in $CDCl_3$, we tentatively assigned the Ser, Hse, and Leu NHs through analysis of their relative $J_{HNC\alpha H}$ values, where coupling constants could be calculated (Tables 2 and 3). Such analysis was not possible in $(CD_3)_2SO$ (see below); therefore, in these solutions the assignments of Ser, Hse, and Leu remain ambiguous.

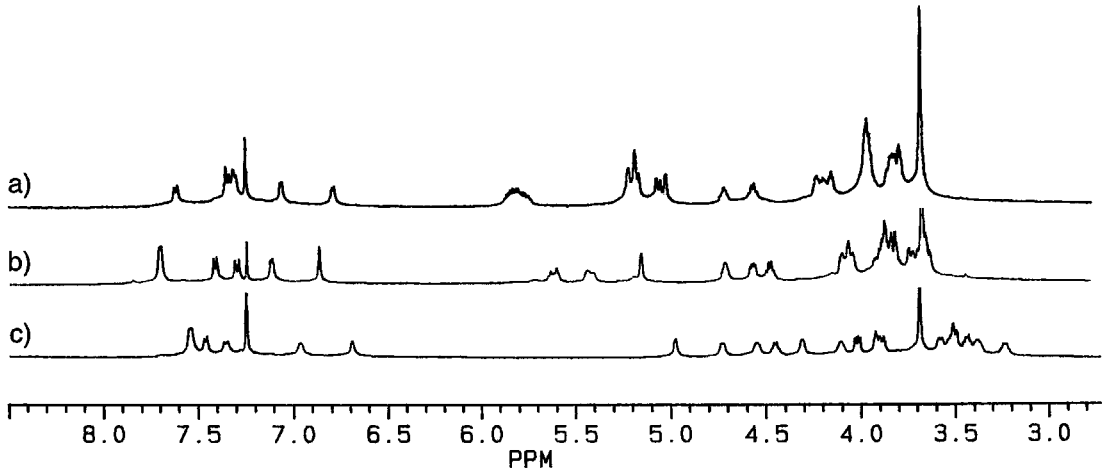


Figure 3. Amide NH, olefin, and C_α-H regions of the 500 MHz ^1H NMR spectra of: a) acyclic peptide **3**, b) cyclic peptide olefins **5**, and c) hydrogenated cyclic peptide **7**. Peptide concentration ~10 mg/mL in $CDCl_3$, 25 °C.

Table 2. NMR paramters of amide NH groups in peptides **3** and **7**.

peptide	residues[a]	$CDCl_3$[b]		$(CD_3)_2SO$[b]		
		δ_{NH} (ppm)	$^3J_{HNC\alpha H}$ (Hz)	δ_{NH} (ppm)	$^3J_{HNC\alpha H}$ (Hz)	$d\delta/dT$[c] (-ppb/K)
3	Val (1)	4.96	---[d]	6.81	8.5	6.9
	Ser (2)	7.05	5.0	7.96	---[e]	5.3
	Leu (3)	7.31	6.1	8.05	7.3	5.3
	Aib (4)	7.34	---	7.93	---	5.5
	Val (5)	6.78	6.3	7.09	7.8	1.7[f]
	Ser (6)	7.62	8.0	7.96	---[e]	4.3
	Leu (7)	7.33	8.6	8.10	7.7	6.7
7	Val (1)	5.00	---[d]	6.78	8.7	6.0
	Ser (2)	6.99	---[d]	7.92	---[e]	4.9
	Leu (3)	7.47	6.6	7.95	7.8	3.0
	Aib (4)	6.74	---	8.02	---	3.9
	Val (5)	7.35	8.9	7.33	7.6	2.2[f]
	Ser (6)	7.54	---[e]	7.90	---[e]	5.1
	Leu (7)	7.54	---[e]	8.10	7.7	5.9

[a] Val, Ser, and Leu NHs were assigned using spin decoupling. Assignment of the Ser and Leu NHs is not unequivocal (see text). [b] Spectra were measured at 298 K and at a 10 mg/ml sample concentration. [c] $d\delta/dT$ is the temperature coefficient of the NH chemical shifts. [d] Peak not resolved-apparent singlet. [e] Peak not resolved due to overlap. [f] This $d\delta/dT$ value is indicative of a solvent shielded NH.

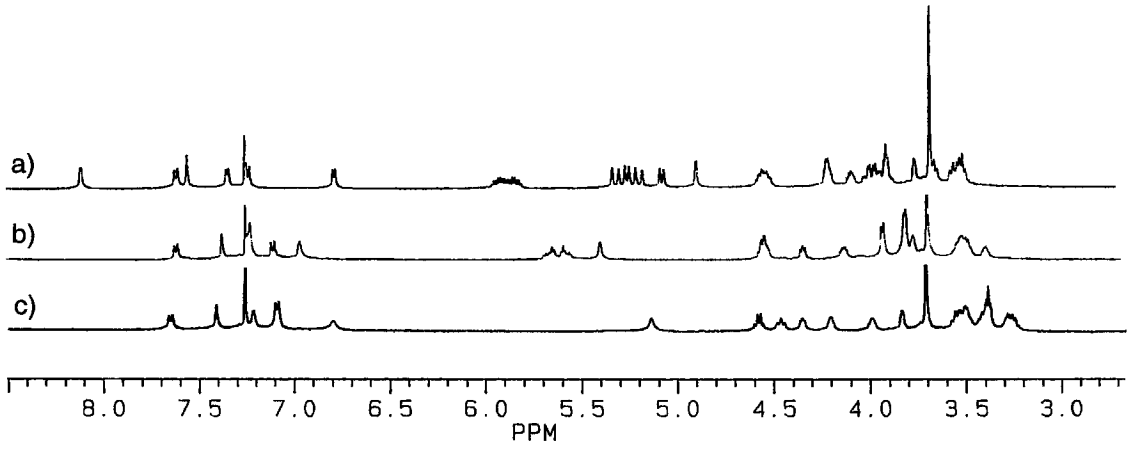


Figure 4. Amide NH, olefin, and C_α-H regions of the 500 MHz ^1H NMR spectra of: a) acyclic peptide **4**, b) cyclic peptide olefins **6**, and c) hydrogenated cyclic peptide **8**. Peptide concentration ~10 mg/mL in $CDCl_3$, 25 °C.

Table 3. NMR paramters of amide NH groups in peptides **4** and **8**.

peptide	residues[a]	$CDCl_3$[b]		$(CD_3)_2SO$[b]		
		δ_{NH} (ppm)	$^3J_{HNC^\alpha H}$ (Hz)	δ_{NH} (ppm)	$^3J_{HNC^\alpha H}$ (Hz)	$d\delta/dT$[c] (-ppb/K)
4	Val (1)	4.85	2.4	6.81	8.2	6.9
	Hse (2)	8.11	3.4	7.82	7.8	4.0
	Leu (3)	7.34	5.7	7.91	---[d]	4.7
	Aib (4)	7.54	---	8.08	---	6.1
	Val (5)	6.77	6.8	7.12	7.3	2.8[e]
	Hse (6)	7.61	8.3	7.96	7.3	3.6
	Leu (7)	7.22	8.3	7.93	---[d]	5.3
8	Val (1)	5.13	---[f]	6.84	7.5	---[g]
	Hse (2)	6.80	---[f]	8.00	---[d]	---
	Leu (3)	7.20	3.0	7.76	---[f]	---
	Aib (4)	7.40	---	8.00	---	---
	Val (5)	7.09	---[d]	7.30	7.8	---
	Hse (6)	7.64	8.4	7.83	7.8	---
	Leu (7)	7.07	---[d]	8.00	---[d]	---

[a] Val, Hse, and Leu NHs were assigned using spin decoupling. Assignment of the Hse and Leu NHs is not unequivocal (see text). [b] Spectra were measured at 298 K and at a 10 mg/ml sample concentration. [c] $d\delta/dT$ is the temperature coefficient of the NH chemical shifts. [d] Peak not resolved due to overlap. [e] This $d\delta/dT$ value is indicative of a solvent shielded NH. [f] Peak not resolved-apparent singlet. [g] $d\delta/dT$ values for peptide **8** could not be obtained due to severe overlap of the C^α-H resonances in $(CD_3)_2SO$ over the measured temperature range.

Despite the ambiguity which remains in the assignment of the amide NH resonances in peptides **3**, **4**, **7**, and **8**, we established the presence of specific intramolecular H-bonds in these peptides by analyzing the solvent dependence of the amide NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures[31,32] and the temperature dependence of these chemical shifts in $(CD_3)_2SO$.[33] The NMR experiments conducted on acyclic peptide **3** and its corresponding macrocyclic product **7** will be discussed first, after which the analogous experiments conducted on the acyclic homologue **4** and cyclic peptide **8** will be presented (Figures 5-6).

Solvent titration experiments on **3** (Figure 5a) indicated that only two NH groups, assigned to Val (1) and Ser (2), move appreciably downfield on addition of the strongly H-bonding solvent, $(CD_3)_2SO$, to solutions of the apolar solvent, $CDCl_3$. Clearly, in solutions of up to 50% $(CD_3)_2SO$, the remaining five NH groups are shielded from solvent, strongly supporting a completely helical conformation in which the NH groups of residues 3-7 are intramolecularly H-bonded. A completely 3_{10}-helical sequence with five 4→1 H-bonds is consistent with this data. However, structures involving both 4→1 and 5→1 H-bonding patterns with the Boc C=O accepting H-bonds from both the Leu (3) and the Aib (4) NH groups can not be ruled out.[34]

The analogous solvent titration experiment on cyclic peptide **7** also established that the corresponding Val (1) and Ser (2) NH groups are significantly solvent exposed (Figure 5b), indicative of a similar helical conformation. Interestingly, however, the Aib (4) NH in peptide **7** also appears to be solvent exposed. This suggests that the helical conformation of **3** is slightly disrupted upon cyclization to give **7**, with the Aib (4) in **7** no longer able to form the 4→1 present in **3**. Notably, the Aib (4) residue is in the center of the macrocyclic sub-structure of **7** and could potentially be conformationally disturbed by the cyclization; this could be a factor in the loss of the Aib (4) H-bond in **7**.

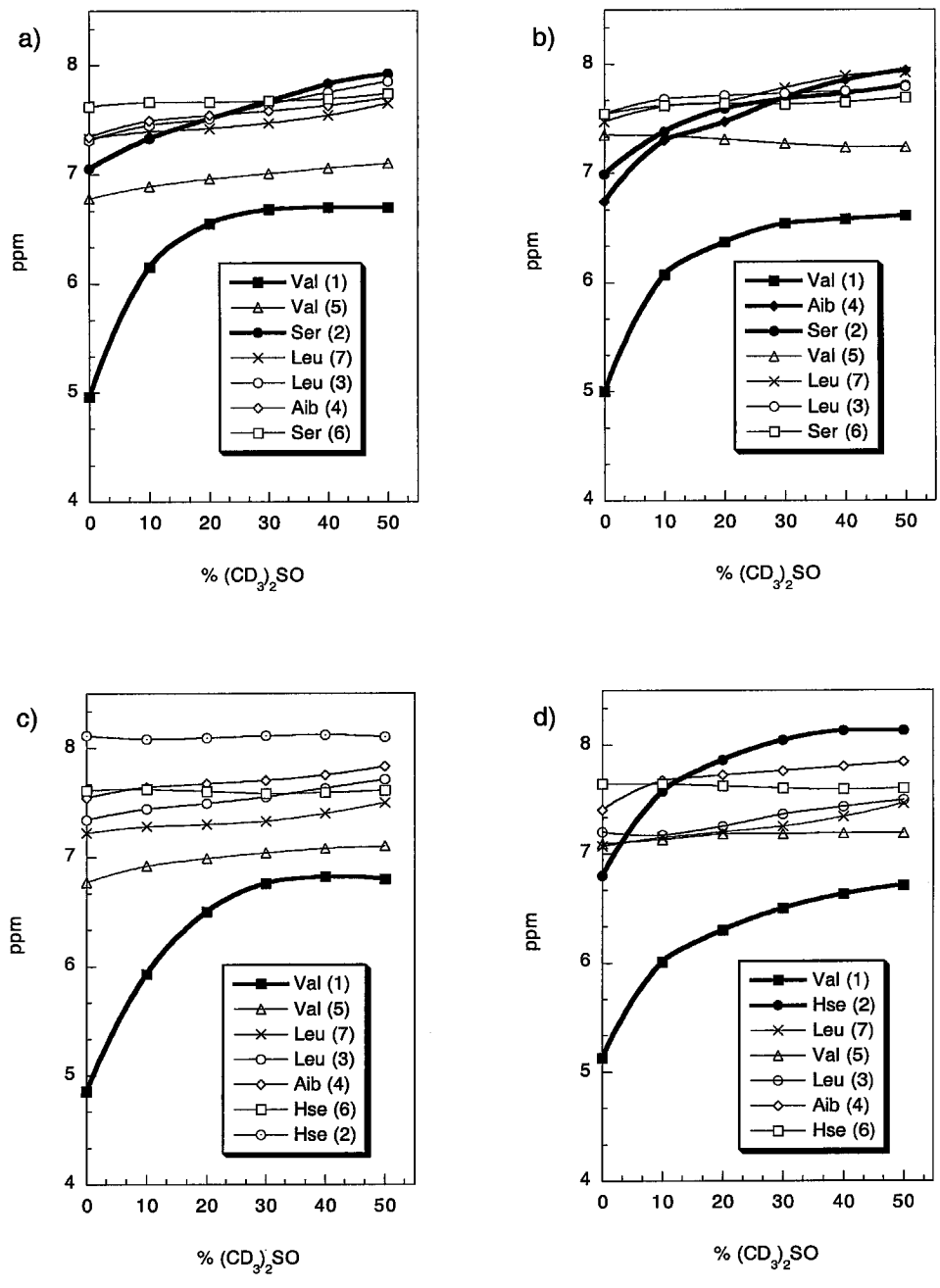


Figure 5. Solvent dependence of NH chemical shifts of: a) acyclic peptide **3**, b) cyclic peptide **7**, c) acyclic peptide **4**, and d) cyclic peptide **8** in $CDCl_3$-$(CD_3)_2SO$ mixtures of varying concentrations. Peptide concentration ~10 mg/mL, 25 °C. Assignments to specific residues are in the legends of each plot. The bold traces correspond to residues believed to be significantly solvent exposed in these solvent mixtures. Assignment of the Ser, Hse, and Leu residues is not unequivocal (see text).

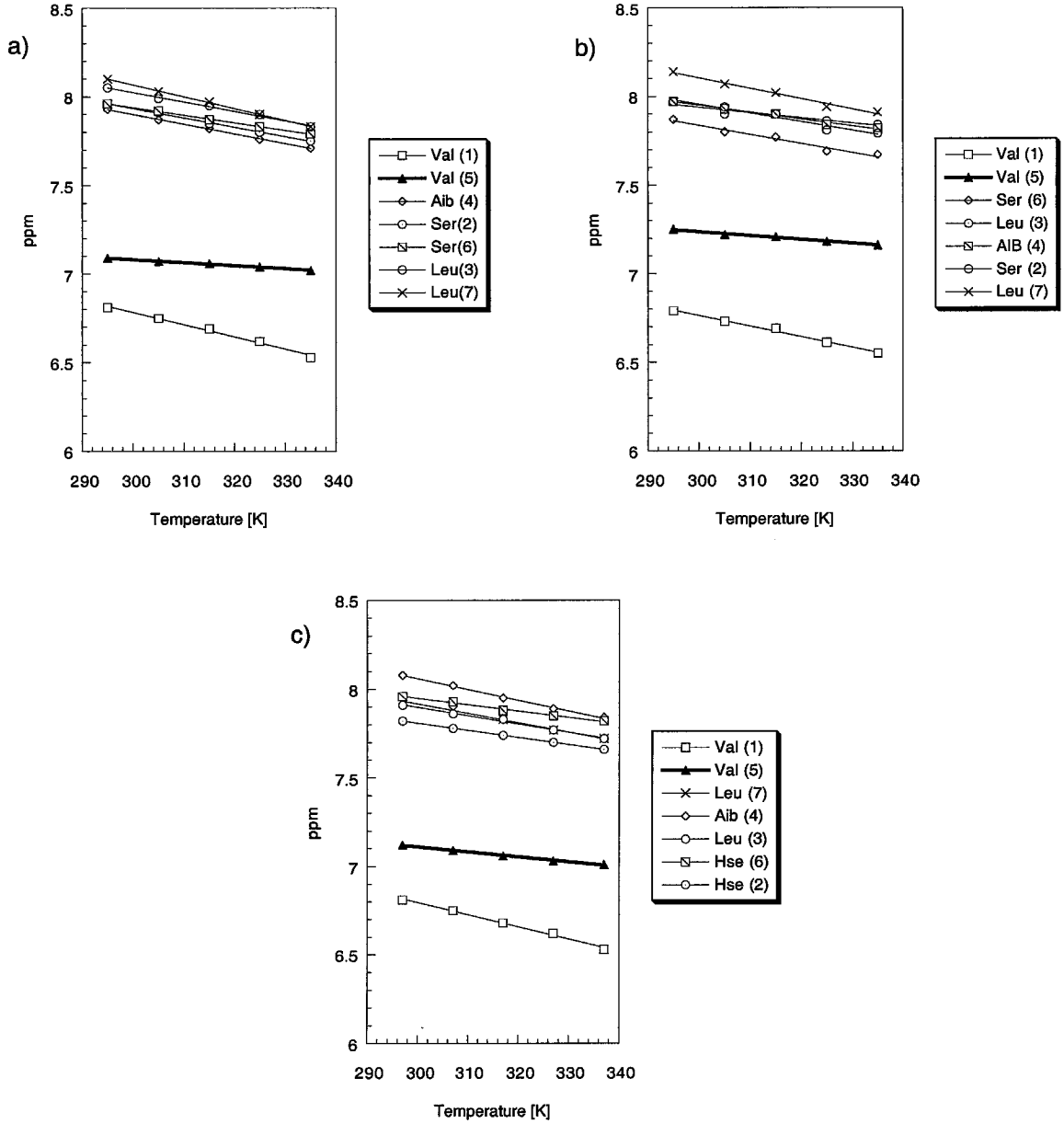


Figure 6. Temperature dependence of NH chemical shifts in $(CD_3)_2SO$: a) acyclic peptide **3**, b) cyclic peptide **7**, and c) acyclic peptide **4**. Peptide concentration ~10 mg/mL. Assignments to specific residues are in the legends of each plot. The bold traces correspond to residues believed to be solvent shielded in $(CD_3)_2SO$. The $d\delta/dT$ values derived from these plots are listed in Tables 2 and 3. Assignment of the Ser, Hse, and Leu residues is not unequivocal (see text).

Temperature gradient NMR experiments in $(CD_3)_2SO$ of **3** and **7** yielded very similar conformational data for both peptides (Table 2, Figure 6a-b). The majority of the temperature coefficients ($d\delta/dT$) of the NH groups in both **3** and **7** are high ($\geq$ 4 ppb/K),

with the exception of those for the Val (5) NHs, which have values of 1.7 and 2.2 ppb/K, respectively (highlighted in Table 2). This is suggestive of a completely non-helical conformation in $(CD_3)_2SO$, with instead an isolated weak Leu (3)-Aib (4) β-turn, stabilized by a 4→1 hydrogen bond between the Ser (2) C=O and the Val (5) NH groups. Such X-Aib β-turns are common features in small Aib-containing peptides, as discussed previously in Chapter 2. While all of the other $d\delta/dT$ values for the NH groups of peptide **3** are high, indicative of full solvent exposure in $(CD_3)_2SO$, the Leu (3) NH of cyclic peptide **7** has a moderately low $d\delta/dT$ value of 3.0 ppb/K. This suggests that the Leu (3) NH of **7** is also involved in weak H-bonding in $(CD_3)_2SO$: we speculate that the Leu (3) NH could be involved in a weak 4→1 β-turn type H-bond with the Boc C=O. Finally, the $J_{HNC\alpha H}$ values for all the NH groups in peptides **3** and **7** are uniformly high (≥ 8 Hz) in $(CD_3)_2SO$, which are characteristic of extended peptide conformations (Table 2).[35] These data, taken together with the $d\delta/dT$ values and the solvent titration data above, are supportive of predominantly 3_{10}-helical conformations for both **3** and **7** in $CDCl_3$, which are completely disrupted in the more strongly solvating medium, $(CD_3)_2SO$.

Turning next to the NMR experiments conducted on acyclic homologue **4** and macrocycle **8**, we observed behavior in $CDCl_3$ and $(CD_3)_2SO$ solutions that was very similar to peptides **3** and **7** above (Figure 5ac-d, Figure 6c). In the solvent titration experiment on acyclic peptide **4**, only the Val (1) NH moved markedly downfield upon the addition of the more polar solvent $(CD_3)_2SO$ (Figure 5c). The remaining six amide NH groups of **4** show very small changes in chemical shift in up to 1:1 mixtures of the $(CD_3)_2SO$ and $CDCl_3$, again characteristic of intramolecularly H-bonded NH groups. This suggests that the five intramolecular 4→1 H-bonds present in acyclic peptide **3** are also maintained in its bis-Hse homologue **4**, plus an additional potential γ-turn like 3→1 H-bond at the N-terminus between the Boc C=O and the Hse (2) NH. Such γ-turn H-bonding patterns were previously discussed in Chapter 2 in the context of β-turns: however, to the best of our knowledge, there have been few reports of this H-bonding

pattern at the N-termini of peptide helices. Although unlikely, we can not rule out the possibility of the Hse (2) NH being involved in an H-bond with one of the oxygens of the two *O*-allyl ether side chains in **4**. The analogous solvent titration experiments were conducted on cyclic peptide **8** (Figure 5d), and the extra Hse (2) H-bond observed in peptide **4** was not apparent. Instead, in analogy to peptide **3** (Figure 5a), only the Val (1) and Hse (2) NHs moved downfield upon addition of $(CD_3)_2SO$, while the other five amide NHs remained constant. Therefore, cyclic peptide **8** was assumed to have a fully helical conformation similar to that of acyclic peptide **3** in solutions containing up to 50% $(CD_3)_2SO$, with five intramolecular amide H-bonds. Interestingly, cyclic peptide **8** lost one H-bond relative to its acyclic precursor **4**, suggesting that **8**, like related cyclic peptide **7**, undergoes a subtle conformational shift upon cyclization. Again, as in the analysis of peptide **3** and **7**, we can not rule out the possibility of 4→1 and/or 5→1 H-bonding patterns at the N-termini of peptides **4** and **8** from these experiments alone.

The temperature gradient NMR experiment conducted on acyclic peptide **4** (Figure 6c) exposed it to assume an extended conformation in $(CD_3)_2SO$ similar to peptides **3** and **7**. All of the observed $d\delta/dT$ values for the NH groups were high (≥ 4 ppb/K) except for Val (5), which had a $d\delta/dT$ value of 2.8 ppb/K (highlighted in Table 3). In analogy to peptides **3** and **7** above, this was believed to be indicative of a weak 4→1 H-bond between the Hse (2) C=O and the Val (5) NH group in the context of a Leu (3)-Aib (4) β-turn. This, along with the fairly high $J_{HN\alpha H}$ values for **4** in $(CD_3)_2SO$ (Table 3), suggests that peptide **4** assumes a very loosely structured conformation in $(CD_3)_2SO$. Unfortunately, an analogous temperature gradient NMR experiment could not be conducted on cyclic peptide **8** due to the severe overlap of its C_α-H resonances over the temperature range 295-335 K; this overlap precluded assignment of the amide NHs by sequential spin decoupling. However, in view of the similar conformational behavior of peptides **3**, **4**, and **7** in $(CD_3)_2SO$ over this temperature range, we are confident that cyclic

peptide **8** would also adopt a fairly random, extended conformation in this medium. The fairly high $J_{HN\alpha H}$ values in $(CD_3)_2SO$ for **8** corroborate well with this theory (Table 3).

The ^1H NMR experiments above strongly suggest that peptides **3**, **4**, **7**, and **8** adopt predominantly 3_{10}-helical structures in $CDCl_3$, and that these helical structures are completely disrupted in the more solvating medium, $(CD_3)_2SO$. The latter trend dispelled our hopes that the covalent link installed in macrocycles **4** and **8** would act to constrain them to helical conformations even in polar media. Importantly, however, these data suggested that acyclic peptides **3** and **4** were preorganized in a helical conformation in $CDCl_3$ for RCM macrocyclization, and that this conformation was maintained in the cyclic products **7** and **8**. Interestingly, the data obtained for Karle's peptide **2** in the analogous NMR experiments was almost identical,[19] suggesting that replacement of the two Ala resides in **2**, residues believed to be strongly helix-promoting,[36] with Ser or Hse (*O*)-allyl ethers did not act to disrupt the 3_{10}-helical predisposition of the peptide backbones.

While qualitatively relevant to peptide conformation, the ^1H NMR analyses of inaccessible amide NH groups only provides an identification of potentially intramolecular H-bonded NH groups, and does not permit identification of the acceptor carbonyl residues. Further conformational characterizations in solution therefore rely on NOE evidence. Work toward the solution phase 2D NMR structures of peptide **3**, **4**, **7**, and **8** in $CDCl_3$ solutions is currently in progress: preliminary structures of acyclic peptide **4** and cyclic peptide **8** strongly suggest that they both adopt 3_{10}-helical conformations in $CDCl_3$ solutions.[37]

X-ray Crystal Structure of Cyclic Peptide 8

Cyclic peptide **8** exhibited high crystallinity, and evaporation from CH_2Cl_2/hexane provided crystals suitable for X-ray crystallographic analysis (Figure 7).[38] Pertinent torsion angles from the structure of **8** are listed below in Table 4.[39] Viewing the peptide

from the N- to C-terminus, it appears that the peptide backbone is helical for the first five residues and then the helix begins to fray for the last two (Hse 6 and Leu 7).[40] The average torsional angles ϕ (about N-Cα) and ψ (about Cα-C') for the first five residues are -57° and -34°, respectively, which approximate those for a right-handed 3_{10}-helix (-57° and -30°).[41] These torsional angles are marginally close to those for the average right-handed α-helix (ϕ = -63°, ψ = -42°), and therefore it is difficult to completely exclude the possibility of the peptide being α-helical or accessing a mixed $\alpha/3_{10}$-helix conformation.

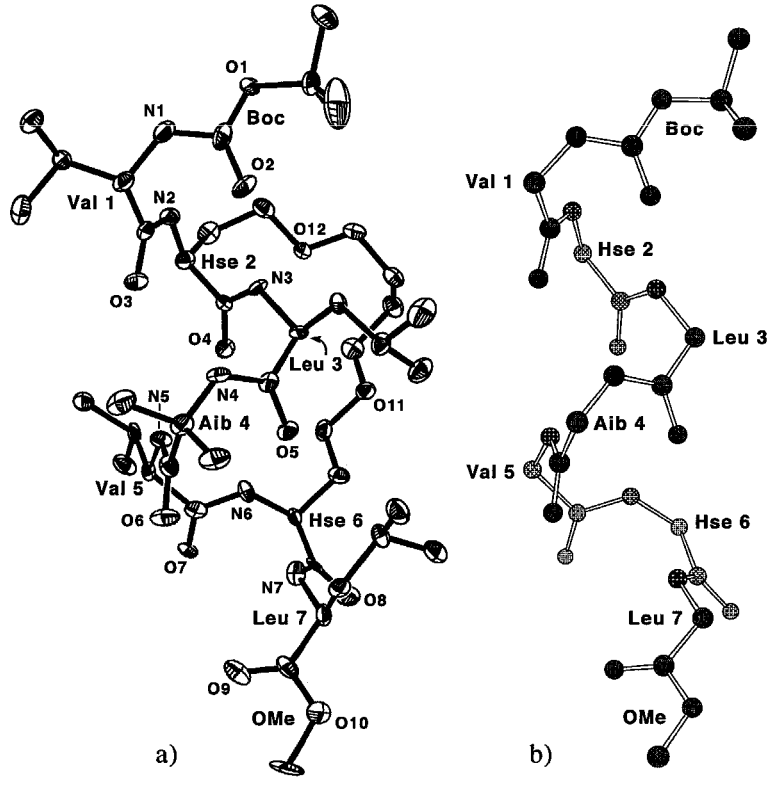


Figure 7. a) ORTEP diagram of X-ray crystal structure of cyclic peptide **8**. Thermal ellipsoids represent 30% probability levels. Hydrogen atoms have been omitted for clarity. b) Rendering of **8** without amino acid side chains.

Table 4. Torsional Angles for **8** (deg)

Residue	ϕ	ψ	ω	χ^1	χ^2
Val (1)	-52[a]	-40	-174	167, -71	
Hse (2)	-53	-28	-178	71	
Leu (3)	-60	-37	-180	176	-178, 61
Aib (4)	-55	-36	-178		
Val (5)	-65	-30	-170	176, -62	
Hse (6)	-85	-15	-167	-62	
Leu (7)	-109	165[b]	-180[c]	-61	-178, -55

[a] C' (0), N (1), C^{α} (1),C' (1). [b] N (7), C^{α} (7), C' (7), O (OMe).
[c] C^{α} (7), C' (7), O (OMe), C (OMe)

Analysis of the amide N to carbonyl O distances (N···O) and respective angles (N···O=C), however, supports the presence of four consecutive 4→1 intramolecular hydrogen bonds (2.96-3.01 Å), involving N(3)-N(6) and O(2)-O(5), which are diagnostic of a 3_{10}-helix (Table 5).[42] This iterative hydrogen bonding pattern involves the carbonyl O and amide NH of amino acids that are two residues apart.[43] We speculate that the Leu 3 carbonyl O(5) could also be involved in a fifth extremely long 5→1 hydrogen bond with the Leu 7 amide N(7) (N···O 3.50 Å), which may be lengthened due to the disorder of the helix at the C-terminus. Thereafter, the 4→1 hydrogen bonding pattern is continued intermolecularly in a head-to-tail fashion, with the Val 5 carbonyl O(7) hydrogen bonding to the Val 1 Boc amide N(1) (N···O 2.91 Å) of the adjacent peptide molecule in the unit cell.[44] The evidence suggests that replacement of the two Ala residues in peptide **2** with tethered Hse residues in peptide **8** has induced the peptide backbone to transform from an α-helix to a predominantly 3_{10}-helix in the solid state. The constraint imposed by the side chain linkage in peptide **8** could be cause for this unique conformational shift.[45]

Table 5. Hydrogen Bonds for **8**.

Type	Donor[a]	Acceptor	N···O (Å)	Angle (deg) C=O···N
Interpeptide	N (1)[b]	O (5')[c]	2.930	158
4 → 1	N (3)	O (0)[d]	3.014	131
4 → 1	N (4)	O (1)	2.993	130
4 → 1	N (5)	O (2)	2.970	117
4 → 1	N (6)	O (3)	2.957	118

[a] Carbonyl oxygens O (4), O (6), and O (7) and amide nitrogens N (2) and N (7) do not appear to be involved in any hydrogen bonding. [b] Numbers correlate with residue number from N- to C-terminus. [c] Val (5) carbonyl of the adjacent peptide in the unit cell. [d] Corresponds to Boc group carbonyl.

Two views comparing the X-ray structures of Karle's peptide **2** and cyclic heptapeptide **8** are shown in Figures 8 and 9. Viewing the two helical structures side-on in Figure 8, it is apparent that cyclic peptide **8** is longer from N- to C-termini than peptide **2**, which corroborates with the longer average length of 3_{10}-helices versus α-helices due to the shorter helical pitch in 3_{10}-helices (3 residues versus 3.6 residues per turn of the helix, respectively).[40] This difference in pitch is apparent in Figure 9, where two views into the helices of peptide **2** and **8** are shown (from the N- to C-termini). Both structure views show a pseudo-circular arrangement of amide NH groups about the axis of the helix, an arrangement frequently observed in helical peptide and protein structures. However, the circumference of the circular core in cyclic peptide **8** is considerably smaller than that of α-helical peptide **2**, which is further evidence of peptide **8** adopting a more tightly-pitched, 3_{10}-helical conformation.

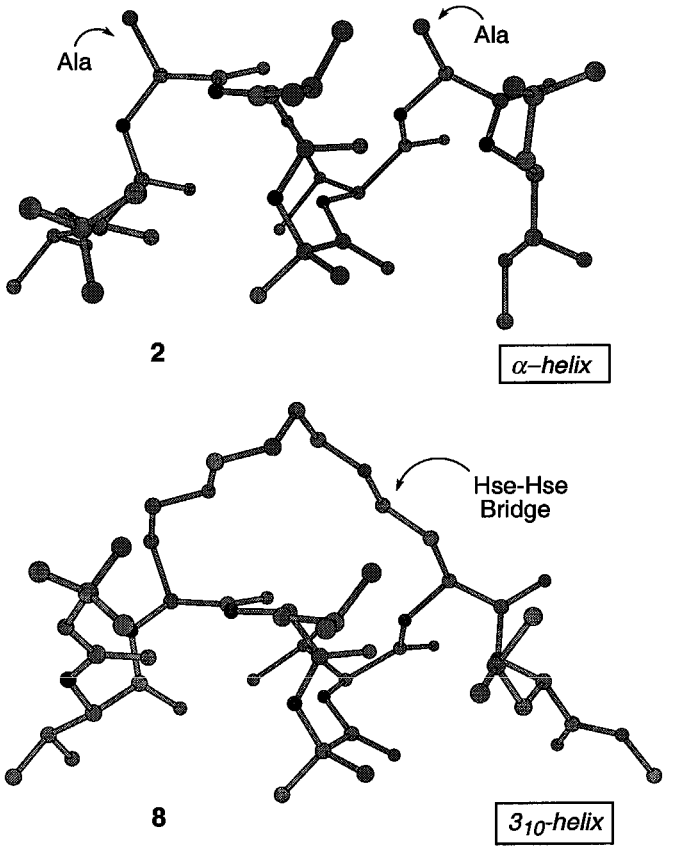


Figure 8. Views of the solid state structures of Karle's peptide **2** and cyclic peptide **8**. The two Ala side chains of **2** and the Hse-Hse C=C bridge of **8** are designated with arrows.

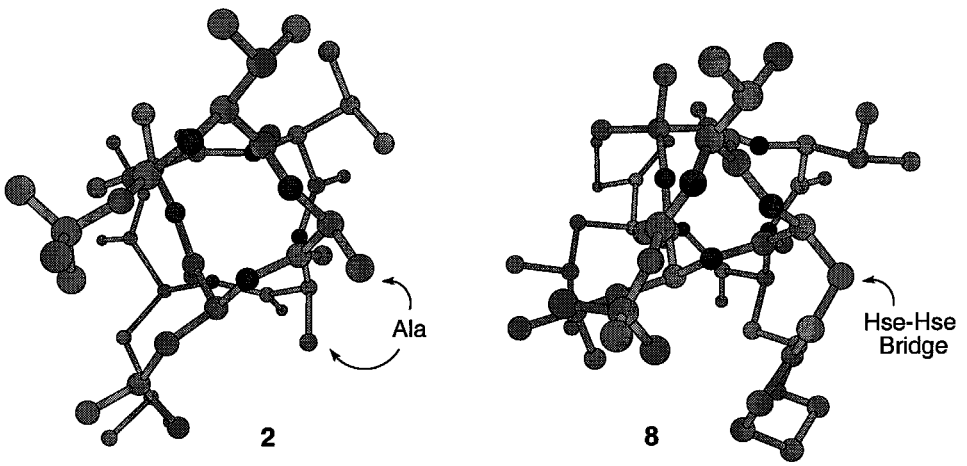


Figure 9. Views of the structures of Karle's peptide **2** and cyclic peptide **8** from N- to C-termini. The two Ala side chains in **2** and the Hse-Hse C=C bridge in **8** are designated with arrows.

Summary and Conclusions

Acyclic dienes incorporated into a helical peptide scaffold have been treated with alkylidene **1** to afford macrocyclic peptide by a remarkably facile ring-closing metathesis reaction. CD, IR, ^{1}H NMR, and X-ray crystallographic studies strongly suggest that peptides **3**, **4**, **7**, and **8** adopt predominantly 3_{10}-helical conformations in solution and/or in the solid state. Interestingly, while Karle's peptide **2** undergoes the subtle conformational shift from a 3_{10}- to an α-helix on going from $CDCl_3$ solution to the solid state, cyclic peptide **8** maintains a 3_{10}-helical conformation in both solution and in the crystal. The relative ease of introducing carbon-carbon bonds into peptide secondary structures *via* RCM and the predicted metabolic stability of these bonds renders olefin metathesis an exceptional methodology for the synthesis of rigidified peptide architectures. Specifically, we believe the macrocyclization of small, hydrophobic peptide helices is uniquely suited to RCM in organic solvents because helical conformations are frequently favored in apolar media.

In view of the success of RCM as a route toward cyclic peptide helices, an exciting future application for RCM is in the synthesis of cyclic/tethered analogs of naturally occurring helical peptide antibiotics (e.g. the trichogin family of lipopeptaibols) with the intent of exposing the requirements of a helical conformation on their activities (as discussed above).[46] Another interesting application for olefin metathesis in the context of peptides is the syntheses of tethered peptide helix bundles and helix-turn-helix motifs by RCM, for potential use as peptide ligands for proteins and DNA. The recent advent of water-soluble olefin metathesis catalysts (introduced in Chapter 3) has accelerated our pursuit toward such complex, biologically relevant structures.[47] While almost all of our work with peptide RCM in organic solvents has relied on the preorganization of acyclic dienes through intramolecular H-bonding, RCM in water may require the introduction of hydrophobic packing as a novel preorganizing factor. The application of RCM to peptide chemistry is enormous in scope; we anticipate that the

work described in Chapters 2-4 of this thesis will serve as a framework for the design and synthesis of more structurally elaborate biomolecules *via* olefin metathesis in the near future.

Acknowledgments

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Experimental Section

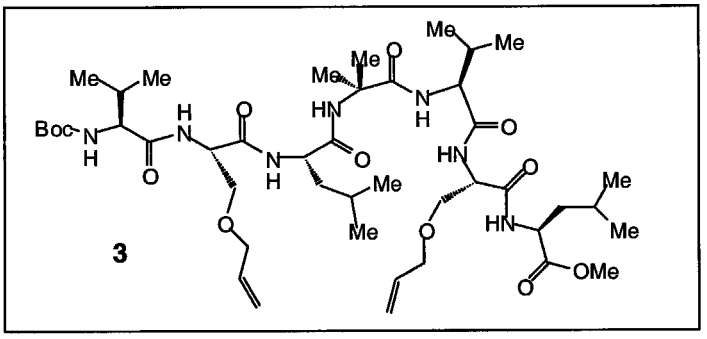
General Experimental Section. NMR spectra were recorded on a Jeol GX-400 or Bruker AM-500 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Infrared spectra were obtained on a Perkin-Elmer 1600 Series FT-IR. Optical rotations were recorded on either a Jasco DIP-181 or DIP-1000 digital polarimeter at 589 nm and are reported as $[\alpha]_D$ (concentration (c) in grams/100 mL of solvent). Low- and high-resolution mass spectra were provided by the Southern California Mass Spectrometry Facility (University of California, Riverside).

Analytical thin-layer chromatography (TLC) was performed using silica gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Flash column chromatography was performed using silica gel 60 (230-400 mesh) from EM Science.[48] Catalyst **1** was prepared according to published procedure.[11] RCM reactions were carried out under an argon atmosphere with dry, degassed solvents under anhydrous conditions.[49]

Peptide Synthesis. *N-tert*-Butyloxycarbonyl-*O*-allyl-L-serine and *N-tert*-butyl-oxycarbonyl-*O*-allyl-L-homoserine were prepared according to a modified literature procedure.[21] Peptides **3** and **4** were synthesized by conventional solution phase synthesis methods using a racemization free fragment condensation strategy.[22] Couplings were mediated by *N,N*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT). The Boc group was used to protect the N-terminus, and the C-terminus was protected as a methyl ester. Deprotections were performed using 1:1 trifluoroacetic acid/CH_2Cl_2 and saponification, respectively. All intermediates were characterized by ^{1}H-NMR (400 or 500 MHz) and TLC, and if necessary purified by column chromatography on silica gel. Prior to the ring-closing metathesis (RCM) reaction, peptides **3** and **4** were purified by column chromatography on silica gel (83% EtOAc/Hexane as eluent) and fully characterized (see below).

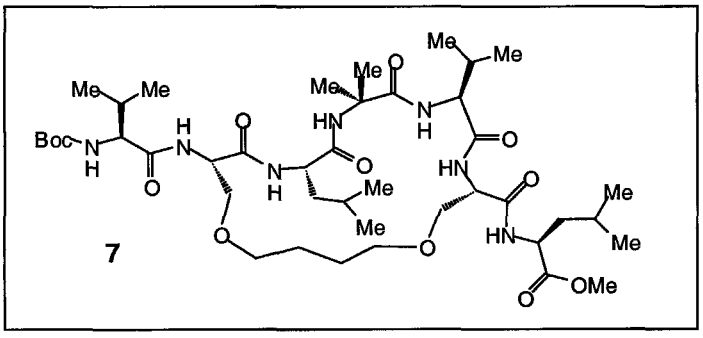
Circular Dichroism Studies. CD spectra were recorded on a Jasco J-600 spectropolarimater equipped with a JFC data processor (J-600 series spectropolarimater system software, Ver. 2.00) using 1 mm pathlength cuvettes. The scan speed was 5 nm/min, and spectra were averaged over 4 scans. Spectral baselines were obtained under analogous conditions as that for the samples. All spectra are baseline subtracted, converted to a uniform scale of molar ellipticity, and replotted. The temperature was maintained at 25 °C and the sample concentration was 1.0 mM.

X-ray Crystallographic Data of Cyclic Peptide 8. Tables of experimental data, distances, angles, torsion angles, stereoview plots, unit cell plots, and position and displacement parameters (13 pages) can be found in the Appendix to this thesis.



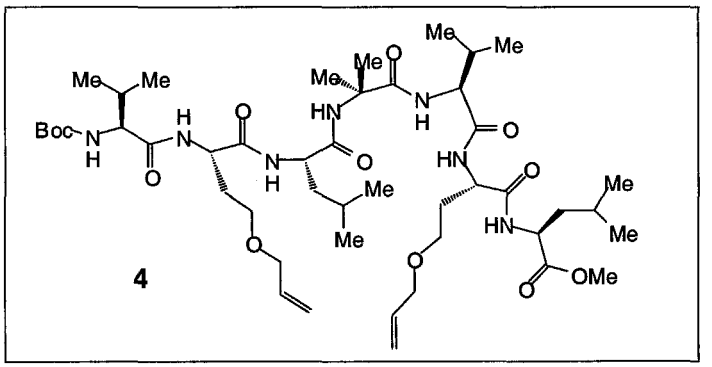
3

Acyclic Peptide (3). Heptapeptide **3** was prepared according to the standard solution phase protocol described in the general experimental above.[22] ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.61 (1H, d, J = 8 Hz), 7.34 (1H, s), 7.32 (1H, d, J = 9 Hz), 7.30 (1H, d, J = 6 Hz), 7.05 (1H, d, J = 5 Hz), 6.78 (1H, d, J = 6 Hz), 5.87-5.77 (2H, br m), 5.23-5.06 (4H, br m), 4.95, (1H, apparent s), 4.73 (1H, m), 4.58 (1H, m), 4.24-4.16 (3H, br m), 3.97 (5H, m), 3.88-3.81 (4H, br m), 3.69 (3H, s), 2.23 (1H, m), 2.03 (1H, m), 1.71-1.38 (6H, br m), 1.50 (3H, s), 1.48 (3H, s), 1.46 (9H, s), 1.06 (3H, d, J = 7 Hz), 1.00 (6H, m), 0.93 (3H, d, J = 6 Hz), 0.88 (9H, d, J = 6 Hz), 0.86 (3H, d, J = 6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.9, 173.3, 173.2, 172.8, 171.8, 171.0, 170.2, 157.5, 134.8, 133.6, 117.6, 116.4, 80.9, 72.1, 71.9, 69.6, 62.4, 60.4, 56.9, 55.9, 54.4, 53.8, 51.9, 50.8, 40.4, 39.7, 29.5, 29.0, 28.2, 27.1, 24.4, 24.3, 23.2, 23.0, 22.8, 21.6, 21.0, 19.2, 19.0, 18.6, 17.1; IR (1.0 mM in $CHCl_3$, cm^{-1}): 3428, 3319, 2960, 2931, 2869, 1738, 1699, 1661, 1525, 1470, 1369, 1239, 1159, 1097; TLC R_f = 0.26 (83% EtOAc/Hexane); $[\alpha]_D$ = -21.7 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{44}H_{77}N_7O_{12}$ $[M+Na]^+$ 918.5528, found 918.5494.

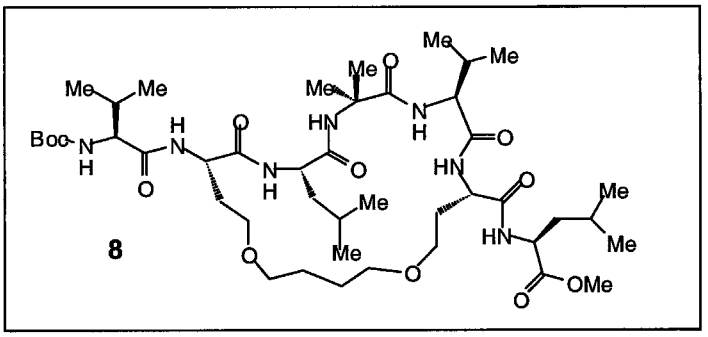


Cyclic Peptide (7). To a solution of acyclic diene **3** (103 mg, 0.115 mmol) in 20 mL of $CHCl_3$ was added *via* syringe a solution of ruthenium catalyst **1** (19 mg, 0.023 mmol) predissolved in 3 mL of $CHCl_3$. Within 10 min, the purple solution became orange-brown, and the solution was stirred an additional 4 h at 25 °C, when TLC analysis showed full disappearance of starting material. Triethylamine (1 mL) was added to the solution to deactivate any remaining catalyst, and the solution was concentrated to afford a brown, crystalline solid. Purification by column chromatography (4 cm x 12 cm silica gel, solvent gradient from 83% EtOAc/Hexane to 100% EtOAc) afforded 85 mg (85%) of cyclic olefin isomers **5** as an off-white powder. Cyclic peptides **5** (20 mg, 0.023 mmol) were then dissolved in 2.9 mL of anhydrous EtOH, and 10% Pd-C (8 mg, 0.4 wt/wt) was added to the stirring solution. The system was purged with hydrogen, and allowed to stir under 1 atm of hydrogen for 2 h. The solution was then filtered (2X) through a pad of Celite and concentrated to afford 19.6 mg (98%) of hydrogenated macrocycle **7** as a white, crystalline solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55 (2H, two amide NHs obscured, apparent m), 7.49 (1H, d, J = 7 Hz), 7.33 (1H, br d), 7.04 (1H, br d), 6.80 (1H, s), 5.07 (1H, br d), 4.72 (1H, m), 4.53 (1H, m), 4.46 (1H, m), 4.31 (1H, m), 4.11 (1H, m), 4.01 (1H, m), 3.93 (1H, m), 3.88 (1H, m), 3.72 (1H, m), 3.70 (3H, s), 3.59 (1H, m), 3.51 (2H, m), 3.43 (1H, m), 3.40 (1H, m), 3.25 (1H, m), 2.43 (1H, m), 2.26 (1H, m), 1.95-1.33 (6H, m), 1.68 (4H, d, J = 6 Hz), 1.48 (3H, s), 1.47 (3H, s), 1.46 (9H, s), 1.05 (4H, d, J = 7 Hz), 0.94 (12H, m), 0.89 (8H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.1, 172.9, 172.5, 172.1, 170.4, 157.1, 81.1, 71.1, 71.0, 70.2, 68.5, 61.6, 59.5, 57.3, 55.5, 55.1, 54.7, 52.2,

51.7, 40.4, 30.2, 29.9, 28.3, 27.5, 25.8, 25.4, 24.7, 23.6, 23.0, 22.8, 22.2, 22.1, 19.7, 19.5, 18.1, 18.0; IR (1.0 mM in $CHCl_3$, cm^{-1}): 3431, 3331, 2960, 2931, 2869, 1737, 1663, 1530, 1465, 1367, 1240, 1160, 1099, 1029; TLC $R_f = 0.10$ (83% EtOAc/Hexane); $[\alpha]_D$ = +2.06 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{42}H_{75}N_7O_{12}$ $[M+Na]^+$ 892.5371, found 892.5361.



Acyclic Peptide (4). Heptapeptide **4** was prepared according to the standard solution phase protocol described in the general experimental above.[22] ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.11 (1H, d, J = 3 Hz), 7.62 (1H, d, J = 8 Hz), 7.55 (1H, s), 7.35 (1H, d, J = 6 Hz), 7.22 (1H, d, J = 8 Hz), 6.79 (1H, d, J = 8 Hz), 5.94-5.82 (2H, br m), 5.33-5.06 (4H, br m), 4.85 (1H, d, J = 2 Hz), 4.55 (2H, m), 4.22 (3H, m), 4.10 (1H, m), 3.97 (2H, m), 3.91 (2H, m), 3.76 (1H, m), 3.68 (3H, s), 3.66 (1H, m), 3.54 (2H, m), 2.50 (1H, m), 2.36 (1H, m), 2.15 (2H, m), 2.01 (2H, m), 1.70-1.35 (6H, br m), 1.50 (3H, s), 1.48 (12H, apparent s), 1.04-0.87 (24H, br m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 186.6, 176.0, 173.4, 173.2, 172.9, 172.4, 171.7, 157.4, 135.4, 134.2, 117.7, 116.5, 81.6, 72.5, 71.8, 69.0, 67.4, 62.38, 62.37, 62.3, 60.2, 57.3, 56.2, 54.3, 52.1, 51.2, 51.1, 40.9, 40.2, 31.3, 30.6, 29.8, 29.1, 28.4, 27.4, 25.0, 24.8, 23.5, 23.1, 23.0, 21.9, 21.6, 19.5, 19.3, 18.3, 17.3; IR (1.0 mM in $CHCl_3$, cm^{-1}): 3439, 3326, 3005, 2962, 2933, 2872, 1742, 1701, 1666, 1525, 1488, 1368, 1283, 1227, 1159, 1100; TLC R_f = 0.39 (83% EtOAc/Hexane); $[\alpha]_D$ = -20.9 (c =1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{46}H_{81}N_7O_{12}$ $[M+H]^+$ 924.6021, found 924.6055.



8

Cyclic Peptide (8). To a solution of acyclic diene **4** (1.00 g, 1.08 mmol) in 200 mL of $CHCl_3$ was added *via* syringe a solution of ruthenium catalyst **1** (178 mg, 0.216 mmol) predissolved in 16 mL of $CHCl_3$. Within 10 min, the purple solution became orange-brown, and the solution was stirred an additional 3 h at 25 °C, when TLC analysis showed full disappearance of starting material. Triethylamine (3 mL) was added to the solution to deactivate any remaining catalyst, and the solution was concentrated to afford a brown, crystalline solid. Purification by column chromatography (5 cm x 15 cm silica gel, solvent gradient from 75% EtOAc/Hexane to 100% EtOAc) afforded 869 mg (90%) of cyclic olefin isomers **6** as an off-white powder. Cyclic peptides **6** (676 mg, 0.756 mmol) were then dissolved in 92 mL of anhydrous EtOH, and 10% Pd-C (270 mg, 0.4 wt/wt) was added to the stirring solution. The system was purged with hydrogen, and allowed to stir under 1 atm of hydrogen for 2 h. The solution was then filtered (2X) through a pad of Celite and concentrated to afford 661 mg (98%) of hydrogenated macrocycle **8** as a white, crystalline solid. ^1H NMR (CD_2Cl_2, 400 MHz): δ 7.54 (1H, d, J = 8 Hz), 7.47 (1H, s), 7.23 (1H, d, J = 4 Hz), 7.09 (1H, d, J = 8 Hz), 7.03 (1H, d, J = 8 Hz), 6.74 (1H, d, J = 4 Hz), 5.17 (1H, apparent s), 4.46 (1H, m), 4.25 (1H, m), 4.17 (2H, m), 3.97 (1H, m), 3.80 (1H, m), 3.67 (3H, br s), 3.53-3.22 (8H, br m), 2.43 (1H, m), 2.33 (1H, m), 2.15 (2H, m), 1.72 (2H, m), 1.63-1.30 (10H, br m), 1.47 (12H, apparent s), 1.45 (3H, s), 1.10-0.85 (24H, br m); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.5, 174.2, 173.6, 173.4, 172.9, 172.6, 172.5, 157.5, 80.9, 71.2, 70.6, 68.1, 66.3, 62.7, 60.4, 57.0, 55.3, 54.4, 52.34, 52.29, 51.1, 40.72, 40.68, 31.9, 31.2, 29.8, 29.1, 28.5, 27.5, 26.6, 25.8, 25.0, 24.7,

23.4, 23.1, 22.8, 22.3, 21.7, 19.7, 19.4, 18.9, 18.0; IR (1.0 mM in $CHCl_3$, cm^{-1}): 3427, 3324, 3001, 2960, 2935, 2871, 1736, 1698, 1665, 1602, 1528, 1369, 1275, 1235, 1157, 1105; TLC R_f = 0.10 (83% EtOAc/Hexane); $[\alpha]_D$ = -2.09 (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{44}H_{79}N_7O_{12}$ $[M+H]^+$ 898.5865, found 898.5893.

References and Notes

† Portions of this chapter were previously reported in a separate publication. See: Blackwell, H. E.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **1998**, *37*, 3281-3284.

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(23) Both RCM reactions appeared quantitative by TLC. The yields are reduced only by the isolation procedure. Lower catalyst loadings (5-10 mol %) gave a reduced yield of macrocyclic products. This is often observed for RCM macrocyclizations, and is believed to be rooted in the apparently accelerated decomposition of the ruthenium methylidene catalyst species at high dilution. For a review of macrocyclization by RCM, see reference 16a.

(24) Each set of olefin isomers proved inseparable on silica gel. The ratio of olefin isomers was estimated by the relative integration of the olefin peaks in the ^1H NMR spectra.

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(34) Such H-bonding patterns with a mixed $3_{10}/\alpha$-helical turn are often found at the N-termini of α-helical peptides. See reference 31.

(35) Wuthrich, K. *NMR of Proteins and Nucleic Acids*, Wiley-Interscience: New York, 1986.

(36) O'Neil, K. T.; DeGrado, W. F. *Science* **1990**, *250*, 646-651.

(37) Blackwell, H. E.; Sampson, J.; Steinmetz, W. E.; O'Leary, D. J.; Grubbs, R. H., **1999**, unpublished results.

(38) Colorless crystals of **8** were grown from CH_2Cl_2/hexane; crystal size 0.3 · 0.5 · 0.5 mm, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 19.30(1)$, $b = 24.73(2)$, and $c = 12.134(7)$ Å, $V = 5791(6)$ Å^3, $\rho_{calcd} = 1.246$ g cm^{-3}; $2\theta = 1.6 - 50.0$ ° ($M_{K\alpha}$, $\lambda = 0.7107$ Å, graphite monochromator, 2θ-θ scan, $T = 156$ K); 4698 reflections measured, 2266 unique data used [$I>3.0\sigma(I)$]; no absorption corrections were applied; direct methods (SHELXTL 86), refinement with Full-Matrix Least-Squares, hydrogen atoms refined isotropically, all other atoms refined anisotropically; $R = 0.066$, $R_W = 0.077$; maximum residual electron density 0.80 e/ Å^3; *GOF* = 2.28 for 633 variables. Crystallographic data (excluding structure factors) for structure **8** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101810. A full copy of the crystallographic data for structure **8** (excluding structure factors) can be found in the Appendix accompanying this thesis.

(39) For standard definitions of the torsion angles for rotations about bonds of peptide backbones (ϕ, ψ, and ω) and amino acid side chains (χ^n), see: IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **1970**, *9*, 3471-3479.

(40) Residues at the ends of peptide helices are often irregular in conformation. This effect is more pronounced at the C-terminus. See: Chothia, C. *Ann. Rev. Biochem.* **1984**, *53*, 537-572.

(41) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350-353.

(42) The 4→1 N···O=C angles for these four hydrogen bonds range from 118 to 131°.

(43) The analogous hydrogen bonding pattern in α-helices is 5→1, spanning three residues. See reference 40.

(44) All peptide helix axes are parallel in the unit cell of **8**. Head-to-tail hydrogen bonding is commonly observed in crystalline hydrophobic peptide helices. See reference 17.

(45) The different medium from which **2** and **8** were crystallized (CH_2Cl_2/hexane for **8**; methanol/H_2O for **2**) may contribute to the disparity in their crystal structures.

(46) (a) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C.; Monaco, V.; Goulard, C.; Rebuffat, S.; Bodo, B. *J. Am. Chem. Soc.* **1996**, *118*, 4952-4958. (b) Toniolo, C.; Peggion, C.; Crisma, M.; Formaggio, F.; Shui, X. Q.; Eggleston, D. S. *Nature Struct. Biol.* **1994**, *12*, 908-914. (c) Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 7951-7954.

(47) Lynn, D. M.; Mohr, B.; Grubbs, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 1627-1628, and references therein.

(48) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(49) CH_2Cl_2 was purified by passage through a solvent column composed of activated alumina (A-2) and supported copper redox catalyst (Q-5 reactant). See: Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

Chapter 5

New Approaches Toward Selective Olefin Cross-Metathesis[†]

Abstract

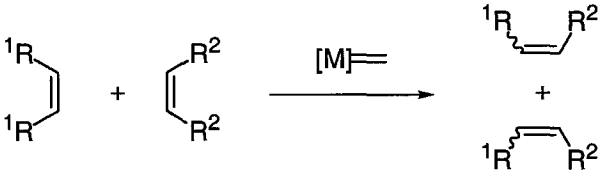
The advent of well-defined ruthenium (**1**) and molybdenum (**2**) metathesis catalysts has generated renewed interest in developing methods for the selective cross-metathesis (CM) of terminal olefins. This chapter describes new methodology for the selective CM of terminal olefins. Our initial approach was inspired by the synthesis of telechelic polymers *via* tandem ROMP coupled with the CM of acyclic disubstituted internal olefins. To probe the viability of this approach for applications in organic synthesis, we have explored the homologation of unhindered terminal alkenes *via* CM with symmetrically disubstituted olefins. Treatment of a terminal olefin such 9-decen-1-yl benzoate (**3**) with 1-2 equivalents of a symmetric internal olefin and 5 mol % **1** provides the desired CM products in good yields. The isolated mixture of olefin regioisomers was predominantly *trans*, and methods for increasing the *trans* selectivity were developed. Due to the limited availability of symmetrical disubstituted olefins, we employed CM in an initial self-metathesis step to synthesize a variety of disubstituted olefins with diverse functionality. These homodimers were processed further by CM with terminal olefins to generate a structurally-diverse pool of heterodimeric products in good yields. In the course of our studies, a new CM application was discovered involving the metathesis of acrolein acetal derivatives with terminal olefins. Acrolein acetals proved to be exceptionally robust substrates for CM and generated predominantly *trans* cross-products. Finally, the general implications of these new CM methodologies on the future of chemo- and regioselective CM will be discussed.

Introduction

Olefin Metathesis

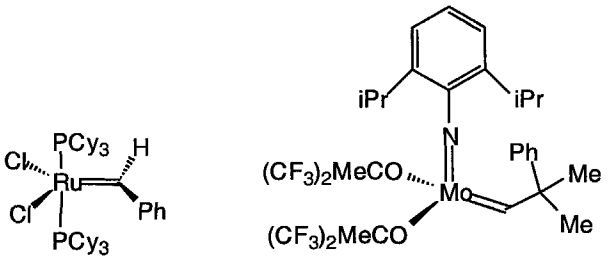
Carbon-carbon bond forming reactions are among the most important reactions in organic synthesis. One particularly interesting carbon-carbon forming reaction is olefin metathesis, which is the metal-catalyzed exchange of alkylidene moieties between alkenes (Eq 1).[1]

Olefin Metathesis:



(Eq 1)

Historically, olefin metathesis has been studied both from a mechanistic standpoint[2] and in the context of polymer synthesis (specifically, in ring opening metathesis polymerization, or ROMP).[3] In contrast, the application of olefin metathesis to the synthesis of complex organic molecules and natural products was limited due to the incompatibility of ill-defined, "classical" catalysts with the diverse functionality encountered in organic synthesis.[1a] Recently, however, ring-closing olefin metathesis (RCM) of acyclic dienes has received a great amount of attention as a highly efficient methodology for the synthesis of functionally diverse carbocycles and heterocycles.[4] This is primarily due to the development of well-defined, transition metal catalysts over the past decade. The two olefin metathesis catalysts that have seen the most use to date are ruthenium benzylidene **1** developed by Grubbs *et al.*,[5] and the molybdenum alkylidene **2** developed by Schrock *et al.*[6] The relatively high activities and functional group tolerance of both catalysts **1** and **2**, coupled with their commercial availability, has dramatically increased their application in organic synthesis.

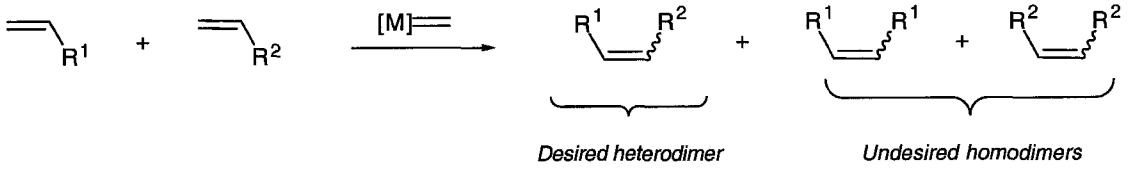


1 **2**

Olefin Cross-Metathesis

The volume of work reported in the areas of RCM, ROMP, and novel combinations thereof has dramatically overshadowed that reported for olefin cross-metathesis (CM). This unique method for the intermolecular formation of carbon-carbon double bonds has not yet found widespread application in organic synthesis because general reaction conditions that give high product and *trans/cis* selectivity have not been developed. The simplified CM reaction between two terminal olefins is depicted in Eq 2.

Terminal Olefin Cross-Metathesis: (Eq 2)

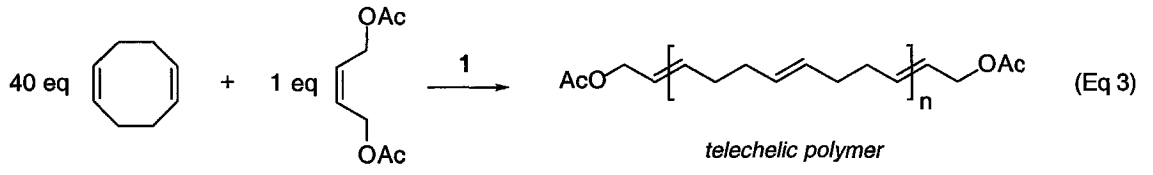


Generally, this reaction proceeds to yield three unique products: one desired heterodimeric product and two undesired homodimeric products, each as a mixture of olefin isomers. The majority of the work reported to date in the area of CM has focused upon terminal olefin substrates, because employing asymmetrically substituted internal olefins as starting materials can add further unwanted complexity to the final product mixture. A predominance of the early reports of CM employing "classical" catalysts[7] involved the synthesis of insect pheromone natural products: these compounds are frequently isolated from natural sources as a specific ratio of *cis* and *trans* isomers, so CM

proved to be a moderately effective route toward synthesizing these product mixtures.[8] However, for application to synthetic organic chemistry in general, some degree of control over *trans/cis* and product selectivity is essential.

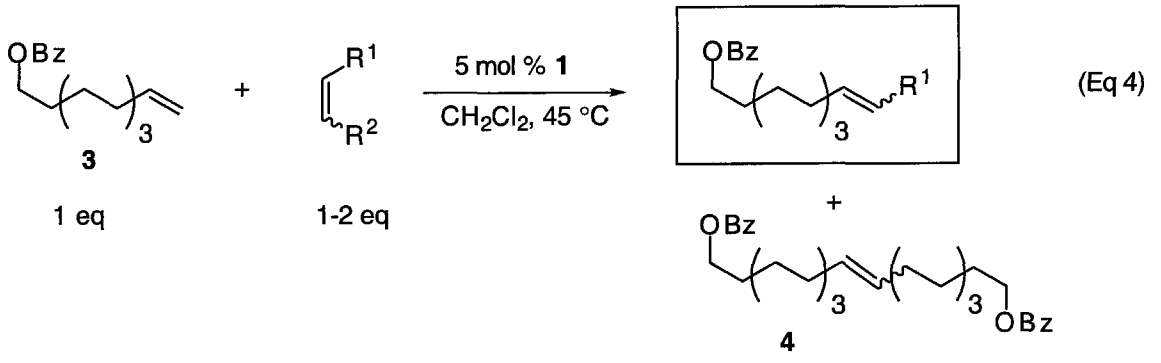
As described in Chapter 1, the recent development of well-defined ruthenium and molybdenum metathesis catalysts **1** and **2** has generated renewed interest in developing new methods for the selective CM of terminal olefins. Crowe *et al.* have demonstrated that π-substituted terminal olefins such as styrene[9] and acrylonitrile[10] can be used to efficiently functionalize terminal olefins employing molybdenum catalyst **2**. Crowe has also reported a useful terminal olefin cross-coupling procedure utilizing nucleophilic alkenes such as allyltrimethylsilane.[11,12] Recently, Blechert *et al.* have shown that certain sterically hindered terminal olefins do not undergo self-metathesis, and can be functionalized with a variety of commercially available terminal olefins using both catalysts **1** and **2**.[13,14] The novel ruthenium-catalyzed homologation of homo-allylglycine derivatives *via* CM has been reported by Gibson *et al.*[15] Efficient crossed yne-ene[16] and ring-opening cross-metathesis (ROM) reactions[17,18] using catalysts **1** and **2** have also been demonstrated. Finally, CM is being employed with increasing frequency in the synthesis of solution-phase combinatorial libraries of highly functionalized dimeric molecules.[19]

Outlined herein is a new method for the selective CM of unhindered terminal olefins. This approach was inspired in part by the synthesis of telechelic polymers[20] *via* tandem ROMP coupled with the CM of disubstituted internal olefins (Eq 3). Blechert *et al.* have previously employed this approach in organic synthesis in the ROM of strained cyclic olefins with symmetrically disubstituted olefins.[17b] To further probe the viability of this approach for applications in organic synthesis, we have explored the homologation of unhindered terminal alkenes *via* CM with functionally diverse, disubstituted internal olefins employing ruthenium carbene **1**.



Results and Discussion

We selected 9-decen-1-yl benzoate (**3**) as our model terminal olefin substrate because its low volatility and UV chromophore significantly aided synthetic manipulations. Treatment of **3** with 1-2 equivalents of a symmetric internal olefin and 5 mol % ruthenium benzylidene **1** in refluxing dichloromethane provided the desired cross-metathesis products in good yields (Eq 4). The CM reactions proceeded largely to completion over 12 hours, and any benzoate homodimer (**4**) side product generated (5-10%) could be easily recovered and recycled in a subsequent cross-metathesis step. In all of the cases examined thus far, the reaction has favored the formation of the *trans* olefin isomer. Higher *trans* selectivity was observed with *cis*-1,4-butenediol derivatives bearing bulky protecting groups.



Our initial efforts focused upon elaborating benzoate **3** to the corresponding allylic alcohol derivatives (Table 1).[21] The commercially available *cis*-2-butene-1,4-diol diacetate (entry 1) provided the homologated allylic acetate **5** in excellent yield (89%, 4.7:1 *E/Z*) using two equivalents of internal olefin in refluxing dichloromethane. When only one equivalent of diacetate was used, the yield of **5** decreased (77%) and no significant change in the *trans/cis* ratio was observed (entry 2). The use of two equivalents of diacetate was

found to be more efficient than simply using one, two, or four equivalents of allyl acetate (entries 3-5).[22] (An explanation for these increased yields employing disubstituted alkenes will be given below.) Employing the diol acetate as solvent (55 equiv., 45 °C, 12 hr) increased the isolated yield of **5** to 91%, although with diminished *trans* olefin content (3:1 *E/Z*). In contrast, the use of neat allyl acetate provided only a marginal amount (10%) of the desired cross-product (data not shown), presumably due to the statistically favored solvent dimerization of allyl acetate by **1** which dominated the catalytic cycle. The diol trifluoroacetate (entry 6) afforded a reduced yield of the homologated allylic trifluoroacetate **6** (63%, 2.8:1 *E/Z*), with an *E/Z* ratio approximating that of the allylic acetate **5**.

Table 1. CM reactions with symmetrically disubstituted olefins.

Entry	Substrate	Equiv.	Product (%)[a]	*E/Z*[b]
1	$R_1 = R_2 = CH_2OAc$ (*cis*)	2	**5**: 89	4.7:1
2	$R_1 = R_2 = CH_2OAc$ (*cis*)	1	**5**: 77	5:1
3	$R_1 = CH_2OAc$, $R_2 = H$	4	**5**: 81	3:1
4	$R_1 = CH_2OAc$, $R_2 = H$	2	**5**: 80	4:1
5	$R_1 = CH_2OAc$, $R_2 = H$	1	**5**: 59	5.7:1
6	$R_1 = R_2 = CH_2OC(O)CF_3$ (*cis*)	4	**6**: 63[c]	2.8:1
7	$R_1 = R_2 = CH_2OH$ (*cis*)	2	**7**: 56[d]	5:1
8	$R_1 = R_2 = CH_2OtBu$ (*cis*)	2	**8**: 90	7:1
9	$R_1 = R_2 = CH_2O$trityl (*cis*)	2	**9**: 75[e]	8:1
10	$R_1 = R_2 = CH_2OCH_2Ph$ (*cis*)	2	**10**: 71[f]	9:1
11	$R_1 = R_2 = CH_2OTBS$ (*cis*)	2	**12**: 77[g]	10:1
12	$R_1 = R_2 = CH_2CH_2CH_3$ (*cis*)	2	**13**: 72	3:1
13	$R_1 = R_2 = CH_2NHBoc$ (*cis*)	4	**14**: 71	3:1
14	$R_1 = R_2 = CH_2C(O)OMe$ (*trans*)	2	**15**: 74	3.3:1
15	$R_1 = R_2 = CH_2C(O)NMe(OMe)$ (*trans*)	4	**16**: 17	1.9:1

[a]Isolated product yields. [b]Determined by ^1H NMR integration. [c]Yield determined after NEt_3 deprotection of the allyl trifluoroacetate ether (to afford allylic alcohol **7**). [d]Reaction run at room temperature. [e]Yield determined after the formic acid deprotection of the allyl trityl ether to afford **7**. [f]Yield determined after H_2/Pd-C hydrogenation-hydrogenolysis of allyl benzyl ether (to afford alcohol **11**). [g]Yield determined after TBAF deprotection of allyl TBS ether to afford **7**.

Direct reaction of benzoate **3** with 1,4-butenediol (entry 7, Table 1) did occur in dichloromethane at room temperature to yield allylic alcohol **7** (54%, 5:1 *E/Z*), in spite of the limited solubility of the diol. Elevating the temperature lead to apparent decomposition of catalyst **1**. No improvement in the isolated yield of **7** was observed when the reaction was conducted as a homogenous mixture in chloroform. Several diether derivatives of *cis*-1,4-butenediol (entries 8-11) were found to provide better CM yields and improved *trans* selectivity. The *trans* selectivity was observed to gradually increase as the ether protecting groups became sterically larger. For example, on going from the bis-*tert*-butyl (entry 8) to the bis-TBS protected diol (entry 11), the *E/Z* ratio increased from 7:1 for cross product **8** to 10:1 for **12**. While no attempts were made to separate the olefin regioisomers in the present study, the increased *trans* selectivity observed in the CM of benzoate **4** with the bis-TBS diol now represents a synthetically useful protocol for the direct installation of allylic alcohol functionality.

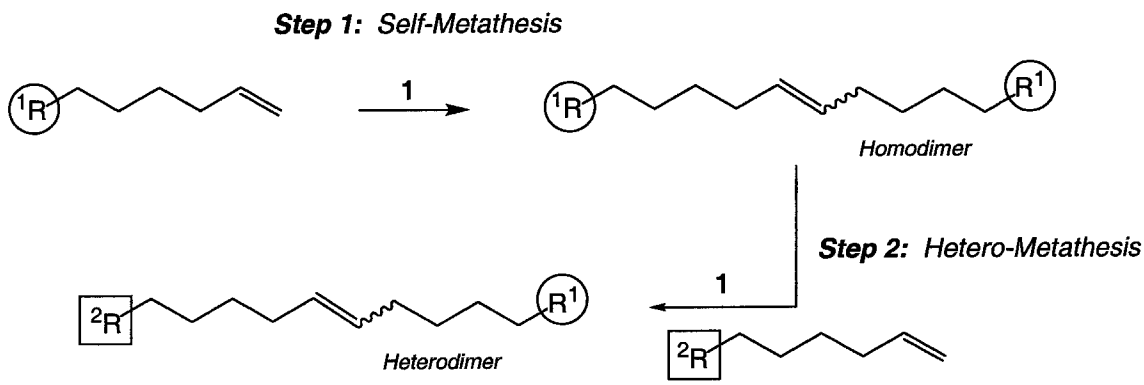
Purely aliphatic functionality could be readily incorporated employing this CM methodology: for example, the CM of *cis*-3-hexene with **4** (entry 12) yielded the ethyl functionalized internal olefin cross-product **13** in good yield (72%, 3:1 *E/Z*). The compatibility of nitrogen-containing substrates was next probed through the CM of Boc-protected *cis*-1,4-diaminobutene (entry 13). Chapters 2-4 of this thesis have described the compatibility of ruthenium catalyst **1** with amide functionality. Boc-protected allylic amine **12** was isolated in good yield (71%, 3:1 *E/Z*), which demonstrates that CM is a straightforward route to the introduction of nitrogen functionality.

All of the CM reactions discussed up this point have involved *cis* disubstituted internal olefins. We chose to employ *cis* olefins from the outset because it had been observed that ruthenium catalyst **1** is more reactive toward the more sterically accessible *cis* olefin.[23] However, *trans* disubstituted internal olefins were also found to be reactive coupling partners for CM with terminal olefins.[24] Dimethyl *trans*-3-hexene-1,6-dioate (entry 14) provided the desired homoallylic ester cross product (**15**) as the major product

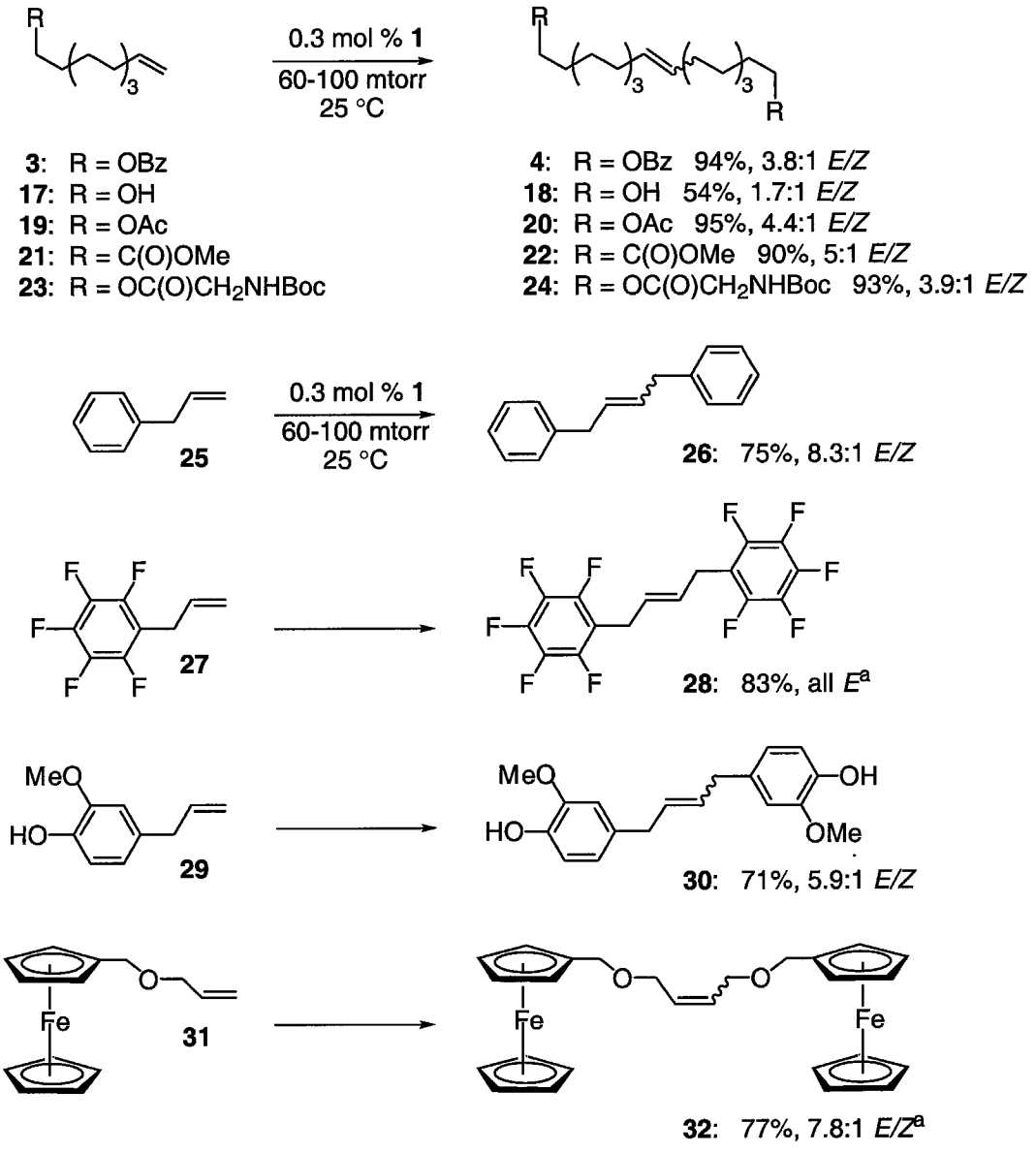
(74%, 3:1 *E/Z*; recovered homodimer **4**: 23%). However, in an attempt to introduce Weinreb amide[25] functionality through CM, we found that *trans*-1,4-bis-[methyl(methoxy)amido]but-2-ene (entry 15) was a poor substrate for CM, affording **16** in only 17 % yield and with poor *trans* selectivity (1.9:1 *E/Z*). As substantial homodimeric cross-product **4** was not generated, we speculate that the coordination of the amide to the catalyst rather than the *trans* geometry of the double bond could be a determining factor.[26]

A Two-Step Procedure for Terminal Olefin Cross-Metathesis

These initial results suggested that *cis* or *trans* disubstituted olefins could be employed as efficient coupling partners in CM reactions. Accordingly, we have investigated the use of a two-step procedure[27] for terminal olefin CM outlined in Scheme 1. First, a terminal olefin was self-metathesized by treatment with catalyst **1**. The mixture of olefin isomers generated was then subjected to CM with another terminal olefin employing the methodology described above. The synthesis of a large pool of functionally diverse, homodimeric internal olefins *via* this first self-metathesis procedure is shown in Scheme 2.[28]



Scheme 1. Two-step procedure for the CM of disubstituted olefins.



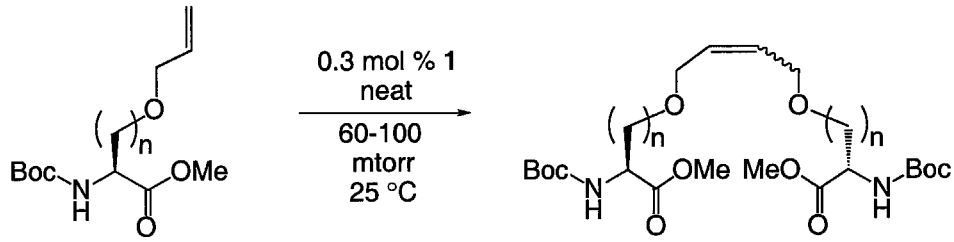
Scheme 2. Synthesis of homodimeric internal olefins *via* CM employing catalyst **1**. *E/Z* ratios determined by ^{1}H and ^{13}C NMR analyses.[29] [a]Homodimers synthesized in solution (0.1 M, 5 mol % **1**, 45 °C).

For almost all of the terminal olefin substrates studied, homodimerization with 0.3 mol % **1** *in vacuo* (25 °C, 24 h) provided predominantly *trans* disubstituted olefins in good to excellent yields (Scheme 2). The solvent-free conditions, low catalyst loading, and high yields make homodimerization *via* CM employing ruthenium alkylidene **1** an exceptional methodology for the synthesis of high molecular weight, symmetrical disubstituted olefins. Furthermore, a majority of the homodimeric products were crystalline solids, which expedited their purification from catalyst **1**.

Performing CM under vacuum has the benefit of removing the stoichiometric gaseous by-product of the reaction, ethylene, and therefore pushes the CM reaction toward completion. This solvent-free method requires, however, that the starting material terminal alkene be of low volatility; this requirement precluded the neat homodimerization of pentafluoroallyl benzene (**27**) and 1-ferrocene methanol (*O*)-allyl ether (**31**). The latter two compounds were efficiently homodimerized, however, employing the standard solution phase CM conditions introduced above (0.1 M, 5 mol % **1**, 45 °C, *ca.* 12 h). CM of the protected derivatives of 9-decen-1-ol (**3**, **19**, **21**, and **23**) afforded the corresponding homodimers (**4**, **20**, **22**, and **24**) in excellent yields. In contrast, homodimerization of neat, unprotected 9-decen-1-ol (**17**) employing **1** generated only a modest yield of diol **18** with low *trans* selectivity. This result is indicative of alcohol **17** potentially sequestering catalyst **1** by chelation, and effectively shutting down the catalytic cycle before a thermodynamic *trans/cis* ratio of products was achiveved.[29] Aromatic and organometallic homodimeric products (**26**, **28**, **30**, and **32**) could be prepared in good yields *via* CM (Scheme 2).[30] Notably, upon treatment with **1**, the more electron withdrawing pentafluoroallyl benzene **27** generated only one detectable olefin regioisomer (by ^{1}H NMR); reasons for this *trans* selectivity remain to be found.

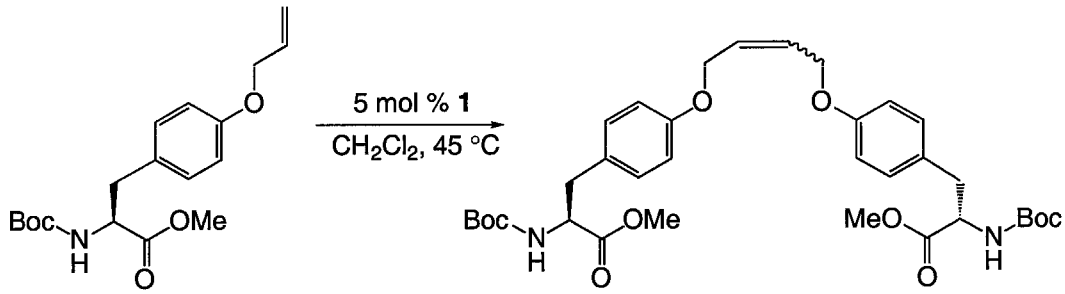
Due to the on-going interest in our laboratory of employing olefin metathesis in the context of peptide and carbohydrate synthesis, we next turned our attention to the synthesis of a series of novel amino acid, carbohydrate, and peptide homodimers by CM (Schemes 3

and 4). These experiments further confirmed the exceptional functional group tolerance of ruthenium alkylidene **1**. Terminal olefin functionality can be readily installed into amino acid side chains by the incorporation of allyl ethers. (*O*)-allyl ethers of protected L-serine (**33**), L-homoserine (**35**), and L-tyrosine (**37**) derivatives were straightforward to prepare (Scheme 3), and upon treatment with catalyst **1** afforded good yields of their respective homodimers with moderate *trans* selectivity (*ca.* 3:1 *E/Z*).[21,31] As amino acid derivatives **33** and **35** were low viscosity oils, self-metathesis was performed *in vacuo* as described above (Scheme 2); the high viscosity of protected tyrosine derivative **37** required for the CM reaction to be performed in solvent for optimal yield of homodimer **38** (71%). While side chain-bridged amino acids **34**, **36**, and **38** could be generated *via* CM, treating Boc-L-allylglycine-OMe under the analogous reaction conditions (*in vacuo* or in CH_2Cl_2) yielded less than 5% of the respective homodimer (data not shown); this data corroborates well with the observations of Gibson *et al.* that longer terminal olefin side chains are required for efficient CM.[15] Finally, in extending CM methodology to carbohydrate substrates, we observed the crystalline 2,3,4,6-tetra-*O*-benzyl-1-α-*C*-allylglucoside[21] **39** to undergo facile CM in solution, affording the novel α,α-linked dimer **40** in high yield (93%, 4:1 *E/Z*).[32]



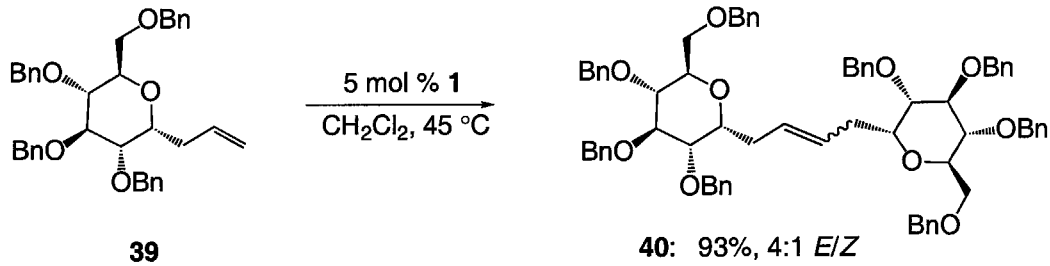
33: n = 1 Boc-L-Ser(O-Allyl)-OMe
35: n = 2 Boc-L-Hse(O-Allyl)-OMe

34: n = 1 73%, 2.6:1 *E/Z*
36: n = 2 75%, 2.7:1 *E/Z*



37: Boc-L-Tyr(O-Allyl)-OMe

38: 71%, 3:1 *E/Z*

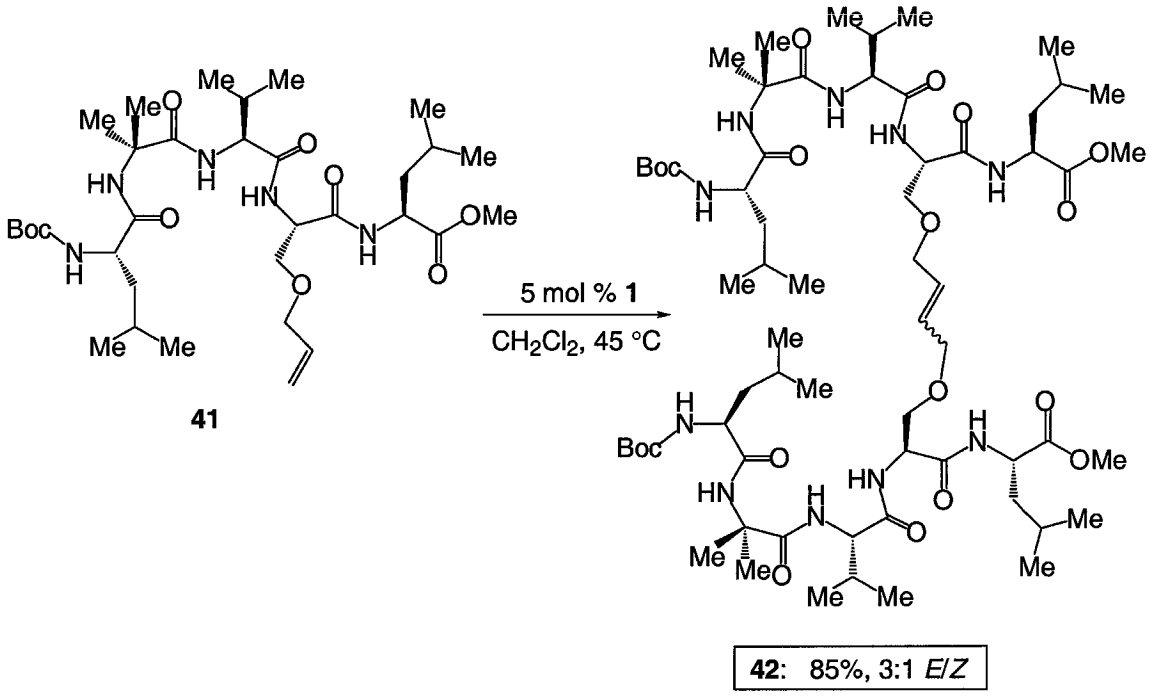


39

40: 93%, 4:1 *E/Z*

Scheme 3. Synthesis of amino acid and carbohydrate homodimers *via* CM employing **1**. *E/Z* ratios determined by ^{1}H and ^{13}C NMR analyses.[29]

In an attempt to probe the general applicability of olefin self-metathesis for the generation of more complex molecular architectures, we introduced terminal olefin functionality into a hydrophobic pentapeptide framework (**41**) through incorporation of L-serine (*O*)-allyl ether.[21] Treatment of pentapeptide alkene **41** with ruthenium alkylidene **1** under standard solution phase CM conditions generated the side chain-bridged homodimer **42** in good yield. Interestingly, the *trans/cis* selectivity appeared to approximate that for the CM of the free amino acid (**35**). The facile synthesis of **42** demonstrates the utility of CM methodology for the synthesis of unique, acyclic peptidic architectures containing non-native C-C linkages; cyclic peptide olefin counterparts have been previously generated employing the intramolecular metathesis variant, RCM.[33]



Scheme 4. CM of pentapeptide **41** to afford side chain linked peptide homodimer **42**. *E/Z* ratio determined by ^{1}H and ^{13}C NMR analyses.[29]

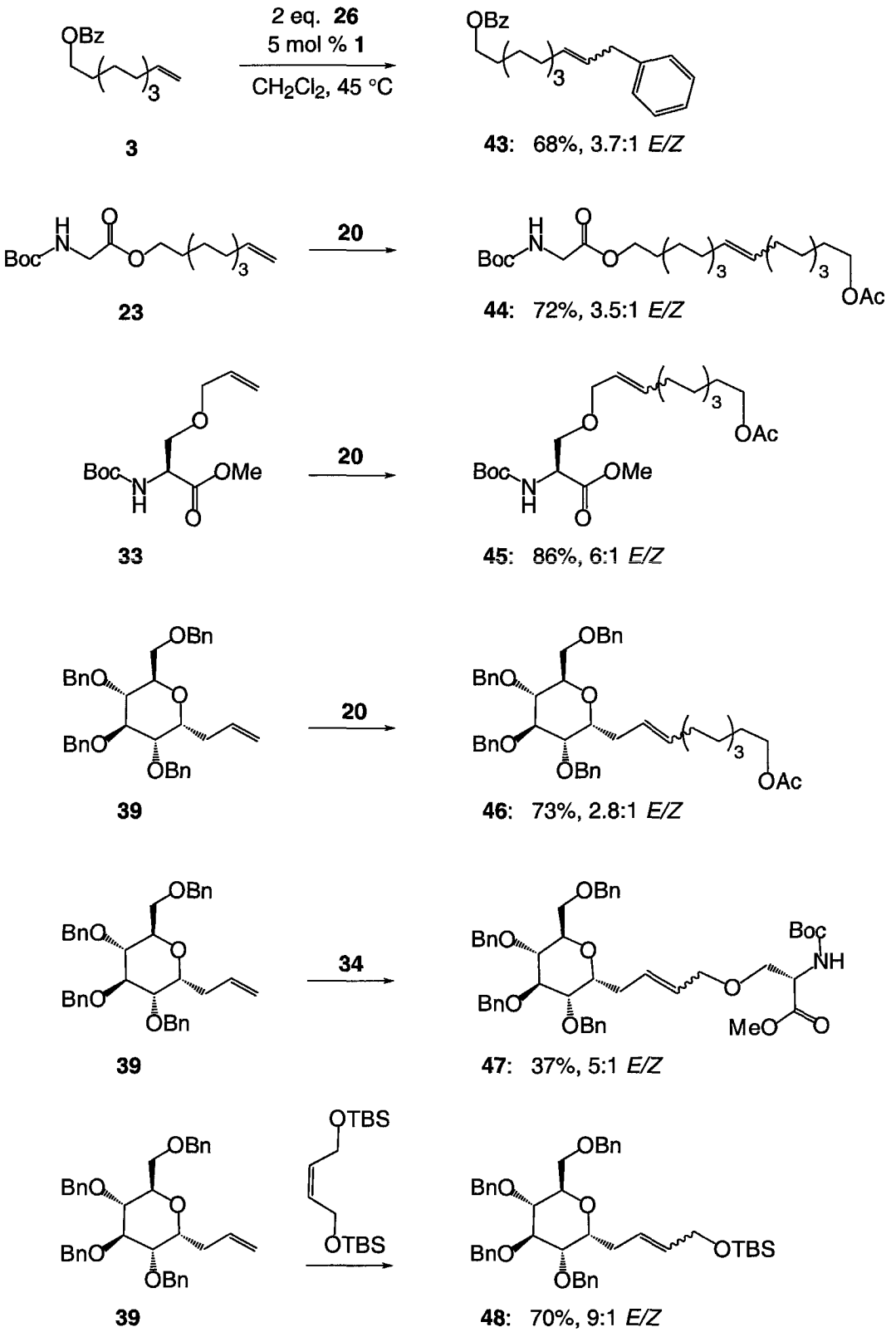
Finally, in order to demonstrate the efficacy of our two-step CM protocol, selected symmetrical disubstituted olefins prepared by self-metathesis (Scheme 2-4) were further processed in the CM with terminal olefins. We selected internal and terminal olefin

substrates which exhibited varied functionalities and sterics to explore the scope and limitations of this two-step procedure (and selective CM overall). Accordingly, many of the substrates were based upon the structurally diverse amino acid, carbohydrate, and peptide structures shown in Schemes 3 and 4.[34] Furthermore, many of the terminal olefins in Schemes 3 and 4 were utilized as substrates for CM, either with self-metathesized homodimers or simple *cis*-1,4-butenediol substrates from Table 1. Representative examples of this second CM processing step are shown in Schemes 5 and 6. Overall, the heterodimeric products were formed selectively and with moderate to good *trans* selectivity. Standard solution phase CM conditions were employed throughout (0.1M in terminal olefin, 2 equiv. disubstituted olefin, CH_2Cl_2, 5 mol % **1**, 45 °C), and the reactions were generally complete in 12 hours.

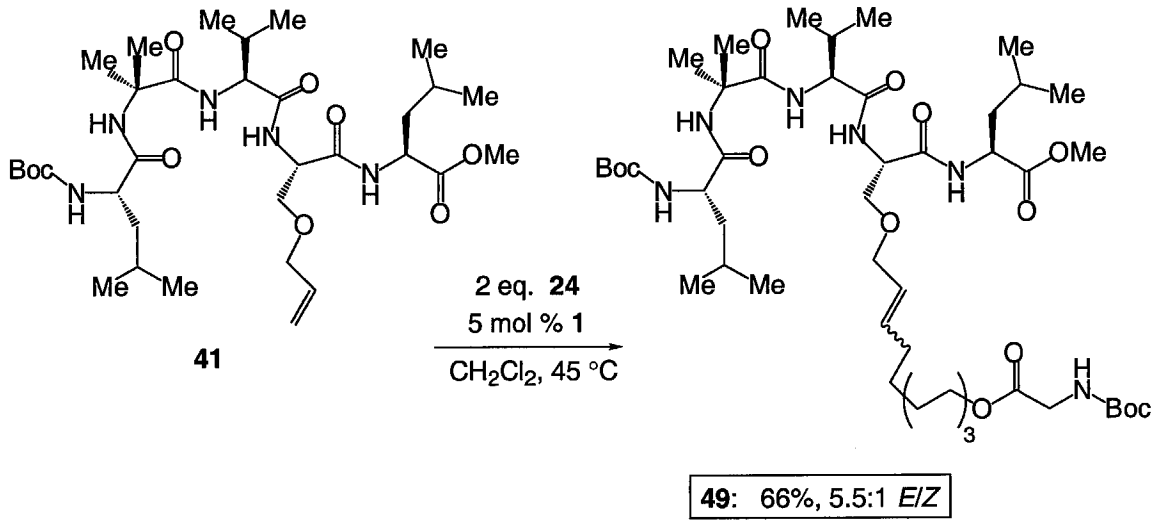
Allyl benzene homodimer **26** was reacted with our standard 9-decen-1-yl benzoate **3** to yield the benzyl-functionalized internal olefin **43** in good yield (68%, 3.7:1 *E/Z*). The corresponding 9-decen-1-yl Boc-glycinate **23** was observed to react with 9-decen-1-yl acetate homodimer **20** to afford the differentially functionalized 9-eicosene (**44**) in moderate yield (72%, 3.5:1 *E/Z*). Treatment of Boc-L-serine(*O*)-allyl ether-OMe (**33**) with bis-acetate **20** generated lipophilic amino acid derivative **45** in high yield with improved *trans* selectivity (86%, 6:1 *E/Z*). The related lipophilic sugar **46** could be prepared in similar fashion through the CM of *C*-allylglucoside **39** with bis-acetate **20**; however, the *trans* selectivity was notably reduced (73%, 2.8:1 *E/Z*). This result, coupled with the high yield and low *trans/cis* ratio of sugar homodimer **40**, suggested that the sterics of tetrabenzyl *C*-allylglucoside **39** do not impede its CM reactivity or influence the *trans* selectivity of the products; this heightened reactivity is in contrast to a jasmonic acid derivative sterically similar to **39** which was reported by Blechert *et al.* not to undergo self-metathesis.[13] Indeed, while the CM of glucoside **39** with the more bulky Boc-L-serine(*O*)-allyl ether-OMe homodimer **34** generated a low yield of the amino acid/sugar heterodimer **47** (37%, 5:1 E/Z), a higher *trans* selectivity was observed, indicating that the sterics of the

internal olefin component most likely effects the outcome of CM. Further evidence for this theory was obtained in the CM of *cis*-1,4-butene diol bis-OTBS with glucoside **39**, the former bulky bis-ether being the most *trans* selective coupling partner studied up to this point (Table 1, entry 11). In direct analogy to benzoate ether **12**, silyl ether derivatized sugar **48** was generated in good yield with pronounced *trans* selectivity (70%, 9:1 *E/Z*), strongly suggesting the *trans/cis* ratios are dependent on the structure of the internal olefin *for these terminal olefin substrates.* Unfortunately, in contrast to the good yield of ether **48**, all efforts to improve the yield of the structurally interesting amino acid/sugar heterodimer **47** failed; the efficient synthesis of carbon-carbon linked glycosyl amino acids and glycopeptides *via* olefin metathesis remains a challenging goal.[35]

To study the scope of CM in the functionalization of more complex substrates, we chose to investigate the CM of pentapeptide **41** with disubstituted internal olefins (Scheme 6). Treatment of **41** with 9-decen-1-yl Boc-glycinate dimer **24** under standard solution phase CM conditions afforded the glycinate functionalized peptide **49** in moderate yield with modest *trans* selectivity (66%, 5.5:1 *E/Z*). This reaction further demonstrated the general applicability of CM as mild methodology for the introduction of diverse functionality tethered through a carbon-carbon bond. In summary, it appeared that our two-step procedure for the CM of selected internal olefins was not only effective, but also, in view of the wide variety of substrates studied, substantially broad in scope.



Scheme 5. Synthesis of novel heterodimers *via* CM of terminal olefins and an excess of internal olefin. *E/Z* ratios determined by ^{1}H and ^{13}C NMR analyses.

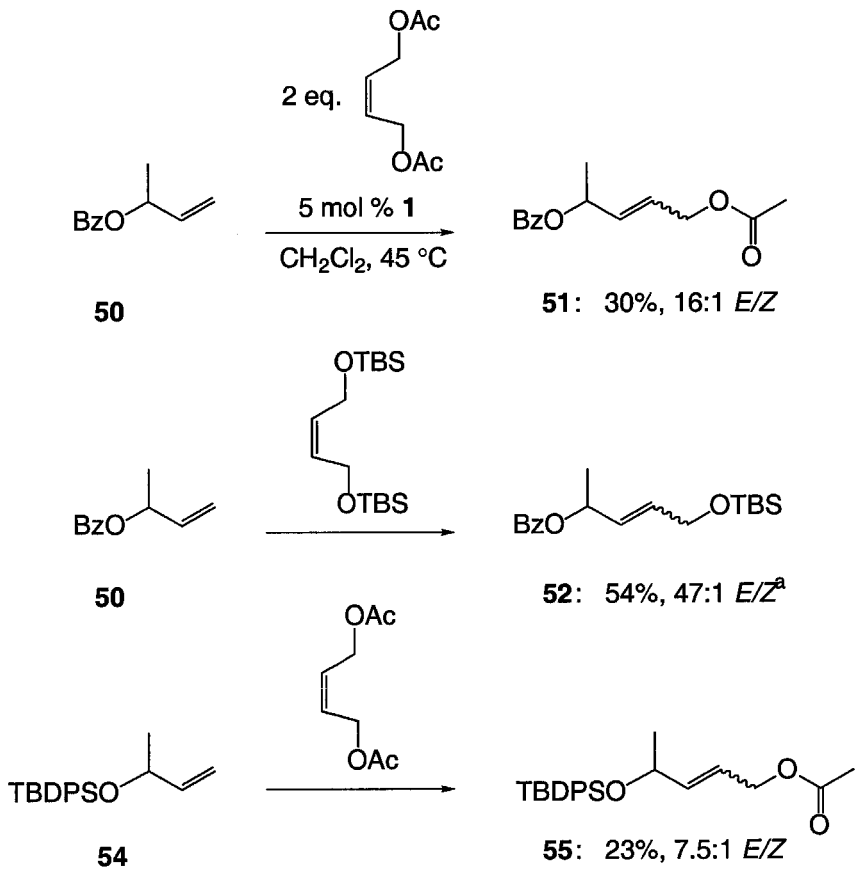


Scheme 6. Synthesis of glycinate functionalized pentapeptide **49** *via* CM. *E/Z* ratios determined by ^{1}H and ^{13}C NMR analyses.

Cross-Metathesis of Terminal Olefins with Allylic Methyl Substituents

To further study the influence of sterics on the *trans/cis* selectivity of CM employing catalyst **1**, a series of CM reactions was conducted on terminal olefins with allylic methyl substituents (Scheme 7).[36] At the outset of our studies, it was anticipated that allylic substitution would introduce steric hindrance close to the olefin group, and potentially direct CM toward the less sterically hindered *trans* product. This was consistently observed in the small group of substrates studied. The increased steric hindrance of the terminal olefin, however, was also observed to significantly reduce the CM yields. 3-Buten-2-yl benzoate (**50**) was selected as the initial model terminal olefin substrate. CM of **50** with two equivalents of *cis*-1,4-butenediol bis-acetate under standard solution phase conditions generated cross-product **51** with high *trans* selectivity, albeit in modest yield (30%, 16:1 *E/Z*). This result was in direct contrast to that observed for the CM of 9-decen-1-yl benzoate (**3**) and the bis-acetate (Table 1, entry 1), where the cross-product **5** was generated in considerably higher yield with lower *trans* selectivity (89%, 4.7:1 *E/Z*), indicating that allylic methyl substituents do direct *trans* selective CM. Notably, the *E/Z* ratio for heterodimer **51** was almost four times greater than that for **5**.

CM of allyl substituted benzoate **50** with the bulky *cis*-1,4-butenediol bis-OTBS gave similar results, affording the coupling product **52** in moderate yield with dramatic *trans* selectivity (54%, 47:1 *E/Z*). Again, the *E/Z* ratio for **52** was approximately four times greater than that observed for the CM of 9-decen-1-yl benzoate (**3**) with the bis-OTBS (compound **12**, 10:1*E/Z*) (Table 1, entry 11). The combination of the allylic methyl substitution on **50** and the bulky TBS protecting groups generated a very selective CM reaction. In attempting to discern how the steric bulk of the allylic substituted terminal olefin effected the regioselectivity of CM, we prepared the 3-buten-2-*O*-*tert*-butyldiphemylsilyl ether (**54**) and examined its reactivity in CM with substituted olefins. CM of **54** with the bis-acetate only generated a low yield of cross-product **55** with a reduced *trans* selectivity. This result suggested that the increased steric bulk of silyl ether **54** in comparison to benzoate **50** had reduced its reactivity with catalyst **1**; the concomitant loss of *trans* selectivity, however, indicated that steric bulk was not the only factor governing regioselective CM. Overall, the results suggested that improved CM *trans* selectivity can be achieved employing terminal olefins bearing allylic methyl group with simultaneous lowered yield of heterodimeric product. CM experiments with terminal olefins containing larger alkyl allylic substituents and cyclic substructures are currently being pursued; preliminary results have indicated that alkyl substituents larger than methyl effectively halt productive CM.

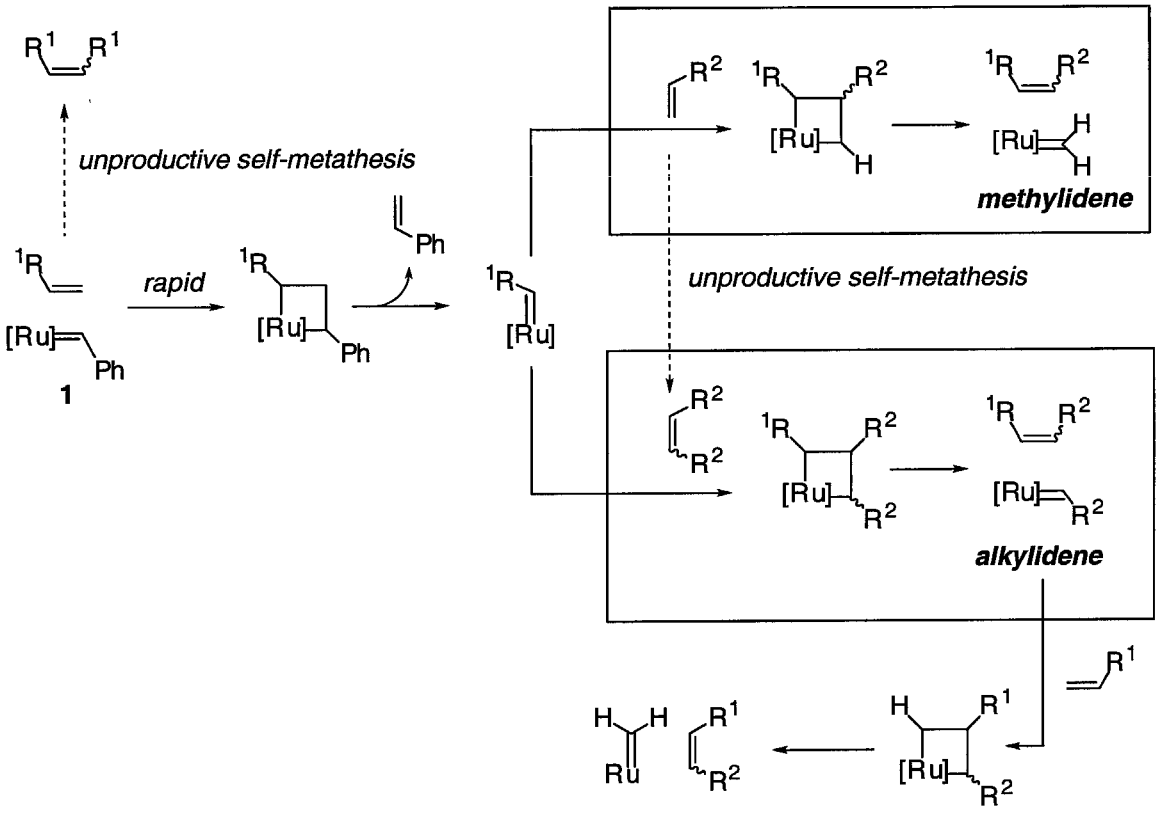


Scheme 7. CM of terminal olefins with allylic methyl substituents employing **1**. *E/Z* ratios determined by ^{1}H and ^{13}C NMR analyses. [a]Yield determined after TBAF deprotection of the TBS ether to afford allylic alcohol **53**.

Reactivity of Disubstituted Olefins versus Monosubstituted Olefins

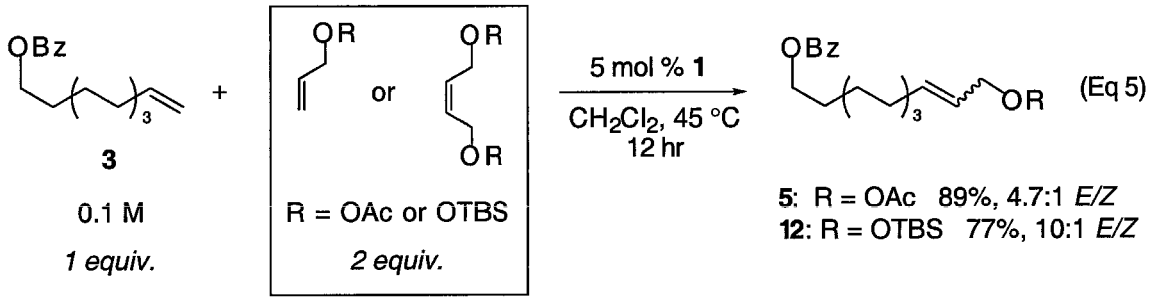
The results presented thus far demonstrate that both *cis* and *trans* disubstituted olefins are effective substrates for CM. Employing an excess of the disubstituted olefin relative to the terminal olefin component in CM was observed to lead to good yields of the desired heterodimeric product. In certain cases, we observed higher yields employing the disubstituted olefins instead of the monosubstituted counterpart. While using an excess of one olefin component should statistically push the reaction toward the heterodimeric product (if both olefins have comparable reactivities), we believed at the outset that employing an excess of the disubstituted olefin component in CM would statistically favor formation of an alkyl substituted ruthenium alkylidene over the unsubstituted ruthenium

methylidene. The simplified metathesis pathways leading to both methylidene and alkylidene formation from ruthenium benzylidene **1** are depicted below in Scheme 8. Because the methylidene formed from **1** had been shown to decompose considerably faster than other ruthenium alkylidene species,[23] we believed that preferential formation of an alkylidene species employing substituted olefins would extend the metathesis activity of **1** and potentially lead to higher yields of the desired heterodimeric product.



Scheme 8. CM pathways with mono- and disubstituted alkenes. The ligands on catalyst **1** have been omitted for clarity.

In order to probe this theory, we reanalyzed two previously described CM reactions (Table 1, entries 1 and 11) and compared the CM results employing a two-fold excess of either mono- or disubstituted olefins (Eq 5). We conducted four side-by-side CM reactions with benzoate **3**, 5 mol % **1**, and two equivalents of: 1) allyl acetate, 2) allyl OTBS, 3) *cis*-1,4,-butenediol bis-acetate, and 4) *cis*-1,4,-butenediol bis-OTBS. Monitoring the reactions by GC-MS analysis of reaction mixture aliquots gave qualitative evidence for the superiority of using disubstituted olefins in CM for these substrates (Figures 1-2).



Reaction profiles for the formation of heterodimeric CM products **5** and **12** and subsequent disappearance of benzoate starting material **3** are shown in Figure 1. For both the acetate (Figure 1(a-b)) and the OTBS (Figure 1(c-d)) series, CM with the disubstituted olefin afforded higher yields of the respective heterodimer product. In the case of the bis-acetate substrate, almost all of benzoate **3** is consumed in 2 hours, while consumption takes almost 6 hours for the allyl acetate reaction. For the silyl ether substrates, the benzoate starting material **3** was consumed considerably faster but the reactivity pattern was similar: **3** was consumed in under 15 minutes for the bis-OTBS reaction, while it took almost 2 hours for the reaction with allyl OTBS to come to completion.

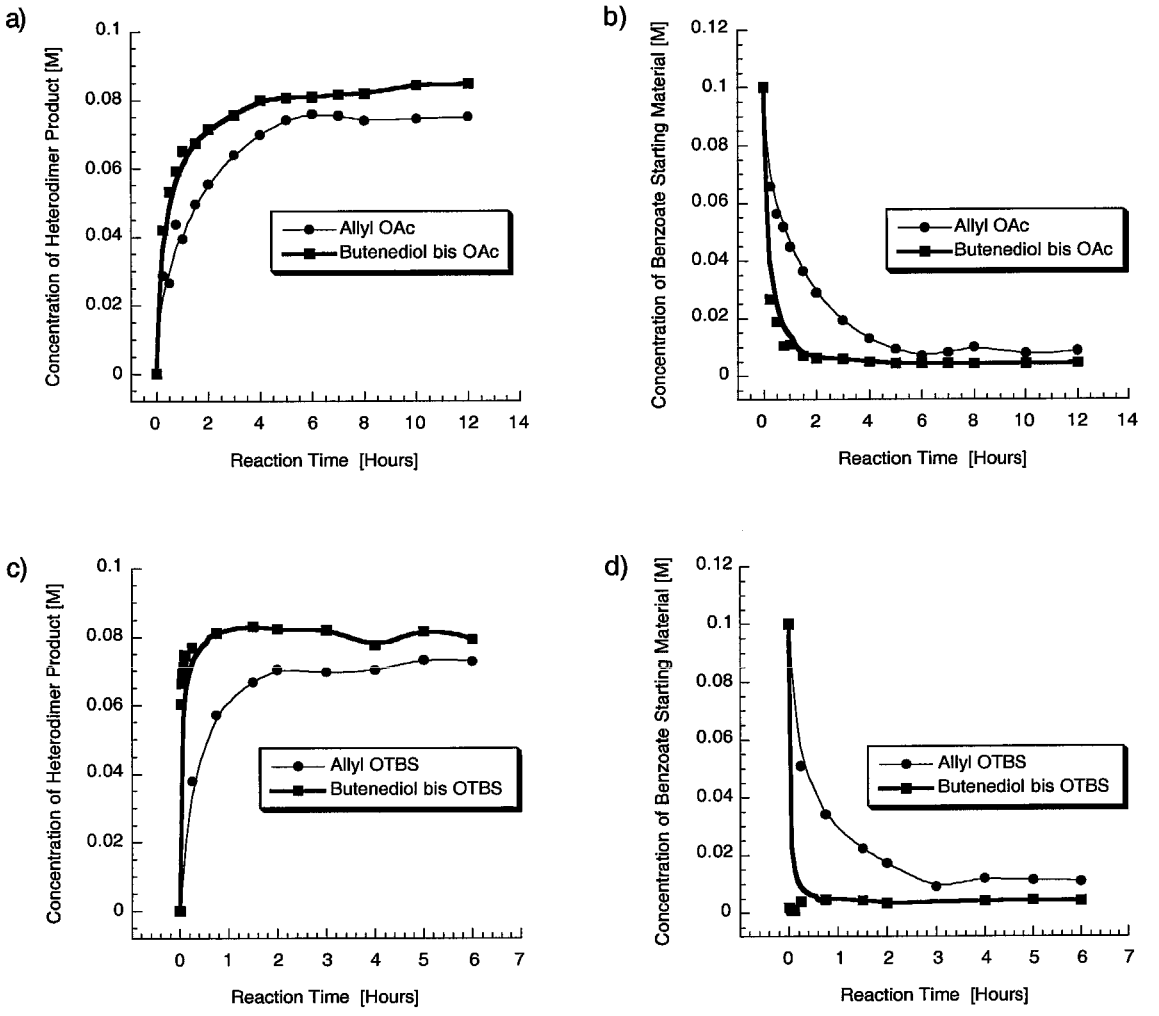


Figure 1. GC-MS reaction profiles for the CM of benzoate **3** with mono- and disubstituted olefins. a) Concentration of heterodimeric product **5** formed vs. reaction time in the CM of **3** and allyl acetate and *cis*-1,4,-butenediol bis-acetate. b) Disappearance of **3** vs. reaction time in the CM of **3** and allyl acetate and *cis*-1,4,-butenediol bis-acetate. c) Concentration of heterodimeric product **12** formed vs. reaction time in the CM of **3** and allyl OTBS and *cis*-1,4,-butenediol bis-OTBS. d) Disappearance of **3** vs. reaction time in the CM of **3** and allyl OTBS and *cis*-1,4,-butenediol bis-OTBS. Data obtained from GC-MS analysis of reaction aliquots (1,4-dichlorobenze as internal standard, data corrected for relative response).

In monitoring the formation of benzoate homodimer **4** for the OTBS reaction series (Figure 2a), a negligible amount of **4** was formed in the reaction with the bis-OTBS substrate, while the formation of homodimer **4** was occurring at a slow, but steady rate in the CM with allyl OTBS. This homodimer formation was consistent with the formation of heterodimer **12**: benzoate **3** was almost entirely consumed at the start of the CM reaction

with the bis-OTBS, while the formation of heterodimer **12** occurred at a more steady rate for the allyl OTBS, allowing benzoate **3** to competitively homodimerize. Analogous results for homodimer formation **4** in the CM of the acetate series were also obtained (data not shown). From these results, it appeared that secondary metathesis of the heterodimers **5** and **12** and homodimer **4** was not occurring at an appreciable rate. Interestingly, the *trans/cis* ratio of heterodimer **12** was observed to be higher throughout the course of the reaction with the bis-OTBS substrate relative to allyl OTBS (Figure 2b). A higher *trans/cis* ratio was also observed in the formation of acetate heterodimer **5**. While definitive reasons for the latter ratio were not realized, the qualitative GC-MS analysis of these four reactions strongly suggested that employing these disubstituted olefins in CM allowed more chemo- and regioselective CM relative to monosubstituted olefins.

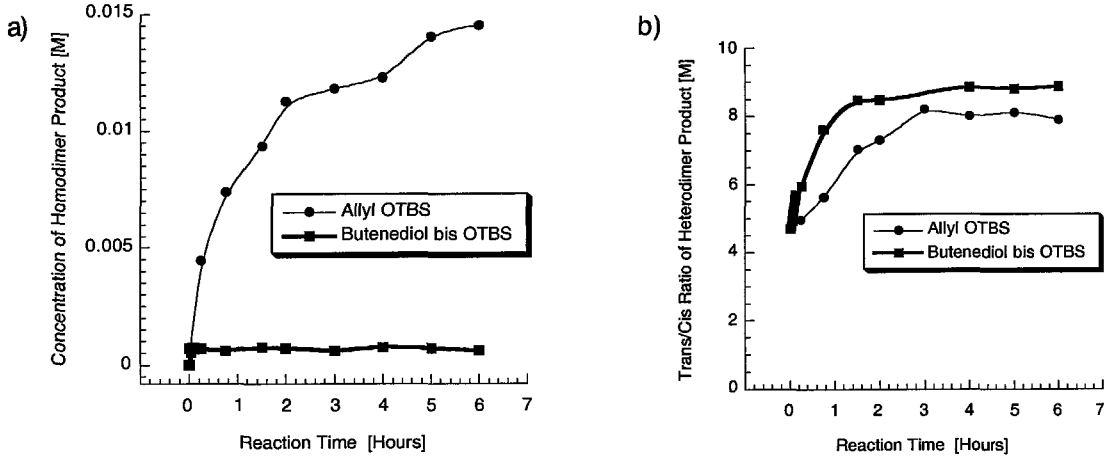
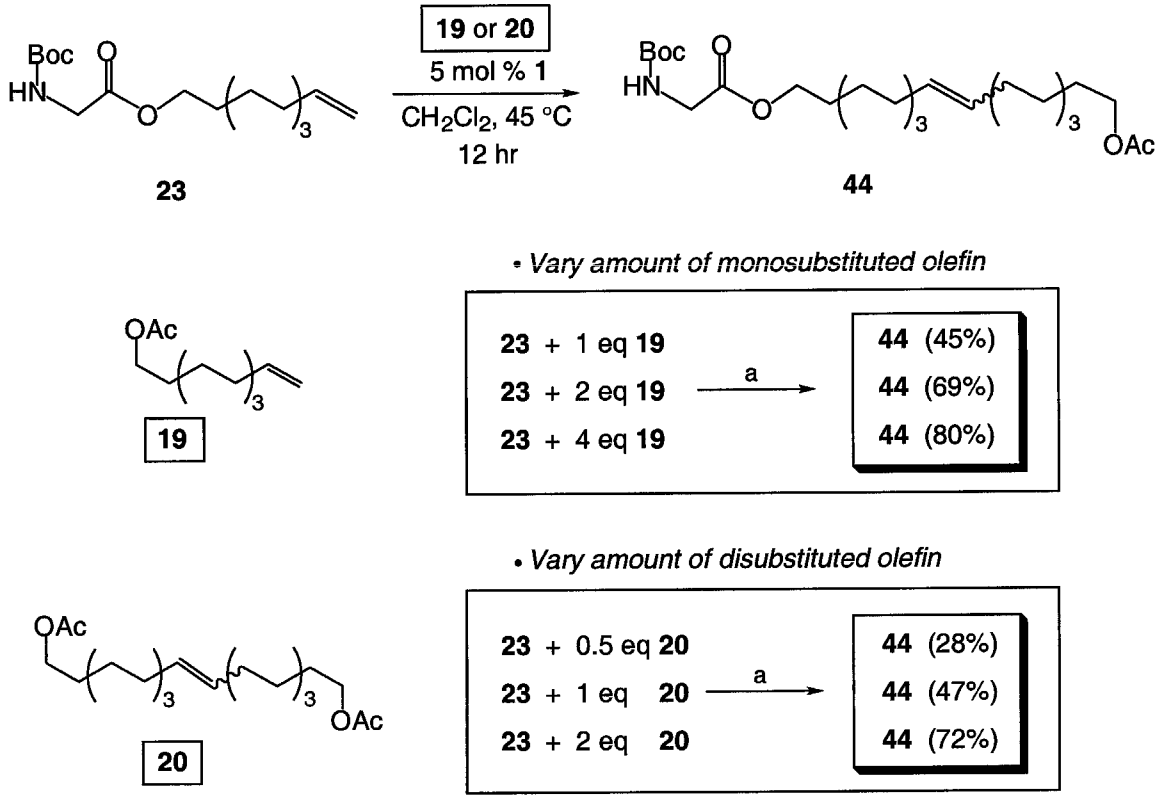


Figure 2. GC-MS reaction profiles for the CM of benzoate **3** with allyl OTBS and *cis*-1,4,-butenediol bis-OTBS. a) Concentration of homodimeric product **4** formed vs. reaction time in the CM of **3** and allyl OTBS and *cis*-1,4,-butenediol bis-OTBS. b) *Trans/cis* ratios of heterodimeric product **12** vs. reaction time in the CM of **3** and allyl OTBS and *cis*-1,4,-butenediol bis-OTBS. Data obtained from GC-MS analysis of reaction aliquots (1,4-dichlorobenze as internal standard, data corrected for relative response).

We discovered in our later studies with more complex substrates, however, that the benefit of employing disubstituted olefins in CM did not appear to be general. While a systematic study was not conducted, we observed that as the number of carbon units between the olefin and any functional or sterically bulky group was increased, CM with the monosubstituted olefin afforded competitive yields of the desired heterodimeric cross-

product. For example, cross coupling reactions using 9-decen-1-yl *N*-Boc glycinate (**23**) and various equivalents of 9-decen-1-yl acetate (**19**) or the internal olefin homodimer **20** demonstrated no benefit to employing the disubstituted olefin for CM instead of the corresponding terminal olefin (Scheme 9). Using 1 or 2 equivalents of olefin **19** or **20** afforded similar yields of heterodimer **44**. Furthermore, employing 0.5 equivalents of disubstituted olefin **20** was not analogous to 1 equivalent of monosubstituted olefin **19** (28% vs. 45% yield of heterodimer **44**), which indicated the lower overall reactivity of the disubstituted olefin. While these data did not invalidate our two-step CM procedure described above, it did suggest that, in the case of structurally "isolated" olefins, the first self-metathesis step was not essential. Finally, the comparable yields of **44** afforded employing equivalent amounts of either the mono- or disubstituted olefin (**19** or **20**) suggested that preferential formation of a ruthenium alkylidene over the methylidene may not be governing the reaction outcome in the CM of structurally "isolated" olefins.

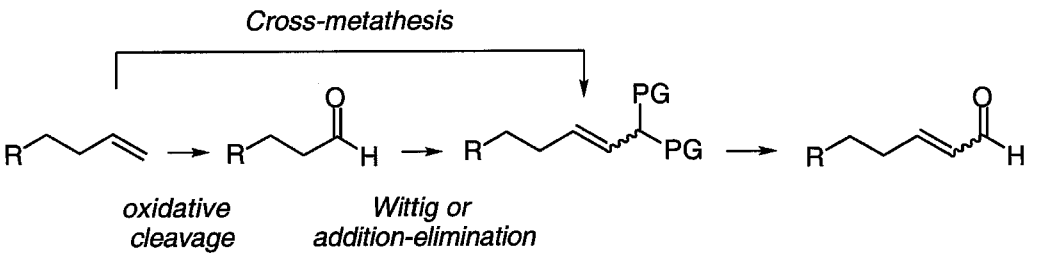
These results were in contrast to those observed in the GC-MS analysis of CM with protected allylic alcohols above, suggesting that functionality allylic to the olefin could be influencing metathesis. We speculate that the heightened heterodimer yields employing disubstituted olefins with electron-donating allylic functionality is related to the alkylidenes generated upon metathesis. Relatively bulky, electron-donating substituents on alkylidenes have been shown previously to accelerate metathesis processes;[23] the preferential formation of a more active alkylidene employing an excess of the disubstituted olefin could thus be reason for the observed higher CM yields.[37] In order to pursue this theory further, detailed kinetic studies of these CM transformations, rigorous ^{1}H NMR analysis of the alkylidenes formed, and attempts at their isolation are currently in progress.



Scheme 9. Synthesis of amide ester heterodimer **44** *via* CM with either terminal olefin acetate **19** or internal olefin bis-acetate **20**. a) 5 mol % **1**, CH_2Cl_2, 45 °C, 12 hr.

Cross-metathesis of Terminal Olefins with Acrolein Acetals

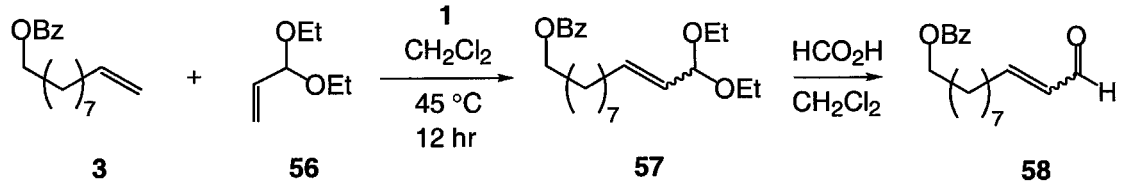
In the course of examining the activity of substrates for CM with allylic oxygen functionality, we discovered that certain acrolein acetals were particularly robust substrates for CM with terminal olefins yielding α,β-unsaturated aldehydes. The preparation of α,β-unsaturated aldehydes has been accomplished previously by Wittig[38] homologation of aldehydes employing reagents such as Ph_3P=CHCHO[39] or with acetal[40] or imine[41] protected two-carbon ylides. Addition-elimination methods have also been used to homologate aldehydes.[42,43,44] In cases where a terminal olefin is serving as an aldehyde precursor, a cross-metathesis approach offers a means for direct homologation (Scheme 10).



Scheme 10. Synthetic methods for terminal olefin and aldehyde homologation. PG = protecting group.

Although acrylonitrile has been successfully employed in molybdenum carbene **2** catalyzed CM reactions,[10] conjugated olefins including acrolein were found to be unreactive in reactions using catalytic ruthenium benzylidene **1**. Unconjugated acrolein acetals, on the other hand, were found to be viable metathesis substrates.[45] Our initial investigations employed the commercially available acrolein diethyl acetal (**56**) and our standard terminal olefin substrate, 9-decen-1-yl benzoate (**3**) (Scheme 11, Table 2).[21] An optimization study led to the following observations: α,β-unsaturated aldehyde **58** was obtained in 75-80% yield using either 2.5 or 5 mol % **1** and two equivalents of acetal **56** (Table 2, entries 2 and 3). Although the acid-sensitive diethyl acetal cross-metathesis product (**57**) could be isolated with chromatography employing Et_3N-treated silica gel, it was more convenient to

recover the α,β-unsaturated aldehyde **58** after formic acid hydrolysis. We observed that reactions using older samples of acrolein diethyl acetal (**56**) gave low yields, presumably due to small amounts of hydrolysis-derived acrolein. Increasing the number of acetal **56** equivalents (entry 4) was found to suppress the formation of aldehyde product **58**, presumably due to engaging the propagating ruthenium alkylidene in unproductive acetal homodimerization.



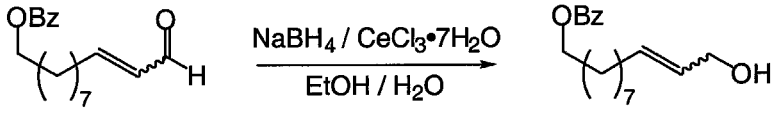
3 **56** **57** **58**

Scheme 11. Synthesis of α,β-unsaturated aldehyde **58** *via* CM of terminal olefin **3** with acetal **56**.

Table 2. Results of CM reactions with acrolein acetal **56**.

entry	[3]	equiv. **56**	mol % **1**	isolated yield **58**
1	0.1 M	4	5	72%
2	0.1 M	2	5	75%
3	0.2 M	2	2.5	81%
4	0.1 M	65 (neat)	5	<10%

Using a Luche reduction,[46] α,β-unsaturated aldehyde **58** was converted to allylic alcohol **7** in a highly *trans*-selective manner (Eq 6). This three-step allylic alcohol synthesis was an improvement, in terms of both yield and *trans* selectivity, upon our CM procedure using protected *cis*-2-butene-1,4-diols described above (Table 1).

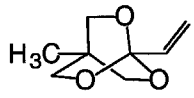


(Eq 6)

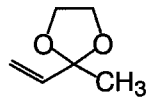
58

7: 94%, 26:1*E/Z*

The reactivity of acetal **56** was unexpected, as it was originally thought that allylic disubstitution would hinder the CM reaction. Extending this methodology to substrates with allylic trisubstitution could, in principle, provide access to additional functional groups such as α,β-unsaturated esters and methyl ketones. However, attempts at ruthenium-catalyzed CM of benzoate **3** with orthoester **59**[47] or ketal **60**[48] proved unsuccessful, potentially due to their relative steric bulk in comparison to acetal **56** above.

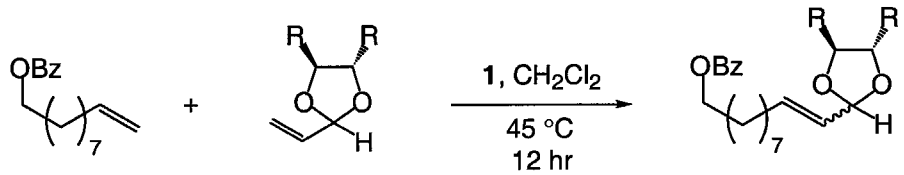


59



60

Cross-metathesis reactions between terminal olefin **3** and 2-vinyl-1,3-dioxolane (**61**), a commercially available acrolein acetal with enhanced acid-stability compared to diethyl acetal **56**, gave good to excellent yields of the dioxolane-protected α,β-unsaturated aldehyde **62** (Scheme 12). Under these conditions, a 74% isolated yield of protected aldehyde **62** was obtained with the catalyst loading reduced to as little as 1 mol % **1** (Table 3, entry 1). Yields of 87-91% (7:1 *E/Z*) were obtained for reactions using catalyst loadings of 2-5 mol % **1** (Table 3, entries 2-4). Notably, replacement of the methyl group of ketal **60** with a hydrogen had transformed a completely inactive substrate to a highly reactive CM coupling partner, indicating again that allylic trisubstitution shuts down CM reactivity for terminal olefins.



3 (0.2 M)

61: R = H
63: R = CO_2Et

62: R = H
64: R = CO_2Et

Scheme 12. CM of 2-vinyl-1,3-dioxolane derivatives employing catalyst **1**.

Table 3. CM with 2-vinyl-1,3-dioxolane derivatives

entry	mol %1	isolated yield
1	1	**62**: 74%
2	2	**62**: 87%
3	2.5	**62**: 93%
4	5	**62**: 91%
5	2.5	**64**: 86%

Extending the scope of the reaction to include asymmetric acrolein acetals was considered worthwhile because chiral α,β-unsaturated acetals are useful synthetic intermediates.[49] Accordingly, diethyl vinylidene-L-tartrate (**63**) was prepared[50] and found to provide a *trans* selective (6.7:1 *E/Z* by ^{1}H NMR) cross-metathesis product **64** in excellent yield (Table 3, entry 5) approximating that of vinyl dioxolane CM product **62**.[51]

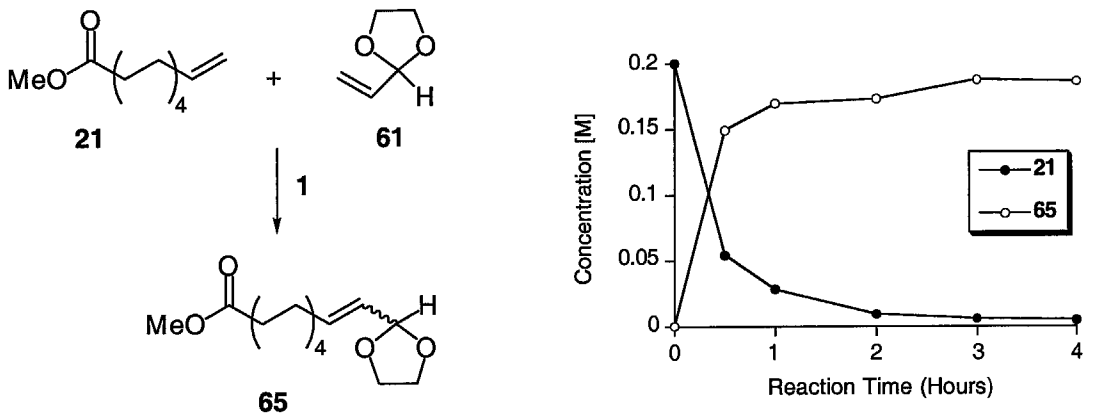
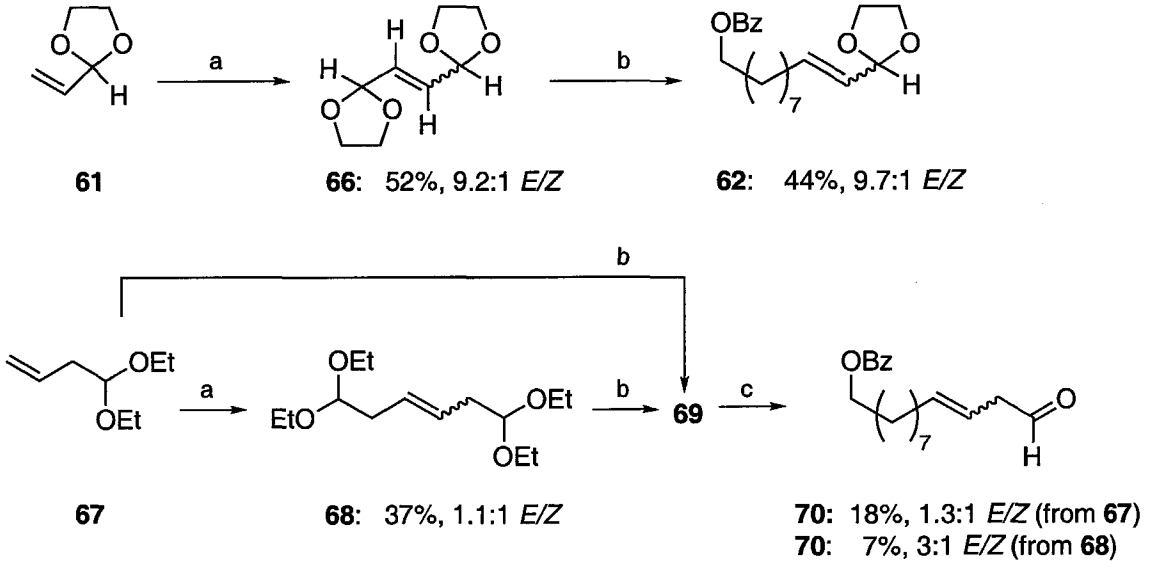


Figure 3. Reaction profile for cross-metathesis reaction employing alkene **21** (0.2 M), 2 equiv. vinyl dioxolane **61**, and 2.5 mol % catalyst **1** (45 °C, CH_2Cl_2). Data obtained from GC-MS analysis of reaction aliquots (1,4-dichlorobenzene as internal standard, data corrected for relative response).

A GC-MS assay analogous to those described above for allylic alcohol derivatives was used to measure the qualitative rate of CM with vinyl dioxolane **61**. Figure 3 shows a representative reaction profile for a standard reaction employing 2.5 mol % **1**, 2 equivalents of vinyl dioxolane **61**, and 0.2 M methyl 10-undecylenate (**21**).[52] The data show that the reaction was essentially complete (>90%) after 3 hours. GC-MS analysis also indicated

that the concentration of product heterodimer **65** consisted of an approximately 7:1 *trans/cis* olefin composition throughout the course of the reaction (data not shown).

Attempting to build upon our earlier work, which demonstrated certain advantages to using symmetrically disubstituted olefins as CM partners (see above), we prepared fumaraldehyde bis(ethylene glycol acetal) (**66**)[53] by homodimerization of vinyl dioxolane **61** employing ruthenium benzylidene **1** under standard solution phase CM reaction conditions (Scheme 13). However, bis-acetal **66** was not as reactive as vinyl dioxolane **61** in CM reactions with terminal olefin **3**, presumably due to steric factors. Interestingly, the *trans/cis* ratio improved when the fumaraldehyde bis-acetal **66** was employed (**66**: *E/Z* = 9.7:1; **61**: *E/Z* = 7:1), which corroborated well with the pattern we had observed previously in the CM of terminal olefins with allylic methyl substituents (Scheme 7). Also of note is that the high *trans* content of bis-acetal **66** was maintained in the heterodimer product **62**.



Scheme 13. Reagents and Conditions: a) 0.2 M in acetal, 5 mol % **1**, CH_2Cl_2, 45 °C. b) 2 equiv. of acetal component, 0.2 M **3**, 5 mol % **1**, CH_2Cl_2, 45 °C. c) HCO_2H-CH_2Cl_2 (1:8), 25 °C.[29]

Extending this CM acetal methodology to the construction of β,γ-unsaturated aldehydes *via* cross-metathesis was also explored (Scheme 13). Unfortunately, the homologue of acetal **56**, 3-butenal diethyl acetal **67**, did not appear to be a promising substrate for CM. CM of benzoate **3** with 3-butenal diethyl acetal **67** or its homodimer **68**, followed by acetal hydrolysis afforded only low yields of the β,γ-unsaturated aldehyde **70** with poor *trans/cis* selectivity. This result correlated with the observations of Crowe *et al.* that certain homoallylic substituents on terminal olefins deactivate catalytic CM.[9,10] Specifically, formation of the alkylidene from acetal **67** allows for the formation of a potential 5-membered chelate between one of oxygens of **67** and the ruthenium **1** metal center, which could be prematurely shutting down of the catalytic cycle.

Finally, acrolein acetals **56**, **61**, and **63** have been shown to be highly reactive for CM with terminal olefins. These results corroborated nicely with our previous work showing that allylic oxygen functionality activates olefins towards CM. This CM method offers a mild alternative to traditional homologative methods for preparing α,β-unsaturated aldehydes. Notably, the use of asymmetric acrolein equivalents, coupled with emergent asymmetric metathesis catalysts,[54] suggests a means for effecting catalytic kinetic resolutions *via* CM.

Summary and Future Prospectives

In conclusion, cross-metathesis reactions involving internal disubstituted olefins and acrolein acetals appear to be a promising method for the direct homologation of terminal olefins. The desired heterodimeric cross-products can be generated in good to excellent yields employing a two-fold excess of the internal olefin or protected acrolein acetal. Furthermore, the cross-metathesis reactions were shown to be systematically more *trans* selective as the steric bulk at the allylic position of the either the internal olefin or the terminal olefin was increased. Details of the current rationale behind the improved chemoselectivity of allylic oxygen functionalized olefins have been presented. The cross-

metathesis methodology described herein should be of particular use for the functionalization of advanced intermediates in organic syntheses, for the synthesis of diverse combinatorial libraries, and for the construction of dimeric molecules for use as tools in molecular biology.[55] The CM homodimerization procedure employing alkylidene **1** also allows rapid access to functionally diverse chain transfer agents for the synthesis of novel telechelic polymers by ROMP. Future work is directed toward the installation of other functional groups *via* CM such as protected boron,[56] phosphorus,[57] sulfur,[58] and alkyne functionality, all of which allow for further post CM synthetic manipulation. Routes toward dendritic architectures *via* selective CM are also being pursued in our laboratory. Finally, the simplicity and power of cross-metathesis as an intermolecular carbon-carbon bond forming reaction is only now being appreciated; we anticipate that as selective cross-metathesis routes are disclosed, the volume of CM applications in synthesis will dramatically escalate.

Acknowledgements

This work was generously supported by grants form the National Institutes of Health (NIH), Zeneca Pharmaceuticals, and the ACS Division of Organic Chemistry. Prof. Daniel J. O'Leary is gratefully acknowledged for his tremendous intellectual and technical contributions to this work. Rebecca A. Washenfelder in acknowledged for her analytical GC-MS work. Dr. Katsukiyo Miura is acknowledged for his assistance in the acrolein acetal cross-metathesis portion of this work.

Experimental Section

General Experimental Section. NMR spectra were recorded on either a JEOL GX-400 or a Bruker AM-500 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q),

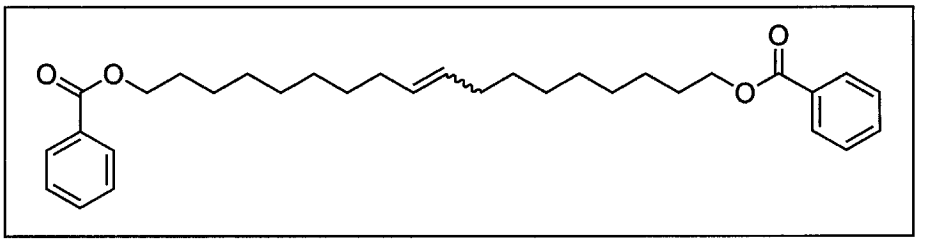
quintet (quint), and multiplet (m). The reported ^{1}H NMR data refer to the major olefin isomer unless stated otherwise. The reported ^{13}C NMR data include all peaks observed and no peak assignments were made. Optical rotations were recorded on a Jasco DIP-1000 digital polarimeter at 589 nm and are reported as $[\alpha]_D$ (concentration in grams/100 mL of solvent). Low- and high-resolution mass spectra were provided by either the Southern California Mass Spectrometry Facility (University of California, Riverside) or the UCLA Mass Spectrometry Facility (University of California, Los Angeles).

Analytical thin-layer chromatography (TLC) was performed using silica gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Flash column chromatography was performed using silica gel 60 (230-400 mesh) from EM Science.[59] *Cis*-3-hexene was purchased from Chemsampco, Gray Court, SC. All other chemicals were purchased from the Aldrich, Strem, or Nova Biochem Chemical Companies, and used as delivered unless noted otherwise. Catalyst **1** was prepared according to published procedure.[5]

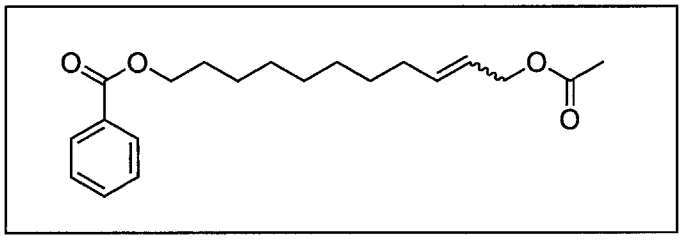
Peptide Synthesis. *N*-Boc-L-serine(*O*-allyl) methyl ester (**33**), *N*-Boc-L-homoserine(*O*-allyl) methyl ester (**35**), and *N*-Boc-L-tyrosine(*O*-allyl) methyl ester (**37**) were prepared according to a modified literature procedure.[60] Peptide **41** was synthesized by conventional solution phase synthesis methods using a racemization free fragment condensation strategy. Couplings were mediated by *N,N*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT).[61] The Boc group was used to protect the N-terminus, and the C-terminus was protected as a methyl ester. Deprotections were performed using 1:1 trifluoroacetic acid/CH_2Cl_2 and saponification, respectively. All intermediates were characterized by ^{1}H NMR and TLC, and if necessary purified by column chromatography on silica gel.

Cross-metathesis reactions were carried out under an argon atmosphere with dry, degassed solvents under anhydrous conditions. CH_2Cl_2 was purified by passage through a solvent column prior to use.[62]

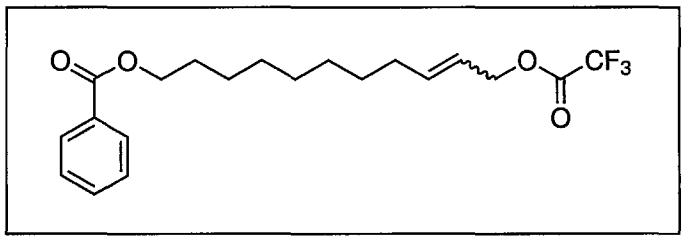
General Procedure for the Solution-Phase Cross-Metathesis Reactions. An oven-dried flask was charged with a magnetic stir bar and ruthenium benzylidene **1** (21 mg, 5 mol %) and capped with a septum under nitrogen atmosphere. CH_2Cl_2 (5 mL) and the disubstituted olefin (1.0 mmol, 2 equiv.) were added in succession. The terminal olefin (0.5 mmol, 1 equiv.) was added and the septum was quickly replaced with a condenser which was connected to a nitrogen bubbler. The flask was immersed in an oil bath and refluxed (oil bath temperature: 45 °C) for a period of 12 hours or until the reaction was judged complete by TLC analysis. The product was then isolated by silica gel column chromatography.



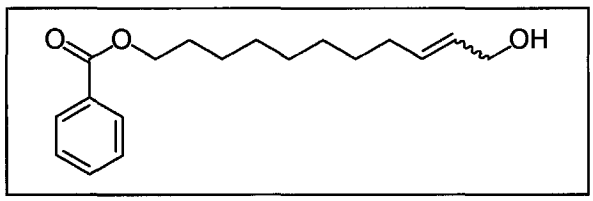
Compound 4. 9-Decen-1-yl benzoate (**3**)[63] (349 mg, 1.34 mmol) and **1** (3.5 mg, 4 μmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 36 hours at room temperature. The thick burgundy-colored oil was observed to steadily produce gas during the course of the reaction. The reaction mixture was dissolved in 1.0 mL CH_2Cl_2 and applied to a silica gel column (2x10 cm, eluting with CH_2Cl_2 (375 mL). Pure fractions were concentrated to give a clear, colorless, viscous oil which formed a white solid over time (312 mg, 94% yield, 3.8:1 *trans/cis* as determined by integration of peaks at 5.38 and 5.35 ppm in the ^1H NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (4H, d, J = 7.2 Hz), 7.53 (2H, t, J = 7.3 Hz), 7.42 (4H, t, J = 7.2 Hz), 5.38 (2H, m), 4.30 (4H, t, J = 6.7 Hz), 2.10-1.90 (4H, m), 1.75 (4H, quint, J = 7.2 Hz), 1.50-1.20 (20H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.3, 132.5, 130.4, 130.2, 129.7, 129.4, 128.1, 64.9, 32.4, 29.6, 29.4, 29.2, 29.1, 28.9, 28.6, 27.0, 25.9. R_f = 0.50 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{32}H_{44}O_4$ $[M+H]^+$ 493.3239, found 493.3318.



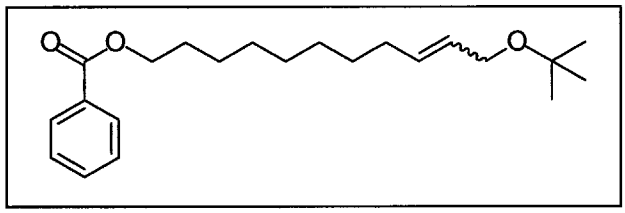
Compound 5. 9-Decen-1-yl benzoate (**3**) (69 μl, 0.25 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(acetyloxy)but-2-ene[64] (79 μl, 0.5 mmol) and **1** (21 mg, 0.025 mmol, 10 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 3 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1, and 2:1 hexane/ethyl acetate (100 mL aliquots). A pale yellow oil was obtained (68 mg, 82% yield, 5:1 *trans/cis* as determined by integration of peaks at 4.50 and 4.61 ppm in the ^1H NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, d, J = 7.2 Hz), 7.53 (1H, t, J = 7.4 Hz), 7.42 (2H, t, J = 7.8 Hz), 5.78-5.72 (1H, broad m), 5.57-5.50 (1H, broad m), 4.50 (2H, d, J = 6.4 Hz), 4.30 (2H, t, J = 6.7 Hz), 2.06-2.02 (2H, broad m), 2.03 (3H, s), 1.75 (2H, m), 1.44-1.31 (10H, broad m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.7, 166.5, 150.5, 136.4, 135.2, 132.6, 130.5, 129.4, 128.2, 123.7, 123.3, 65.1, 64.9, 60.2, 32.1, 29.2, 29.1, 28.9, 28.7, 28.6, 27.4, 25.9, 20.9. R_f = 0.36 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{20}H_{28}O_4$ $[M-H]^+$ 333.2066, found 333.2067.



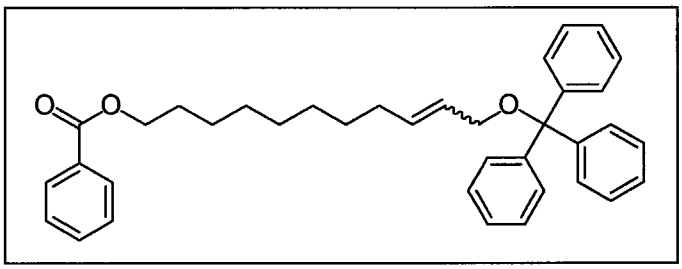
Compound 6. 9-Decen-1-yl benzoate (**3**) (69 µl, 0.25 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(trifluoroacetyloxy)but-2-ene[65] (188 µl, 1.0 mmol) and **1** (11 mg, 0.013 mmol, 5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to a thick brown oil and redissolved in 2 mL methanol to which 1 mL of NEt_3 was added to effect cleavage of the trifluoroacetate moiety.[65] The mixture was stirred for 2 hours at room temperature until TLC analysis indicated that deprotection was complete. The solution of alcohols (**7**) was concentrated and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1, 2:1 and 1:1 hexane/ethyl acetate (100 mL aliquots). A pale yellow, viscous oil was obtained that exhibited spectral properties identical to alcohol **7** (45 mg, 63% yield over two steps, 2.8:1 *trans/cis* as determined by integration of peaks at 4.07 and 4.18 ppm in the ^{1}H NMR spectrum).



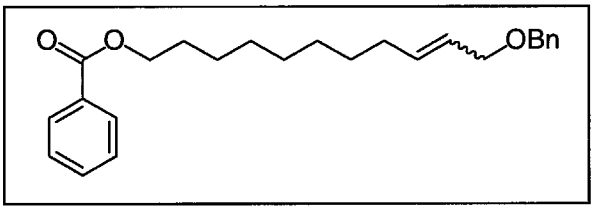
Compound 7. 9-Decen-1-yl benzoate (**3**) (130 mg, 0.5 mmol) was added *via* syringe to a stirring solution of 1,4-butenediol (95% *cis*, 88 mg, 1.0 mmol) and **1** (21 mg, 0.026 mmol, 5 mol %) in CH_2Cl_2 (5.0 mL). The reaction was stirred at room temperature under a nitrogen atmosphere for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2 (100 mL) followed by 4:1 CH_2Cl_2:ethyl acetate (150 mL). A clear, colorless, viscous oil was obtained (80 mg, 56% yield, 5:1 *trans/cis* as determined by integration of peaks at 4.07 and 4.18 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, app d, J = 7.0 Hz), 7.53 (1H, tt, J = 7.4, 1.4 Hz), 7.41 (2H, t, J = 8.0 Hz), 5.70-5.50 (2H, m), 4.30 (2H, t, J = 6.7 Hz), 4.07 (2H, dd, J = 0.7, 5.5 Hz), 2.02 (2H, q, 6.8 Hz), 1.78 (1H, s), 1.77 (2H, quint, J = 7.9 Hz), 1.45-1.28 (10H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.6, 133.2, 132.9, 132.7, 130.5, 129.5, 128.9, 128.5, 128.2, 65.0, 63.7, 58.5, 32.1, 29.5, 29.3, 29.1, 29.0, 28.99, 28.7, 27.3, 25.9. R_f = 0.23 (3:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{18}H_{26}O_3$ $[M-H]^+$ 290.1882, found 289.1804.



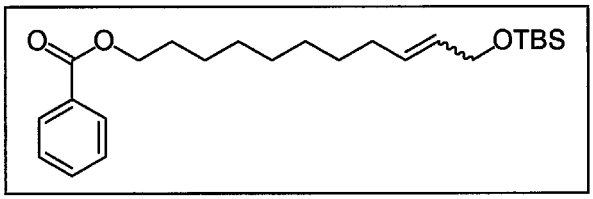
Compound 8. 9-Decen-1-yl benzoate (**3**) (130 mg, 0.5 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(tert-butoxy)but-2-ene[66] (200 mg, 1 mmol) and **1** (21 mg, 0.026 mmol, 5 mol %) in CH_2Cl_2 (5.0 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x8 cm), eluting with CH_2Cl_2 (300 mL). Fractions containing the desired product were concentrated to an oil (164 mg, 94% yield, 7:1 *trans/cis* as determined by integration of peaks at 3.80 and 3.93 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, app d, J = 7.0 Hz), 7.52 (1H, tt, J = 7.4, 1.4 Hz), 7.40 (2H, t, J = 8.0 Hz), 5.7-5.6 (1H, m), 5.57-5.47 (1H, m), 3.80 (2H, d, J = 5.9 Hz), 2.10 (2H, m), 1.74 (2H, quint, J = 7.0 Hz), 1.45-1.25 (10H, m), 1.19 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.5, 133.0, 132.6, 132.0, 130.5, 130.2, 129.4, 128.2, 127.6, 72.8, 64.9, 62.8, 57.6, 32.4, 32.2, 29.5, 29.4, 29.2, 29.1, 29.0, 28.9, 28.6, 27.5, 27.4, 25.9. R_f = 0.46 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{22}H_{34}O_3$ $[M+H]^+$ 347.2508, found 347.2586.



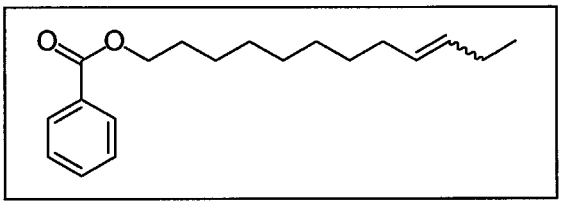
Compound 9. 9-Decen-1-yl benzoate (**3**) (66 mg, 0.25 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(O-trityl)but-2-ene[67] (285 mg, 0.5 mmol) and **1** (11 mg, 0.013 mmol, 5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2 (250 mL). Fractions containing the desired product and homodimer were concentrated to an oil which was dissolved in CH_2Cl_2 (8 mL) and treated with 98% formic acid (2.0 mL, 53 mmol) at room temperature for 1 hour.[68] The reaction was quenched by dilution with ethyl acetate (40 mL) followed by slow addition of aqueous sodium bicarbonate solution (7 g $NaHCO_3$ in 40 mL H_2O). The biphasic mixture was allowed to stir for two hours, after which the organic layer was removed, washed with brine and dried with sodium sulfate. This brown solution was concentrated, redissolved in 2.0 mL CH_2Cl_2 and applied to a silica gel column (2x10 cm), eluting with 9:1 hexane/ethyl acetate (100 mL) followed by 1:1 hexane/ethyl acetate (100 mL). A clear, colorless, viscous oil was obtained with spectral properties identical to alcohol **7** (55 mg, 75% yield over 2 steps, 8:1 *trans/cis* as determined by integration of peaks at 4.07 and 4.18 ppm in the ^1H NMR spectrum).



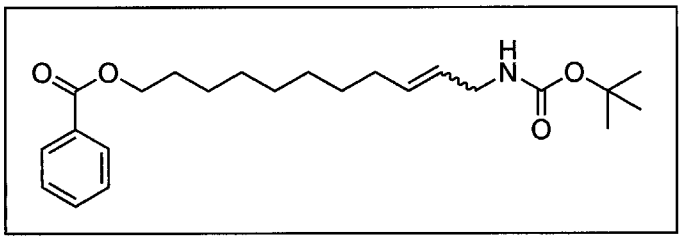
Compound 10. 9-Decen-1-yl benzoate (**3**) (130 mg, 0.5 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(benzyloxy)but-2-ene[69] (270 mg, 1 mmol) and **1** (21 mg, 0.026 mmol, 5 mol %) in CH_2Cl_2 (5.0 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2 (300 mL). Fractions containing the desired product and unreacted terminal alkene were concentrated to an oil which was dissolved in ethyl acetate (15 mL) and hydrogenated (1 atm H_2 balloon) over 10% Pd-C (20 mg) for 6 hours.[70] The reaction mixture was filtered through a glass frit and concentrated to an oil. The alcohol product (**11**) was isolated by silica gel chromatography (2x10 cm), eluting with 9:1 hexane/ethyl acetate (100 mL) followed by 1:1 hexane/ethyl acetate (100 mL). A clear, colorless, viscous oil (**11**) was obtained (104 mg, 71% yield). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, m), 7.53 (1H, tt, J = 7.4, 1.4 Hz), 7.42 (2H, t, J = 7.4 Hz), 4.29 (2H, t, J = 6.7 Hz), 3.6 (2H, t, J = 6.7 Hz), 2.03 (1H, br s), 1.75 (2H, quint, J = 6.8 Hz), 1.54 (2H, quint, J = 6.6 Hz), 1.4 (2H, m), 1.38-1.22 (12H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.6, 132.7, 130.4, 129.4, 128.2, 65.0, 62.8, 32.7, 29.4, 29.4, 29.3, 29.1, 28.6, 25.9, 25.7. R_f = 0.34 (3:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{18}H_{28}O_3$ $[M+H]^+$ 293.2038, found 293.2113.



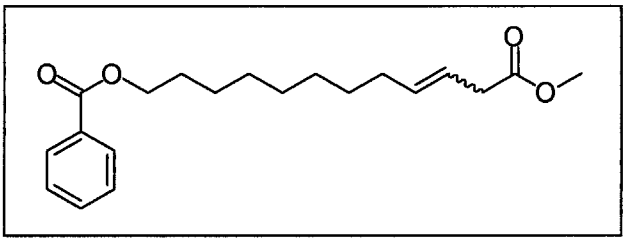
Compound 12. 9-Decen-1-yl benzoate (**3**) (130 mg, 0.5 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(tert-butyldimethylsilyloxy)but-2-ene[71] (317 mg, 1 mmol) and **1** (21 mg, 0.026 mmol, 5 mol %) in CH_2Cl_2 (5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 0.75 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2 (300 mL). Fractions containing the desired product and unreacted terminal alkene were concentrated to an oil which was dissolved in THF (5 mL) and treated with TBAF (1.6 mL of a 1.0M THF solution) at room temperature for 2 hours.[71] The reaction mixture was concentrated, redissolved in 2.0 mL CH_2Cl_2 and applied to a silica gel column (2x10 cm), eluting with 9:1 hexane/ethyl acetate (100 mL) followed by 1:1 hexane/ethyl acetate (100 mL). A clear, colorless, viscous oil was obtained that exhibited spectral properties identical to alcohol **7** (113 mg, 77% yield over 2 steps, 10:1 *trans/cis* as determined by integration of peaks at 4.07 and 4.18 ppm in the ^1H NMR spectrum).



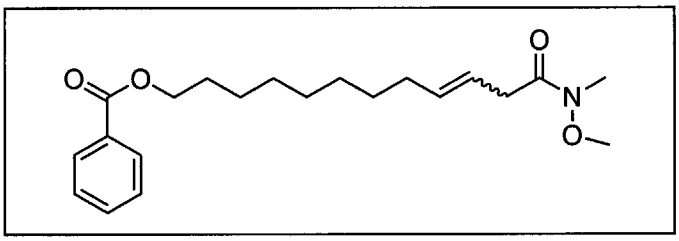
Compound 13. 9-Decen-1-yl benzoate (**3**) (130 mg, 0.5 mmol) was added *via* syringe to a stirring solution of *cis*-3-hexene (84 mg, 1 mmol) and **1** (21 mg, 0.026 mmol, 5 mol %) in CH_2Cl_2 (5.0 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2 (300 mL). Fractions containing the desired product were concentrated to an oil (104 mg, 72% yield, 3:1 *trans/cis* as determined by analysis of methyl intensities at 0.97 and 0.96 ppm in the ^1H NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, app d, J = 7.0 Hz), 7.52 (1H, tt, J = 7.4, 1.4 Hz), 7.40 (2H, t, J = 8.0 Hz), 5.50-5.30 (2H, m), 4.32 (2H, t, J = 6.7 Hz), 2.10-1.90 (4H, m), 1.75 (2H, quint, J = 7.2 Hz), 1.43 (2H, m), 1.40-1.25 (10H, m), 0.97 (3H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.5, 132.7, 131.9, 131.5, 130.6, 129.2, 129.16, 128.2, 65.0, 32.5, 29.7, 29.5, 29.3, 29.2, 29.1, 29.0, 28.7, 27.0, 26.0, 25.5, 20.4, 14.3, 13.92. R_f = 0.63 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{19}H_{28}O_2$ $[M+H]^+$ 289.2167, found 289.2171.



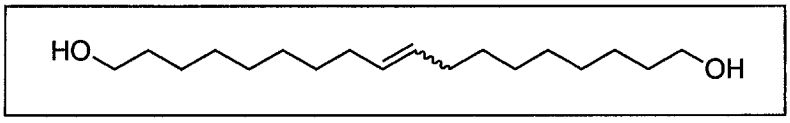
Compound 14. 9-Decen-1-yl benzoate (**3**) (69 μl, 0.25 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(*N*-Boc)but-2-ene[72] (286 mg, 1.0 mmol) and **1** (11 mg, 0.013 mmol, 5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1, 2:1 and 1:1 hexane/ethyl acetate (100 mL aliquots). A pale yellow, viscous oil was obtained (70 mg, 71% yield, 2.9:1 *trans/cis* as determined by integration of peaks at 3.64 and 3.73 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.01 (2H, d, J = 7.2 Hz), 7.52 (1H, t, J = 7.3 Hz), 7.40 (2H, t, J = 7.7 Hz), 5.56-5.52 (1H, broad m), 5.41-5.37 (1H, broad m), 4.58 (1H, m), 4.28 (2H, t, J = 6.6 Hz), 3.64 (2H, app s), 1.97 (2H, m), 1.73 (2H, quint, J = 6.7 Hz), 1.41 (9H, s), 1.47-1.27 (10H, broad m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.8, 155.9, 133.2, 133.16, 132.9, 131.0, 129.7, 128.5, 127.7, 126.6, 126.3, 79.3, 65.2, 62.4, 43.0, 38.1, 32.3, 29.7, 29.5, 29.4, 29.33, 29.29, 29.2, 29.0, 28.6, 28.5, 27.5, 26.2. R_f = 0.30 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{23}H_{35}NO_4$ $[M-H]^+$ 390.2644, found 390.2635.



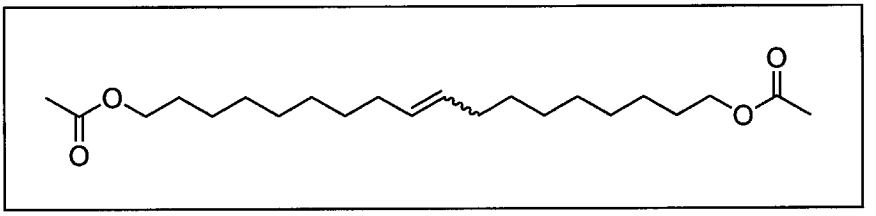
Compound 15. 9-Decen-1-yl benzoate (**3**) (130 mg, 0.5 mmol) was added *via* syringe to a stirring solution of dimethyl trans-3-hexene-1,6-dioate[73] (84 mg, 1 mmol) and **1** (21 mg, 0.026 mmol, 5 mol %) in CH_2Cl_2 (5.0 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2 (300 mL). Fractions containing the desired product were concentrated to an oil (124 mg, 74% yield, 3:1 *trans/cis* as determined by integration of peaks at 3.00 and 3.07 ppm in the ^1H NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, app d, J = 7.0 Hz), 7.52 (1H, tt, J = 7.4, 1.4 Hz), 7.40 (2H, t, J = 8.0 Hz), 5.50-5.30 (2H, m), 4.32 (2H, t, J = 6.7 Hz), 3.63 (3H, s), 3.00 (2H, d, J = 5.5 Hz), 1.98 (2H, q, J = 7.1 Hz), 1.74 (2H, quint, J = 6.8 Hz), 1.45-1.20 (10H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 172.4, 166.5, 131.7, 133.4, 132.7, 130.5, 129.4, 128.2, 121.4, 120.7, 65.0, 51.6, 51.5, 37.8, 32.7, 32.3, 29.2, 29.1, 29.0, 28.9, 28.7, 27.3, 25.9. R_f = 0.38 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{20}H_{28}O_4$ $[M+H]^+$ 333.2066, found 333.2059.



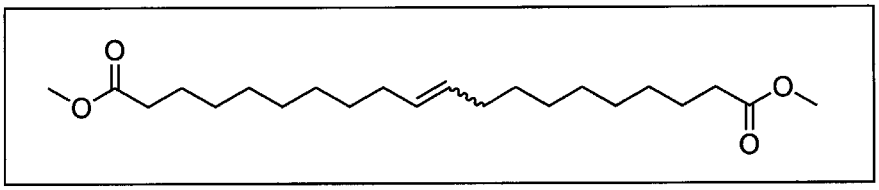
Compound 16. 9-Decen-1-yl benzoate (**3**) (69 μl, 0.25 mmol) was added *via* syringe to a stirring solution of trans-1,4-bis-(methyl(methoxy)amido)but-2-ene[74] (230 mg, 1.0 mmol) and **1** (11 mg, 0.013 mmol, 5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 5:1, and 1:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained (15 mg, 17% yield, 1.9:1 *trans/cis* as determined by the relative intensities of peaks at 65.35 and 65.33 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, d, J = 7.3 Hz), 7.54 (1H, t, J = 7.4 Hz), 7.43 (2H, t, J = 7.8 Hz), 5.56 (2H, m), 4.30 (2H, t, J = 6.6 Hz), 3.67 (3H, s) , 3.20 (2H, m), 3.16 (3H, s), 2.06-2.02 (2H, broad m), 1.75 (2H, quint, J = 6.7 Hz), 1.42-1.24 (10H, broad m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 134.6, 133.2, 132.9, 129.8, 128.5, 122.7, 121.9, 65.35, 65.33, 61.5, 36.4, 32.7, 31.4, 29.62, 29.58, 29.47, 29.46, 29.3, 29.0, 27.8, 26.3. R_f = 0.09 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{21}H_{31}NO_4$ $[M]^+$ 362.2331, found 362.2337.



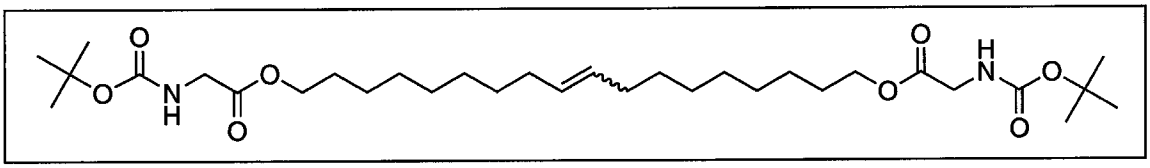
Compound 18. 9-Decen-1-ol (**17**) (1.14 mL, 6.4 mmol) and **1** (15.8 mg, 0.019 mmol, 0.3 mol %) were combined in a 5 mL one-neck round-bottom flask. A magnetic stir bar was added to the flask and it was equipped with a vacuum adapter. The flask was held under vacuum (60-100 mtorr) accompanied by stirring for 36 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction, and after 24 hours a white solid was visible. The reaction mixture was then dissolved in 1.0 mL CH_2Cl_2 and purified directly on a silica gel column (5x25 cm), eluting with 9:1, 4:1, and 3:1 CH_2Cl_2:ethyl acetate (250 mL aliquots). A fluffy white, crystalline solid was obtained (494 mg, 54% yield, 1.7:1 *trans/cis* as determined by integration of peaks at 5.36 and 5.33 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 5.36 (2H, m), 3.61 (4H, t, J = 6.6 Hz), 2.00-1.94 (4H, br m), 1.54 (4H, quint, J = 6.5 Hz), 1.30-1.27 (20H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 130.6, 130.1, 63.3, 33.0, 32.8, 29.9, 29.8, 29.68, 29.66, 29.6, 29.4, 29.3, 27.4, 25.9. R_f = 0.18 (9:1 CH_2Cl_2:ethyl acetate); HRMS (EI) calcd for $C_{18}H_{36}O_2$ $[M]^+$ 284.2715, found 284.2713.



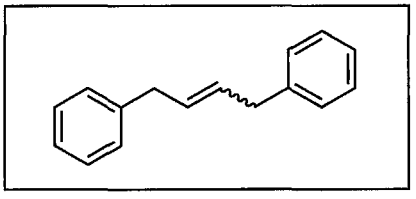
Compound 20. 9-Decen-1-yl acetate (**19**)[64] (1.0 g, 5.0 mmol) and **1** (13 mg, 0.015 mmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 16 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction, which subsided as the product began to crystallize. The reaction mixture was then purified directly on a silica gel column (2x15 cm), eluting with 9:1, 2:1, and 1:1 hexane/ethyl acetate (200 mL aliquots). A pale yellow, viscous oil that crystallized over time was obtained (878 mg, 95% yield, 4.4/1 *trans/cis* as determined by the relative intensities of peaks at 130.2 and 129.6 ppm in the ^{13}C NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 5.17 (2H, m), 3.85 (4H, t, J = 6.8 Hz), 1.83 (6H, s), 1.85-1.78 (4H, broad m), 1.43 (4H, m) , 1.11 (20H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.3, 130.2, 129.6, 61.2, 32.4, 29.6, 29.4, 29.2, 29.1, 29.0, 28.9, 28.6, 27.0, 25.8, 20.5. R_f = 0.29 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{22}H_{40}O_4$ $[M-H]^+$ 369.3005, found 369.2993.



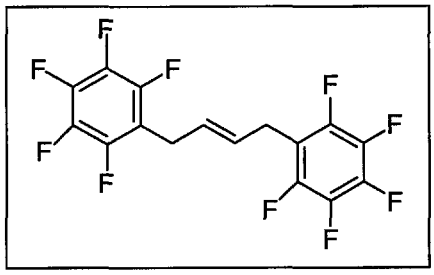
Compound 22. Methyl 10-undecylenate (**21**) (2.26 g, 11.4 mmol) and **1** (46 mg, 0.06 mmol, 0.5 mol %) were combined in a round-bottom flask. A magnetic stir bar was added to the flask, and the flask was equipped with a vacuum adapter. The flask was held under vacuum (100-200 mtorr) and stirred for 36 hours at room temperature. The thick burgundy-colored oil was observed to steadily produce gas during the course of reaction, and after two hours a thick precipitate formed. The solidified reaction mixture was melted by warming the flask with a heat-gun. This procedure was repeated three times over a three hour period. The reaction mixture was dissolved in 3 mL CH_2Cl_2 and applied to a silica gel column (10x10 cm), eluting with CH_2Cl_2 (700 mL). Pure fractions were concentrated to give a white solid upon standing (1.9 g, 90% yield, 5:1 *trans/cis* as determined by integration of olefinic peaks while decoupling allylic protons at 2.24 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 5.31 (2H, m), 3.58 (6H, s), 2.24 (4H, t, J = 7.5 Hz), 1.90 (4H,m), 1.55 (4H, quint, J = 7.2 Hz), 1.30-1.15 (20H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 174.0, 130.2, 129.7, 51.2, 33.9, 32.4, 29.6, 29.5, 29.2, 29.1, 29.0, 28.9, 27.1, 24.8. R_f = 0.66 (3:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{32}H_{44}O_4$ $[M+H]^+$ 368.2928, found 368.2927.



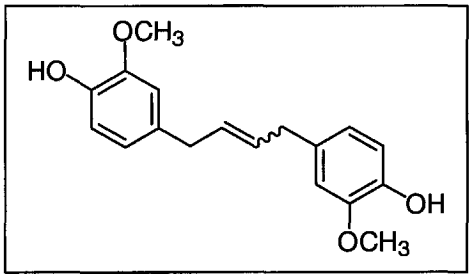
Compound 24. 9-Decen-1-yl *N*-Boc-glycinate (**23**)[75] (173 mg, 0.55 mmol) and **1** (1.4 mg, 2.0 μmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 36 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction. The reaction mixture was then purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1, and 2:1 hexane/ethyl acetate (100 mL aliquots). A clear, colorless viscous oil was obtained (153 mg, 93% yield, 3.9:1 *trans/cis* as determined by the relative intensities of peaks at 130.5 and 130.0 ppm in the ^{13}C NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 5.33 (2H, m), 5.07 (2H, m), 4.09 (4H, t, J = 6.7 Hz), 3.85 (4H, d, J = 5.0 Hz), 1.92 (4H, m), 1.58 (4H, m), 1.53-1.24 (20H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.5, 155.8, 130.5, 130.0, 80.1, 65.6, 42.9, 32.7, 29.9, 29.8, 29.52, 29.48, 29.3, 29.2, 28.8, 28.5, 27.4, 26.0. R_f = 0.31 (3:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{32}H_{58}N_2O_8$ $[M-H]^+$ 599.4271, found 599.4271.



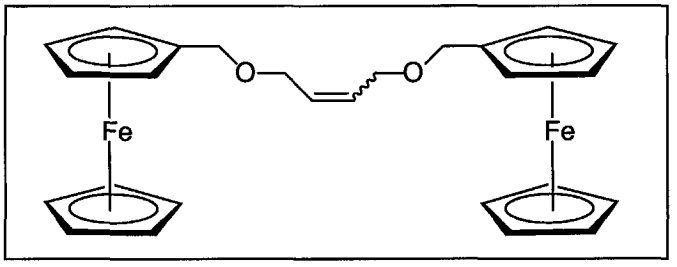
Compound 26. Allyl benzene (**25**) (1.0 g, 8.5 mmol) and **1** (15.8 mg, 0.019 mmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 24 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction, and after 24 hours the reaction mixture became a pale brown, crystalline mass. The crude reaction mixture was then purified directly by Kugel-Rohr distillation (140°C, 60 mtorr). A clear, viscous oil was obtained which slowly crystallized over time (664 mg, 75% yield, 8.3:1 *trans/cis* as determined by the relative intensities of peaks at 39.1 and 33.7 ppm in the ^{13}C NMR spectrum). This product could not be characterized by TLC. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 7.31 (5H, m), 7.21 (5H, m), 5.68 (2H, m), 3.37 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 141.0, 140.9, 130.6, 129.3, 128.7, 128.67, 128.6, 126.2, 39.1, 33.7. HRMS (EI) calcd for $C_{16}H_{16}$ $[M]^+$ 208.1252, found 208 1250.



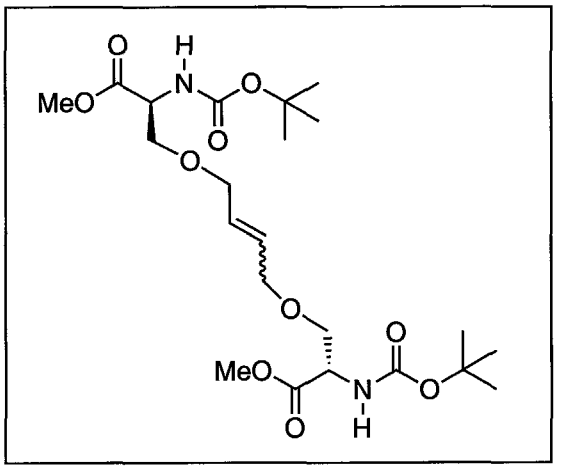
Compound 28. Allyl pentafluorobenzene (**27**) (147 μl, 0.96 mmol) was added *via* syringe to a stirring solution of **1** (40 mg, 0.048 mmol, 5 mol %) in CH_2Cl_2 (9.6 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction was monitored by ^1H NMR. The reaction mixture was reduced in volume to 0.5 mL and purified directly by addition of 10 equivalents of $P(CH_2OH)_3H^+Cl$ and NEt_3, respectively, in 3.0 mL CH_2Cl_2 to effect removal of the ruthenium catalyst **1** and/or catalyst decomposition products.[76] The brown homogenous solution was stirred at room temperature for 30 minutes. The solution gradually became clear light yellow over this time period. The organic layer was then washed with 10 mL of brine, 10 mL of 10% citric acid solution, and 10 mL of deionized water to remove the water soluble phosphine derivative complexed to any residual catalyst and/or catalyst decomposition products. The colorless organic layer was dried over $MgSO_4$ and concentrated to give an off-white crystalline solid (155 mg, 83% yield, all *trans*). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.57 (2H, app br s), 3.37 (4H, app br s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 146.1, 144.2, 141.1, 139.1, 138.7, 136.7, 127.7, 113.3, 113.1, 25.2. R_f = 0.60 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{16}H_6F_{10}$ $[M]^+$ 388.0310, found 388.0315.



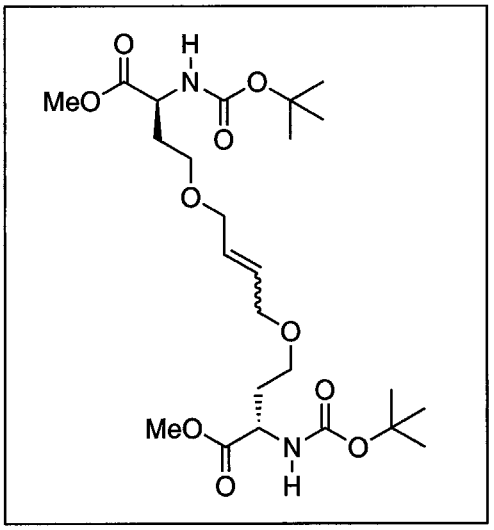
Compound 30. Eugenol (**29**) (1.0 mL, 6.5 mmol) and **1** (16 mg, 0.02 mmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 24 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction, gradually turning into a pale brown, crystalline mass. The reaction mixture was then purified directly by on a silica gel column, eluting with 9:1, 4:1, 3;1, and 1:1 hexane/ethyl acetate. A cream, crystalline solid was obtained which slowly turned maroon in color over time (697 mg, 71% yield, 5.9:1 *trans/cis* as determined by integration of peaks at 3.29 and 3.44 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 6.83 (2H, d, J = 7.9 Hz), 6.69 (2H, masked d, J = 7.7 Hz), 6.68 (2H, s), 5.63 (2H, m), 5.49 (2H, s), 3.84 (6H, s), 3.29 (4H, d, J = 5.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ146.6, 144.0, 132.9, 130.8, 129.4, 121.2, 121.1, 114.5, 114.4, 111.3, 111.28, 111.2, 56.0, 38.8, 33.2. R_f = 0.21 (3:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{18}H_{20}O_4$ $[M]^+$ 300.1362, found 300.1364.



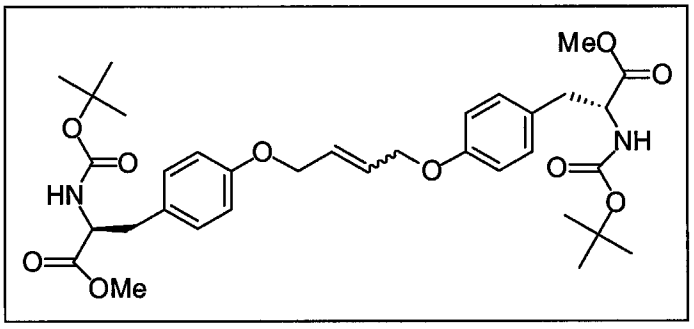
Compound 32. 1-Ferrocene methanol *O*-allyl ether (**31**)[77] (200 mg, 0.78 mmol) was added to a stirring solution of **1** (32 mg, 0. 039 mmol, 5 mol %) in CH_2Cl_2 (7.8 mL). The flask was fitted with a condenser and refluxed under nitrogen for 14 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x15 cm), eluting with 9:1, 7:1, 4:1, and 3:1 hexane/ethyl acetate (100 mL aliquots). An orange crystalline solid was obtained (145 mg, 77% yield, 7.8:1 *trans/cis* as determined by the relative intensities of peaks at 129.73 and 129.69 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.78 (2H, app br s), 4.27 (4H, app br s), 4.23 (4H, app br s), 4.15 (4H, app s obscured), 4.13 (10H, app s), 3.82 (4H, d, J = 7.2 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 129.73, 129.69, 83.6, 70.0, 69.6, 68.7, 68.6. R_f = 0.57 (3:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{26}H_{28}Fe_2O_2$ $[M]^+$ 484.0788, found 484.0802.



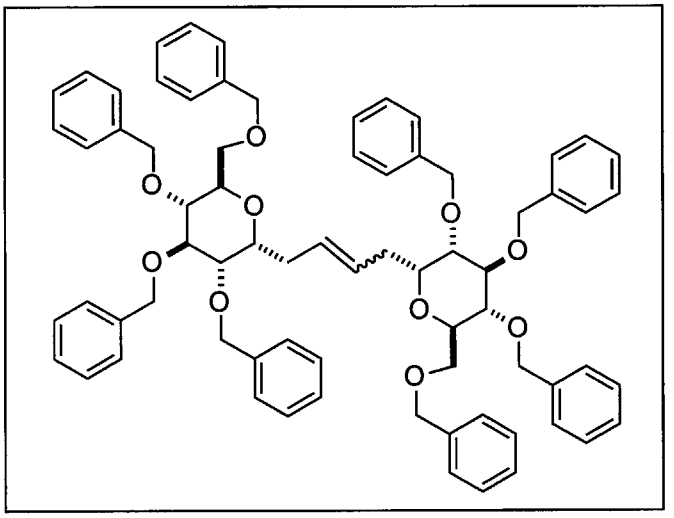
Compound 34. *N*-Boc-L-serine(*O*-Allyl) methyl ester (**33**) (160 mg, 0.62 mmol) and **1** (2.0 mg, 2 μmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 48 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction. The reaction mixture was then dissolved in 0.5 mL CH_2Cl_2 and purified directly on a silica gel column (2x10 cm), eluting with 4:1, 2:1, and 1:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained (110 mg, 73% yield, 2.6:1 *trans/cis* as determined by the relative intensities of peaks at 129.3 and 129.2 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.59 (2H, m), 5.34 (2H, d, J = 8.3 Hz), 4.33 (2H, m), 3.93-3.81 (4H, broad m), 3.74 (2H, m), 3.64 (6H, s), 3.53 (2H, m), 1.36 (18H, s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 171.1, 155.4, 129.3, 129.2, 80.0, 71.3, 70.5, 70.4, 67.3, 54.7, 52.1, 28.5. R_f = 0.21 (2:1 hexane/ethyl acetate); $[\alpha]_D$ = +16.11 (CH_2Cl_2, c = 0.36); HRMS (FAB) calcd for $C_{22}H_{38}N_2O_{10}$ $[M-H]^+$ 491.2605, found 491.2605.



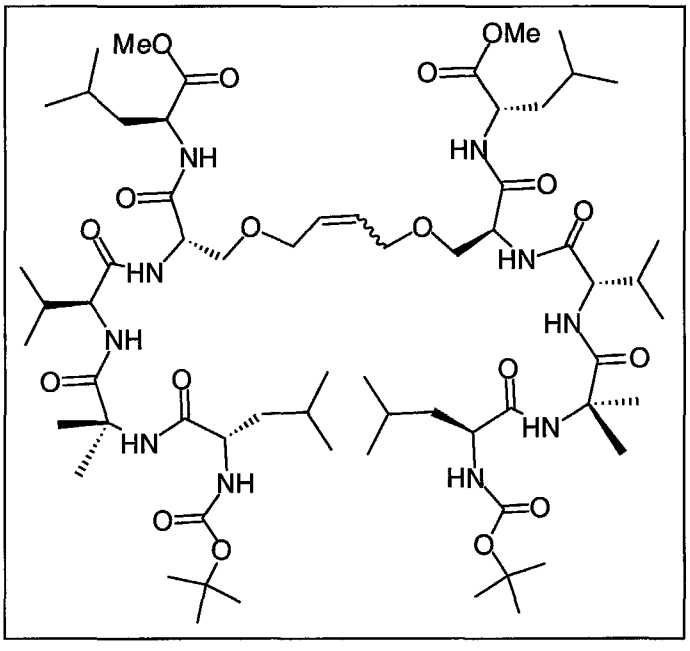
Compound 36. *N*-Boc- L-homoserine(*O*-Allyl) methyl ester (**35**) (223 mg, 0.82 mmol) and **1** (2.0 mg, 2.0 μmol, 0.3 mol %) were combined in a 1 dram vial. A magnetic stir bar was added to the vial, which was placed inside a vacuum chamber and held under vacuum (60-100 mtorr) accompanied by stirring for 48 hours at room temperature. The thick burgundy-colored oil steadily produced gas during the course of reaction. The reaction mixture was then dissolved in 0.5 mL CH_2Cl_2 and purified directly on a silica gel column (2x10 cm), eluting with 4:1, 2:1, and 1:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil that slowly crystallized over time was obtained (159 mg, 75% yield, 2.7:1 *trans/cis* as determined by the relative intensities of peaks at 129.4 and 129.3 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.65 (2H, m), 5.39 (2H, broad s), 4.29 (2H, m), 3.84 (4H, app s), 3.60 (6H, s), 3.44-3.38 (4H, broad m), 1.98-1.90 (4H, broad m), 1.34 (18H, broad s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 172.9, 155.4, 129.4, 129.3, 79.8, 71.0, 66.8, 66.6, 52.1, 32.3, 28.4. R_f = 0.12 (2:1 hexane/ethyl acetate); $[\alpha]_D$ = +2.14 (CH_2Cl_2, c = 0.35); HRMS (FAB) calcd for $C_{24}H_{42}N_2O_{10}$ $[M-H]^+$ 519.2918, found 519.2921.



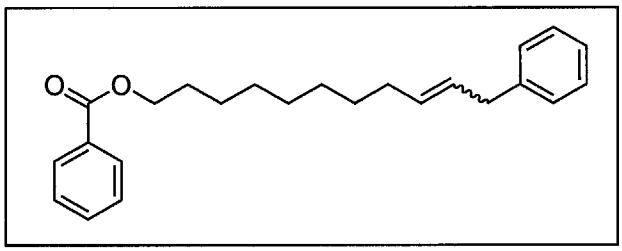
Compound 38. *N*-Boc-L-tyrosine(*O*-Allyl) methyl ester (**37**) (115 mg, 0.34 mmol) was added to a stirring solution of **1** (14 mg, 0.017 mmol, 5 mol %) in CH_2Cl_2 (3.3 mL). The flask was fitted with a condenser and refluxed under nitrogen for 14 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x12 cm), eluting with 8:1, 4:1, 2:1, and 1:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained which slowly crystallized over time (78.2 mg, 71% yield, 3:1 *trans/cis* as determined by integration of peaks at 6.04 and 5.89 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 7.01 (4H, d, J = 8.2 Hz), 6.81 (4H, d, J = 8.5 Hz), 6.04 (2H, app br s), 4.99 (2H, d, J = 7.7 Hz), 4.51 (6H, app br s), 3.68 (6H, s), 2.99 (4H, m), 1.39 (9H, br s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 172.5, 157.8, 157.6, 155.2, 130.5, 130.4, 128.7, 128.51, 128.47, 114.9, 101.8, 80.0, 67.8, 64.3, 62.2, 54.7, 52.3, 37.6, 28.4. R_f = 0.31 (2:1 hexane/ethyl acetate); $[\alpha]_D$ = +49.16 (CH_2Cl_2, c = 0.52); HRMS (FAB) calcd for $C_{34}H_{46}N_2O_{10}$ $[M+H]^+$ 643.3231, found 643.3220.



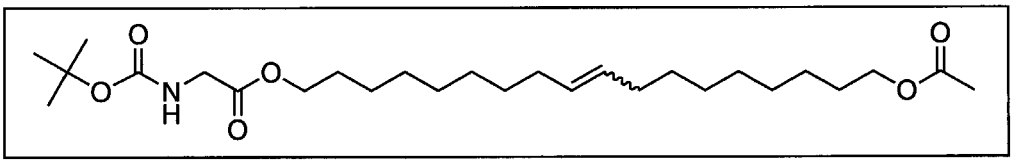
Compound 40. 2,3,4,6-tetra-*O*-benzyl-1-α-*C*-allylglucoside (**39**)[78] (133 mg, 0.24 mmol) was added to a stirring solution of **1** (9.7 mg, 0.012 mmol, 5 mol %) in CH_2Cl_2 (2.4 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x15 cm), eluting with 9:1, 5:1, 4:1, and 2:1 hexane/ethyl acetate (100 mL aliquots). A pale yellow, crystalline solid was obtained (121 mg, 93% yield, 2.5:1 *trans/cis* as determined by the relative intensities of peaks at 82.6 and 82.5 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 7.60-7.21 (40 H, br m), 5.61 (2H, m), 5.02 (2H, m), 4.89 (4H, m), 4.73 (6H, m), 4.54 (4H, m), 4.17 (2H, m), 3.84-3.70 (12H, br m), 2.52 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 138.9, 138.4, 138.3, 138.2, 128.55, 128.51, 128.1, 128.03, 127.99, 127.91, 127.87, 127.82, 127.76, 127.7, 127.5, 82.6, 82.5, 80.2, 80.1, 78.2, 75.6, 75.5, 75.2, 74.3, 74.2, 73.6, 73.2, 73.1, 71.5, 71.3, 69.0, 29.8, 28.8, 23.8. R_f = 0.59 (3:1 hexane/ethyl acetate); $[\alpha]_D$ = +58.85 (CH_2Cl_2, c = 0.53); LRMS (FAB) calcd for $C_{72}H_{76}O_{10}$ $[M-H]^+$ 1100.5, found 1102.0.



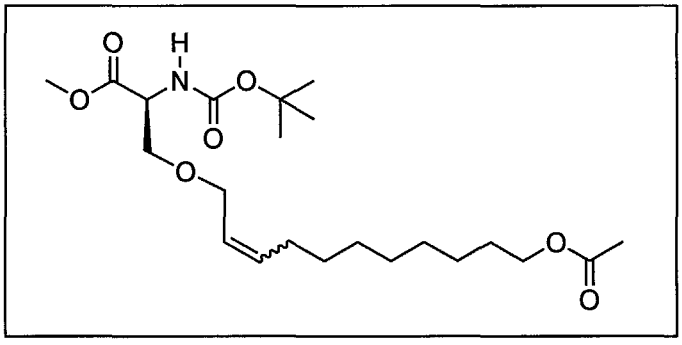
Compound 42. Pentapeptide **41** (100 mg, 0.10 mmol) was added to a stirring solution of **1** (1.2 mg, 1.0 μmol, 1 mol %) in CH_2Cl_2 (0.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 20 hours. The reaction mixture was then reduced in volume to 0.25 mL and purified directly on a silica gel column (2x10 cm), eluting with 1:3, 1:5 and 1:9 hexane/ethyl acetate (100 mL aliquots), and finally with 100% ethyl acetate (200 mL). An off-white crystalline solid was obtained (61 mg, 62% yield, 2.8:1 *trans/cis* as determined by the relative intensities of peaks at 129.1 and 129.0 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 7.47 (2H, d, J = 6.6 Hz), 7.27 (2H, d, J = 9.1 Hz), 7.11 (2H, d, J = 6.3 Hz), 6.83 (2H, s), 5.66 (2H, m), 5.37 (2H, m), 4.66 (2H, m), 4.54 (2H, m), 4.24 (2H, m), 4.05-3.93 (6H, br m), 3.81 (2H, m), 3.70 (8H, s), 2.42 (2H, m), 1.70-1.59 (12H, br m), 1.46 (30H, m), 0.99-0.89 (36H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 175.7, 175.6, 173.6, 173.5, 172.9, 171.7, 170.4, 170.3, 156.6, 129.1, 129.0, 80.8, 71.1, 69.7, 66.8, 60.4, 57.0, 55.0, 54.1, 52.3, 51.1, 40.7, 40.3, 29.8, 29.3, 28.4, 27.1, 24.8, 24.6, 24.0, 23.0, 22.97, 22.0, 21.8, 19.4, 17.3. R_f = 0.08 (3:1 ethyl acetate/hexane); $[\alpha]_D$ = -13.67 (CH_2Cl_2, c = 0.34); LRMS (FAB) calcd for $C_{64}H_{114}N_{10}O_{18}$ $[(M)\text{-Boc}]^+$ 1211.8, found 1211.9.



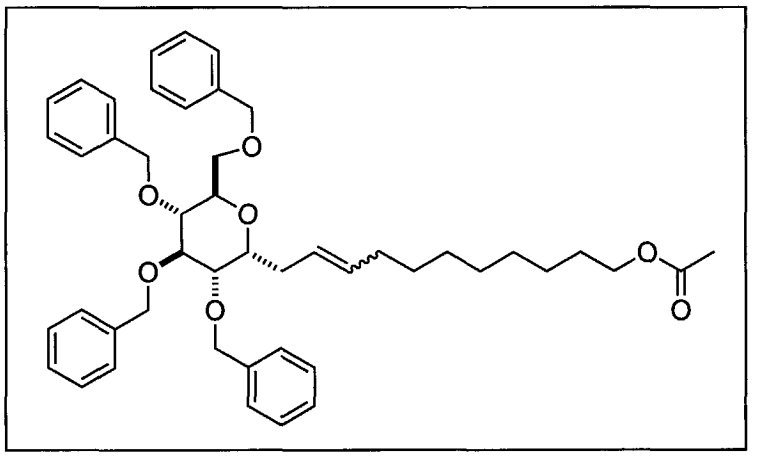
Compound 43. 9-Decen-1-yl benzoate (**3**) (75 µl, 0.27 mmol) was added *via* syringe to a stirring solution of allyl benzene homodimer **26** (114 mg, 0.55 mmol) and **1** (11.3 mg, 0.014 mmol, 5 mol %) in CH_2Cl_2 (2.7 mL). The flask was fitted with a condenser and refluxed under nitrogen for 20 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x10 cm), eluting with 99:1, 49:1, and 20:1 hexane/ethyl acetate (100 mL aliquots). A clear, colorless oil was obtained (64.7 mg, 68% yield, 3.7:1 *trans/cis* as determined by integration of peaks at 3.33 and 3.40 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.06 (2H, d, J = 7.1 Hz), 7.55 (1H, t, J = 7.4 Hz), 7.44 (2H, t, J = 7.8 Hz), 7.28 (3H, m), 7.19 (2H, apparent d, J = 2.6 Hz), 5.59-5.49 (2H, br m), 4.32 (2H, t, J = 6.7 Hz), 3.33 (2H, d, J = 6.1 Hz), 2.02 (2H, m), 1.76 (2H, m), 1.43-1.26 (10H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.8, 141.3, 132.9, 132.2, 131.1, 130.7, 129.7, 128.9, 128.63, 128.55, 128.5, 128.2, 126.1, 126.02, 125.98, 112.3, 65.3, 39.2, 33.7, 32.7, 29.9, 29.8, 29.6, 29.5, 29.4, 29.3, 28.9, 27.4, 26.2. R_f = 0.57 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{24}H_{30}O_2$ $[M]^+$, 350.2246, found 350.2806.



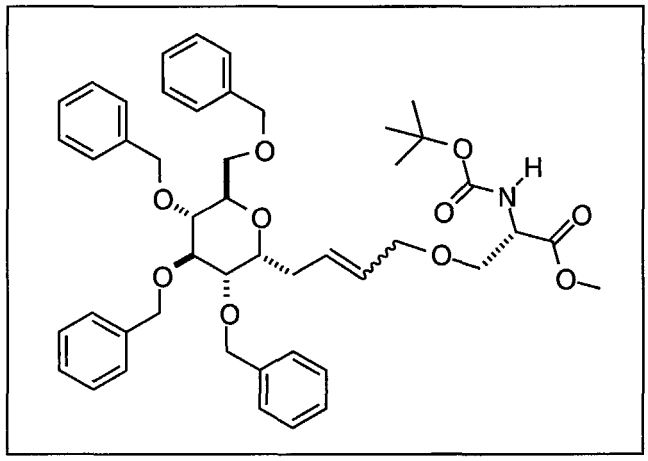
Compound 44. 9-Decen-1-yl *N*-Boc-glycinate (**23**) (61.7 mg, 0.20 mmol) was added to a stirring solution of bis-acetate **20** (147 mg, 0.40 mmol) and **1** (7.9 mg, 0.010 mmol, 5 mol %) in CH_2Cl_2 (1.9 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1 and 2:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained (68.2 mg, 72% yield, 3.5:1 *trans/cis* as determined by the relative intensities of peaks at 130.46 and 129.99 in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.33 (2H, m), 5.04 (1H, br s), 4.10, (2H, t, J = 6.7 Hz), 4.01 (2H, t, J = 6.8 Hz), 3.86 (2H, d, J = 5.2 Hz), 2.02 (3H, masked s), 2.04-1.93 (4H, br m), 1.56 (4H, m), 1.45 (9H, masked s), 1.35-1.25 (20H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 171.3, 170.6, 155.9, 130.48, 130.46, 130.01, 129.99, 80.0, 65.6, 64.8, 42.6, 32.7, 29.9, 29.73, 29.66, 29.55, 29.53, 29.5, 29.48, 29.4, 29.34, 29.32, 29.2, 28.8, 28.7, 28.5, 27.3, 26.2, 26.0, 25.96, 21.1. R_f = 0.23 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{27}H_{49}NO_6$ $[(M-H)-Boc]^+$ 384.3114, found 384.3114.



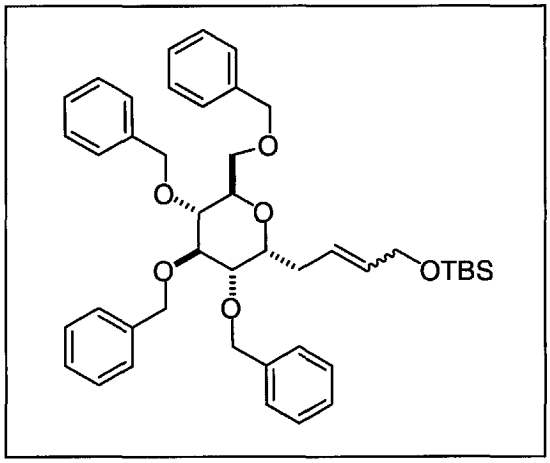
Compound 45. *N*-Boc-serine(*O*-Allyl) methyl ester (**33**) (100 mg, 0.39 mmol) was added to a stirring solution of bis-acetate **20** (284 mg, 0.77 mmol) and **1** (16 mg, 0.019 mmol, 5 mol %) in CH_2Cl_2 (3.9 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x10 cm), eluting with 4:1, 2:1 and 1:1 hexane/ethyl acetate (100 mL aliquots). A pale yellow, viscous oil was obtained (142 mg, 86% yield, 6:1 *trans/cis* as determined by the relative intensities of peaks at 135.0 and 134.1 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.58-5.52 (1H, broad m), 5.39-5.32 (1H, broad m), 5.34 (1H, d), 4.31 (1H, m), 3.95 (2H, t, J = 6.7 Hz), 3.82 (2H, m), 3.70 (1H, m), 3.65 (3H, s), 3.52 (1H, m), 1.94 (3H, s), 1.97-1.92 (2H, broad m), 1.53-1.48 (2H, broad m), 1.35 (9H, broad s), 1.38-1.20 (10H, broad m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 171.2, 170.8, 155.5, 135.0, 134.1, 125.9, 125.6, 79.8, 72.1, 69.9, 69.7, 67.0, 64.5, 62.3, 54.4, 52.2, 32.8, 32.2, 29.5, 29.3, 29.2, 29.1, 28.7, 28.4, 27.6, 26.0, 20.8. R_f = 0.12 (9:1 hexane/ethyl acetate); $[\alpha]_D$ = +7.59 (CH_2Cl_2, c = 0.34); HRMS (FAB) calcd for $C_{22}H_{39}NO_7$ $[M-H]^+$ 430.2805, found 430.2810.



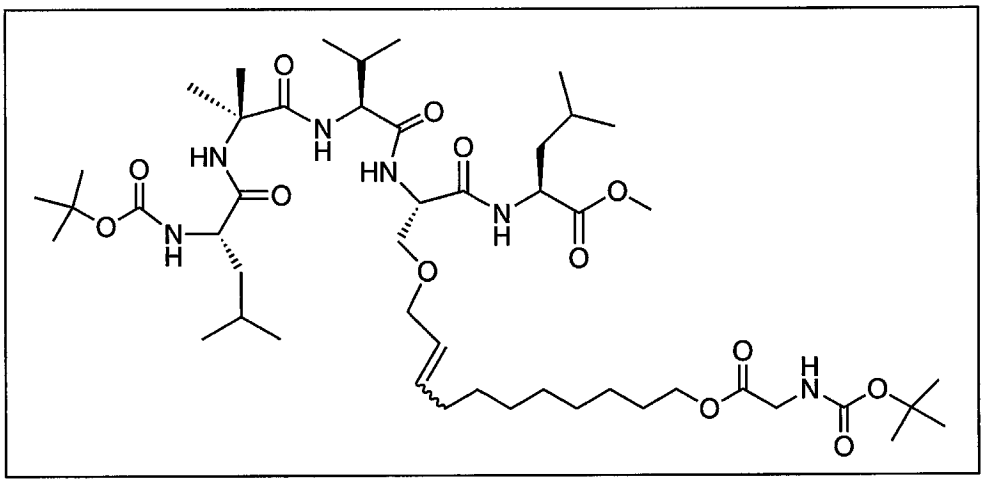
Compound 46. 2,3,4,6-tetra-*O*-benzyl-1-α-*C*-allylglucoside (**39**) (75 mg, 0.13 mmol) was added to a stirring solution of bis-acetate **20** (96 mg, 0.26 mmol) and **1** (5.4 mg, 6.6 μmol, 5 mol %) in CH_2Cl_2 (1.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 6 hours. The reaction mixture was then reduced in volume to 0.25 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1 and 2:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained (72 mg, 73% yield, 2.8:1 *trans/cis* as determined by analysis of methyl intensities at 2.04 and 2.03 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 7.52-7.22 (18H, br m), 7.14 (2H, m), 5.56-5.37 (2H, br m), 4.94 (1H, m), 4.81 (2H, m), 4.70-4.61 (3H, br m), 4.49-4.46 (2H, br m), 4.11-4.03 (3H, br m), 3.82-3.69 (3H, br m), 3.67-3.60 (3H, br m), 2.45-2.41 (2H, br m), 2.04 (3H, masked s), 1.98 (2H, apparent masked q, J = 6.4 Hz), 1.62 (2H, quint, J = 6.7 Hz), 1.32-1.28 (10H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 171.4, 139.1, 138.6, 138.5, 138.4, 133.2, 132.1, 128.6, 128.5, 128.2, 128.1, 128.0, 127.93, 127.90, 127.8, 126.0, 125.5, 82.7, 82.67, 80.5, 78.5, 75.7, 75.6, 75.3, 74.6, 74.3, 73.7, 73.4, 73.2, 71.6, 71.3, 69.3, 64.8, 32.9, 29.9, 29.7, 29.6, 29.5, 29.3, 28.9, 27.9, 26.2, 23.5, 21.2. R_f = 0.14 (9:1 hexane/ethyl acetate); HRMS (FAB) calcd for $C_{47}H_{58}O_7$ $[M+H]^+$ 735.4261, found 735.4242.



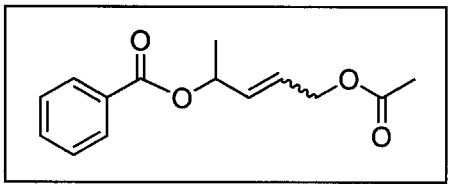
Compound 47. 2,3,4,6-tetra-*O*-benzyl-1-α-*C*-allylglucoside (**39**) (58 mg, 0.01 mmol) was added to a stirring solution of *N*-Boc-L-serine(*O*-Allyl)-OMe dimer **34** (100 mg, 0.02 mmol) and **1** (4.2 mg, 5 μmol, 5 mol %) in CH_2Cl_2 (1.0 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.25 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 4:1 and 2:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained (30.1 mg, 37% yield, 5:1 *trans/cis* as determined by the relative intensities of peaks at 28.8 and 28.6 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 7.32-7.25 (18H, broad m), 7.11 (2H, m), 5.64 (1H, m), 5.55 (1H, m), 5.34 (1H, d, J = 8.4 Hz), 4.92 (1H, d, J = 10.9 Hz), 4.79 (2H, d, J = 10.7 Hz), 4.69 (1H, d, J = 11.6 Hz), 4.60 (2H, d, J = 11.1 Hz), 4.46 (1H, d, J = 4.0 Hz), 4.44 (1H, d, J = 2.2 Hz), 4.39 (1H, m), 4.07 (1H, m), 3.88 (2H, app d, J = 5.8 Hz), 3.73 (3H, s), 3.79-3.69 (4H, broad m), 3.68-3.56 (4H, broad m), 2.46 (2H, m), 1.44 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 171.4, 159.8, 139.2, 138.8, 138.6, 130.9, 128.6, 128.53, 128.47, 128.1, 128.02, 127.97, 127.81, 127.76, 127.7, 82.6, 80.4, 78.7, 75.4, 75.1, 74.4, 74.1, 73.8, 73.6, 73.4, 72.2, 71.9, 70.0, 69.7, 67.5, 54.6, 52.4, 28.8, 28.6, 27.2. R_f = 0.50 (2:1 hexane/ethyl acetate); $[\alpha]_D$ = +39.95 (CH_2Cl_2, c = 0.33); HRMS (FAB) calcd for $C_{47}H_{57}NO_{10}$ $[M-H]^+$ 796.4061, found 796.4059.



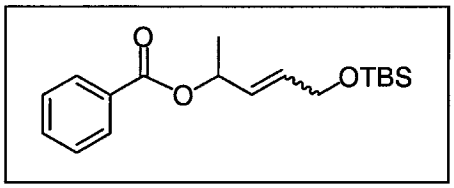
Compound 48. 2,3,4,6-tetra-O-benzyl-α-C-allyl glucoside (**39**) (100 mg, 0.18 mmol) was added to a stirring solution of *cis*-1,4-bis-(tert-butyldimethylsilyloxy)but-2-ene (130 μL, 0.36 mmol) and **1** (7.2 mg, 0.0087 mmol, 5 mol %) in CH_2Cl_2 (2.0 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 1.0 mL and applied to a silica gel column (2x10 cm), eluting with CH_2Cl_2, followed by 4:1 CH_2Cl_2:ethyl acetate (100 mL aliquots). Fractions containing the desired product were pooled and evaporated to yield a clear, colorless, viscous oil (89 mg, 70% yield, 9:1 *trans/cis* as determined by integration of peaks at 4.07 and 4.18 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.03 (2H, app d, J = 7.0 Hz), 7.53 (1H, tt, J = 7.4, 1.4 Hz), 7.41 (2H, t, J = 8.0 Hz), 5.70-5.50 (2H, m), 4.30 (2H, t, J = 6.7 Hz), 4.07 (2H, dd, J = 0.7, 5.5 Hz), 2.02 (2H, q, J = 6.8 Hz), 1.78 (1H, s), 1.77 (2H, quint, J = 7.9 Hz), 1.45-1.28 (10H, m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.6, 133.2, 132.9, 132.7, 130.5, 129.5, 128.9, 128.5, 128.2, 65.0, 63.7, 58.5, 32.1, 29.5, 29.3, 29.1, 29.0, 28.99, 28.7, 27.3, 25.9. R_f = 0.64 (3:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{18}H_{26}O_3$ $[M-H]^+$ 290.1882, found 289.1804.



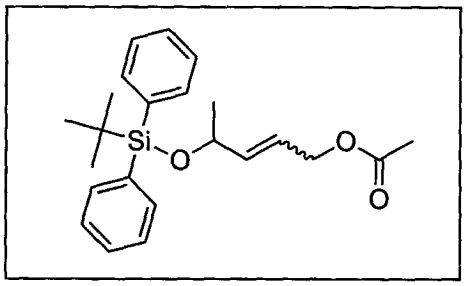
Compound 49. Pentapeptide **41** (50 mg, 0.075 mmol) was added to a stirring solution of 9-Decen-1-yl *N*-Boc-glycinate dimer **24** (89 mg, 0.149 mmol) and **1** (3.1 mg, 0.037 mmol, 5 mol %) in CH_2Cl_2 (0.75 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.25 mL and purified directly on a silica gel column (2x8 cm), eluting with 3:1, 2:1, 1:1, and 1:2 hexane/ethyl acetate (100 mL aliquots), and finally flushing with 100% ethyl acetate (200 mL). A pale yellow oil was obtained (46.9 mg, 66% yield, 5.5:1 *trans/cis* as determined by the relative intensities of peaks at 134.7 and 133.4 in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 7.45 (1H, d, J = 5.7 Hz), 7.23 (1H, d, J = 8.6 Hz), 7.07 (1H, d, J = 5.9 Hz), 6.85 (1H, s), 5.58 (1H, m), 5.44 (1H, m), 5.32 (1H, br s), 5.05 (1H, br s), 4.64 (1H, m), 4.53 (1H, m), 4.18 (1H, m), 4.09 (2H, m), 4.03 (1H, m), 3.88 (4H, m), 3.77 (1H, m), 3.69 (1H, m), 3.67 (3H, masked s), 2.64 (1H, m), 1.94 (2H, m), 1.63-1.55 (6H, br m), 1.47-1.42 (26 H, br m), 1.35-1.21 (10H, br m), 0.98-0.86 (18H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 174.8, 173.3, 172.9, 171.5, 170.5, 170.2, 156.4, 134.7, 133.4, 126.4, 80.98, 71.9, 69.5, 69.3, 67.0, 65.6, 60.1, 57.6, 54.6, 53.4, 52.1, 51.3, 42.9, 41.3, 40.8, 32.4, 29.8, 29.7, 29.5, 29.3, 29.29, 28.8, 28.6, 28.5, 27.8, 26.6, 26.0, 25.2, 25.0, 24.8, 23.1. R_f = 0.42 (3:1 ethyl acetate/hexane); $[\alpha]_D$ = -13.72 (CH_2Cl_2, c = 0.38); HRMS (FAB) calcd for $C_{48}H_{86}N_6O_{13}$ $[(M-H)-Boc]^+$ 855.5807, found 855.5800.



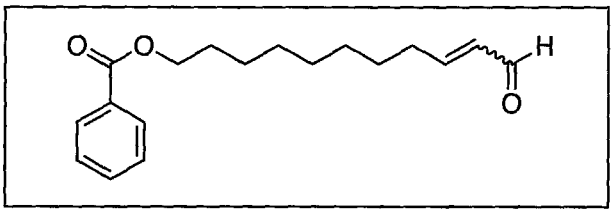
Compound 51. 3-Buten-2-yl benzoate (**50**)[63] (80 μl, 0.49 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(acetyloxy)but-2-ene (155 μl, 0.98 mmol) and **1** (20 mg, 0.025 mmol, 5 mol %) in CH_2Cl_2 (4.9 mL). The flask was fitted with a condenser and refluxed under nitrogen for 15 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x12 cm), eluting with 9:1, 7:1, 4:1, and 3:1 hexane/ethyl acetate (100 mL aliquots). A clear, colorless oil was obtained (36.5 mg, 30% yield, 16:1 *trans/cis* as determined by the relative intensities of peaks at 70.6 and 67.7 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.04 (2H, d, J = 7.9 Hz), 7.54 (1H, t, J = 7.3 Hz), 7.43 (2H, t, J = 7.8 Hz), 5.87 (2H, m), 5.63 (1H, m), 4.57 (2H, d, J = 3.6 Hz), 2.06 (3H, s), 1.44 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.9, 165.9, 133.8, 133.1, 130.6, 129.8, 128.5, 126.3, 125.9, 70.6, 67.7, 64.2, 60.9, 29.8, 21.1, 21.0, 20.4. R_f = 0.29 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{14}H_{16}O_4$ $[M]^+$ 248.1049, found 248.1041.



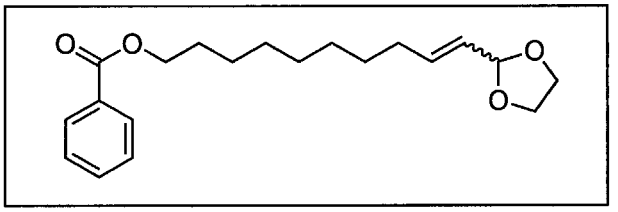
Compound 52. 3-Buten-2-yl benzoate (**50**) (80 μl, 0.49 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(tert-butyldimethylsilyloxy)but-2-ene (361 μl, 0.98 mmol) and 1 (20 mg, 0.025 mmol, 5 mol %) in CH_2Cl_2 (4.9 mL). The flask was fitted with a condenser and refluxed under nitrogen for 24 hours. The crude reaction mixture was then reduced in volume to 0.5 mL and applied directly to a silica gel column (2x12 cm), eluting with 9:1, 7:1, 4:1, and 3:1 hexane/ethyl acetate (100 mL aliquots). A pale brown, viscous oil was obtained, which was directly treated with TBAF (0.98 mL of a 1M solution in THF) to effect cleavage of the TBDMS group. The solution was stirred for one hour at 25° C until TLC analysis indicated that the deprotection was complete. The reaction mixture was reduced in volume to 0.3 mL and purified directly on a silica gel column (2x10 cm), eluting with 9:1, 3:1, 2:1, and 1:1 hexane/ethyl acetate. A clear, viscous oil (**53**) was isolated (55 mg, 54% yield over 2 steps, 47:1 *trans/cis* as determined by the relative intensities of the peaks at 20.5 and 20.9 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 8.02 (2H, d, J = 8.4 Hz), 7.52 (1H, t, J = 7.5 Hz), 7.40 (2H, t, J = 7.8 Hz), 5.92 (1H, m), 5.81 (1H, m), 5.59 (1H, quint, J = 6.1 Hz), 4.14 (2H, d, J = 4.9 Hz), 2.31 (1H, br s), 1.43 (3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.1, 133.1, 132.7, 132.3, 131.9, 131.2, 130.7, 130.67, 130.1, 129.7, 128.5, 128.2, 127.8, 127.7, 127.5, 127.3, 124.4, 121.7, 115.0, 71.1, 62.8, 55.6, 29.8, 20.9, 20.5. R_f = 0.24 (3:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{12}H_{14}O_3$ $[M]^+$ 206.0943, found 206.0939.



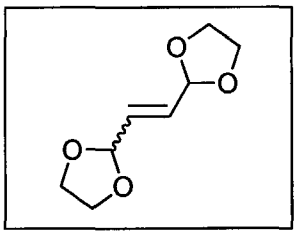
Compound 55. 3-Buten-2-*O*-tert-butyldiphenylsilyl ether (**54**)[79] (80 mg, 0.258 mmol) was added *via* syringe to a stirring solution of *cis*-1,4-bis-(acetyloxy)but-2-ene (81 µl, 0.516 mmol) and **1** (10.6 mg, 0.013 mmol, 5 mol %) in CH_2Cl_2 (2.58 mL). The flask was fitted with a condenser and refluxed under nitrogen for 20 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x12 cm), eluting with 9:1, 4:1, and 2:1 hexane/ethyl acetate (100 mL aliquots). A clear, viscous oil was obtained (23 mg, 23% yield, 7.5:1 *trans/cis* as determined by the relative intensities of peaks at 69.6 and 66.1 ppm in the ^{13}C NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 7.67-7.62 (4H, br m), 7.41-7.33 (6H, br m), 5.73 (1H, m), 5.56 (1H, m), 4.45 (2H, d, J = 6.0 Hz), 4.43 (1H, m), 2.04 (3H, s), 1.14 (3H, d, J = 6.3 Hz), 1.05 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.9, 139.0, 138.9, 136.1, 136.06, 136.0, 134.6, 134.2, 129.8, 129.76, 129.7, 127.8, 127.7, 127.66, 122.8, 122.0, 112.9, 69.9, 66.1, 64.7, 60.5, 29.9, 27.2, 27.1, 24.6, 24.2, 21.2, 19.4. R_f = 0.49 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{23}H_{30}O_3Si$ $[M-H]^+$ 381.1886, found 381.1883.



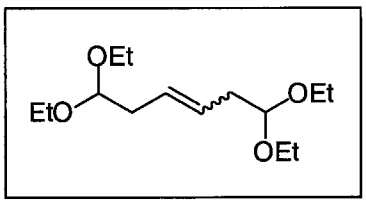
Compound 58. 9-Decen-1-yl benzoate (**3**) (140 µl, 0.50 mmol) and acrolein diethyl acetal (**56**) (153 µl, 1.0 mmol) were added *via* syringe to a stirring solution of **1** (10.4 mg, 0.013 mmol, 2.5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was allowed to come to room temperature and excess 98% formic acid (0.5 mL, 13 mmol) was added. The orange brown solution was allowed to stir for 1.5 hrs until TLC analysis indicated complete cleavage of the acetal group.[80] The reaction mixture was then reduced in volume to 0.75 mL and purified directly on a silica gel column (2x15 cm), eluting first with CH_2Cl_2 (90 mL), and then 9:1 and 4:1 CH_2Cl_2:ethyl acetate (100 mL aliquots). A clear yellow liquid **58** was obtained (120.5 mg, 82% yield). Aldehyde **58** (110 mg, 0.38 mmol) was dissolved 2:1 EtOH:H_2O (7.5 mL) at room temperature. $CeCl_3 \cdot 7H_2O$ (142 mg, 0.38 mmol) was added and the solution was stirred for 10 minutes to effect dissolution. The solution was then cooled to 0 °C and $NaBH_4$ (25 mg, 0.66 mmol) was added slowly in portions. The reaction mixture was allowed to stir at 0 °C for 30 minutes after which TLC analysis showed complete reduction of aldehyde **58** to allylic alcohol **7**.[46] The reaction was quenched by dilution with Et_2O (10 mL), followed by slow addition of a saturated aqueous $NaHCO_3$ solution (10 mL). The biphasic mixture was allowed to stir for two hours, after which the organic layer was removed, washed with brine, dried over $MgSO_4$, and concentrated to yield a light yellow oil. The oil was diluted to 0.5 mL in CH_2Cl_2 and purified directly on a silica gel column (2x15 cm), eluting with 2:1 CH_2Cl_2:ethyl acetate (300 mL). A clear, colorless oil was obtained that exhibited spectral properties identical to alcohol **7** (107 mg, 80% yield over 2 steps, 26:1 *trans/cis* as determined by integration of peaks at 4.07 and 4.18 ppm in the ^1H NMR spectrum).



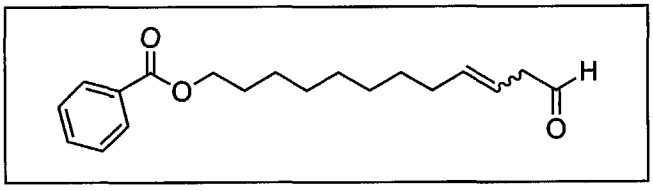
Compound 62. 9-Decen-1-yl benzoate (**3**) (140 mL, 0.50 mmol) and 2-vinyl-1,3-dioxolane (**61**) (100 mL, 1.0 mmol) were added *via* syringe to a stirring solution of 1 (10.4 mg, 0.013 mmol, 2.5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 0.75 mL and purified directly on a silica gel column (2x15 cm), eluting first with CH_2Cl_2 (90 mL), and then 9:1 and 4:1 CH_2Cl_2:ethyl acetate (100 mL aliquots). A clear yellow liquid was obtained (156.4 mg, 93% yield, 8.6:1 *trans/cis* as determined by integration of peaks at 1.91 and 2.12 ppm in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.19 (2H, m), 7.16-7.06 (3H, br m), 5.87 (1H, m), 5.71 (1H, m), 5.27 (1H, d, J = 6.1 Hz), 4.21 (2H, t, J = 6.1 Hz), 3.60 (2H, m), 3.43 (2H, m), 1.91 (2H, m), 1.51 (2H, quint, J = 6.8 Hz), 1.40-1.02 (10H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.3, 136.5, 132.7, 131.3, 129.8, 128.5, 128.2, 128.0, 127.8, 127.4, 104.5, 99.7, 65.0, 64.8, 32.2, 29.7, 29.6, 29.4, 29.3, 29.2, 29.0, 28.9, 28.1, 26.2. R_f = 0.35 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{20}H_{28}O_4$ $[M]^+$ 332.1988, found 332.1982.



Compound 66. 2-Vinyl-1,3-dioxolane (**61**) (1 g, 9.99 mmol) was added *via* syringe to a stirring solution of **1** (205 mg, 0.25 mmol, 2.5 mol %) in CH_2Cl_2 (49 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (4x20 cm), eluting with 4:1, 2:1 and 1:1 hexane/ethyl acetate (250 mL aliquots). An off-white crystalline solid was obtained (451 mg, 52% yield, 9.2:1 *trans/cis* as determined by the relative intensities of peaks at 131.5 and 124.1 ppm in the ^{13}C NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 5.89 (2H, m), 5.32 (2H, m), 4.01-3.85 (8H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 131.5, 124.1, 102.4, 72.8, 65.2, 65.1, 47.1. R_f = 0.08 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_8H_{14}O_4$ $[M-H]^+$ 171.0657, found 171.0659.



Compound 68. 3-Butenal diethyl acetal (**67**) (450 μl, 2.65 mmol) was added to a stirring solution of **1** (60.7 mg, 0.074 mmol, 2.8 mol %) in CH_2Cl_2 (14.8 mL). The flask was fitted with a condenser and refluxed under nitrogen for 12 hours. The reaction mixture was then reduced in volume to 0.5 mL and purified directly on a silica gel column (2x15 cm), eluting with 19:1, 9:1, and 4:1 hexane/ethyl acetate (100 mL aliquots, ca. 2 mL of Et_3N added to each aliquot to prevent deprotection of the acetal on the column). A pale yellow oil was obtained (127 mg, 37% yield, 1.1:1 *trans/cis* as determined by integration of peaks at 2.28 and 2.32 ppm in the ^1H NMR spectrum). ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 5.44 (2H, m), 4.42 (2H, m), 3.46-3.40 (8H, br m), 2.28 (4H, t, J = 3.9 Hz), 1.13 (12H, t, J = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 127.8, 126.4, 102.7, 102.6, 61.4, 61.2, 37.4, 32.4, 29.8, 15.4. R_f = 0.30 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{14}H_{28}O_4$ $[M-H]^+$ 259.1909, found 259.1904.



Compound 70. 9-Decen-1-yl benzoate (**3**) (140 μl, 0.50 mmol) was added *via* syringe to a stirring solution of 3-butenal diethyl acetal (**67**) (171 μl, 1.0 mmol) and **1** (10.4 mg, 0.013 mmol, 2.5 mol %) in CH_2Cl_2 (2.5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 hours. The reaction mixture was then allowed to come to room temperature, after which excess 98% formic acid (340 μl, 5.0 mmol) was added to effect cleavage of the diethyl acetal. The reaction was stirred at room temperature for 2 hours until the reaction was judged complete by TLC. The reaction was quenched by dilution with CH_2Cl_2 (10 mL), followed by slow addition of a saturated aqueous $NaHCO_3$ solution (10 mL). The biphasic mixture was allowed to stir for two hours, after which the organic layer was removed, washed with brine, dried over $MgSO_4$, and concentrated to yield a light brown oil. The reaction mixture was then diluted to 0.5 mL with CH_2Cl_2 and purified directly on a silica gel column (2x12 cm), eluting with 19:1, 9:1, 6:1, and 4:1 hexane/ethyl acetate (100 mL aliquots). A clear viscous oil (**70**) was obtained (28 mg, 18% yield over 2 steps, 1.3:1 *trans/cis* as determined by integration of peaks at 3.10 and 3.17 ppm in the ^1H NMR spectrum), which gradually isomerized to the α,β-unsaturated aldehyde over time. ^1H NMR (500 MHz, $CDCl_3$, ppm): δ 9.64 (1H, t, J = 2.1 Hz), 8.03 (2H, d, J = 7.1 Hz), 7.54 (1H, t, J = 7.4 Hz), 7.43 (2H, t, J = 7.8 Hz), 5.62-5.45 (2H, br m), 4.31 (2H, t, J = 7.0 Hz), 3.10 (2H, d, J = 6.0 Hz), 2.04 (2H, quint, J = 6.6 Hz), 1.75 (2H, quint, J = 6.8 Hz), 1.43-1.24 (10H, br m). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 166.9, 137.1, 135.7, 133.0, 129.8, 128.5, 119.3, 118.3, 65.3, 47.5, 42.8, 32.9, 29.6, 29.5, 29.4, 29.36, 29.3, 29.2, 28.9, 27.8, 26.2. R_f = 0.33 (9:1 hexane/ethyl acetate); HRMS (EI) calcd for $C_{19}H_{26}O_3$ $[M]^+$ 302.1882, found 302.1874.

References and Notes

† Portions of this chapter were previously reported in separate publications. See: (a) O'Leary, D. J.; Blackwell, H. E.; Washenfelder, R. A.; Grubbs, R. H. *Tetrahedron Lett.* **1998**, *39*, 7427-7430. (b) O'Leary, D. J.; Blackwell, H. E.; Washenfelder, R. A.; Miura, K.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 1091-1094.

(1) For general olefin metathesis references, see: (a) Ivin, K. J.; Mol, J. C. *Olefin Metathesis and Metathesis Polymerization*; 2nd Ed.; Academic: San Diego, 1997. (b) Grubbs, R.H.; Pine, S.H. In *Comprehensive Organic Synthesis*; Trost, B.M., Ed.; Pergamon, New York: 1991, Volume 5, Chapter 9.3.

(2) For details of the present accepted mechanism of olefin metathesis involving formation of a metallocyclobutane intermediate, see: Herrison, J. L.; Chauvin, Y. *Makromol. Chem.* **1971**, *141*, 161-176.

(3) For leading references, see: (a) reference 1a. (b) Feldman, J.; Schrock, R. R. *Prog. Inog. Chem.* **1991**, *39*, 1-74. (c) Grubbs, R. H.; Tumas, W. *Science* **1989**, *243*, 907-915.

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(7) Issues of poor selectivity plagued early CM efforts employing "classical" catalysts. For a recent review of CM employing "classical" catalysts, see: Finkel'shtein, E. S.; Bykov, V. I.; Portnykh, E. B. *J. Mol. Catal.* **1992**, *76*, 33-52.

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(18) For a novel variant of ROM including a tandem RCM reaction, see: Stragies, R.; Blechert, S. *Synlett* **1998**, 169-170.

(19) (a) Boger, D. L.; Chai, W; Ozer, R. S.; Anderson, C.- M. *Biorg. Med. Chem. Lett.* **1997**, *7*, 463-468. (b) Boger, D. L.; Chai, W. *Tetrahedron* **1998**, *54*, 3955-3970. (c) Boger, D. L.; Chai, W.; Jin, Q. *J. Am. Chem. Soc.* **1998**, *120*,

7220-7225. (d) Giger, T.; Wigger, M.; Audétat, S.; Benner, S. A. *Synlett* **1998**, 688-691. (e) Brändli, C.; Ward, T. R. *Helv. Chim. Acta* **1998**, *81*, 1616-1621.

(20) For a recent report from these laboratories, see: Hillmyer, M. A.; Nguyen, S. T.; Grubbs, R. H. *Macromolecules* **1997**, *30*, 718-721, and references cited therein.

(21) Details of the origin or synthesis of all of the starting materials, full experimental details of the CM reactions, and full characterization of the heterodimeric CM products (^{1}H NMR, ^{13}C NMR, and HRMS) can be found in the Experimental Section.

(22) This is the exact opposite effect that Blechert *et al.* observed in the ROM of cyclic olefins with monosubstituted olefins versus disubstituted olefins. A large excess (up to 10-fold) of the less reactive disubstituted olefin was required to suppress the ROMP of the strained cyclic olefin substrates, while only 1 equivalent of the corresponding monosubstituted olefin was required to effect analogous yields. See reference 17b.

(23) Ullman, M.; Grubbs, R. H. *Organometallics* **1998**, *17*, 2484-2489.

(24) This corroborated well with the observation of Blechert *et al.* that *trans* disubstituted internal olefins are reactive substrates for ROM. See reference 17b.

(25) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815-3818.

(26) To our knowledge, this is the first example of employing a Weinreb amide functionalized substrate in an olefin metathesis reaction with catalyst **1**.

(27) A similar two-step procedure has been implemented for telechelic polymer syntheses *via* ROMP coupled with CM. See: (a) reference 20. (b) Nubel, P. O.; Yokelson, H. B.; Lutman, C. A.; Bouslog, W. G.; Behrends, R. T.; Runge, K. D. *J. Mol. Catal. A* **1997**, *115*, 43-50.

(28) The self-metathesis of terminal olefins employing "classical" olefin metathesis catalysts has been utilized previously in the synthesis of symmetrically substituted

olefins. The majority of these applications involved the synthesis of structurally simple, aliphatic internal alkenes. See: (a) Marciniec, B.; Gulinski, J. *J. Organomet. Chem.* **1984**, *266*, C19-C21. (b) Marciniec, B.; Maciejewski, H.; Gullinski, J.; Rzejak, Z. *J. Organomet. Chem.* **1989**, *362*, 273-279. (c) Marciniec, B.; Pietraszuk, C.; Foltynowicz, Z. *J. Organomet. Chem.* **1994**, *474*, 83-87.

(29) Moderate *trans* selectivity has been consistently observed in almost all metathesis accounts employing **1** to date. This selectivity is consistent with preferential formation of *trans*-α,β-disubstituted metallocyclobutane intermediates. We have made the assumption that the predominant olefin regioisomer for these symmetrical homodimers is *trans* in our NMR spectroscopic analyses.

(30) Allyl benzene (**25**) has been previously reported by Benner *et al.* to be an excellent substrate for the synthesis of combinatorial libraries *via* CM. See reference 19d.

(31) (*O*)-allyl ethers of L-serine, L-homoserine, and L-tyrosine have been previously employed in the synthesis of peptide macrocycles *via* RCM. See: (a) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606-9614 (b) Blackwell, H. E.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **1998**, *37*, 3281-3284.

(32) For recent CM and RCM applications in carbohydrate synthesis, see: (a) reference 12. (b) Feng, J.; Schuster, M.; Blechert, S. *Synlett* **1997**, 129-130. (c) El Sukkari, H.; Gesson, J.- P.; Renoux, B. *Tetrahedron Lett.* **1998**, *39*, 4043-4046. (d) Fürstner, A.; Müller, T. *J. Org. Chem* **1998**, *63*, 424-425. (e) Calimente, D.; Postema, M. H. D. *J. Org. Chem.* **1999**, *64*, 1770-1771. For a recent example of sugar dimer synthesis *via* Suzuki coupling, see: (f) Johns, B. A.; Johnson, C. R. *Tetrahedron Lett.* **1998**, *39*, 749-752.

(33) For leading references to peptide RCM, see references 4a and 31b.

(34) Terminal olefin derived sugars and amino acids have previously been employed in CM with other terminal olefins. See references 12 and 32b.

(35) For recent syntheses of carbon-carbon linked glycosyl amino acids, see: (a) Dondoni, A.; Marra, A.; Massi, A. *Chem Commun.* **1998**, 1741-1742. (b) Dondoni, A.; Massi, A.; Marra, A. *Tetrahedron Lett.* **1998**, *39*, 6601-6604.

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(45) For a recent example of an acrolein acetal used in an RCM reaction, see: (a) Crimmins, M. T.; King, B. W. *J. Am. Chem. Soc.* **1998**, *120*, 9084-9085. For the recent report of the ROM of cyclopropenone ketal with terminal olefins, see: (b) Michaut, M.; Parrain, J.- L.; Santelli, M. *Chem. Commun.* **1998**, 2567-2568.

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(48) Prepared *via* a literature procedure. See: Gassman, P. G.; Burns, S. J.; Pfister, K. B. *J. Org. Chem.* **1993**, *58*, 1449-1457.

(49) For an example of an asymmetric Simmons-Smith reaction, see: Mori, A.; Arai, I.; Yamamoto, H. *Tetrahedron* **1986**, *42*, 6447-6458.

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(51) The *trans/cis* ratio was determined by ^{1}H NMR of the crude tartrate CM product **64**. The yield of tartrate CM product **64** was determined after acid hydrolysis of the acetal to afford aldehyde **58**.

(52) Methyl 10-undecylenate (**21**) was employed for this study because it was observed to be more amendable to rapid GC-MS analysis (i.e. significantly lower retention time) than 9-decen-1-yl benzoate (**3**). Heterodimer **65** was not completely characterized.

(53) Cyclic diacetals of fumaraldehyde have been prepared previously. See: Sokolov, G. P.; Hillers, S. *Khim. Geterotsikl. Soedin.* **1969**, *1*, 32-35.

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permeable protein dimerizer that activates signal transduction and gene expression *in vitro*. See: Diver, S. T.; Schreiber, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 5106-5109.

(56) For a recent example of the synthesis of cyclic alkenylboronates *via* RCM employing ruthenium catalyst **1**, see: Renaud, J.; Ouellet, S. G. *J. Am. Chem. Soc.* **1998**, *120*, 7995-7996.

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(62) The solvent columns are composed of activated alumina (A-2) and supported copper redox catalyst (Q-5 reactant). See: Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

(63) Prepared according to a general literature procedure: Schlessinger, R. H.; Lopes, A. *J. Org. Chem.* **1981**, *46*, 5252-5253.

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(65) Prepared according to a standard literature procedure: Lardon, A.; Reichstein, T. *Helv. Chim. Acta.* **1954**, *37*, 443-450.

(66) Prepared using a general method: Alexakis, A.; Gardette, M.; Colin, S. *Tetrahedron Lett.* **1988**, *29*, 2951-2954.

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(68) For a reference to this cleavage protocol, see: Bessodes, M.; Komiotis, D.; Antonakis, K. *Tetrahedron Lett.* **1986**, *5*, 579-580.

(69) Prepared by a general procedure: Forster, R. C.; Owen, L. N. *J. Chem. Soc., Perkin Trans. I* **1978**, 822-829.

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(72) Prepared according to a modified literature procedure. See: Zuwen, H.; Durgesh, V. N.; Lawrence, M. S.; Frederick, T. G. *Biochim. Biophys. Acta* **1995**, *1257*, 117.

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(74) Prepared *via* a DCC coupling between *cis*-2-butene-1,4-diol and *N,O*-dimethylhydroxylamine hydrochloride. See: (a) reference 61. (b) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815-3818. .

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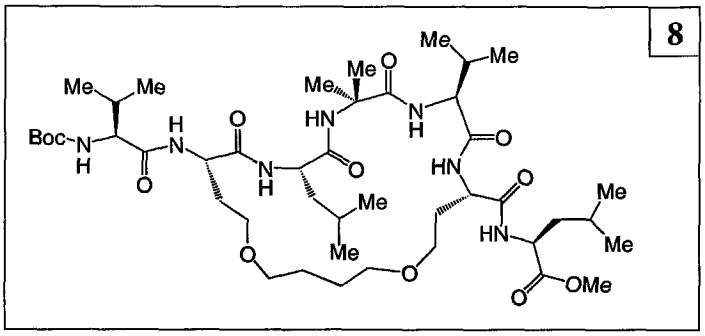
Appendix

X-ray Crystallographic Data for Chapter 4



General X-ray Crystallographic Data for Cyclic Peptide 8. Clear, colorless crystals of cyclic peptide **8** (Chapter 4) were obtained by slow diffusion from CH_2Cl_2/hexane at room temperature (20-25°C). The dimensions of the crystal analyzed were: 0.3 x 0.5 x 0.5 mm. The crystals belong to the orthorhombic system with unit cell parameters at 156 K: a = 19.30(1) Å, b = 24.73(2) Å, c = 12.134(7) Å, and V = 5791(6) Å^3. The space group is $P2_12_12_1$ with Z = 4 formula units/unit cell and ρ (calcd) = 1.246 g/cm^3. Intensity data (4698 total) was collected on a Picker (Crystal Logic) diffractometer system using monchromatized MoK$_\alpha$ radiation (λ = 0.7107 Å) *via* an θ-2θ scan technique. Those 2266 reflections with $|I_o| > 3\sigma(|I_o|)$ were considered observed. An absorption correction was not applied.

The structure was solved by Direct Methods using the UCLA Crystallographic Computing Package[1] and SHELXTL 86 program set,[2] and refined by full-matrix least-squares techniques. There are two molecules of CH_2Cl_2 and one molecule of H_2O present per one peptide molecule. Hydrogen atoms were included using a riding model with d(C-H) = 0.96 Å and U(iso) = 0.08 Å^2. At convergence, R_F = 6.6%, R_{wF} = 7.7% and *GOF* = 2.28 for 633 variables.

Crystallographic data (excluding structure factors) for structure **8** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101810. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Additional Included X-ray Crystallographic Data. Tables of experimental data, distances, angles, torsion angles, stereoview plots, and unit cell plots (13 pages).

Table 1. Details of data collection and structure refinement for Compound **8**.

Formula	C46 H85 Cl4 N7 O13
fw	1086.03
cryst syst	orthorhombic
space group	P212121
cryst color	colorless
cryst habit	irregular
a, Å	19.30(1)
b, Å	24.73(2)
c, Å	12.134(7)
Z	4
V, $Å^3$	5791(6)
ρ(calcd), g cm^{-3}	1.246
radiation, λ	Mo k_α .7107
abs coeff(μ), mm^{-1}	0.263
F(000), e	2328
temp, K	156
diffractometer	Picker (Crystal Logic)
scan mode, speed(deg/min)	θ-2θ, 6.0
2θ range, deg	1.6 - 50.0
total data collcd, unique data used	4698, 2266(I> 3σ(I))
no. of parms refined	633
final shift/error, max and avg	0.070, 0.004
max resid density, $e/Å^3$	0.80
$R = \Sigma\|\|F_o\|-\|F_c\|\|/\Sigma\|F_o\|$	0.066
$R_w = (\Sigma w(\|F_o\|-\|F_c\|)^2/\Sigma w(\|F_o\|)^2)^{1/2}$	0.077

Table 2 Distances, angles and torsion angles for Compound **8**.

O1	C4	1.41(2)	O1	C5	1.31(2)	O2	C5	1.26(2)
O3	C10	1.25(2)	O4	C12	1.241(16)	O5	C18	1.25(2)
O6	C22	1.22(2)	O7	C27	1.23(2)	O8	C29	1.24(2)
O9	C35	1.21(2)	O10	C35	1.35(2)	O10	C36	1.46(2)
O11	C38	1.46(2)	O11	C39	1.43(2)	O12	C42	1.40(2)
O12	C43	1.44(2)	N1	C5	1.35(2)	N1	C6	1.47(2)
N2	C10	1.34(2)	N2	C11	1.46(2)	N3	C12	1.31(2)
N3	C13	1.536(16)	N4	C18	1.35(2)	N4	C19	1.50(2)
N5	C22	1.37(2)	N5	C23	1.41(2)	N6	C27	1.34(2)
N6	C28	1.47(2)	N7	C29	1.37(2)	N7	C30	1.46(2)
C1	C4	1.53(2)	C2	C4	1.54(3)	C3	C4	1.50(3)
C6	C7	1.55(2)	C6	C10	1.51(2)	C7	C8	1.51(2)
C7	C9	1.53(2)	C11	C12	1.53(2)	C11	C44	1.57(2)
C13	C14	1.55(2)	C13	C18	1.46(2)	C14	C15	1.48(2)
C15	C16	1.55(2)	C15	C17	1.54(2)	C19	C20	1.54(2)
C19	C21	1.54(2)	C19	C22	1.58(2)	C23	C24	1.52(2)
C23	C27	1.56(2)	C24	C25	1.53(2)	C24	C26	1.57(2)
C28	C29	1.50(2)	C28	C37	1.52(2)	C30	C31	1.58(2)
C30	C35	1.49(2)	C31	C32	1.52(2)	C32	C33	1.53(2)
C32	C34	1.53(2)	C37	C38	1.52(2)	C39	C40	1.51(2)
C40	C41	1.52(2)	C41	C42	1.49(2)	C43	C44	1.51(2)

C4	O1	C5	121.5(13)	C35	O10	C36	115.1(12)
C38	O11	C39	111.0(10)	C42	O12	C43	113.0(10)
C5	N1	C6	123.3(12)	C10	N2	C11	118.7(11)
C12	N3	C13	115.7(11)	C18	N4	C19	122.9(11)
C22	N5	C23	119.2(11)	C27	N6	C28	121.9(11)
C29	N7	C30	119.6(11)	O1	C4	C1	104.7(13)
O1	C4	C2	111.4(14)	O1	C4	C3	112.8(14)
C1	C4	C2	106.5(15)	C1	C4	C3	111.9(15)
C2	C4	C3	109.3(15)	O1	C5	O2	127.3(15)
O1	C5	N1	112.9(14)	O2	C5	N1	119.7(14)
N1	C6	C7	109.0(11)	N1	C6	C10	111.8(12)
C7	C6	C10	114.2(12)	C6	C7	C8	112.4(11)
C6	C7	C9	112.4(12)	C8	C7	C9	108.5(12)
O3	C10	N2	122.9(13)	O3	C10	C6	120.2(13)
N2	C10	C6	116.6(13)	N2	C11	C12	112.5(11)
N2	C11	C44	108.7(11)	C12	C11	C44	112.9(11)
O4	C12	N3	124.2(12)	O4	C12	C11	118.5(12)
N3	C12	C11	117.1(12)	N3	C13	C14	106.3(10)
N3	C13	C18	111.1(11)	C14	C13	C18	113.8(11)
C13	C14	C15	113.4(11)	C14	C15	C16	111.3(12)
C14	C15	C17	111.7(12)	C16	C15	C17	108.3(12)
O5	C18	N4	119.7(13)	O5	C18	C13	121.5(12)
N4	C18	C13	118.7(12)	N4	C19	C20	109.8(12)
N4	C19	C21	110.7(11)	N4	C19	C22	110.1(11)
C20	C19	C21	110.3(12)	C20	C19	C22	106.0(12)
C21	C19	C22	109.9(12)	O6	C22	N5	125.8(13)
O6	C22	C19	119.2(13)	N5	C22	C19	115.0(12)
N5	C23	C24	108.9(11)	N5	C23	C27	113.4(11)
C24	C23	C27	110.5(11)	C23	C24	C25	109.4(11)
C23	C24	C26	111.2(11)	C25	C24	C26	109.8(11)
O7	C27	N6	123.0(13)	O7	C27	C23	123.0(12)
N6	C27	C23	113.9(12)	N6	C28	C29	113.7(11)

Table 2 (continued) Page 2
Compound 8.

N6	C28	C37	109.3(10)	C29	C28	C37	109.1(11)
O8	C29	N7	122.1(13)	O8	C29	C28	120.7(12)
N7	C29	C28	117.2(12)	N7	C30	C31	110.7(11)
N7	C30	C35	111.3(12)	C31	C30	C35	108.8(12)
C30	C31	C32	112.3(11)	C31	C32	C33	108.8(11)
C31	C32	C34	112.3(12)	C33	C32	C34	110.9(12)
O9	C35	O10	122.5(15)	O9	C35	C30	127.4(15)
O10	C35	C30	110.0(13)	C28	C37	C38	113.0(11)
O11	C38	C37	108.5(11)	O11	C39	C40	108.3(12)
C39	C40	C41	115.4(12)	C40	C41	C42	114.9(12)
O12	C42	C41	110.7(11)	O12	C43	C44	108.6(11)
C11	C44	C43	115.3(12)				

C4	O1	C5	O2	4(2)	C4	O1	C5	N1	-172.6(14)
C38	O11	C39	C40	172.3(12)	C42	O12	C43	C44	-175.5(12)
C5	N1	C6	C7	-179.4(14)	C5	N1	C6	C10	-52(2)
C10	N2	C11	C12	-55.9(17)	C10	N2	C11	C44	178.3(13)
C12	N3	C13	C14	176.7(12)	C12	N3	C13	C18	-59.1(15)
C18	N4	C19	C20	-171.7(13)	C18	N4	C19	C21	66(2)
C18	N4	C19	C22	-55(2)	C22	N5	C23	C24	171.4(12)
C22	N5	C23	C27	-65.2(16)	C27	N6	C28	C29	-84.6(16)
C27	N6	C28	C37	153.2(13)	C29	N7	C30	C31	129.8(13)
C29	N7	C30	C35	-109.0(15)	C5	O1	C4	C1	-176.0(15)
C5	O1	C4	C2	-61(2)	C5	O1	C4	C3	62(2)
O1	C5	N1	C6	-180.0(13)	O2	C5	N1	C6	3(2)
N1	C6	C7	C8	-70.5(15)	N1	C6	C7	C9	166.8(12)
N1	C6	C10	O3	145.4(14)	N1	C6	C10	N2	-40(2)
C7	C6	C10	O3	-90(2)	C7	C6	C10	N2	84.4(16)
C10	C6	C7	C8	163.7(13)	C10	C6	C7	C9	41(2)
O3	C10	N2	C11	1(2)	C11	N2	C10	C6	-173.9(13)
N2	C11	C12	O4	156.3(12)	N2	C11	C12	N3	-28(2)
N2	C11	C44	C43	71.2(15)	C12	C11	C44	C43	-54.4(17)
O4	C12	N3	C13	-2(2)	O4	C12	C11	C44	-80.2(16)
C13	N3	C12	C11	-178.3(11)	N3	C12	C11	C44	95.9(15)
N3	C13	C14	C15	176.1(11)	N3	C13	C18	O5	145.5(13)
N3	C13	C18	N4	-37(2)	C14	C13	C18	O5	-94.7(16)
C14	C13	C18	N4	83.2(16)	C18	C13	C14	C15	53.6(16)
C13	C14	C15	C16	-177.9(12)	C13	C14	C15	C17	60.9(16)
O5	C18	N4	C19	-2(2)	C19	N4	C18	C13	-179.9(13)
N4	C19	C22	O6	146.7(13)	N4	C19	C22	N5	-35.9(17)
C20	C19	C22	O6	-94.6(16)	C20	C19	C22	N5	82.7(16)
C21	C19	C22	O6	24(2)	C21	C19	C22	N5	-158.2(13)
O6	C22	N5	C23	-1(2)	C23	N5	C22	C19	-178.3(12)
N5	C23	C24	C25	-62.2(14)	N5	C23	C24	C26	176.3(11)
N5	C23	C27	O7	154.8(13)	N5	C23	C27	N6	-30(2)
C24	C23	C27	O7	-83(2)	C24	C23	C27	N6	92.7(15)
C27	C23	C24	C25	172.6(12)	C27	C23	C24	C26	51.1(15)
O7	C27	N6	C28	5(2)	C28	N6	C27	C23	-170.2(12)
N6	C28	C29	O8	166.9(12)	N6	C28	C29	N7	-15(2)
N6	C28	C37	C38	-62.1(15)	C29	C28	C37	C38	173.0(12)
O8	C29	N7	C30	11(2)	O8	C29	C28	C37	-70.8(16)
C30	N7	C29	C28	-167.0(12)	N7	C29	C28	C37	107.1(14)
N7	C30	C31	C32	-60.6(15)	N7	C30	C35	O9	-11(2)
N7	C30	C35	O10	165.3(12)	C31	C30	C35	O9	111(2)

C31	C30	C35	O10	-72.4(15)	C35	C30	C31	C32	176.8(13)
C30	C31	C32	C33	-177.7(12)	C30	C31	C32	C34	-54.5(16)
O9	C35	O10	C36	-3(2)	C36	O10	C35	C30	-179.5(13)
C28	C37	C38	O11	173.2(11)	C39	O11	C38	C37	-178.3(12)
O11	C39	C40	C41	-67.2(16)	C39	C40	C41	C42	-85.1(16)
C40	C41	C42	O12	62.0(16)	C43	O12	C42	C41	-179.3(12)
O12	C43	C44	C11	75.0(15)					

Note: Units of distances are Angstroms, of angles degrees
E.s.d. in parentheses, in units of least significant digit
of the corresponding value

TABLE 3. Position and Displacement Parameters for Compound **8**.

Atom	x	y	z	U11 or <u Sq>	U22	U33	U12	U13	U23	Equiv <u Squared>
O1	0.1915(5)	0.4750(4)	0.7831(10)	0.035(6)	0.035(7)	0.103(10)	-0.021(5)	0.031(7)	-0.023(7)	0.058
O2	0.1876(5)	0.3821(4)	0.7930(8)	0.041(6)	0.044(7)	0.061(8)	0.021(5)	0.000(6)	-0.015(6)	0.049
O3	0.0516(5)	0.2982(4)	0.8664(8)	0.060(7)	0.025(6)	0.060(8)	0.013(5)	0.007(6)	0.007(6)	0.048
O4	0.0373(4)	0.2398(4)	0.5991(7)	0.030(6)	0.038(6)	0.044(7)	-0.002(5)	0.000(5)	-0.009(5)	0.037
O5	0.1727(4)	0.1557(4)	0.6479(7)	0.043(6)	0.034(6)	0.032(6)	-0.003(5)	-0.002(5)	-0.016(5)	0.037
O6	0.0775(5)	0.0740(4)	0.8301(8)	0.073(8)	0.027(6)	0.060(8)	0.010(6)	-0.014(7)	0.004(6)	0.053
O7	-0.0353(5)	0.0372(4)	0.5887(9)	0.046(6)	0.022(6)	0.086(9)	-0.019(5)	0.027(6)	-0.004(6)	0.051
O8	0.1387(5)	-0.0079(4)	0.4052(8)	0.069(8)	0.037(6)	0.037(7)	-0.007(6)	-0.003(6)	-0.008(6)	0.048
O9	0.1289(6)	-0.0727(4)	0.7158(9)	0.060(8)	0.057(7)	0.054(8)	-0.016(7)	-0.009(7)	0.025(6)	0.057
O10	0.2315(6)	-0.1065(4)	0.6632(9)	0.073(8)	0.044(7)	0.058(8)	0.003(6)	0.012(7)	0.003(6)	0.058
O11	0.1112(5)	0.1988(4)	0.2972(8)	0.059(7)	0.033(6)	0.036(6)	0.010(6)	0.008(6)	0.007(5)	0.043
O12	0.0750(5)	0.3413(3)	0.4293(8)	0.035(6)	0.030(6)	0.041(6)	-0.008(5)	0.003(5)	-0.003(5)	0.035
N1	0.1091(7)	0.4349(4)	0.8802(10)	0.055(8)	0.040(8)	0.048(9)	0.013(7)	0.010(8)	-0.007(7)	0.048
N2	0.0314(5)	0.3662(4)	0.7439(10)	0.031(7)	0.030(7)	0.038(8)	-0.006(6)	-0.002(7)	-0.006(7)	0.033
N3	0.1245(6)	0.2954(4)	0.6501(9)	0.029(7)	0.023(6)	0.040(8)	0.009(6)	0.001(6)	-0.012(6)	0.031
N4	0.1438(6)	0.2079(4)	0.7923(10)	0.055(9)	0.020(6)	0.034(8)	0.003(6)	-0.008(7)	-0.003(7)	0.036
N5	0.0230(6)	0.1482(4)	0.7558(10)	0.031(7)	0.027(7)	0.044(8)	0.003(6)	-0.008(7)	0.007(6)	0.034
N6	0.0510(6)	0.0971(4)	0.5604(10)	0.026(7)	0.047(8)	0.035(8)	-0.012(6)	0.002(6)	0.006(7)	0.036
N7	0.1503(6)	0.0186(5)	0.5845(9)	0.037(8)	0.057(8)	0.016(7)	0.000(7)	-0.007(6)	-0.002(6)	0.037
C1	0.2631(9)	0.5375(7)	0.6960(20)	0.062(13)	0.069(14)	0.170(22)	-0.044(11)	0.047(15)	-0.028(14)	0.100
C2	0.3119(9)	0.4494(10)	0.7508(18)	0.045(12)	0.225(27)	0.093(17)	-0.003(16)	0.006(13)	0.005(19)	0.121
C3	0.2271(9)	0.4528(7)	0.5971(16)	0.079(14)	0.054(12)	0.084(15)	-0.010(11)	0.024(13)	0.009(11)	0.072
C4	0.2460(9)	0.4773(6)	0.7062(15)	0.034(10)	0.058(12)	0.070(13)	-0.004(9)	0.019(11)	-0.012(11)	0.054
C5	0.1662(8)	0.4289(7)	0.8169(14)	0.027(10)	0.067(13)	0.049(12)	0.009(10)	0.005(9)	-0.011(11)	0.048
C6	0.0709(7)	0.3889(6)	0.9283(12)	0.048(10)	0.038(10)	0.043(11)	-0.001(8)	0.005(9)	-0.017(9)	0.043
C7	0.0084(7)	0.4109(5)	0.9944(12)	0.045(10)	0.032(8)	0.030(10)	0.002(8)	0.005(8)	0.005(7)	0.035
C8	0.0302(7)	0.4395(6)	1.0988(12)	0.047(10)	0.045(9)	0.043(11)	0.015(8)	0.000(9)	-0.004(9)	0.045
C9	-0.0432(8)	0.3664(7)	1.0248(13)	0.062(11)	0.076(12)	0.045(12)	0.030(11)	0.018(10)	0.016(10)	0.061
C10	0.0522(7)	0.3472(6)	0.8423(14)	0.032(9)	0.033(10)	0.049(12)	0.005(8)	0.015(9)	-0.006(9)	0.038
C11	0.0061(7)	0.3278(5)	0.6618(13)	0.025(8)	0.034(8)	0.050(11)	0.003(7)	-0.001(8)	-0.005(8)	0.036
C12	0.0587(7)	0.2835(5)	0.6365(11)	0.031(9)	0.026(8)	0.035(10)	-0.006(7)	0.005(8)	0.001(8)	0.030
C13	0.1761(7)	0.2503(5)	0.6206(12)	0.036(9)	0.015(8)	0.045(10)	0.007(7)	0.005(8)	0.005(8)	0.032
C14	0.2493(7)	0.2747(5)	0.6362(12)	0.016(8)	0.040(9)	0.051(11)	-0.003(7)	0.006(8)	0.000(8)	0.036
C15	0.3059(7)	0.2351(6)	0.6183(13)	0.027(9)	0.049(10)	0.049(11)	-0.004(8)	0.004(9)	-0.014(9)	0.042
C16	0.3777(8)	0.2609(7)	0.6390(13)	0.054(11)	0.081(12)	0.064(13)	-0.004(10)	-0.010(11)	-0.038(11)	0.066
C17	0.3049(7)	0.2123(6)	0.5003(15)	0.035(9)	0.058(11)	0.078(13)	0.011(9)	0.001(9)	-0.018(11)	0.057
C18	0.1636(7)	0.2020(6)	0.6864(12)	0.034(9)	0.039(10)	0.024(10)	-0.007(8)	-0.006(8)	-0.016(8)	0.032
C19	0.1299(8)	0.1609(6)	0.8669(12)	0.058(11)	0.040(9)	0.034(11)	0.018(9)	-0.001(9)	0.009(9)	0.044
C20	0.0983(10)	0.1811(6)	0.9757(13)	0.111(16)	0.055(11)	0.031(11)	0.024(11)	-0.005(11)	-0.002(8)	0.066
C21	0.1969(8)	0.1290(6)	0.8895(13)	0.069(11)	0.039(9)	0.050(11)	0.017(9)	-0.023(10)	0.004(9)	0.053
C22	0.0739(8)	0.1225(6)	0.8138(12)	0.043(10)	0.045(11)	0.028(10)	-0.011(9)	0.002(9)	-0.003(8)	0.038
C23	-0.0304(7)	0.1174(5)	0.7077(12)	0.018(8)	0.037(8)	0.035(10)	0.014(7)	0.009(7)	0.015(8)	0.030
C24	-0.0859(7)	0.1556(6)	0.6637(12)	0.021(8)	0.048(10)	0.050(11)	0.004(8)	0.019(8)	0.013(9)	0.040
C25	-0.1169(8)	0.1878(6)	0.7592(14)	0.055(10)	0.037(9)	0.059(12)	0.012(8)	0.016(10)	0.015(9)	0.051
C26	-0.1447(7)	0.1237(6)	0.6021(14)	0.030(9)	0.046(10)	0.094(14)	-0.001(8)	0.004(10)	0.026(10)	0.056

TABLE 3 (CONTINUED) PAGE 2

Atom	x	y	z	U11 or <u Sq>	U22	U33	U12	U13	U23	Equiv <u Squared>
C27	-0.0047(8)	0.0787(5)	0.6147(12)	0.037(10)	0.030(9)	0.036(10)	0.010(8)	-0.014(9)	0.015(8)	0.034
C28	0.0764(6)	0.0709(5)	0.4593(11)	0.027(8)	0.031(9)	0.023(9)	-0.005(7)	0.007(7)	0.004(7)	0.027
C29	0.1235(7)	0.0237(5)	0.4803(12)	0.052(10)	0.018(9)	0.034(11)	-0.024(8)	0.015(9)	0.000(8)	0.035
C30	0.2071(7)	-0.0190(6)	0.6041(12)	0.028(9)	0.047(10)	0.038(10)	0.007(8)	0.012(9)	0.012(8)	0.038
C31	0.2679(7)	0.0097(5)	0.6686(11)	0.038(9)	0.033(9)	0.034(10)	0.014(8)	-0.005(8)	0.007(7)	0.035
C32	0.2978(7)	0.0572(6)	0.6056(12)	0.023(8)	0.049(10)	0.033(10)	-0.008(8)	-0.003(8)	0.006(8)	0.035
C33	0.3582(8)	0.0810(6)	0.6717(13)	0.052(11)	0.057(11)	0.052(12)	0.000(9)	0.008(10)	0.021(9)	0.054
C34	0.3211(8)	0.0414(6)	0.4893(14)	0.052(11)	0.046(10)	0.065(13)	-0.017(9)	0.009(10)	0.001(9)	0.054
C35	0.1836(9)	-0.0669(6)	0.6687(14)	0.042(11)	0.042(11)	0.048(12)	-0.022(9)	0.002(10)	0.004(9)	0.044
C36	0.2139(10)	-0.1560(6)	0.7230(14)	0.120(17)	0.015(8)	0.079(14)	-0.018(9)	-0.006(13)	0.014(10)	0.071
C37	0.1144(7)	0.1123(5)	0.3892(12)	0.048(10)	0.028(8)	0.040(10)	-0.009(8)	-0.002(9)	-0.001(8)	0.039
C38	0.0683(7)	0.1584(6)	0.3518(12)	0.051(10)	0.050(10)	0.037(10)	0.011(9)	0.016(9)	0.005(9)	0.046
C39	0.0705(8)	0.2442(6)	0.2628(13)	0.049(10)	0.053(10)	0.048(11)	-0.004(9)	-0.007(9)	-0.002(9)	0.050
C40	0.1191(8)	0.2877(6)	0.2214(12)	0.047(10)	0.052(11)	0.043(11)	-0.002(9)	0.000(9)	0.013(9)	0.047
C41	0.1654(7)	0.3134(5)	0.3087(12)	0.035(9)	0.042(9)	0.035(10)	-0.010(7)	-0.007(8)	0.008(8)	0.037
C42	0.1332(8)	0.3591(5)	0.3709(12)	0.054(10)	0.027(9)	0.044(10)	-0.007(8)	-0.005(9)	0.004(8)	0.041
C43	0.0413(7)	0.3841(6)	0.4900(13)	0.035(9)	0.039(9)	0.060(12)	-0.006(9)	-0.003(9)	0.008(9)	0.045
C44	-0.0168(7)	0.3599(6)	0.5568(13)	0.038(9)	0.054(10)	0.047(10)	0.006(8)	-0.008(9)	-0.007(9)	0.046
O1W	0.5062(7)	0.5262(4)	0.1928(9)	0.175(13)	0.043(7)	0.050(8)	0.046(8)	0.016(9)	0.008(6)	0.089
CL1	0.503(1)	0.465(1)	0.827(1)	0.07(1) *						
CL1A	0.526(1)	0.447(1)	0.841(2)	0.06(1) *						
CL1B	0.467(5)	0.483(4)	0.842(8)	0.23(1) *						
CL2	0.420(1)	0.417(1)	0.999(1)	0.13(1) *						
CL2A	0.440(2)	0.370(1)	0.920(3)	0.08(1) *						
CL2B	0.413(1)	0.463(1)	1.013(2)	0.04(1) *						
CL2C	0.435(1)	0.389(1)	0.972(2)	0.04(1) *						
C1S	0.483(1)	0.459(1)	0.964(2)	0.10(1) *						
CL3	0.749(1)	0.137(1)	0.958(1)	0.05(1) *						
CL3A	0.754(2)	0.198(1)	1.027(3)	0.24(1) *						
CL3B	0.745(1)	0.177(1)	0.959(2)	0.07(1) *						
CL4	0.867(1)	0.238(1)	1.053(2)	0.20(1) *						
CL4A	0.789(1)	0.238(1)	0.998(2)	0.12(1) *						
CL4B	0.819(1)	0.244(1)	1.033(2)	0.06(1) *						
C2S	0.814(2)	0.169(2)	1.083(4)	0.29(1) *						
H1A	0.313	0.547	0.686	0.08 *						
H1B	0.243	0.559	0.758	0.08 *						
H1C	0.237	0.547	0.627	0.08 *						
H2A	0.314	0.413	0.715	0.09 *						
H2B	0.309	0.444	0.832	0.09 *						
H2C	0.356	0.469	0.733	0.09 *						
H3A	0.175	0.453	0.593	0.06 *						
H3B	0.245	0.415	0.590	0.06 *						
H3C	0.246	0.475	0.536	0.06 *						
H6	0.101	0.370	0.982	0.04 *						
H7	-0.014	0.437	0.941	0.04 *						

Atom	x	y	z	<u Sq>	
H8A	0.072	0.461	1.080	0.05	*
H8B	0.044	0.409	1.149	0.05	*
H8C	-0.006	0.462	1.134	0.05	*
H9A	-0.031	0.332	0.986	0.06	*
H9B	-0.092	0.377	1.007	0.06	*
H9C	-0 039	0.360	1.106	0.06	*
H11	-0.032	0.307	0.698	0.04	*
H13	0.172	0.240	0.541	0.03	*
H14A	0 253	0.287	0.715	0.04	*
H14B	0 256	0.307	0.587	0.04	*
H15	0 302	0.202	0.666	0.06	*
H16A	0 386	0.289	0.581	0.06	*
H16B	0.417	0.234	0.640	0.06	*
H16C	0.374	0.279	0.713	0.06	*
H17A	0.336	0.180	0.493	0.06	*
H17B	0.321	0.242	0.450	0.06	*
H17C	0.256	0.202	0.482	0.06	*
H20A	0 086	0.149	1.024	0.07	*
H20B	0.056	0.204	0.966	0.07	*
H20C	0.135	0.203	1.013	0.07	*
H21A	0.235	0.153	0.919	0.05	*
H21B	0.213	0.111	0.821	0.05	*
H21C	0 185	0.101	0.946	0.05	*
H23	0.052	0.094	0.767	0.04	*
H24	-0.059	0.179	0.611	0.03	*
H25A	-0 133	0.164	0.820	0.06	*
H25B	-0.157	0.210	0.731	0.06	*
H25C	-0.081	0.213	0.789	0.06	*
H26A	-0.123	0.116	0.529	0.06	*
H26B	-0.185	0.148	0.590	0.06	*
H26C	-0.161	0.089	0.634	0.06	*
H28	0.037	0.055	0.417	0.03	*
H30	0.222	-0.028	0.527	0.04	*
H31A	0.305	-0.018	0.680	0.04	*
H31B	0.249	0.022	0.742	0.04	*
H32A	0.264	0.087	0.592	0.04	*
H33A	0.366	0.120	0.660	0.04	*
H33B	0.402	0.061	0.659	0.04	*
H33C	0.343	0.075	0.749	0.04	*
H34A	0.362	0.018	0.505	0.05	*
H34B	0.337	0.077	0.459	0.05	*
H34C	0.286	0.023	0.440	0.05	*
H36A	0.257	-0.174	0.752	0.06	*
H36B	0.189	-0.182	0.674	0.06	*
H36C	0.183	-0.146	0.786	0.06	*

Atom	x	y	z	<u Sq>	
H37A	0.135	0.094	0.323	0.04	*
H37B	0.152	0.129	0.434	0.04	*
H38A	0.034	0.144	0.297	0.04	*
H38B	0.043	0.175	0.415	0.04	*
H39A	0.042	0.258	0.325	0.04	*
H39B	0.039	0.233	0.201	0.04	*
H40A	0.094	0.318	0.186	0.05	*
H40B	0.150	0.270	0.166	0.05	*
H41A	0.210	0.326	0.276	0.04	*
H41B	0.175	0.285	0.365	0.04	*
H42A	0.168	0.379	0.417	0.04	*
H42B	0.115	0.384	0.313	0.04	*
H43A	0.019	0.409	0.437	0.06	*
H43B	0.075	0.405	0.537	0.06	*
H44A	-0.048	0.390	0.582	0.04	*
H44B	-0.043	0.334	0.508	0.04	*
H1N	0.093	0.466	0.916	0.04	*
H2N	0.034	0.406	0.727	0.04	*
H3N	0.137	0.333	0.688	0.04	*
H4N	0.126	0.237	0.828	0.04	*
H5N	0.038	0.184	0.754	0.04	*
H6N	0.085	0.128	0.581	0.04	*
H7N	0.141	0.046	0.649	0.04	*

DISPLACEMENT PARAMETERS ARE COMMONLY CALLED VIBRATION PARAMETERS
UNITS OF U(I,J) AND ISOTROPIC <u Squared> ARE ANGSTROMS SQUARED
UNITS OF EACH E.S.D., IN PARENTHESES, ARE THOSE OF THE
LEAST SIGNIFICANT DIGIT OF THE CORRESPONDING PARAMETER
ISOTROPIC VALUES ARE [1/(8 PI-SQUARED)] TIMES THE "EQUIVALENT
B VALUE" DEFINED BY W. C. HAMILTON (1959) ACTA CRYST. 12, 609-610
"ANISOTROPIC TEMPERATURE FACTOR" DEFINED AS:
EXP[-2.0x(PI SQUARED)x(U11xA*xA*xHxH + U22xB*xB*xKxK + U33xC*xC*xLxL + 2.0xU12xA*xB*xHxK
+ 2.0xU13xA*xC*xHxL + 2.0xU23xB*xC*xKxL)]
* DENOTES AN ATOM REFINED ISOTROPICALLY, HYDROGEN ATOMS WERE CALCULATED AND NOT REFINED.

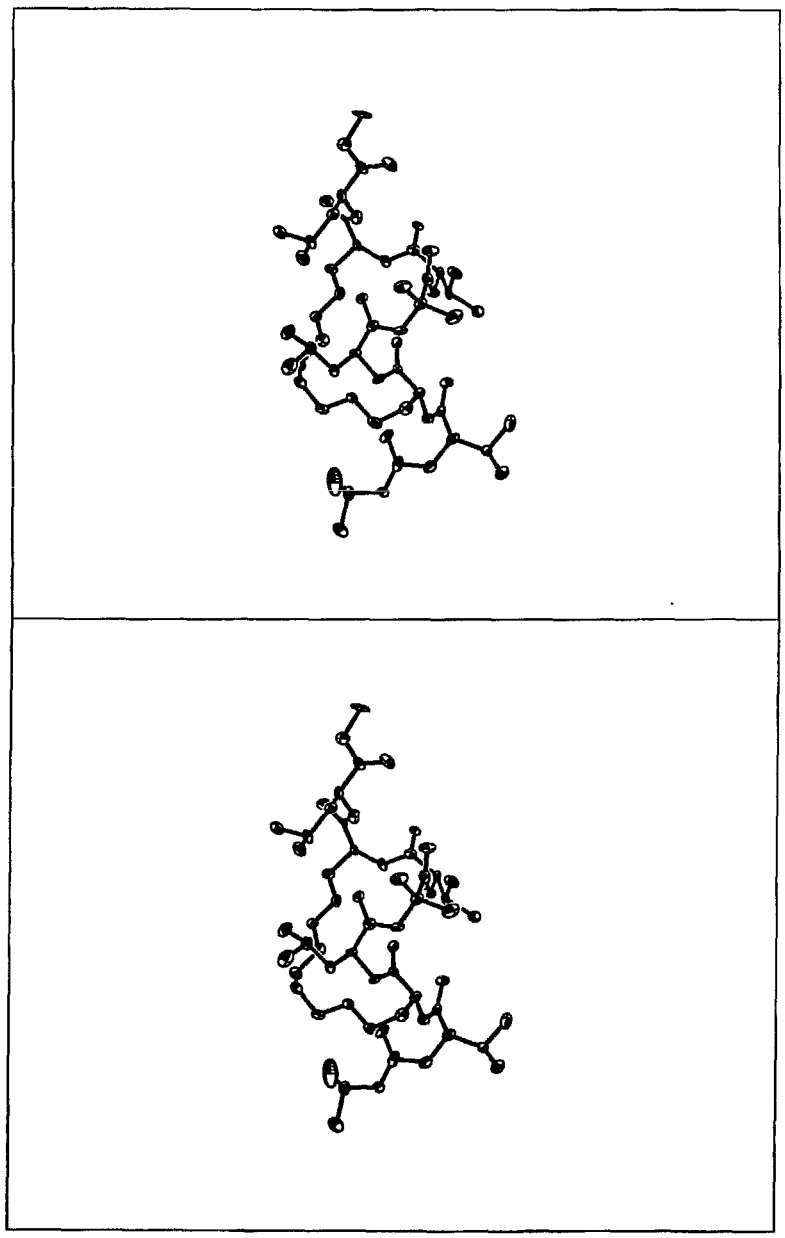


Figure 1. Stereoview of ORTEP diagram of compound **8**.

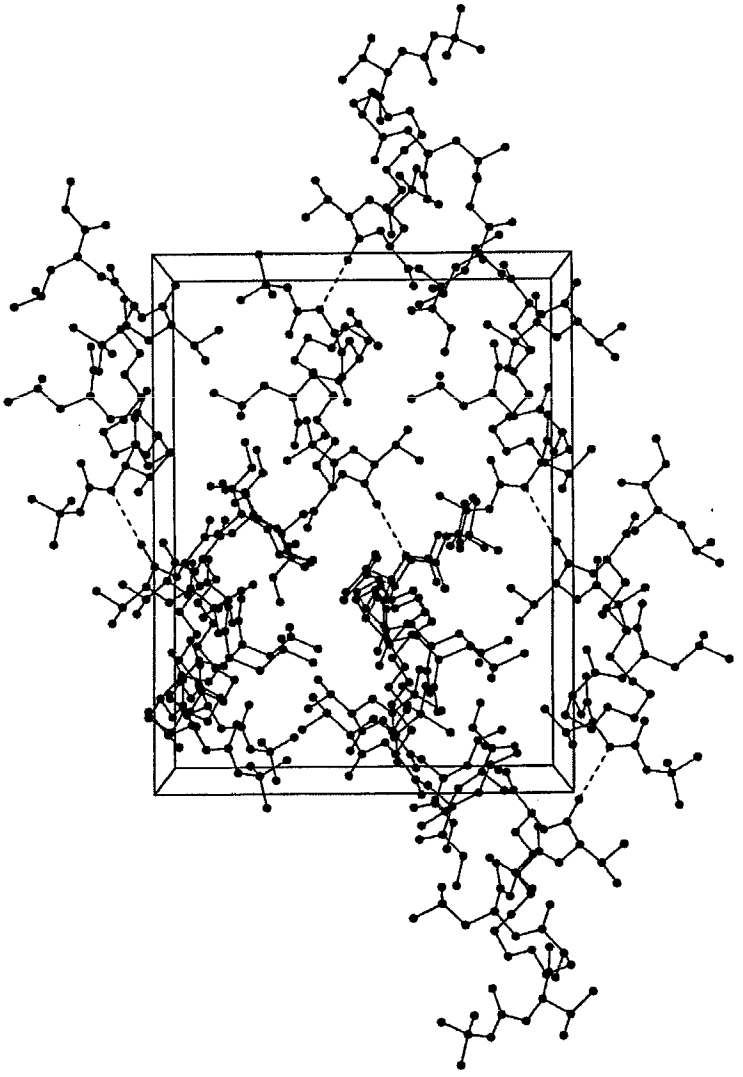


Figure 2. Unit cell of compound 8.

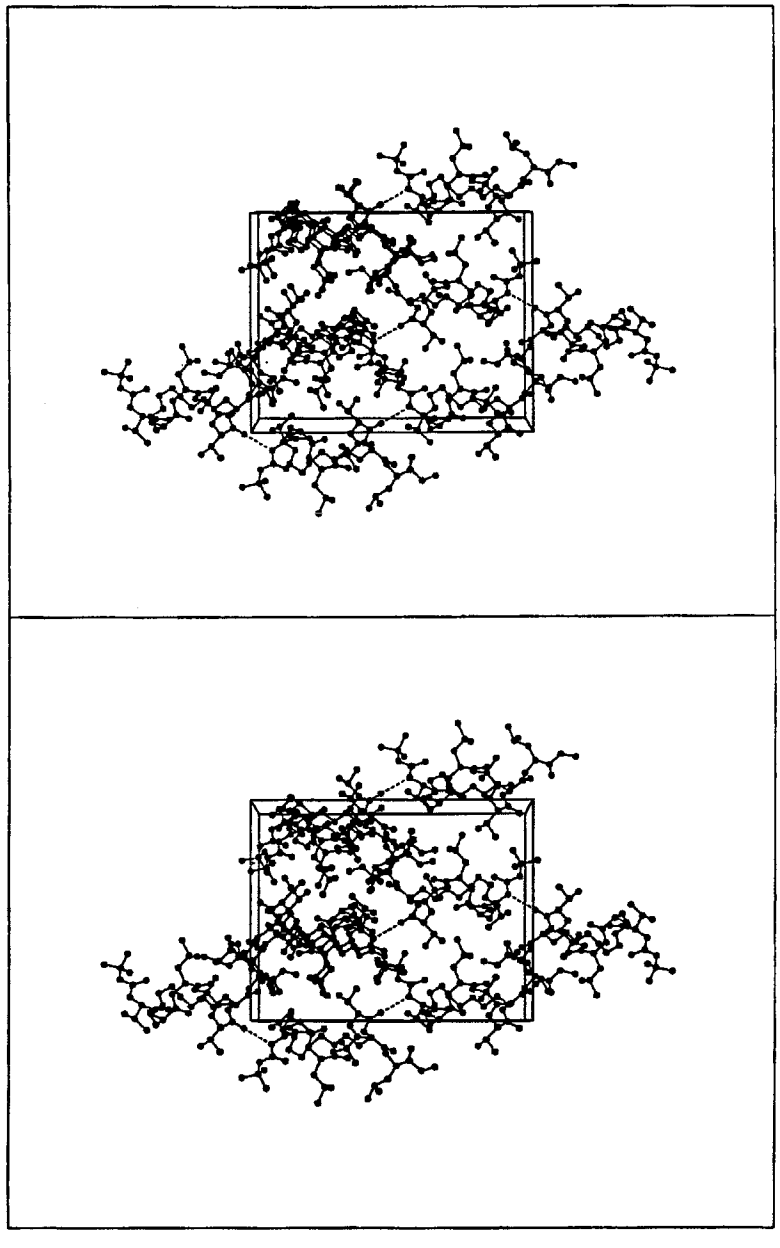


Figure 3. Stereoview of unit cell of compound 8.

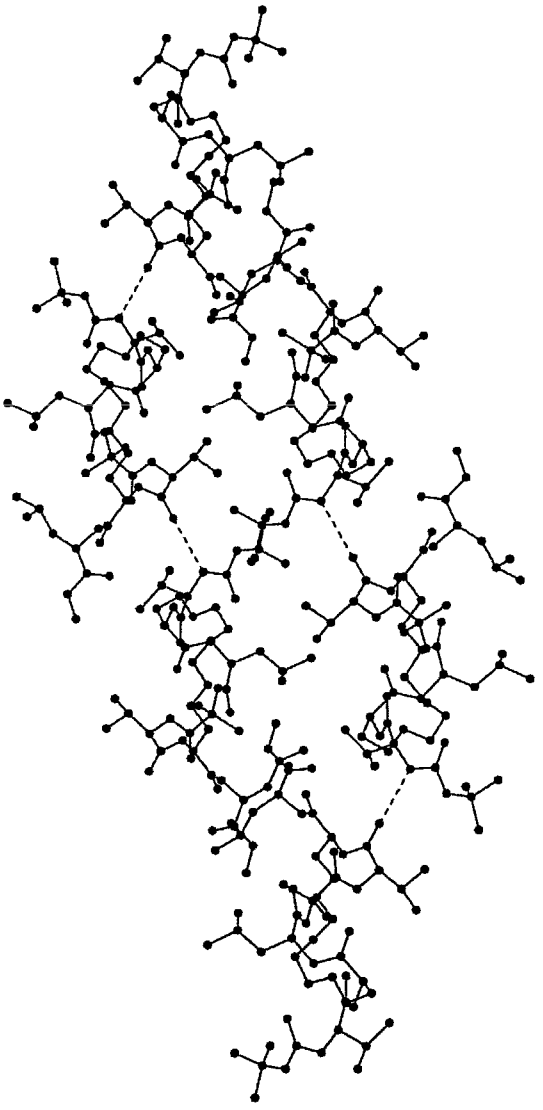


Figure 4. Six molecules from unit cell of compound **8**.

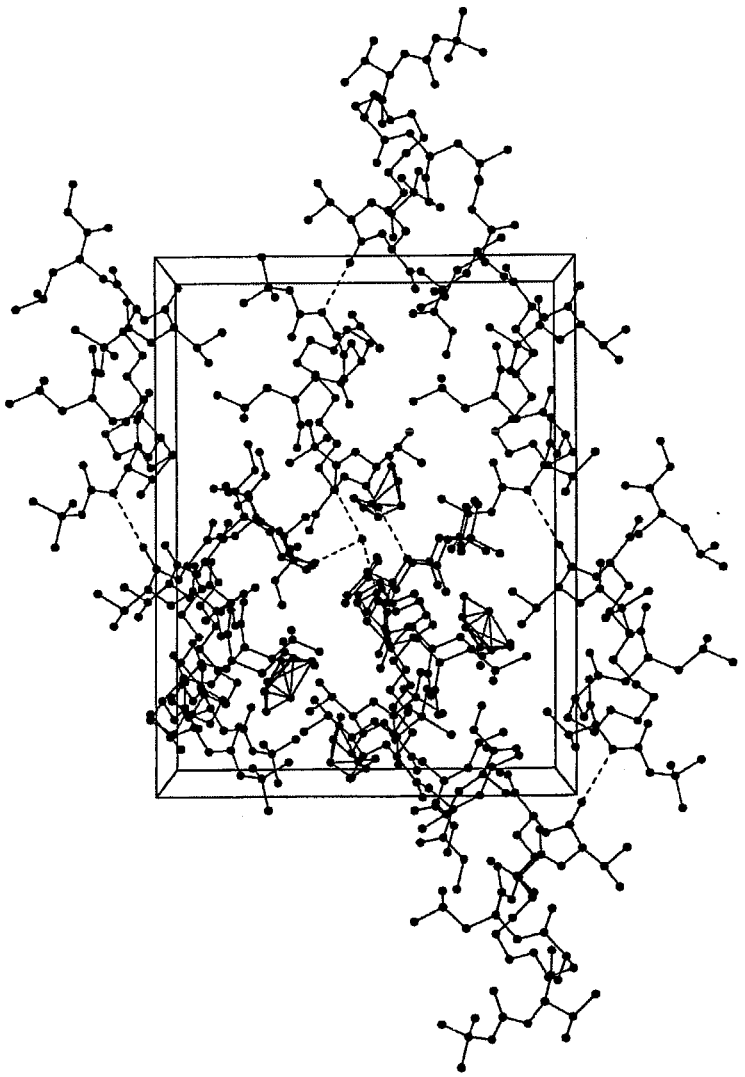


Figure 5. Unit cell of compound **8** including solvent molecules.

References

(1) UCLA Crystallographic Computing Package, University of California, Los Angeles, 1981.

(2) Sheldrick, G. M.; SHELXS-86 *Acta Crystallogr.* **1990**, *A46*, 467-473.