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# Metathesis in Peptides and Peptidomimetics

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Dedicated to Professor Chi-Huey Wong on his 60th birthday.

**Abstract:** This review is intended to cover the applications of olefin metathesis, with its variations, to the synthesis and manipulation of peptides and peptidomimetics. The examples presented here emphasize the use of metathesis in several aspects of peptides and peptidomimetics, from amino acid synthesis, design and synthesis of secondary structure elements, and manipulation of pharmaceutically active peptides and peptidomimetics to improve their stability and activity. These examples testify to the power of the ruthenium-based catalysts, which are particularly useful in the synthesis of complex molecules such as peptides, due to their high stability in various media, high chemoselectivity, and tolerance to a variety of functional groups that decorate the peptide side chains. Another observation one could make from surveying these studies is that ring-closing metathesis (RCM) is the most used variation of olefin metathesis in these fields. Finally, metathesis, which leads to the formation of carbon-carbon bonds with various hybridization states, i.e., alkyne, alkene, and alkane allows for great diversity and flexibility in the synthesis leading to novel structures and function properties. Unarguably, metathesis is a fascinating reaction that continues to have its impact in various fields including peptides and peptidomimetics.



**Keywords:** disulfide bond surrogate; olefin metathesis; peptides; peptidomimetics; ring-closing metathesis; turn mimic

## 1 Introduction

Metathesis is one of the most useful reactions for carbon-carbon bond formation catalyzed by transition metal-based carbene complexes.[1] The reaction with its variations, i.e., ring-closing metathesis (RCM), cross-metathesis (CM), and ring-opening metathesis polymerization (ROMP) has been applied successfully for the synthesis of small molecules and macromolecules.[2] Unarguably, the ruthenium-based complexes **1a**–**1d**[1b,3] (Figure 1) are particularly useful in the synthesis and modification of complex molecules due to their high stability in various media, high chemoselectivity, and tolerance to a variety of functional groups.[4] Complex molecules, such as peptides, have also been manipulated structurally and functionally by applying metathesis reactions.

Peptides are oligomers composed of amino acids, which are known to exhibit a wide range of important biological activities ranging from cell toxins to antibacterial activities. However, due to their peptidic nature, several drawbacks often emerge when they

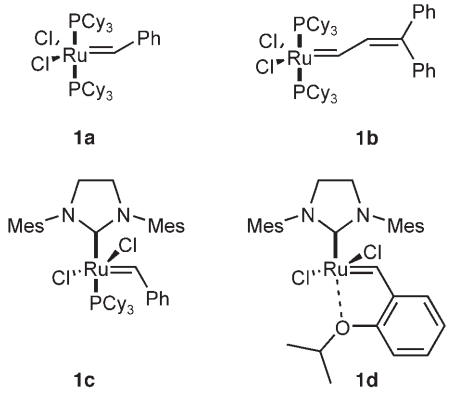


**Figure 1.** Ruthenium carbene catalysts.

  

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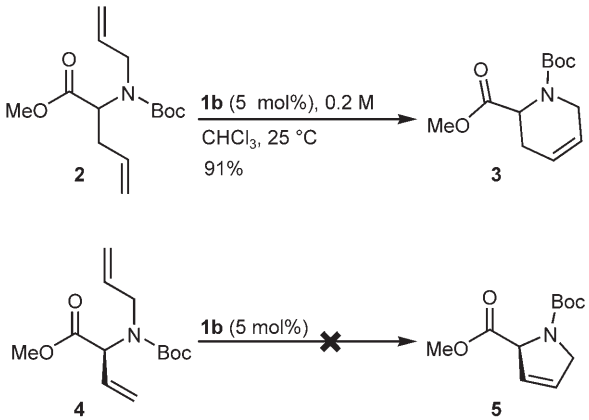
are considered as drug candidates. Peptides are known to have low bioavailability, high potential of immunogenicity, and poor metabolic stability *in vivo*. Yet, chemists are persistent in chasing these molecules due to their relative ease of preparation, the ability to fine-tune their selectivity, and their high binding affinity toward biological targets. As a result, the novel field of peptidomimetics has appeared, and continues to have its impact in many research areas.[5]

In the field of peptidomimetics, the focus is on the creation of a non-natural peptide linkage within the active sequence, in order to remove disadvantageous peptide characteristics, while retaining the original activity. Moreover, these unnatural modifications could lead to a stabilization of the peptide secondary structure with reduced conformational flexibility, therefore resulting in a better affinity to its receptor. Several successful examples have already been introduced to achieve these goals as is the case with peptoids[6] and β-peptides.[7] These "second generation" peptides often exhibit an improved stability towards proteases and an increased bioavailability when compared to the parent sequence. Here, metathesis has also contributed to the field and continues to surprise us with its great potential towards the design of stable peptidic structures with novel properties. In this review, we aim to cover the various aspects of olefin metathesis in the field of peptides and peptidomimetics.

## 2 Metathesis in the Synthesis of Unusual Amino Acids

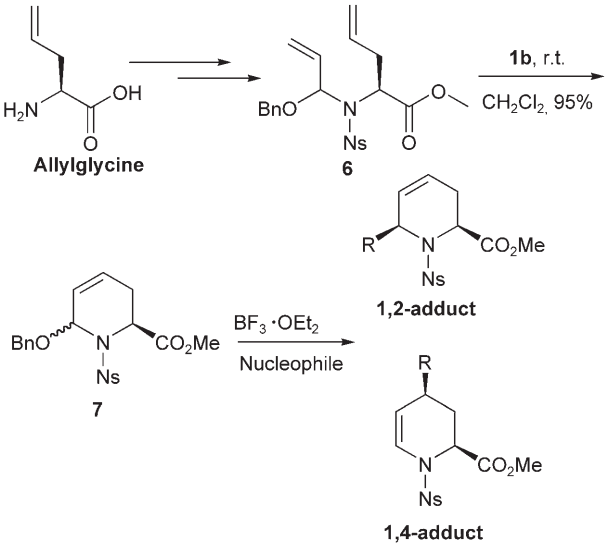
The contribution of olefin metathesis in this area of research is mainly devoted to the synthesis of unusual α- and cyclic β-amino acids. Both types of amino acids are particularly useful in modulating the activity and stability of therapeutic peptides as well as in the synthesis of peptide materials with novel properties.[7] The emerging chemical and biochemical approaches to prepare proteins with well-defined structures and activities from readily available building blocks have added another important aspect to the synthesis of non-proteinogenic amino acids.[8]

RCM, where the fission of two double bonds occurs with a simultaneous formation of a new double bond to produce a cyclic compound, served as an efficient reaction in the synthesis of cyclic α- and β-amino acids. The early work by the Grubbs group on the synthesis of simple amino acid derivatives containing various ring sizes, inspired several research groups to contribute to this field.[9] In these studies, the modified amino acid **2**, for example, was treated with **1b** to furnish the protected amino acid **3** in 91% yield (Scheme 1). Under similar reaction conditions, the vinylglycine derivative **4** failed to deliver the desired cyclized product **5**, rather affording the acyclic α,β-unsaturated esters (Scheme 1). Apparently, the acidity of the $C_\alpha$-proton in the vinylglycine structure hampers the reaction outcome.




**Scheme 1.** Synthesis of cyclic α-amino acids.



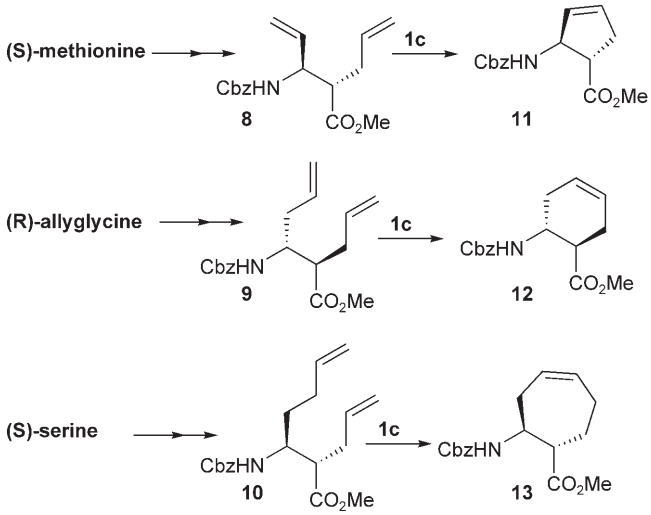

| Entry | Nucleophile | Product | Ratio (1,2:1,4) | Yield [%] |
|---|---|---|---|---|
| 1 | $Et_3SiH$ | R = H | 100:0 | 88 |
| 2 | $SnBu_3$ | R = Allyl | 98:2 | 94 |
| 3 | $Me_3Si$-CN | R = CN | 100:0 | 89 |

**Scheme 2.** Derivatives of restricted cyclic amino acids prepared by using RCM.

Rutjes and co-workers have implemented a similar RCM approach to synthesize novel enantiopure, conformationally restricted cyclic amino acids. Allylglycine was derivatized to give the Ns (4-nitrobenzenesulfonyl) protected allylglycine **6**. RCM conditions were then applied using **1b** catalyst to afford **7** in *ca.* 1:1 mixture of the *E*/*Z* isomers. This mixture was treated with a variety of nucleophiles in the presence of a Lewis acid for a second C–C bond formation to give a set of enantiomerically pure cyclic amino acids (Scheme 2).[10]

β-Amino acids, the building blocks of β-peptides, have been a desirable synthetic target for organic chemists.[7] By applying RCM, cyclic β-amino acids with various ring sizes were successfully synthesized. For example, 5-, 6-, and 7-membered cyclic β-amino esters were constructed from simple amino acids such as serine. In this study, the nature of the starting amino acid determined the size of the carbocyclic ring. Thus, methionine, allylglycine, and serine were converted to the corresponding diene methyl esters, **8**, **9**, and **10** followed by RCM employing **1c** to give the desired cyclized products **11**, **12**, and **13**, respectively, in over 90% yield (Scheme 3).[11]

Stereoselective preparations of cyclic β-amino acids bearing 5- and 6-membered rings were achieved using the diastereoselective thioester enolate/imine condensation reaction and RCM.[12] Cyclic β-amino acids




**Scheme 3.** Cyclic β-amino acids with various ring sizes prepared by using RCM.

substituted at the α-position with alkyl groups[13] and fluorinated seven-membered β-amino acids were efficiently prepared using RCM and CM as the key reactions in the synthetic scheme.[14]

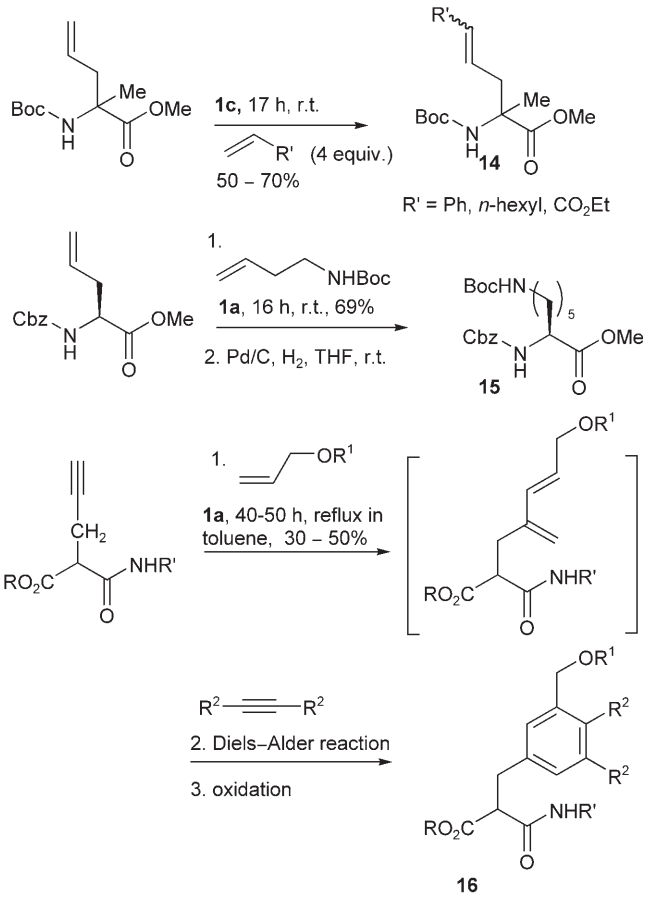
In addition to RCM, CM has also been used in the synthesis of cyclic β-amino acids, albeit to a lesser extent. For example, an efficient synthesis of 5,5-dimethylproline which is known to exclusively adopt the *cis* conformation in peptide sequences, was accomplished using CM as the key synthetic step.[15]

On the other hand, CM has been used more frequently to functionalize α-amino acids, as was shown in the derivatization of α-methyl α-substituted amino acids (**14**, Scheme 4),[16] (*S*)-lysine (**15**, Scheme 4**)** and (*S*)-arginine,[17] glutamic acid,[18] and in the synthesis of highly functionalized phenylalanine derivatives (**16**, Scheme 4) applying cross-enyne metathesis.[19]

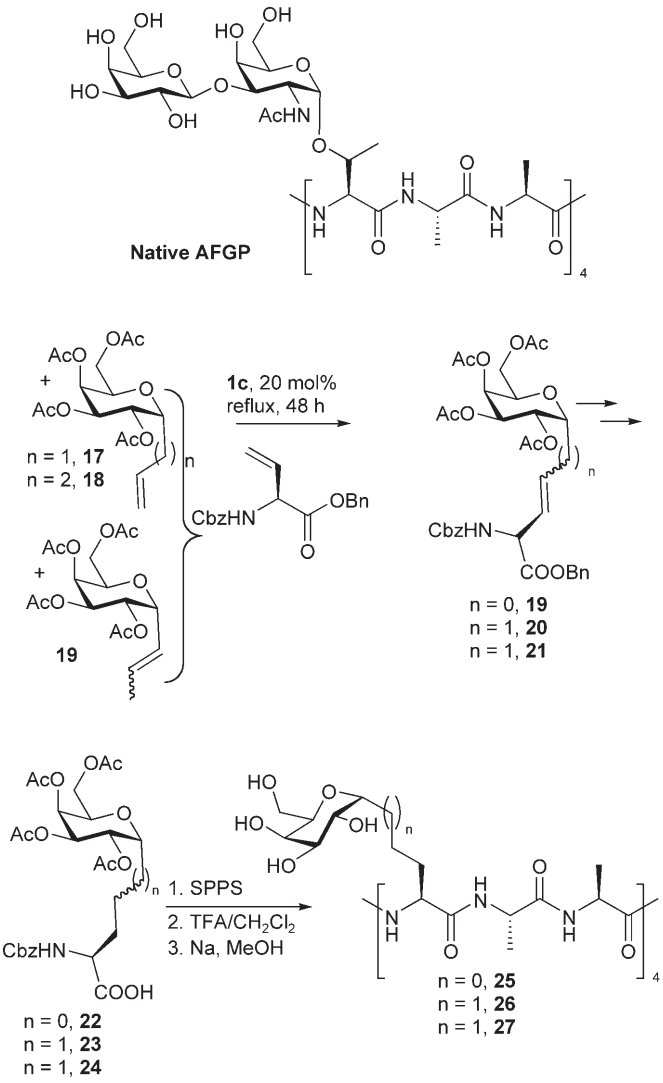
Neoglycopeptides are another important class of molecules that were prepared by CM. They are a family of glycopeptide mimetics, which are considered an easier synthetic target than the parent glycopeptide molecules. Often in this type of structure, the natural glycosidic C–O or C–N bond is replaced with a similar bond, which is more convenient to install. As a result, the new generation of glycopeptide mimics, bearing the C–S or C–C bond, for example, confers resistance to enzymatic hydrolysis. In return, this may lead to an important implication in the drug discovery area.[20] The following example illustrates the contribution of CM in the synthesis of a modified antifreeze glycopeptide to achieve neoglycopeptides with new properties.

Antifreeze glycoproteins (AFGPs), a subclass of biological antifreezes, inhibit the growth of ice and protect living organisms in sub-zero environments (Scheme 5).[21] As a result, these compounds have




**Scheme 4.** Applying CM to functionalize α-amino acids.




**Scheme 5.** Modifying the AFGPs by CM to generate C-linked glycopeptides.

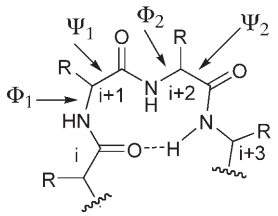
many potential uses in medical and industrial applications. Yet, the development of such a system, which takes advantage of the AFGPs properties, is hampered by their limited bioavailability and the inherent instability of the C–O glycosidic bond. Hence, the substitution of the C–O bond by the more stable C–C bond holds great potential in stabilizing these glycopeptides. In this regard, CM was used to prepare C-linked building blocks starting from C-allylated galactose **17**–**19** to give C-linked amino acids (Scheme 5).[22] Solid phase peptide synthesis (SPPS) based on the Fmoc strategy was then used to incorporate these amino acids to generate C-linked AFGP analogues. Neoglycopeptide **25** was found to be the most potent recrystallization inhibitor resembling the native AFGP.

In the previous example and in several other studies,[23] a preglycosylated amino acid was synthesized in solution using CM. Following the synthesis, the amino acid was coupled to the growing peptide chain by applying SPPS. Alternatively, peptides were also equipped with the alkene functionality at the N-terminal or the side-chain and coupled with a carbohydrate-bearing alkene partner *via* CM, to generate the C-glycopeptides.[24] Each of these approaches has its pros and cons. The strategy that is adopted depends on the ultimate target and several other synthetic considerations. For example, chemoselectivity could be an issue in the second approach if two different modifications of the target peptide are desired. On the other hand, modifications of a peptide bearing the alkene moiety can be completely accomplished on a solid support without the exhausting solution phase synthesis of the preglycosylated amino acid.

## 3 β-Turn Mimic *via* RCM

The β-turn is a ubiquitous secondary structural element that is found in all proteins, comprising 25% of the amino acid residues in most proteins. Besides its structural role in the protein fold, it serves as a key recognition motif for protein-protein and protein-



| β-turn type | $\Phi_1$ | $\Psi_1$ | $\Phi_2$ | $\Psi_2$ |
|---|---|---|---|---|
| I | −60 | −30 | −90 | 0 |
| I' | 60 | 30 | 90 | 0 |
| II | −60 | 120 | 80 | 0 |
| II' | 60 | −120 | −80 | 0 |
| VIa | −60 | 120 | −90 | 0 |
| VIb | −120 | 120 | −60 | 0 |
| IV | −60 | 10 | −50 | 20 |
| VIII | −60 | −30 | −120 | 120 |

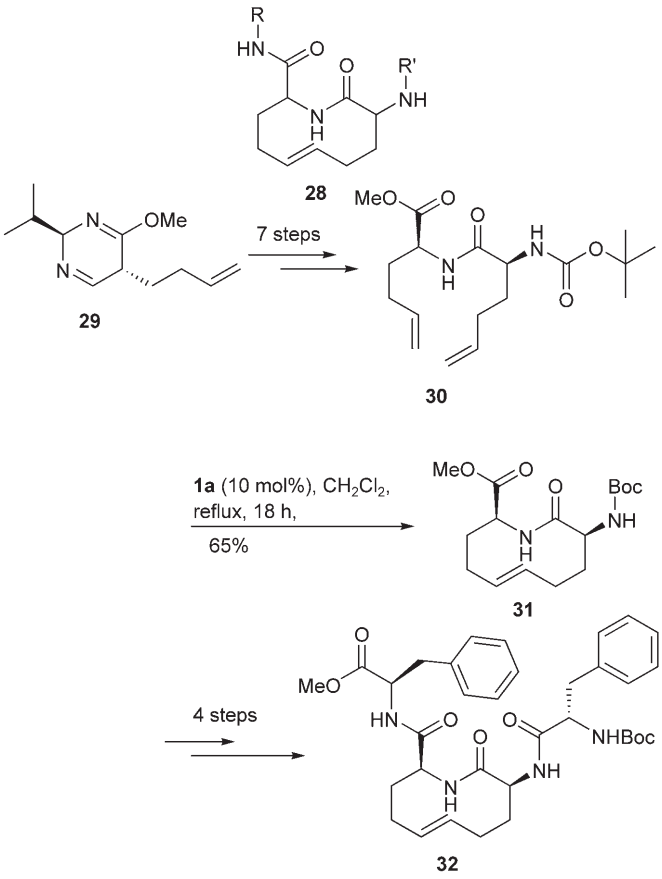
**Figure 2.** Torsion angles of classical β-turns.

ligand interaction.[25] Structurally, β-turns consist of four amino acids in which the peptide chain reverses direction by approximately 180°. An intramolecular hydrogen bond between the carbonyl group oxygen of the first residue (i) and the amide NH proton of the fourth residue (i+3) forming a 10-membered ring, distinguishes most β-turns. The Φ and Ψ peptide bond backbone torsion angles of residues i+1 and i+2 are used to classify the different types of β-turns (Figure 2).

Because of their important role in proteins, potential use in stabilizing short peptides, and protein-protein interactions, numerous studies have been carried out in an effort to develop β-turn mimetics in order to enrich our knowledge on their structures and functions.[26] With its power in constructing rings with various sizes, RCM has made several contributions to this field.[27] Katzenellenbogen and co-workers, used a molecular dynamic conformational search to design type I β-turns, for which the 10-membered lactam **28**, was found to be a good mimic (Scheme 6).[28]

To validate their principles, the group took a synthetic route that combines RCM as the synthetic key step. Starting from Schöllkopf's auxiliary **29**, the dipeptide **30** was synthesized after seven steps in good yield and stereoselectivity, ready for the RCM. Precursor **30** was then subjected to RCM under reflux and high dilution (0.2 mM) conditions to furnish the desired dipeptide **31** in 65% yield (Scheme 6). Evolving **31** to various peptidic structures, combined with careful NMR spectroscopy and X-ray crystallography analysis, revealed that the tetrapeptide **32** restricted the central Φ and Ψ torsion angles ($\Phi_1 = -82°$, $\Psi_1 = -20°$, $\Phi_2 = -107°$, $\Psi_2 = -18°$) to within 30° of the ideal angles of a type I β-turn.

Using similar tools as in the previous example for predicting β-turn structures, Gmeiner and co-workers reported the design of a lactam-bridged type VIa β-turn mimetic.[29] In this example, the researchers used



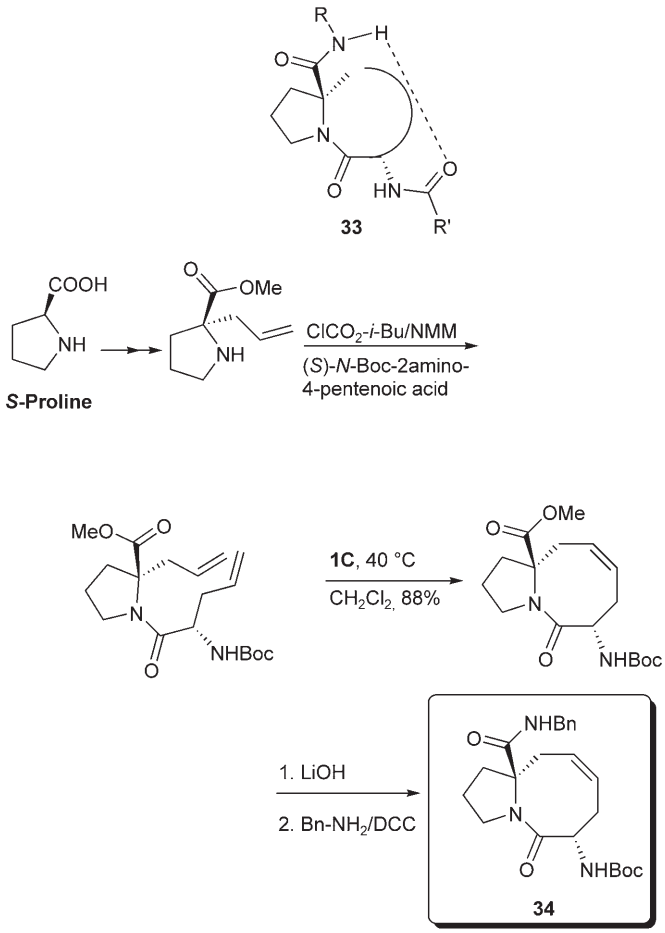

**Scheme 6.** Synthesis of type I β-turn mimic *via* RCM.

the bicyclic *cis*-peptidyl proline surrogates **33** as a potential mimic to the conformation of pseudoproline, which is known to induce a *cis*-peptide bond in the V3 loop of GP120 of HIV-1 (Scheme 7). Several derivatives based on scaffold **33** were prepared using RCM as the key step to construct the various ring sizes of the bicyclic systems. Lactam-bridged peptidomimetic **34** with the *trans* configuration (Scheme 7) showed by NMR spectroscopy (NOEs) the conformation characteristic of VIa β-turns. This confirmed the prediction made in this study by molecular dynamic simulation.

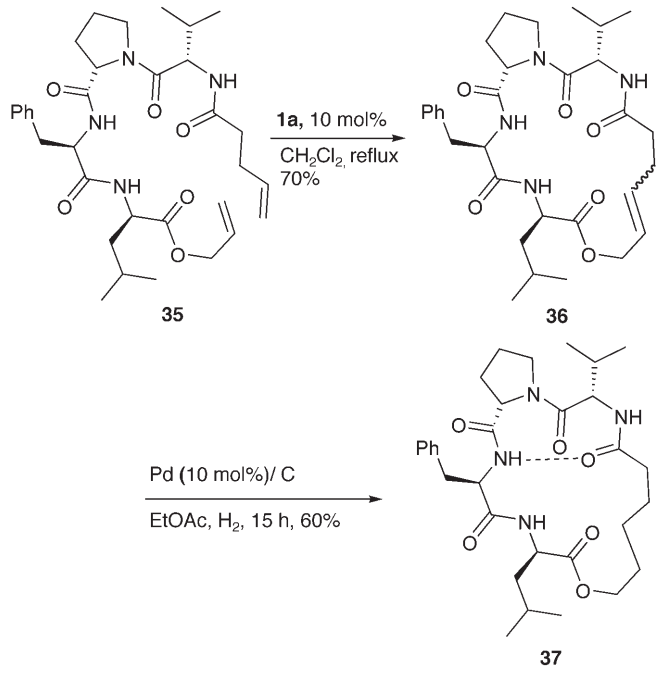
The Gmeiner group has also adopted a similar strategy for the fused azacyclononenone scaffold to fine-tune the dihedral Ψ for achieving superior reverse-turn structures.[30] Eight-membered cyclic peptidomimetics were also prepared by Reitz and co-workers using RCM to give VIa β-turn-like structures.[31]

In proteins, proline is the only amino acid that has the ability to undergo *cis-trans* isomerization about the amide bond linking this residue and the preceding amino acid. Despite its low abundance, the *cis*-proline residue is known to alter the backbone chain direction by the formation of a VI β-turn. RCM was also used to synthesize the *cis*-proline-derived small cyclic type VI β-turn mimic **37** from a precursor **35** that




**Scheme 7.** Synthesis of type VI β-turn mimic *via* RCM.




**Scheme 8.** Proline-derived cyclic VI β-turn mimic *via* RCM.

contains only proteinogenic amino acids functionalized with the alkene moieties (Scheme 8).[32]

While the acyclic peptide **35** showed no organized structure, as indicated by CD, NMR and molecular dynamics, the cyclic tetrapeptide **37** exhibited the presence of a type VI β-turn structure involving also a *cis*-proline-amide bond geometry. Notably, the cyclic peptide **36** did not show the presence of any significant organized structure due to the constraints imposed by the double bond which, upon reduction, was released to give **37** with the desired conformation.

The early work of the Grubbs group in stabilizing β-turns in small cyclic peptides,[33] has ignited this research area for many years to follow.[34] It is worth mentioning, that most of the work presented in this area relied exclusively on ruthenium-based catalysis. However, Overhand and co-workers have used tungsten-based catalysis to perform ring alkyne closing metathesis (RACM) to synthesize conformationally restricted β-turn mimics. A tripeptide containing two acetylenic amino acids was used as the precursor for this synthesis.[35]

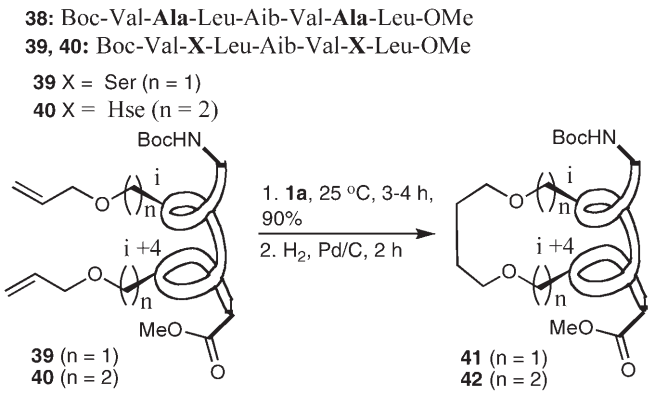
## 4 Cross-Linked Peptide Helices by RCM

The alpha-helix is an important and common secondary motif in protein and peptide structures. The α-helical conformation is adopted by 40% of all residues in proteins.[36] Driven by their important role in protein structures and in pharmaceutically relevant peptides, vast amounts of research have been invested towards designing small helix mimetics.[37] Moreover, short α-helix peptides were modified with various linkages, such as lactams and disulfide bonds, between the side-chains of constituent amino acids (i and i + 4), in order to stabilize the helix structure.[38] As in the case of the β-turn, RCM was utilized to cross-link peptide helices to obtain new structures with novel properties.

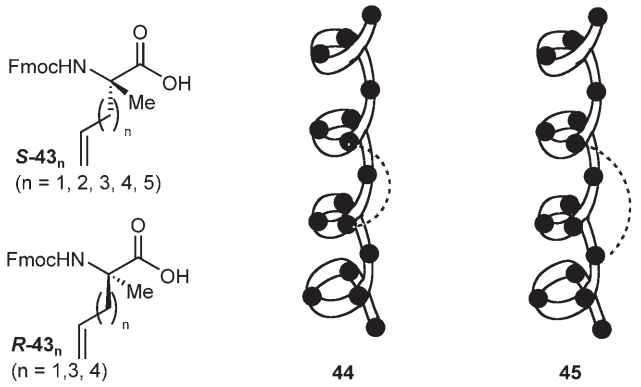
Grubbs and co-workers were the first to use RCM to modify an α-helix, by including the carbon-carbon tether between amino acid side-chains.[39] In this early work, the heptapeptide **38**, which is known to adopt an α-helical conformation was used as a model system. To allow for RCM, alanines (Ala) at positions i and i+4 were replaced with L-serine (Ser) and L-homoserine (Hse) modified with O-allyl ethers to give peptides **39** and **40**. Having these precursors in hand, RCM was applied using the catalyst **1a**, followed by catalytic hydrogenation to afford **41** and **42** in excellent yields (Scheme 9).

The high yield and the relatively fast rate of the RCM (3–4 h) could be attributed to the preorganization of the acyclic dienes in an organic solvent, as the CD spectrum indicated, thus facilitating the RCM reaction. Importantly, the CD measurements of pep-




**Scheme 9.** Cross-linked peptide helices *via* RCM.




**Scheme 10.** Cross-linked helices at i and i+4 (**44**), or i+7 (**45**) positions *via* RCM.

tides **41** and **42** showed similar ellipticities as in the case of the acyclic molecules, which could suggest that no significant conformational changes have occurred due to cyclization. However, X-ray crystallography studies on peptide **42** indicated a similar hydrogen bond network when compared with the characteristic hydrogen bond pattern in the $3_{10}$-helix (four consecutive 4→1 intramolecular hydrogen bonds). This suggests that the replacement of the two Ala with the tethered Hse residue in **40** had induced the peptide backbone to switch from an α-helix to a predominantly $3_{10}$-helix.[39,40]

More recently, Grubbs and O'Leary reported RCM in a $3_{10}$-helix system where the olefin moieties were positioned at the i and i+3 rather than at the i and i+4 or i+7 positions as was previously established.[41] As a result, a facile and *E*-selective (>20:1) intramolecular RCM was achieved, contrary to previous system where *E*/*Z* mixtures are the usual products of RCM. The high selectivity could be attributed to the high content of α-aminoisobutyric acids (Aib) in the peptide sequence, thereby imposing conformational restrictions on the peptides secondary structure and affecting the *E*/*Z* ratio.

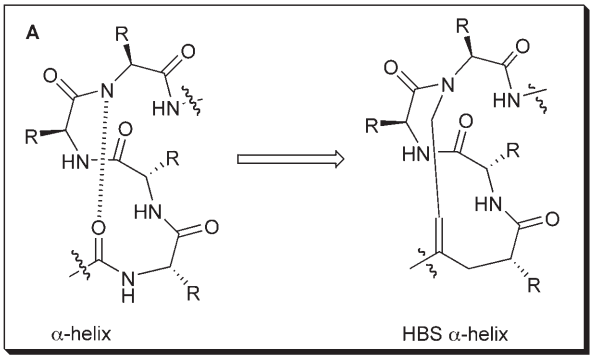
Following the previous work by the Grubbs group, Verdine and co-workers applied RCM to enhance the helicity and the metabolic stability of C-peptide sequences from ribonuclease A.[42] The unnatural amino acids **43** with either the *R* or the *S* stereochemistry, bearing alkyl tethers of various lengths, were synthesized and incorporated across either one or two turns (i and i+4, or i+7 positions respectively). The olefinic moieties with the various lengths, at i and either i+4, or i+7 positions, were then linked *via* RCM to cross-link one (**44**, Scheme 10) or two turns (**45**, Scheme 10), respectively. Several interesting results have emerged from this systematic work on applying RCM in the context of helix peptide.

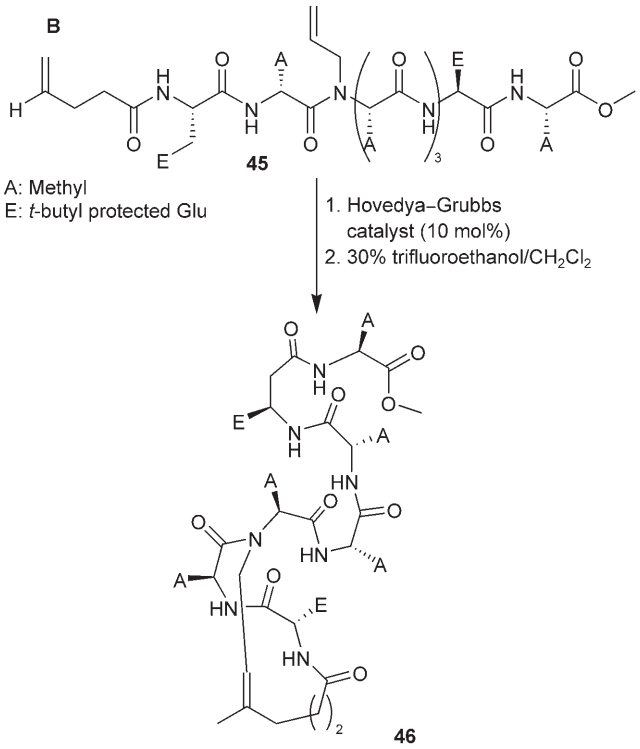
Firstly, the RCM efficiency was highly dependent on the length of the carbon tether produced: RCM conversion increases with the increasing length. For example, despite being the largest ring closed by RCM, the 34-membered macrocyclic ring was formed rapidly and efficiently. Secondly, the helical propensity studied by CD showed that peptides that underwent RCM at the i and i+4 positions were neither stabilized nor destabilized with respect to the uncrossed-linked modified peptides.[42] However, cross-linking of the i and i+7 positions produced an effect ranging from 21% destabilization to significant stabilization depending on the size of the ring and the chirality of the amino acid used. Hydrogenation of the olefin did not have any effect on the helical content; however, in certain cases the *cis* double bond was more stabilizing than the *trans* isomer. Thirdly, the peptide bearing the i and i+7 cross-linked unit with 11 carbons, generated from the peptide with **R-43**, showed a 41-fold increased stability towards trypsin digestion.[42]

In the previous examples, the olefin moiety was attached to the side-chain of the amino acid in order to facilitate RCM and stabilize helices while maintaining hydrogen bond networks similar to the unmodified peptide.[43] Recently, a different approach was reported by Arora and co-workers, in which the double bonds required for RCM were introduced through the backbone amide bond. This was done to substitute the C=O···H–N hydrogen bonds of the i and i+4 residues in the α-helix, with covalent linkages (Scheme 11, **A**). In this case, the carbon-carbon bond acts as a hydrogen bond surrogate (HBS). Notably, the parent peptide (Ac-GEAAAAEA-OMe, **44)** did not exhibit any α-helicity, while the modified peptide **46** generated by applying RCM on **45** exhibited a remarkably strong α-helix structure over a wide temperature range (5–95 °C), as was confirmed by CD and NMR studies (Scheme 11, **B**).

This approach was further extended to solid-phase peptide synthesis,[44] for the synthesis of a peptide mimic that inhibits gp41-mediated cell fusion, and for the design of an artificial Bak BH3 α-helix that binds



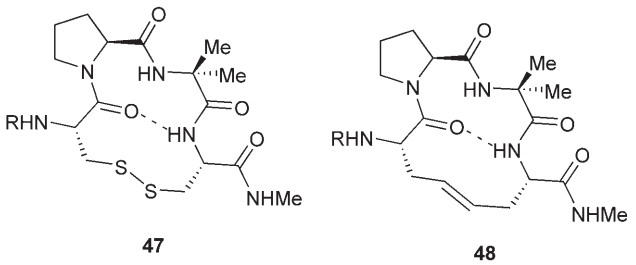




**Scheme 11.** Hydrogen-bond surrogate-derived α-helix *via* RCM.

the Bcl-Xl antiapoptotic protein with higher affinity and proteolysis stability than the unmodified Bak BH3 peptide.[45]

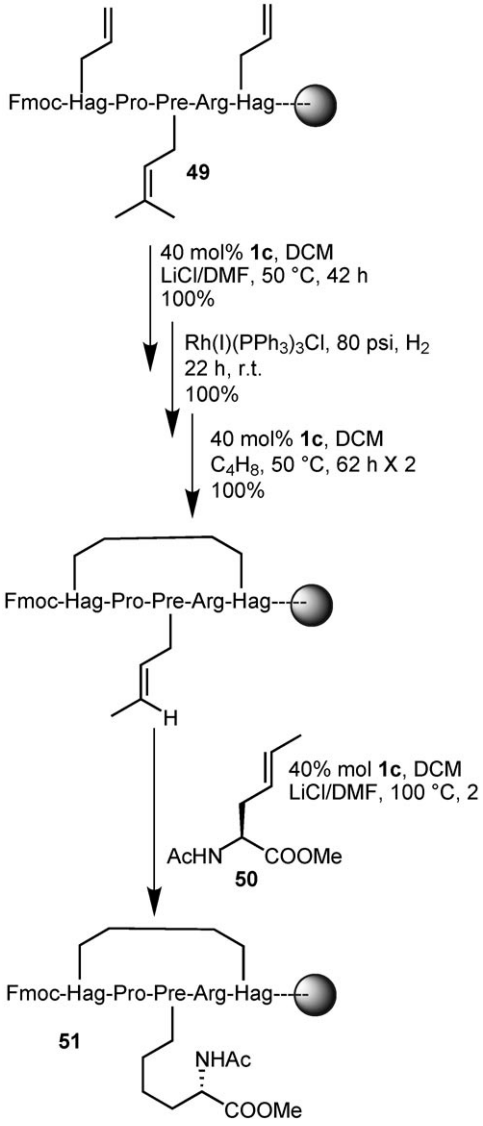
## 5 Disulfide Bond Surrogates by RCM

Although one of the disulfide bonds' functions is to stabilize the secondary and tertiary structures of peptides and proteins, they are known to be intrinsically fragile under a reducing environment. As a result, several studies aimed at replacement of the disulfide bridges in biologically active peptides with covalent cross-links that are inert to reducing conditions. One such modification, which was examined in peptidomimetic research, is the carbon-carbon bond called a "dicarba analogue".[46] Inspired by these studies, the




**Scheme 12.** Dicarba analogue **48** as a replacement for disulfide bond in **47**.

Grubbs group has replaced two cysteine residues in the known cyclic tetrapeptide **47** (Scheme 12) with allylglycine and RCM instead of thiol oxidation.[9]




**Scheme 13.** Construction of dicarba analogues of multicysteine containing peptides.

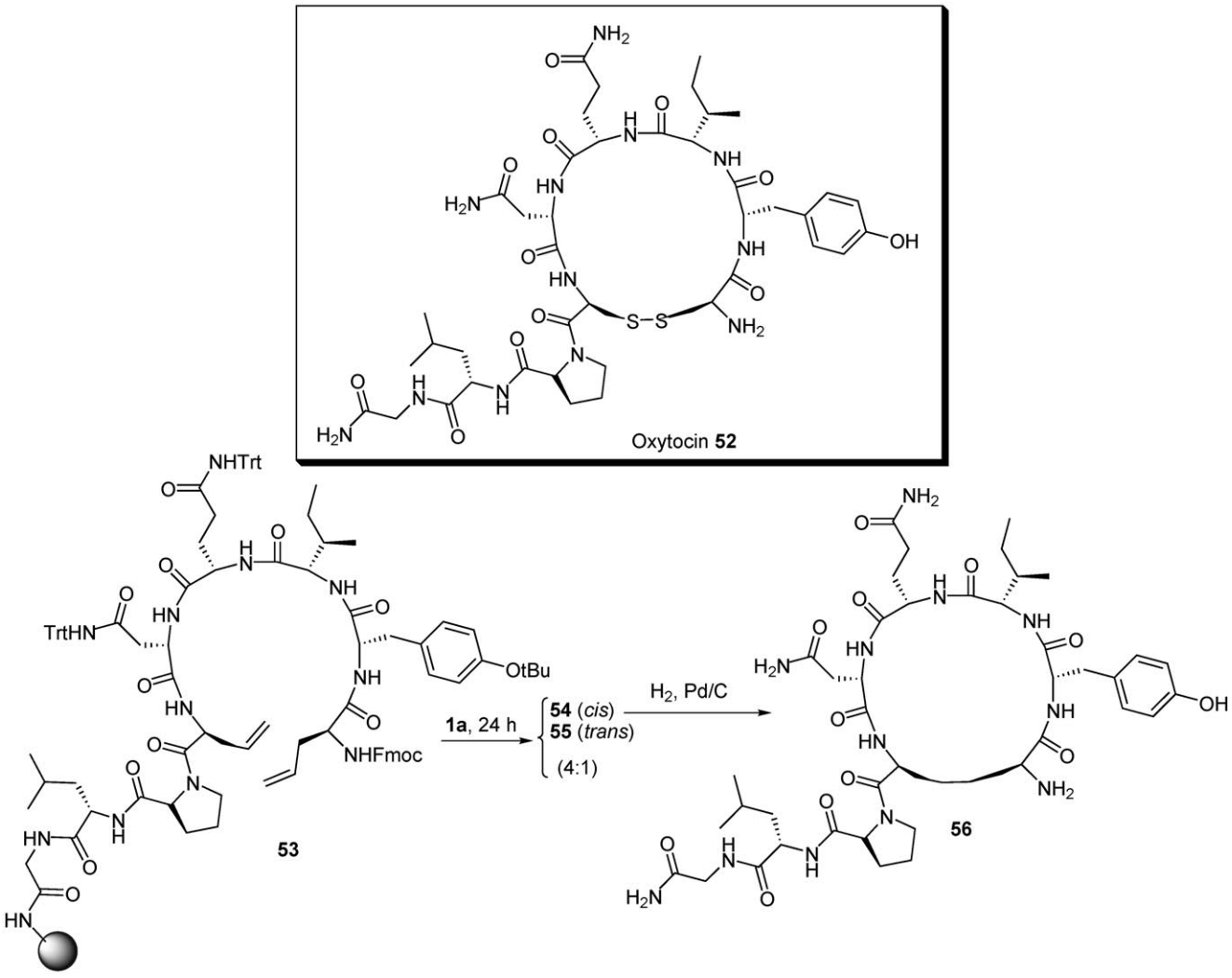
  

In this work, cyclic tetrapeptides with carbon-carbon double bonds as mimics of naturally occurring disulfides were synthesized using RCM. NMR and IR analysis of the cyclic tetrapeptide **48** (Scheme 12) revealed the presence of intramolecular H-bonding similar to the reported disulfide-bridged system. Following this development, various studies were reported in naturally occurring systems and pharmaceutically relevant peptides and peptidomimetics, which will be reviewed in the following section.

While the synthesis of dicarba analogues could be accomplished in a straightforward manner when only one replacement is desired, having multiple substitutions complicates the regioselective formation of analogues of multicysteine-containing peptides. Recently, Robinson and co-workers have described a preliminary study on the regioselective formation of multiple dicarba isosters of cysteine.[47] The method relies on the inert nature of the prenylglycine in peptide **49** to both Grubbs and Wilkinson's catalyzed RCM and hydrogenation of allylglycine under mild conditions. However, under microwave irradiation conditions with an excess of Grubbs catalyst and cortylglycine **50**, the prenylglycine underwent CM to form the second unsaturated bond, which after hydrogenation gave the two-dicarba bridges **51** (Scheme 13). The usefulness of this approach remains to be tested in the construction of peptides with more than one dicarba analogue in an intramolecular fashion.

## 6 Modifying Pharmaceutically Relevant Peptides and Peptidomimetics using RCM

Constraining peptide flexibility, so that only certain shapes could be adopted, has been one of the successful strategies of medicinal chemists to achieve high binding ligands and high *in vivo* stability. Nature performs this task through using a variety of elegant




**Scheme 14.** Replacing the disulfide bond in oxytocin **52** with the dicarba analogues.

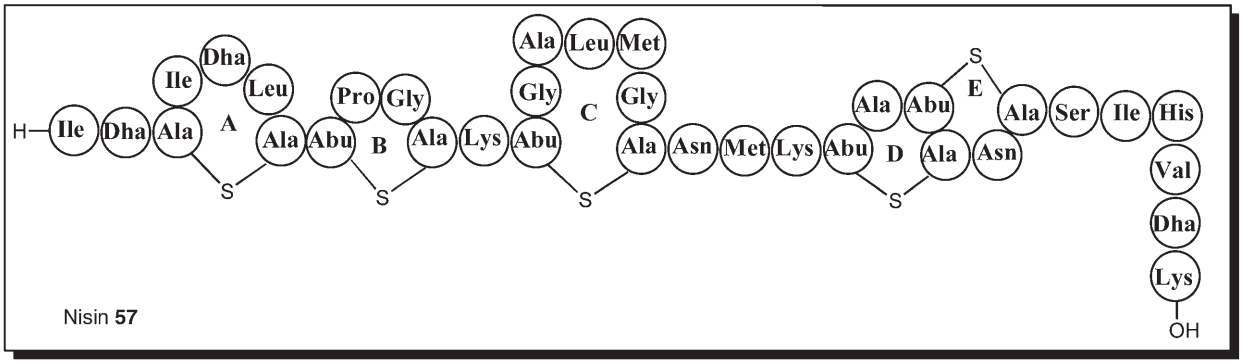
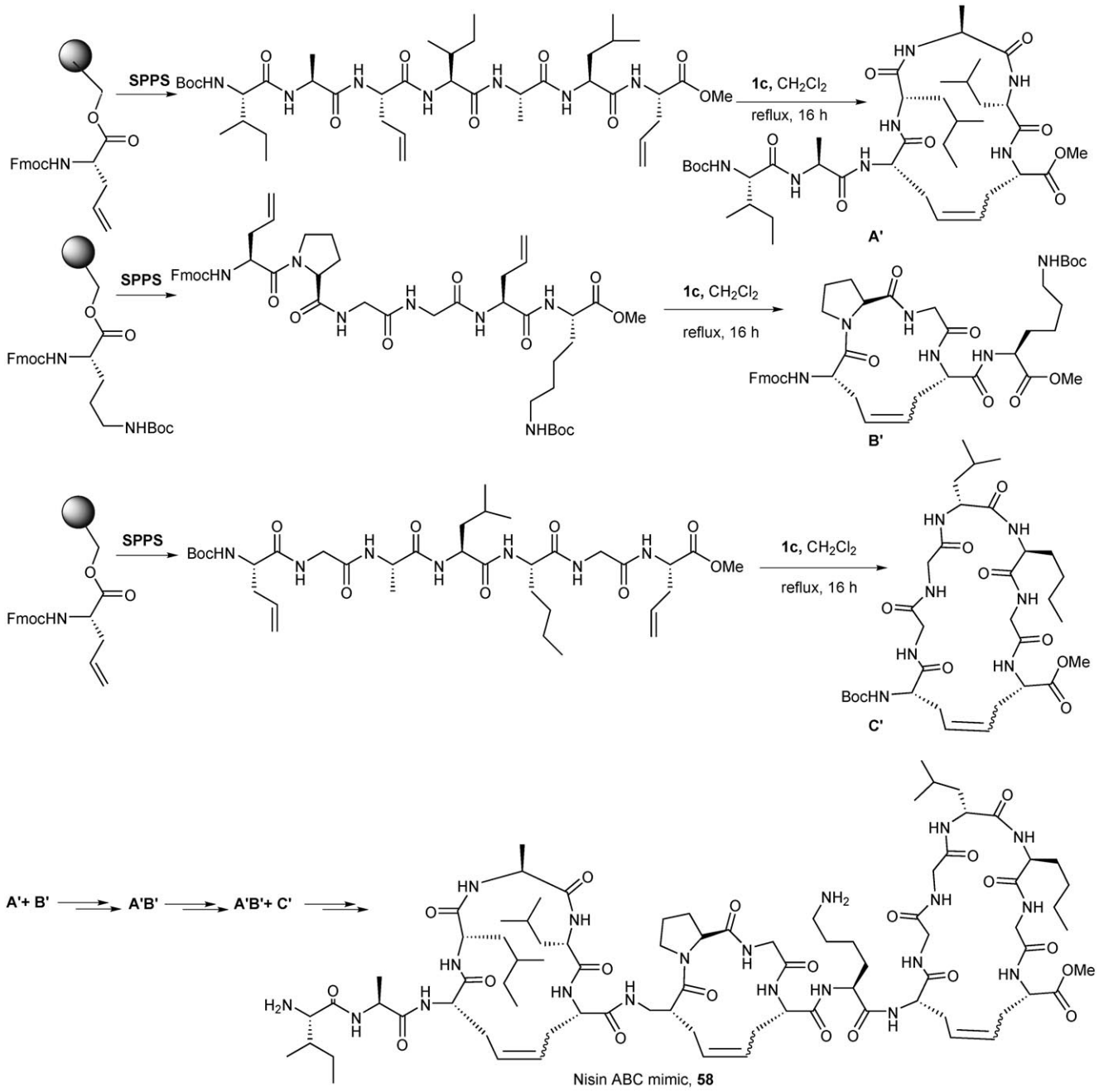



**Figure 3.** Schematic presentation of the nisin structure showing the amino acid sequence and the thioether linkages to form the A–E rings.




**Scheme 15.** Construction of A, B, C ring mimics of nisin *via* RCM.

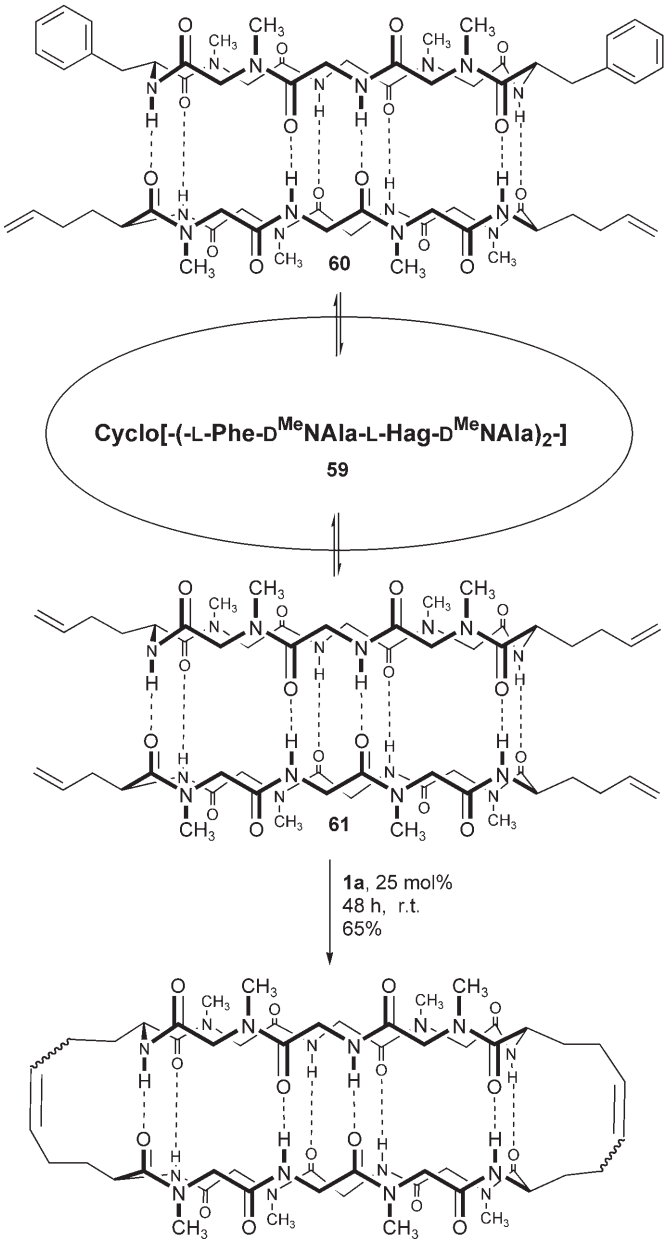
ways such as disulfide bridges and multiple side-chain knotting. The introduction of carbon-carbon bonds to restrict peptide conformation *via* their side chain is one example of the artificial methods to achieve this goal.

Oxytocin **52** (Scheme 14) is a nine amino acid cyclic peptide that is synthesized in hypothalamic neurons.[48] It has been used to induce labor and control postpartum hemorrhage. However, because of its peptidic nature it has a short half-life (2–5 min*) in vivo* and is administered intravenously. As a result several studies were conducted in order to improve its stability *in vivo*.[49] One such approach is replacing the disulfide bond in oxytocin with the dicarba analogue. Vederas and co-workers synthesized the linear peptides of **52** on solid support, where the two cysteine residues were replaced with allylglycine, to give resin-bound linear peptide **53**.[50] On-resin cyclization using **1a** followed by acid cleavage from solid support gave a 4:1 mixture of *cis*/*trans* isomers, **54** and **55**, respectively (Scheme 14). Reduction of the **54** and **55** mixture afforded the saturated cyclic analogue **56** in quantitative yield.

Testing the biological activities of the three derivatives **54**–**56** showed that *cis*-isomer **54** exhibited the most potent activity with an $EC_{50}$ value of 38 ng mL$^{-1}$, which is approximately 14-fold less than oxytocin with an $EC_{50}$ of 2.7 ng mL$^{-1}$. Similar analogues of oxytocin with a larger ring size (i.e., >20 members) were also prepared and tested, which resulted in some analogues with potent activities and with increased stability.[51] Using similar approaches, dicarba analogues of enkephalin, Bowman–Birk inhibitors, and α-conotoxine have also been prepared.[52] Interestingly, the *trans*-isomer of the enkephalin analogue was a μ agonist/δ agonist with subnanomolar potency at both receptors.[52c]

Mimicking the thioether linkage in pharmaceutically relevant peptides and peptidomimetics with the dicarba analogue has also been a goal of several research groups. Liskamp and co-workers have extensively modified the antimicrobial peptide nisin **57** (Figure 3), using RCM[53] and ring-closing alkyne metathesis[54] to replace its natural thioether moieties in rings (A–E) with the alkene or alkane bridges. For example, the synthesis of the alkene and alkane-bridged AB(C)-ring mimics of the nisin was accomplished *via* a combination of SPPS of the individual ring precursors, cyclization by RCM in solution, and an assembly of the alkene-bridged macrocycles using the carbodiimide-synthesis protocol to give the tricyclic alkene-bridged ABC-ring mimic **58** (Scheme 15).

The synthesis of alkene- and alkane-bridged AB ring mimics with the stereochemistry of the native nisin were also accomplished.[53b] These derivatives were tested for their binding with lipid II, an essential precursor for cell-wall synthesis, and compared with




**Scheme 16.** Covalent peptide cylinder *via* RCM.

the native fragments. Generally, the native fragments showed a slightly higher activity than the alkene- and alkane-bridged nisin AB(C) mimics. On the other hand, the alkane-bridged derivatives were better than the alkene counterparts with at least a comparable activity to the natural ones. These results may indicate that the alkane moiety is suitable thioether surrogate and could be used in the design of novel peptide based-antibiotics based on nisin.

The use of RCM as a tool for restricting the conformation of known helix-threading peptides (HTP), which target duplex RNA structures selectively by intercalation, has also been reported.[55] This study demonstrated the effectiveness of RCM in obtaining mac-

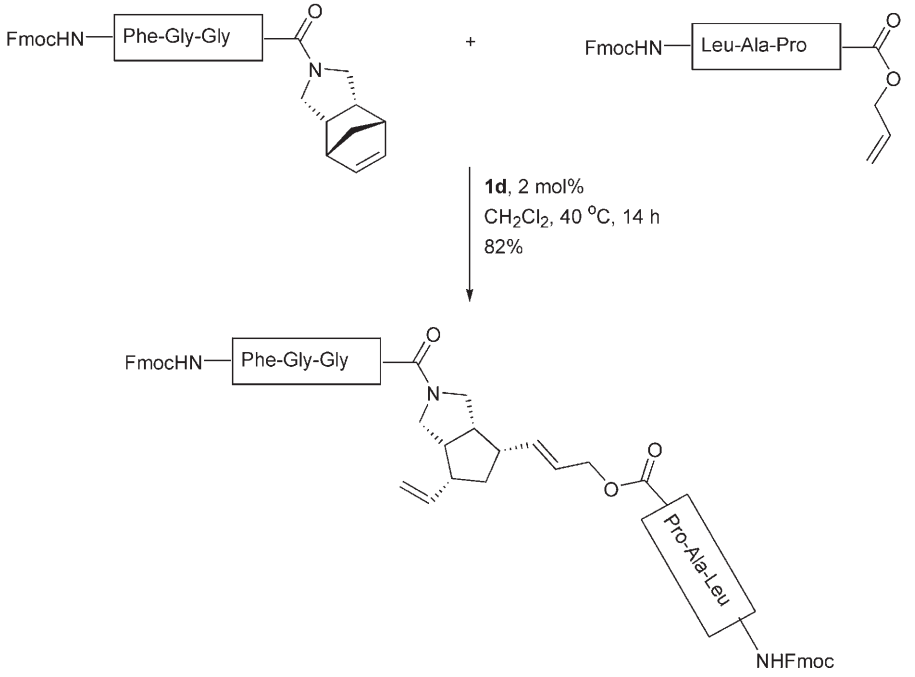
rocyclic HTP with improved affinity and higher RNA sequence selectivity compared to the linear precursor. A small library of peptidomimetic analogues based on the antimalarial inhibitor apicidin were prepared using RCM.[56] Potent peptidomimetic inhibitors of the hepatitis C virus NS3 protease were synthesized using RCM as the key step to achieve macrocyclic structures.[57] Recently, RCM was also applied for the synthesis of 15-membered macrocycles as Grb SH2 domain binding tetrapeptide mimetics with low nanomolar affinities.[58] Similarly, a novel cyclic peptide MC4-ligand was achieved by RCM.[59] Interestingly, in the case of the antimicrobial leucocin polypeptide, the acyclic diallyl-leucocin was one order of magnitude more active than the dicarba analogue obtained by RCM. These results were observed despite the fact that the natural leucocin has a disulfide bridge instead of the allyglycine residues.[60]

## 7 Metathesis in Miscellaneous Peptidic Systems

One of the first applications of olefin metathesis in the context of peptide synthesis and manipulation, originated from the Ghadiri group.[61] In this study, the design of a peptide cylinder *via* hydrogen-bond-promoted intermolecular olefin metathesis was achieved. The design principles of such a system relied on the cyclic peptide **59** equipped with the homoallyglycine residues (Hag), which is known to self-assemble in non-polar solvents to give ensembles **60** and **61** (Scheme 16). Due to the proper orientation of the allylic residues in **61**, only this structure could be converted to the stable tricyclic product **62** *via* olefin metathesis. As a result, the assembly equilibrium shifts toward the productive ensemble **61** and eventually to the covalently stabilized structure **62** as *cis-cis*, *trans-trans*, and *cis-trans* mixtures.

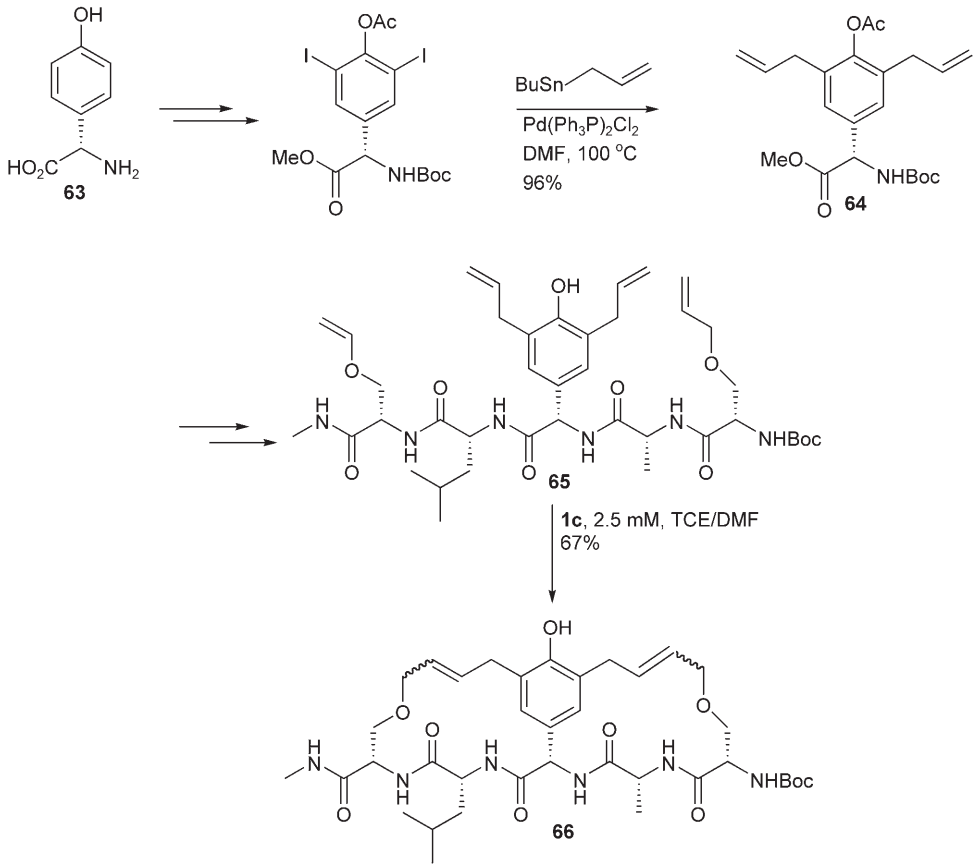
Small peptides and peptidomimetics were efficiently synthesized using olefin metathesis. For example, the Pro-Gly dipeptide alkene isostere was synthesized using CM.[62] Here, the alkene moiety acts as an amide bond surrogate as was suggested in various previous studies. Combining metathesis and amino hydroxylation, small tripeptide molecules were also synthesized.[63] Peptide synthesis by applying ring-opening cross-metathesis (ROCM) was recently reported.[64] Here, a metathesis approach was used to achieve peptide assembly similar to peptide ligation; where two uniquely functionalized peptides are used. In these examples, peptides were decorated with olefin moieties *via* the N- or/and C-terminus to facilitate peptide ligation *via* ROCM (Scheme 17). A similar approach was also reported, however using CM, in which a pentapeptide was prepared from two fragments bearing the alkene moieties.[65]

In the antibiotic area, the drug vancomycin is considered the last resort for the treatment of many Gram-positive infections. Due to the rise of vancomycin resistance, a vast amount of research has been done in order to tackle the drug resistance problem. Searching for highly effective derivatives of vancomy-




**Scheme 17.** Peptide ligation *via* ring-opening cross-metathesis.




**Scheme 18.** Side-chain knotted pentapetide *via* RCM.

cin, Nicolaou and co-workers reported a method for dimerizing vancomycin *via* CM to generate libraries of vancomycin dimers.[66] Inspired by the vancomycin structure, Liskamp and co-workers reported a synthesis of bicyclic side chain knotted peptides to achieve conformationally restricted peptide side chains similar to vancomycin.[67] In this synthesis, RCM served to generate the bicyclic pentapeptide bearing similar side chains with a connectivity pattern as observed in vancomycin. The synthesis started with 4-hydroxyphenylglycine **63**, which was derivatized *via* Stille coupling to give the bisallyl derivative **64**. Further elaboration led to the desired precursor **65** ready for RCM. Using Grubbs catalyst **1c** for the RCM gave the desired bicyclic peptide **66** in 67% yield (Scheme 18).[67]

## 8 Summary and Outlook

Since the discovery of ruthenium-based catalysts by Grubbs, a wide range of examples has been reported using olefin metathesis in the context of peptides and peptidomimetics. Synthesis of a variety of unnatural amino acids, design and synthesis of turns mimics, helix stabilization, and manipulations of pharmaceutically active peptides were achieved by applying this powerful reaction. In general, olefin metathesis has found important applications in various complex systems ranging from polymer and natural products synthesis, and as witnessed in this review, in the modifications of peptides and peptidomimetics. It remains to be seen whether this useful reaction can be applied in the context of proteins under physiological conditions. The new generation of water-compatible ruthenium-based catalysts[68] combined with the ability to introduce unnatural functionalities into proteins (e.g., alkene), using synthetic and biochemical approaches,[8] encourages us to suggest that such a goal might not be far from our reach.

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