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Synthesis of Cyclic Peptides and Peptidomimetics by Metathesis Reactions

Dirk T.S. Rijkers

Abstract This review surveys developments in the field of ring-closing metathesis and cross-metathesis reactions applied to the synthesis of constrained amino acids, peptides, and peptidomimetics. Examples, in which metathesis is used as one of the synthetic tools to arrive at the desired peptide molecules as well as examples that describe in-depth optimized metathesis protocols, will be discussed. Currently, metathesis reactions are well-accepted synthetic tools within the field of peptide chemistry and provide peptides unprecedented properties like conformational, metabolic, and chemical stability and improved bioactivity.

Keywords Conformational constraints · Cross metathesis · Cyclic constraints · Depsipeptides · Dicarba peptides · Macrocyclization · Peptide-based therapeutics · Peptides and peptidomimetics · Rigidification · Ring-closing metathesis · Stapled peptides · Stitched peptides · Structure-inducing scaffolds · *Z*-Selective ring-closing metathesis · α-Helix inducing · α-Helix stabilization · β-Turn inducing

Contents

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1 Introduction

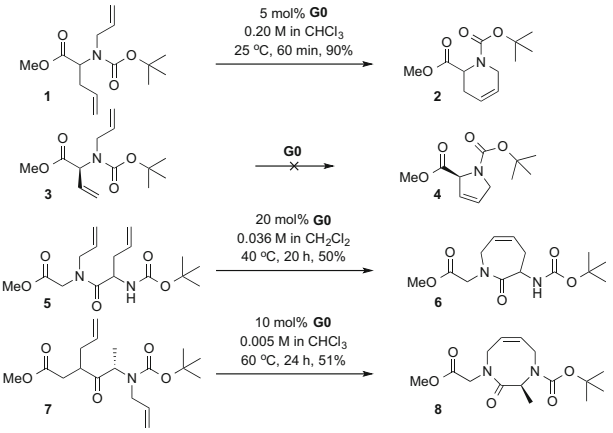
Since the earliest scientific contributions of ruthenium-based metathesis reactions on amino acids and peptides, an enormous increase in synthesis routes and applications was realized. Metathesis on amino acids and peptides was regarded in first instance as rather exceptional, due to the large number of relatively polar functional groups; however, due to the development of more robust ruthenium catalysts [1–3], it became a more common and well-accepted tool in the synthesis of modified as well as constrained amino acids, peptides, and peptidomimetics [4–8]. This contribution does not aim to provide an extensive review of the current literature but rather emphasizes the most important and high-impact examples of metathesis reactions on peptides and peptidomimetics.

2 Application of Olefin Metathesis to Rigidify Amino Acids and Peptides

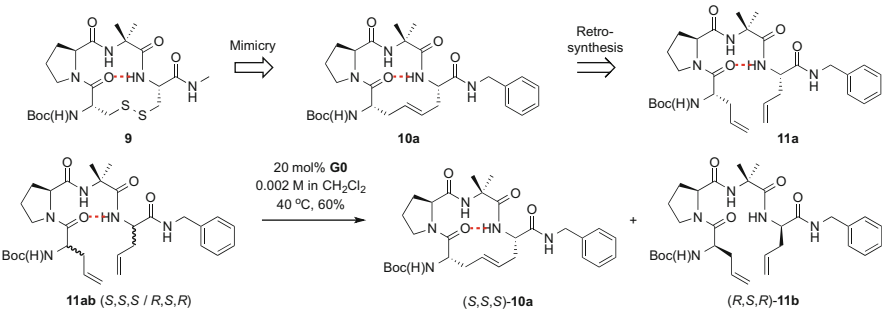
The ruthenium-based catalysts that have been most often used in the olefin metathesis reaction on peptides and peptidomimetics are abbreviated as follows: **G0** for the first well-defined catalyst [9, 10], **G1** for the first-generation Grubbs' catalyst [11], **G2** for the second-generation Grubbs' catalyst [12], and **HG2** for the Hoveyda-Grubbs' catalyst [13].

2.1 The First Applications of Olefin Metathesis in Peptide Synthesis

The first two seminal contributions to apply ruthenium-based olefin metathesis reactions to amino acids and peptides were reported in 1995. In order to synthesize conformationally restricted amino acids and peptides, Miller and Grubbs [14] reported the successful ring-closing metathesis of diene substrates **1**, **5**, and **7**, which were efficiently converted into their six-, seven-, and eight-membered cyclic amino acid derivatives **2**, **6**, and **8**, respectively, in yields varying between 50 and



Scheme 1 Ring-closing metathesis to obtain cyclic amino acid residues



Scheme 2 Mimicking a covalently disulfide-stabilized β-turn by a carbon-carbon double bond, in red a putative hydrogen bond that facilitates ring closure of the "natural" configuration

90%, as shown in Scheme 1. However, under the same reaction conditions, the vinyl glycine derivative **3** did not cyclize into the five-membered dehydroproline derivative **4** (Scheme 1). It was assumed that the acidic α-proton at the chiral carbon atom resulted in a shift of the double bond with the ultimate result that this double bond was unreactive toward catalyst **G0** [15].

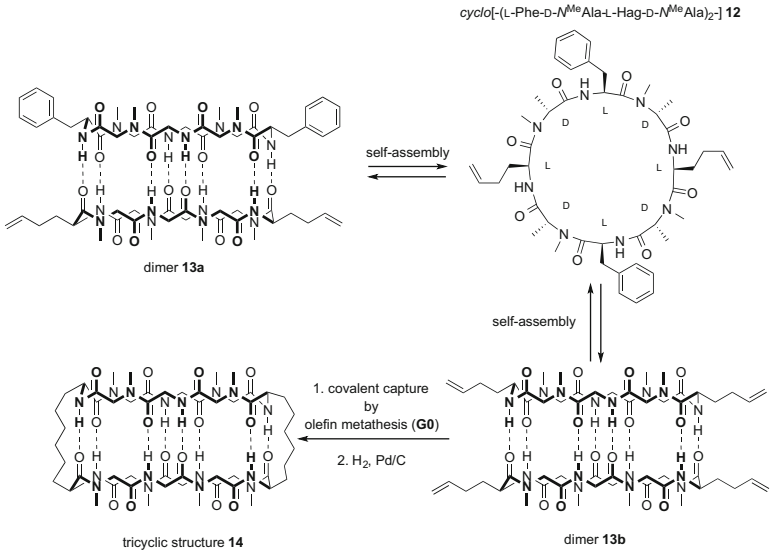
In the same paper [14], Miller and Grubbs reported the synthesis of a conformationally restricted tetrapeptide to mimic a disulfide-stabilized β-turn by replacing both cysteines by allylglycine derivatives (as shown in Scheme 2).

It turned out that the cyclization process is highly stereospecific since the (*S*,*S*,*S*)-diastereomer **11a** smoothly cyclizes into macrocyclic peptide **10a** (in 60% yield), while the (*R*,*S*,*R*)-diastereomer **11b** could be recovered unreacted. It is assumed that the observed stereospecificity is based on the hydrogen bond that facilitates ring closure of the (*S*,*S*,*S*)-diastereomer by preorganization, while it leads to a destabilizing transannular interaction in case of the other diastereomers making macrocyclization less likely to occur. In a follow-up paper [15], Grubbs and

co-workers explored the scope of RCM on these tetrapeptide substrates by replacing the rigidifying (turn-inducing) sequence Pro-Aib by Pro-Tyr and finally by Leu-Leu, in which the conformationally constrained amino acids were absent, and found that these linear tetrapeptides could be smoothly converted into their cyclic congeners in a yield of 60, 80, and 60%, respectively [15]. These results clearly indicated that for the synthesis of cyclic tetrapeptides (as **10**) by RCM, there is no strict requirement to incorporate the Pro-Aib as a preorganization motif.

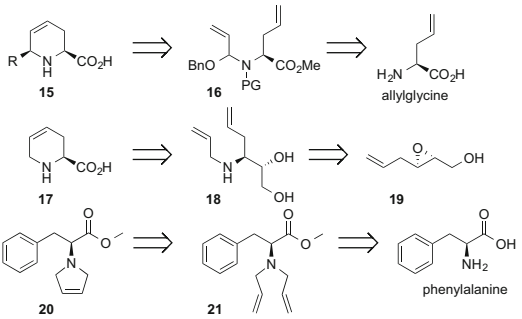
The second seminal contribution was reported by Clark and Ghadiri [16] in which they used olefin cross metathesis for the covalent stabilization of self-assembled dimers of cyclic octapeptides (as shown in Scheme 3).

They prepared a cyclic octapeptide *cyclo*[-L-Phe-D-N^{Me}Ala-L-Hag-D-N^{Me}-Ala)$_2$-] **12** that consisted of an even number of alternating L- and D-amino acid residues and specific *N*-methylated amide bond functionalities, while two homoallylglycine residues were present for covalent capture by olefin metathesis. Such peptides form cylindrical structures driven by hydrogen-bonded self-assembly [17–19], like dimers **13a,b**. Only in case of dimer **13b**, with the correct juxtaposition of the allylic side chains, cross metathesis would be successful, leading, after hydrogenation of the double bonds, to the kinetically stable tricyclic structure **14**, thereby shifting the equilibrium toward **13b**.



Scheme 3 The cyclic peptide **12** self-assembles by hydrogen bond interactions into an equal mixture of dimers **13a** and **13b**. Only **13b** is reactive toward metathesis and shifts the equilibrium to the stable tricyclic structure **14**. *Note*: for clarity side-chain functionalities are omitted

Scheme 4 Examples of conformationally restricted amino acids and peptidomimetics



2.2 *Synthesis of Conformationally Restricted Amino Acids and Peptidomimetics*

Ring-closing metathesis is a versatile approach to the synthesis of enantiopure and conformationally restricted cyclic amino acid derivatives. The group of Rutjes reported an elegant combination of palladium-catalyzed *N*,*O*-acetal formation, ruthenium-catalyzed ring-closing metathesis, and *N*-sulfonyliminium ion-mediated carbon-carbon bond formation approach [20] to synthesize a series of enantiomerically pure 2,6-disubstituted unsaturated pipecolic acid derivates like **15**, as shown in Scheme 4.

Herein, allylglycine was the starting compound, and after conversion into its corresponding *N*-tosyl-protected methyl ester, and reaction with benzyl propadienyl ether in the presence of $Pd(OAc)_2$/dppp/Et_3N in acetonitrile as solvent, the desired *N*,*O*-acetal **16** was isolated in 85% yield. Diolefin **16** underwent ring-closure metathesis using the Grubbs Ru-benzylidene catalyst **G1** in an excellent yield. Functionalization of this scaffold by different R groups (to give molecular entity **15**) was performed with a variety of nucleophiles in the presence of $BF_3{\cdot}Et_2O$ as Lewis acid. In case of Et_3SiH (R=H) as the nucleophile, unsaturated pipecolic acid derivative **17** (baikiain [21]) was obtained in 88% yield [20]. Pipecolic acid derivatives are present in several natural products with important medicinal properties like the immunosuppressants FK506 [22] and rapamycin [23].

Alternative RCM-based syntheses of **17** have been reported by Riera and co-workers [24, 25]. They started with chiral epoxide **19** which underwent a nucleophilic ring opening using allylamine to afford aminodiol **18**, and only after protecting the secondary amine with a Boc group, RCM in the presence of **G1** was successful. Finally, the desired compound **17** (in its Boc-protected form) was isolated in 72% yield [24].

Pyrrolidines that contain a basic and nucleophilic nitrogen atom are rather difficult to prepare by ring-closing metathesis starting from diallylamines. These substrates require a proper deactivation by conversion into, among others, carbamates, e.g., by Boc protection as was the case with aminodiol **18**. To remedy this shortcoming, Yu and co-workers developed a versatile approach for an efficient RCM of chiral diallylamines by virtue of the presence of a strong Lewis acid

[26]. They found that diallylamine **21** (which was prepared from phenylalanine, Scheme 4) could be converted into pyrrolidine **20** (in 92% yield; an almost fourfold improvement) in the presence of **G2** as the RCM catalyst with 20 mol% $Ti(O^iPr)_4$ as the Lewis acid. This reaction was rather insensitive toward variations of the amino acid side-chain functionalities, since general yields varying between 78% and 91% were achieved, in case of alanine, leucine, aspartic acid, tryptophan, and glutamic acid, thus broadening the scope of this pyrrolidine scaffold synthesis.

2.3 Secondary Structure-Inducing Peptide Mimetic Scaffolds Synthesized via Ring-Closing Olefin Metathesis

The central tripeptide motif in Fig. 1 indicates the positions within the peptide backbone that have been used for modification with alkene-based moieties to arrive at cyclic covalent constraints to induce secondary structures in medium-sized peptides and peptidomimetics [27].

Type **I** scaffolds, as represented by **22**, employ the N^i to N^{i+1} connectivity pattern, and in case of *N*-allyl derivatives, Grubbs showed that RCM proceeded smoothly in the presence of **G0**, while **22** was isolated in 51% yield (R^i=CH_3 (alanine), R^{i+1}=H (glycine)) [15]. Reichwein and Liskamp broadened the scope of this reaction to amino acids other than glycine [28]; for example, Boc-N^{All}Phe-N^{All}Phe-OMe was cyclized into **22** (R^i=R^{i+1}=Bn (phenylalanine)) in the presence of **G1** in 1,1,2-trichloroethane at 115°C in 27% isolated yield. Moreover, in a follow-up paper, the same authors described the synthesis of a series of "amide to

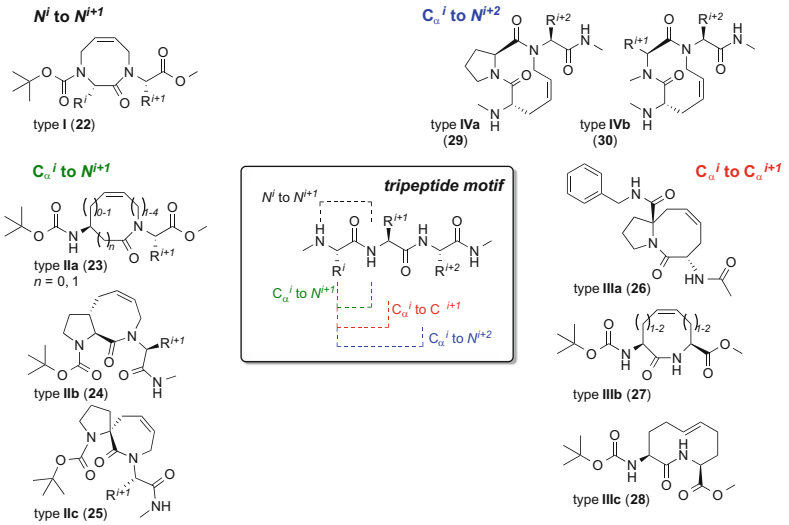


Fig. 1 Secondary structure-inducing peptide mimetic scaffolds synthesized via RCM

amide" cyclized peptides in order to define a set of rules for RCM (in the presence of Ru-catalyst **G1**) as function of the length of the alkene substituent versus the length/nature of the peptide sequence [29]. From their studies the following "rules" for cyclization of bis-*N*-alkenylated peptides by **G1**-mediated RCM could be inferred: cyclic dipeptides – involving two amide bonds – could be obtained starting from bis-*N*-allyl (or longer alkene) substituents. Cyclic tripeptides – involving three amide bonds – could be obtained starting from bis-*N*-4-pentenyl (or longer alkene) substituents. While cyclic tetra-, penta-, and hexapeptides – involving four, five, or six amide bonds, respectively – could be obtained starting from *N*-homoallyl (or longer alkene) substituents. In addition to this, the introduction of either proline or a single D-amino acid in the peptide sequence increased the overall yield of the cyclization reaction [29, 30]. These insights were used to develop the so-called rolling loop scan to probe the bioactive conformation of cyclic peptides [31].

Type **IIa** cyclic constraints, as represented by **23**, are known as Freidinger lactams [32] in which the $C_\alpha{}^i$ is connected to N^{i+1} leading to seven-membered macrocycles ($n = 0$). Their synthesis by ring-closing metathesis will result in dehydro lactams and was elaborated by Grubbs and co-workers [15] (**6**=**23** if $n = 0$, R^{i+1}=H, glycine) and Piscopio et al. (**23**, $n = 0$, R^{i+1}=Bn (phenylalanine)/^{i}Pr (valine)/Ph (phenylglycine)/$PhCH_2CH_2$ (homophenylalanine)) in yields varying between 16 and 62% [33]. The group of Gmeiner explored the synthetic accessibility of this type of constraints further by the preparation of eight-, nine-, and ten-membered macrocycles (**23**, $n = 0$) and by studying the conformational properties of these derivatives [34]. In addition to this, β-amino acids (**23**, $n = 1$) were also found to be versatile RCM substrates to access homo-Freidinger lactams [35].

Incorporation of proline as the R^i residue will lead to type **IIb** (represented by **24**) and eventually in spirocyclic type **IIc** (represented by **25**) fused lactams to tune the ψ^{i+1} dihedral angle to mimic the bioactive conformation of prolyl residues in medium-sized peptides [36]. Of special interest are type **IIc** cyclic constraints since they constitute potent type II β-turn-inducing molecular scaffolds [37]. In this context it is important to mention the contributions of the Moeller [38], respectively, Wagner [39] and Schmalz groups since they showed that stereo- and regiospecific functionalization of the proline ring with suitable alkene moieties is a powerful tool to define the conformation of the fused pyrrolidine ring as polyproline type II (PPII) helix mimic [40] or as an α-helix-nucleating motif [41] to stabilize the secondary structure of relatively small peptide sequences (vide infra).

Type **III** scaffolds, exemplified by structures like **26**–**28**, represent compounds in which $C_\alpha{}^i$ is connected to $C_\alpha{}^{i+1}$ resulting in eight- to ten-membered macrocycles (Fig. 1). Compound **26** (type **IIIa**) was synthesized by RCM and was a highly efficient proline-based type VI β-turn mimetic [42]. A series of eight- to ten-membered enantiomerically pure macrolactams (**27**, type **IIIb**) was synthesized by Lubell and co-workers [43] based on an earlier communication by Banfi et al. [44], who reported the synthesis of a racemic nine-membered lactam derivative via a combination of a Ugi 4-MCR [45] followed by RCM. Finally, compound **28** (type **IIIc**) was synthesized by Katzenellenbogen and co-workers [46] and

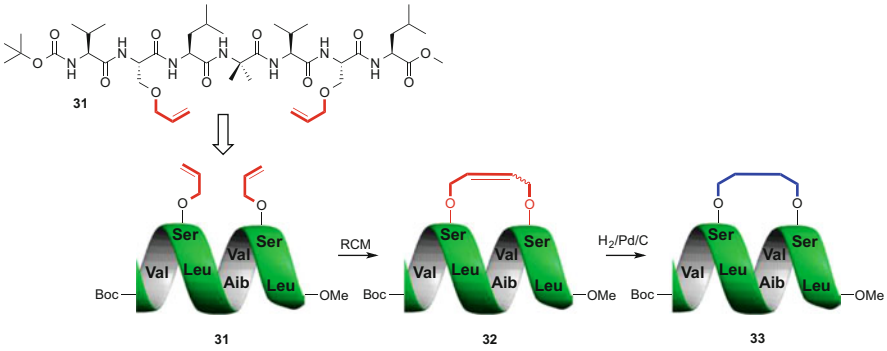
represents a (3*S*,10*S*)-(6*E*)-2-azacyclodec-6-enone constraint as a dipeptide structural mimic (vide infra).

As the last example in this series, the $C_\alpha^{\ i}$ to N^{i+2} connectivity pattern is used in type **IV** constraints and is represented by structures **29** and **30**, as shown in Fig. 1. These molecular entities have been designed by the group of Gmeiner [47] with the idea to replace a hydrogen bond by a covalent alkene constraint in Asn-Pro/Asp-Pro turn motifs, thereby mimicking type I β-turn structures, and form important scaffolds to enable molecular recognition processes between pharmacologically relevant receptor-ligand interactions.

2.4 *Stabilization of α-Helices and β-Turns in Peptides*

For covalent stabilization of α-helices, the incorporation of disulfide and lactam bridges into peptide sequences belongs to the classical toolbox of the peptide chemist. The first application of RCM to stabilize helical peptides was described by Blackwell and Grubbs [48]. They used a hydrophobic heptapeptide, Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe, that was known to adopt an α-helical conformation both in the solid state and in solution. The alanine residues were replaced by serine (compound **31**) or homoserine *O*-allyl ethers to apply RCM for macrocyclization, as shown in Scheme 5. Precursor peptide **31** was treated with catalyst **G1** (20 mol% cat, 5 mM peptide in CH_2Cl_2 at 25°C for 3 to 4 h), to yield a 21-membered cyclic peptide **32** as a mixture of *E*/*Z* isomers (approximately 5:1) in 85% yield. Hydrogenation of the double bond resulted in an alkane-bridged peptide macrocycle **33** in an almost quantitative yield.

It turned out that the ellipticities as measured with circular dichroism did not differ much for the macrocyclic peptide compared to its linear precursor peptide. This was explained by the fact that diene **31** was highly preorganized in the organic solvent used for RCM, and cyclization did not result in a significant conformational change [49]. Although a noticeable helical stabilization was not observed, the



Scheme 5 The *O*-allyl ethers form the cyclic constraint to stabilize an α-helical conformation

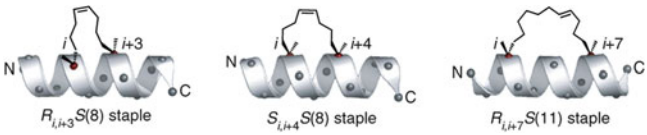
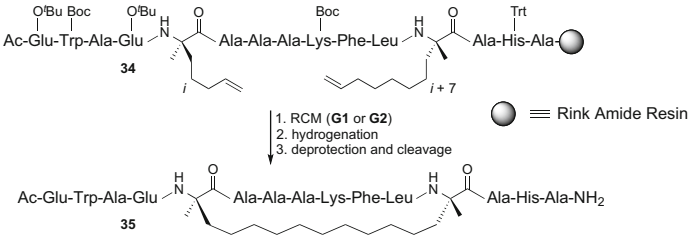


Fig. 2 Schematic representation of three stapled peptides [51]. The nomenclature $R_{i,i+3}S(8)$ refers to an eight-carbon metathesized cross-link with *R*-configuration at *i* and *S*-configuration at $i+3$ position [52]; $S_{i,i+4}S(8)$, eight-carbon tether with *S*-configuration at both *i* and $i+4$ positions; $R_{i,i+7}S(11)$ 11-carbon tether with *R*-configuration at *i* and *S*-configuration at $i+7$ position. Reprinted by permission from Macmillan Publishers Ltd. Nature Protocols (Nat Prot (2011) 6:761–771), © 2011. See [51]. *Note*: the double-bond geometry is a mixture of *E* and *Z*



Scheme 6 Example of peptide stapling for stabilizing the α-helix through all-hydrocarbon cross-linking [50]

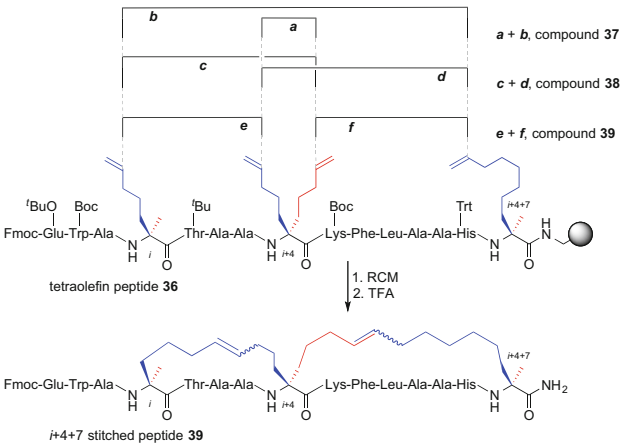
relatively easy incorporation of carbon-carbon bonds for rigidification of peptides and thereby also increasing their metabolic stability paved the way for a new era of constraining therapeutically relevant peptides.

Based on this concept, Verdine and co-workers screened a library of multiple configurations of all-hydrocarbon cross-links differing in position of attachment, stereochemistry, and cross-linker length [50]. This strategy was termed "peptide stapling" to stabilize the α-helical conformation of a peptide, while the "staple" consists of an all-hydrocarbon macrocyclic bridge connecting adjacent turns of the helix, flanked on each end by an α-methyl group, as shown in Fig. 2 [51]. The staple was incorporated into a peptide by the introduction of two appropriately α-amino-protected α-methyl-α-alkenyl amino acids via SPPS protocols; subsequently peptide macrocyclization was performed on the solid support by Ru-based RCM, followed by hydrogenation and treatment with acid to remove protecting groups and to cleave the peptide from the solid support (Scheme 6).

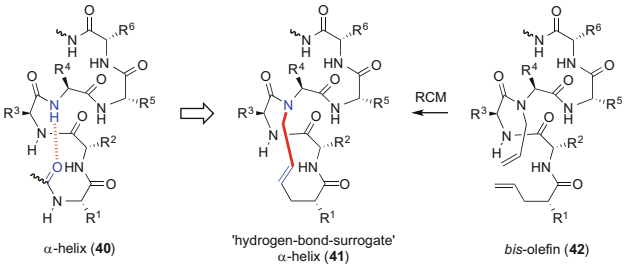
The peptide from the example shown in Scheme 6 represents the C-terminal sequence from RNAse A, and the original amino acid residues threonine at position *i* and alanine at position $i+7$ have been replaced by α-methyl-α-alkenyl amino acids as in the fully protected and resin-bound peptide **34**. This sequence was chosen since it adopts in aqueous solution a partial α-helical conformation, which allows to observe both increases and decreases in helical character. The unmodified RNAse A peptide is ~40% helical in water, while the RCM-precursor peptide is ~60% helical. Macrocyclic peptide **35**, with an 11-carbon cross-link, adopts in

water a conformation that corresponds to 84% helical character and thus helix stabilization by 44% [50]. Besides the noticeable helix stabilization, peptide **35** was ~40-fold more resistant against trypsin digestion than its linear (unmetathesized) counterpart [50]. To address the need for a more in-depth understanding on how peptide stapling stabilizes peptide helices, all-atom Monte Carlo folding simulations were performed [53]. These insights are currently explored as tools in chemical biology or to improve the drug-like properties of therapeutically relevant stapled peptides [54–60]. A recent overview of hydrocarbon-stapled peptides, which highlights their use as biomedical research tool and prototype therapeutics, is recommended to the reader who is interested in more biological applications [61].

Recently, the next generation of hydrocarbon-stapled peptide systems was reported [62] in which two hydrocarbon staples are formed from a single amino acid precursor −*bis*-pentenylglycine(2-(amino)-2-(pent-4-enyl)hept-6-enoic acid)−, as shown in Scheme 7, thereby creating a spiro-macrocyclic connectivity pattern of the amino acid side chains which is known to be exceptionally rigidifying the bicyclic peptide. This approach of introducing contiguous hydrocarbon staples along one side of the α-helix has been coined "stitching" which provides peptides superior helix induction, thermal stability, resistance against proteolysis, and cell permeability properties relative to peptide stapling. Tetraolefin peptide **36** was synthesized by Fmoc/^{t}Bu SPPS (Scheme 7), with (*S*)-α-methyl-α-pentenylglycine on position *i*, *bis*-pentenylglycine on position $i+4$, and (*S*)-α-methyl-α-octenylglycine on position $i+7$. Ring-closing metathesis was performed on the solid support in the presence of **G1** (20 mol%) in 1,2-dichloroethane for 2 h at room temperature. RCM of **36** could lead to the formation of three bis-metathesized peptides, **37**–**39**. Based on model studies it turned out (see Table 1 in [62]) that intraresidue RCM (reaction ***a***; to form cyclononenylglycine) was disfavored as was the undesired reaction ***b***, suggesting that compound **37** would be highly unlikely. Reaction ***c*** on the other hand would



Scheme 7 Tetraolefin peptide **36** designed to undergo *bis*-RCM: peptide stitching [62]



Scheme 8 α-Helix inducing by replacement of an *i* and *i* + 4 hydrogen bond by covalent carbon-carbon constraint

lead to an $S_{i,i+4}R(8)$ staple (see Fig. 2), which is known to be highly disfavored [52, 53]. These model reactions also showed that reaction ***d*** is kinetically disfavored compared to ***f***, while reaction ***e*** and ***f*** lead to well-established $S_{i,i+4}S(8)$- and $R_{i,i+7}S$ (11) staples, respectively. Thus, there is a high degree of regio- and stereochemical control over the RCM reactions on these peptides to yield compound **39** rather than **38**.

Another approach for α-helix stabilization was elegantly pioneered by Arora and co-workers [63, 64]. In their strategy they replace an *i* and *i* + 4 hydrogen bond (C=O···H-N) in an α-helix by a covalent constraint (C=X-Y-N) in which X and Y are carbon atoms introduced via olefin metathesis, as shown in Scheme 8.

Thus, α-helix sequence **40** is mimicked by its covalent congener **41**, which is synthesized from the linear *bis*-olefin precursor peptide **42** by ring-closing metathesis. This concept of α-helix stabilization was coined "hydrogen-bond surrogate" (HBS) and is a general and versatile strategy for generation of short α-helical peptide ligands to interact with therapeutically relevant receptors since the important amino acid side chains for binding interactions are still available [65]. Based on an X-ray crystal structure [66] of a short α-helix peptide (**43**; {HBS}Gln-Val-N^{All}Ala-Arg-Gln-Leu-Ala-Glu-Ile-Tyr-NH_2), it was found that this mimic indeed adopts an α-helical conformation with an RMS deviation of 0.74 Å upon superimposition of the backbone onto a model α-helical peptide, as shown in Fig. 3 [65, 66].

The synthesis of peptide **44** was carried out in solution, and RCM was performed with Hoveyda-Grubbs' catalyst (**HG2**) to afford (in its protected form) HBS α-helix **45** in 55% yield, as shown in Scheme 9. Finally, treatment with TFA removed the ^{t}Bu groups, and peptide **45** was studied by NMR and CD for structural analysis [63, 64]. For these peptides, a solid-phase synthesis approach was also developed (Scheme 8) [67]. Herein, Fmoc-Ile-N^{All}Ala-OH (indicated in red in peptide **46**) was used as a dipeptide building block for an efficient introduction of the *N*-allyl functionality. RCM was performed on the solid support with several Ru-based catalysts, since macrocyclization was far from trivial. Refluxing in CH_2Cl_2 in the presence of **G1** or **G2** did not yield significant amounts of the cyclic peptide. RCM in the presence of Ciba-Ruthenium [68] or Grubbs **G3** [69] (see insert Scheme 9) did also not exceed 20% cyclization yield; only in the presence of the Hoveyda-Grubbs' catalyst **HG2** in dichloroethane at 60°C with 25 mol% catalyst loading

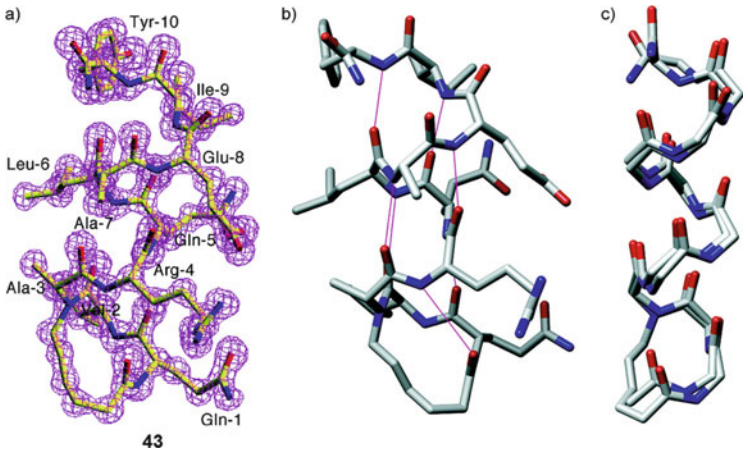
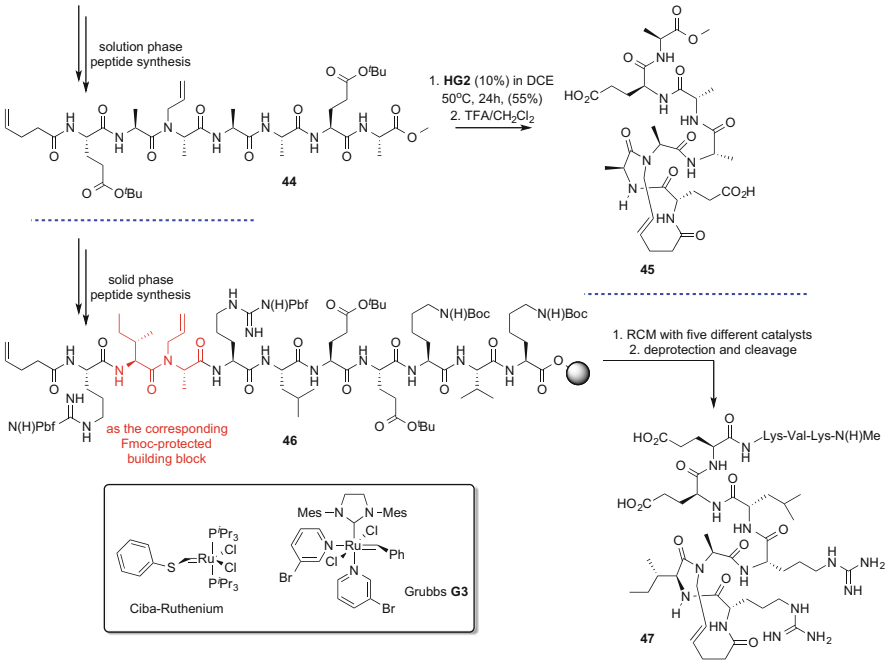


Fig. 3 (**a**) Crystal structure of the HBS α-helix with electron density superimposed onto the refined molecular model, (**b**) putative *i* and *i*+4 hydrogen bonds in crystal structure-derived molecular model of HBS helix, (**c**) overlay of crystal structure and a model of an idealized α-helix. Reprinted with permission from Acc Chem Res (2008) 41:1289–1300, © 2008 American Chemical Society. See [65]



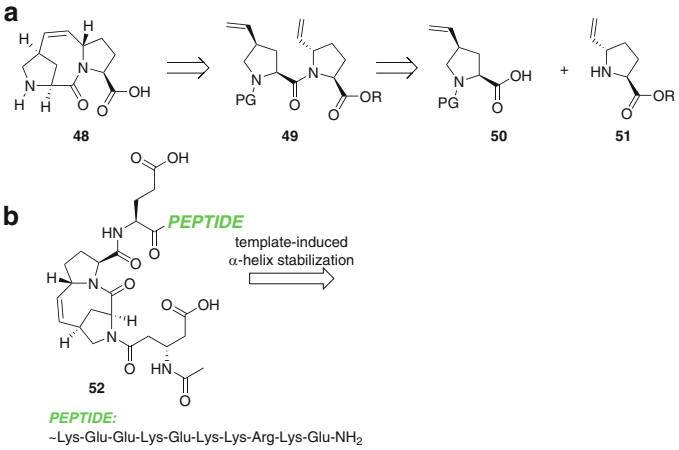
Scheme 9 Solution-phase synthesis and solid-phase synthesis of peptides **44** and **46** and HBS α-helices **45** and **51**

during 48 h, macrocyclization was efficient and cyclic peptide **47** could be isolated in approximately 90% yield [67].

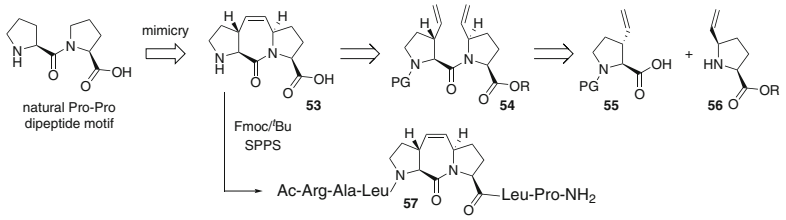
The template-induced folding of short peptide sequences is a challenging approach to the design of folded peptides with a well-characterized secondary structure [70]. α-Helix-nucleating scaffolds are rather difficult to design; however, protein helices often contain proline residues especially in the N-cap region, e.g., at the N-terminus of the protein sequence. The Pro-Pro dipeptide segment forms a partial helical structure by two intramolecular hydrogen bonds [71] and might be used to nucleate helical folding [72, 73]. Based on Kemp's Pro-Pro derivative designed to function as an α-helix-inducing N-cap motif [74, 75], the groups of Kühne and Schmalz [41] designed a tricyclic diproline scaffold **48** for efficient α-helix induction in a linear peptide, as shown in Scheme 10.

Scaffold **48** is a rigidified diproline derivative by means of an ethylidene bridge and was synthesized from precursor **49** by ring-closing metathesis using **G2** in 59% yield. Dipeptide **49** was synthesized starting with two suitably protected vinylproline building blocks **50** (*cis*-4-vinylproline) and **51** (*trans*-5-vinylproline), as shown in Scheme 10. Scaffold **48** was protected at the secondary amine with an Fmoc group for application in Fmoc/^{t}Bu SPPS to afford template-peptide **52**. As a control peptide, the tricyclic Pro-Pro dipeptide was replaced by Pro-Pro, and both peptides were analyzed by circular dichroism on their helical content. It turned out that the Pro-Pro control peptide was random coiled (conformationally unordered), while peptide **52** adopted a high degree of α-helical character [41].

The same group employed a differently annulated tricyclic diproline scaffold [40] (compound **53**, as shown in Scheme 11) to induce a polyproline type II (PPII) helical conformation in short peptides to mimic proline-rich motif-recognition domain (PRD) ligands to interact with proteins containing proline-rich motifs



Scheme 10 (**a**) Retrosynthesis of tricyclic diproline scaffold **48**, (**b**) model of an N-capped α-helical peptide. Based on Angew Chem Int Ed (2013) 52:9539–9543. © Professor H. G. Schmalz, Universität zu Köln, Germany



Scheme 11 Retrosynthesis of tricyclic diproline scaffold **53** to induce a polyproline type II (PPII) helical conformation in short peptides to mimic a proline-rich motif-recognizing domain (PRD) ligand

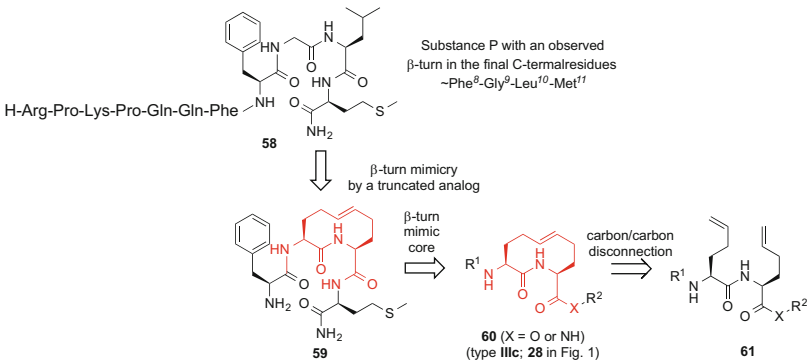
(PRMs). These interactions play an important role in nature and are involved in several biologically relevant processes, among others, tyrosine kinase receptor signaling, endocytosis, transcription, and splicing. Their studies showed that the dipeptide motif Pro-Pro is perfectly mimicked by scaffold **53** since replacing Pro-Pro in the natural ligand peptide (Ac-Arg-Ala-Leu-*Pro-Pro*-Leu-Pro-NH_2) by **53** to give **57** did not affect the binding affinity toward the target Fyn-SH3 as measured with isothermal titration calorimetry (ITC), the K_D being 18 ± 5 versus 62 ± 13 μM, respectively [40]. The retrosynthesis of tricyclic scaffold **53** is shown in Scheme 11. Ring-closing metathesis of **54** mediated by **G2** (5 mol%) in refluxing CH_2Cl_2 (15 h) gave **53** in 91% yield, while dipeptide **54** was obtained by peptide bond formation starting with *trans*-3-vinylproline (**55**) and *cis*-5-vinylproline (**56**) [40].

The synthesis of β-turn mimetics by ring-closing metathesis has already been described in Chapter 2.3 and is illustrated in Fig. 1. In the next section three examples will be discussed that make use of RCM-mediated β-turn mimetics to stabilize the conformation of the peptide, while in most cases this will also result in an increase of the biological activity of the rigidified peptidomimetic.

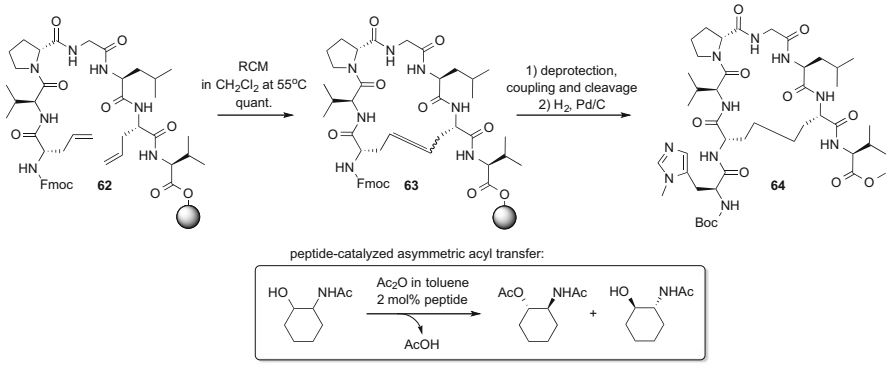
The first example refers to the work of Katzenellenbogen, as shown in Scheme 12 [46]. Herein, they mimicked the C-terminal tetrapeptide (~Phe-Gly-Leu-Met~) of Substance P (**58**, a bioactive undecapeptide that belongs to the tachykinins and plays important roles in pain sensation, inflammation, asthma, and vasodilatation [76]) by cyclic peptide **59**. By NMR studies [77] it was shown that Phe^8-Gly^9-Leu^{10}-Met^{11} adopted a β-turn, and to confirm this observation, it was envisioned that the truncated Substance P analog **59** might shed light on its bioactive conformation.

The versatile cyclic constraint **60** (indicated in red in compound **59**) allows selective functionalization with amino acids to design other tachykinin-selective receptor (ant)agonists. Its synthesis is relatively easily performed, starting with the properly protected (*S*)-2-aminohex-5-enoic acid to afford dipeptide **61**, which undergoes a smooth RCM in the presence of **G1** as the catalytic species to afford **60** in a good yield of 65% [46].

In the second example, ring-closing metathesis was applied to stabilize an octapeptide β-hairpin that exhibits enantioselective acylation activity, as shown in



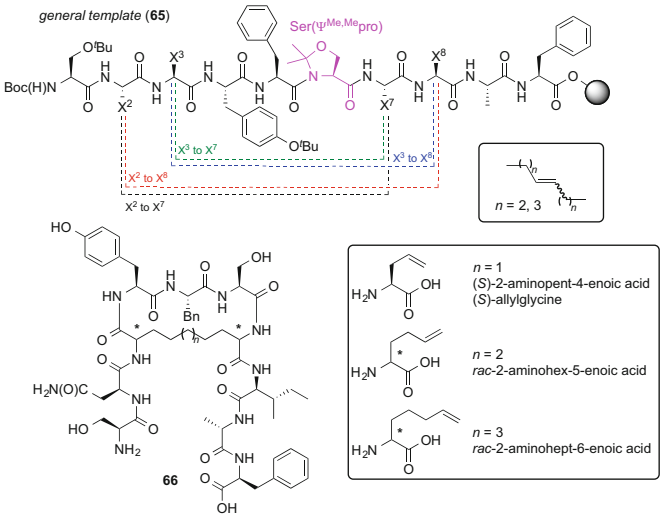
Scheme 12 The incorporation of a β-turn mimic in Substance P to define its bioactive conformation [46]



Scheme 13 Synthesis of rigidified peptide **64** as an asymmetric acyl transfer catalyst [78]

Scheme 13 [78]. The linear peptide, Boc-N^{Im}MeHis-*Val*-Val-D-Pro-Gly-Leu-*Val*-Val-OMe was found to be active as a catalyst in the asymmetric acyl transfer reaction with *rac*-2-acetylaminocyclohexanol as the substrate with a $k_{rel} = 51$ (see insert Scheme 13) [78]. To be active as a catalyst, it should adopt a β-hairpin conformation. In a biomimetic approach, Miller and co-workers synthesized solid-phase-bound peptide **62**, in which two valine residues were replaced by allylglycine, and applied RCM mediated by **G1** to arrive at macrocyclic peptide **63** in quantitative yield. After Fmoc removal, Boc-N^{Im}MeHis-OH coupling, resin cleavage by transesterification, and catalytic hydrogenation, rigidified peptide **64** was obtained. It turned out that macrocyclic peptide **64** as well as its acyclic analog were less enantioselective than the original octapeptide in the kinetic resolution of *rac*-2-acetylaminocyclohexanol, $k_{rel} = 12$ and 20, respectively [78]. This was explained by assuming "that substituents along the peptide backbone may be more important than covalent stabilization of a structural motif" [78].

The third example describes a general synthesis approach to apply ring-closing metathesis to resin-bound peptides [79]. In their approach, Schmiedeberg and



Scheme 14 Solid-phase RCM approach of backbone-protected peptides [79]

Kessler found that RCM was only successful by the introduction of a secondary structure disrupting reversible backbone protection, Ser($\psi^{Me,Me}$pro). Their decapeptide (H-Ser^1-Asn-Lys-Tyr-Phe^5-Ser-Asn-Ile-His-Trp^{10}-OH) was derived from the serine protease urokinase-type plasminogen activator (uPA) and was identified as a receptor-binding domain mimic. However, the C-terminal dipeptide ~ His-Trp ~ was replaced by ~ Ala-Phe ~ to remedy the chemical instability of the former dipeptide sequence against the reductive reaction conditions due to long reaction times. This modified peptide was used as the general cyclization template (**65**), as shown in Scheme 14.

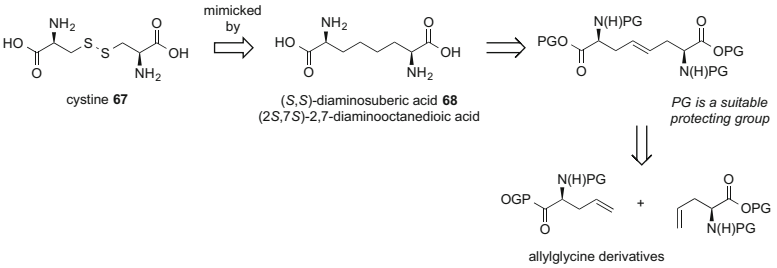
Generally, the solid-phase synthesis of peptides that contain β-turn- or β-hairpin-like secondary structure as introduced by ring-closing metathesis often proceeds with good conversions. However, some peptides were found to be notoriously difficult to cyclize, independent of their ring size and stereochemical orientation, position, and number of carbon atoms of the olefinic amino acid residues. This failure against ring closure originates from the formation of inappropriate secondary structures that prevents the peptides from macrocyclization by RCM. As shown by Reichwein and Liskamp [28, 29], incorporation of *N*-alkenyl amino acids or proline residues will foster the efficiency of the cyclization reaction. Alternatively, reversible backbone protection by, among others, amide protection by either a 2,4-dimethoxybenzyl functionality [80] or *tert*-butyloxycarbonyl group [81] or by incorporation of pseudoprolines [82] is a versatile approach to overcome these difficulties during RCM-mediated cyclization reactions [79]. Schmiedeberg and Kessler [79] synthesized a series of uPA-derived peptides (general template, **65**) in which Ser^6 was replaced by pseudoproline Ser($\psi^{Me,Me}$pro) as introduced via building block Fmoc-Phe-Ser($\psi^{Me,Me}$pro)-OH [82], and the four combinations, Asn^2/Asn^7, Asn^2/Ile^8, Lys^3/Asn^7, and Lys^3/Ile^8, were substituted by the racemic amino acids

2-aminohex-5-enoic acid and 2-aminohept-6-enoic acid (see insert Scheme 14). RCM by **G1** proceeded smoothly, generally in yields varying between 56 and 84%. It should be noted, however, that incorporation of (*S*)-allylglycine as the olefinic amino acid was considered as not suitable, since too large amounts of unreacted peptide were found in the reaction mixture. After cleavage from the resin, the protected peptides with *cis*/*trans* double bonds were subjected to reduction, and remarkable differences in reactivity were found, strongly dependent on the number of carbon atoms spanning the bridge between the peptide backbone. As a representative example, cyclic peptide **66**, with an $X^3 \rightarrow X^7$ connectivity pattern was isolated in 8% ($n = 2$) and 6% ($n = 3$) yield, respectively, after reduction, deprotection, trituration, and HPLC purification [79].

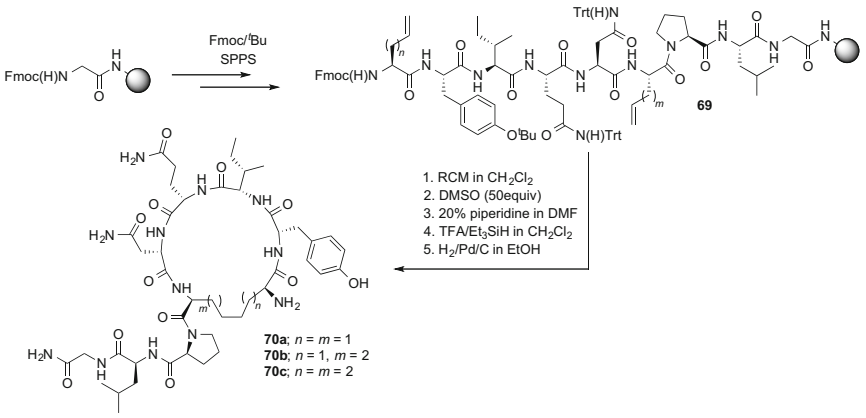
2.5 *Dicarba Analogs by RCM to Mimic Disulfide and Thioether Bridges*

In their seminal contribution to RCM-based peptide cyclization [14, 15], Grubbs and co-workers described the successful mimicry of peptide disulfides by dicarba analogs, as shown in Scheme 2, compounds **9**–**11**, to stabilize β-turn conformational constraints. The disulfide bridge in peptides and proteins is an important entity to stabilize the secondary structure of peptide hormones. Since disulfides are rather sensitive toward reducing conditions, nucleophiles, and bases, several approaches have been developed to mimic the disulfide moiety by dicarba bridges. Thus, cystine **67**, can be replaced by an isosteric unit, like the all-carbon ~ $(CH_2)_4$ ~ fragment, resulting in the non-reducible (2*S*,7*S*)-2,7-diaminooctanedioic acid ((*S*,*S*)-diaminosuberic acid) **68** (Scheme 15), which is easily accessible by olefin metathesis-based syntheses starting from suitably protected allylglycine derivatives [83–85].

One of the first applications to replace disulfide bonds in peptide hormones featuring the cyclization of bis-allylglycine-containing peptides was reported by Vederas and co-workers [86]. In their study, they synthesized a linear oxytocin-derived sequence **69** (in which $n = m = 1$, as shown in Scheme 16), in which the



Scheme 15 Synthesis of (*S*,*S*)-diaminosuberic acid by metathesis approaches

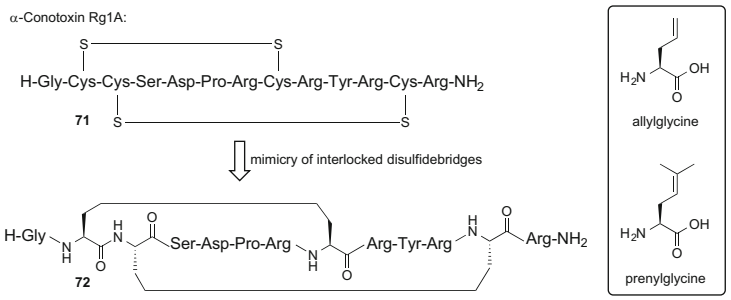


Scheme 16 Synthesis of dicarba oxytocin analogs using RCM on the solid support

cysteines were replaced by L-allylglycine residues. After cyclization, mediated by Ru-catalyst **G1**, the resin-bound peptide was treated with DMSO, an essential step to remove isomerization products and ruthenium-containing contaminants. Subsequent Fmoc removal, acidic deprotection and cleavage from the resin, and hydrogenation resulted in the isolation of oxytocin dicarba analog **70a** in an acceptable yield (8 to 28%). The higher cyclization yield was obtained when the second-generation Grubbs' catalyst **G2** was used. The biological activity of these oxytocin derivatives were tested, and the *cis*-intermediate was the most active oxytocin mimic ($EC_{50} = 38$ ng/mL), while the *trans*-intermediate ($EC_{50} = 242$ ng/mL) and the saturated analog **70a** ($EC_{50} = 338$ ng/mL) were two order of magnitude less active than native oxytocin ($EC_{50} = 2.7$ ng/mL) [86]. In a follow-up study by the same authors, the influence of the geometry of the double bond and the size of the peptide macrocycle were investigated for oxytocin (ant)agonistic activity to design long-lasting and commercially available oxytocin-derived peptide therapeutics for the initiation of parturition and treatment of preterm labor [87]. Their main conclusion was that 20-membered macrocycles, as in **70a**, were the most active, while the 21-membered oxytocin derivative **70b** was a very weak agonist ($EC_{50} = 1{,}400$ ng/mL), and the 22-membered congener **70c** was virtually inactive. However, half-lives of the dicarba analogs were found considerably increased (8 to 11 min longer) compared to their parent disulfide peptides.

Other examples of hydrocarbon bridges as isosteric replacements of disulfides in bioactive peptides have been reviewed [4, 6] and reported in the literature, among others, to arrive at redox-stable analogs of sunflower trypsin inhibitors [88], cyclic dermorphin tetrapeptides [89], cyclic enkephalin peptides [90, 91], octreotide- [92–94] and somatostatin-derived peptides [95], and an antimicrobial human β-defensin-1 derivative [96] and leucocin A analogs [97].

The regioselective formation of interlocked dicarba bridges as exemplified by the peptide toxin α-conotoxin Rg1A **71** (Scheme 17) required olefinic substrates with tunable reactivity against the ruthenium-based metathesis catalysts. Thus, the



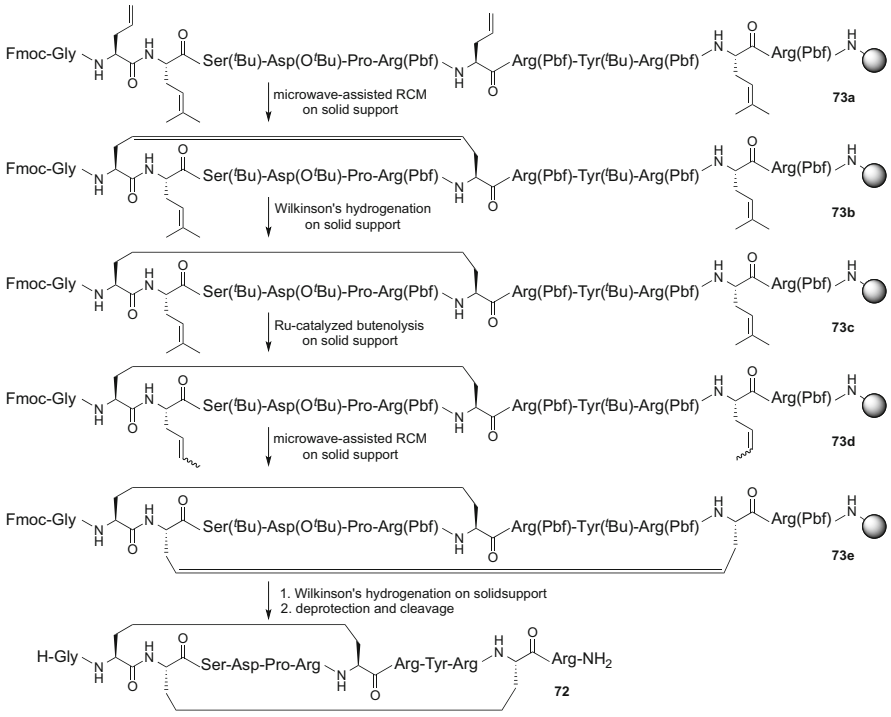
Scheme 17 α-Conotoxin Rg1A **71** and its interlocked dicarba analog **72**

incorporation of allylglycine (Alg) and prenylglycine (Pre) (insert Scheme 17), two metathesis-active olefinic amino acids, facilitates the regioselective formation of the intramolecular dicarba bridges in peptide **72**, as shown in Scheme 18 [98].

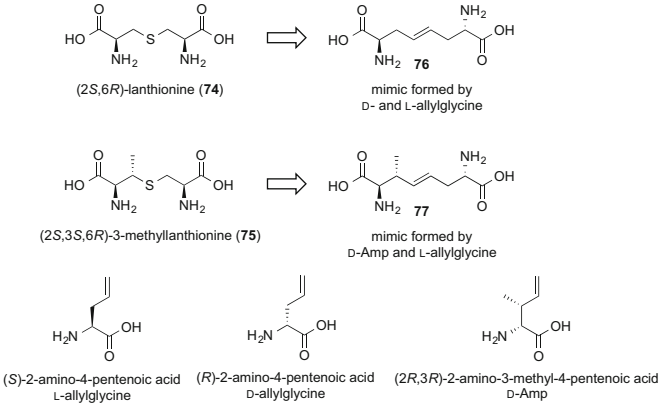
The linear peptide **73a** was synthesized on the solid support via well-established Fmoc/^{t}Bu protocols. Initially, ring-closing metathesis of resin-bound peptide **73a** that was performed in CH_2Cl_2 with Ru-catalyst **G2** (20 mol%) in the presence of 5% 0.4 M LiCl/DMF at 40°C for 40 h under conventional heating did not result in any conversion toward carbocycle **73b**. However, microwave irradiation of resin-bound peptide **73a** in CH_2Cl_2 with Ru-catalyst **G2** (20 mol%) in the presence of 5% 0.4 M LiCl/DMF at 100°C [99] resulted in complete ring closure to **73b** in only 2 h. Apparently, microwave irradiation is able to disrupt peptide aggregation and facilitates cyclization toward peptide carbocycles that cannot be obtained via conventional heating methods [100]. Gratifyingly, the prenyl side chains remain unaffected under these microwave-assisted reaction conditions. Selective hydrogenation of carbocycle **73b** with homogeneous Wilkinson's catalyst ([Rh(I)$(PPh_3)_3Cl$]) and 80 psi H_2 in CH_2Cl_2/MeOH at room temperature resulted in macrocyclic resin-bound peptide **73c**. Subsequently, the prenyl moieties that resisted hydrogenation were activated by butenolysis by exposing construct **73c** to an atmosphere of *cis*-butene and Ru-catalyst **G2** (20 mol%) at 50°C for 72 h, and the formation of *bis*-crotylglycine-containing peptide construct **73d** was confirmed by mass spectrometry. Then, resin-bound peptide **73d** was subjected to microwave-assisted RCM under the same reaction conditions as for **73a**, which resulted in the correct bicyclic conotoxin framework **73e**. After hydrogenation and deprotection/cleavage, the saturated *bis*-dicarba conotoxin analog **72** was obtained [98].

Sulfide bridges (thioethers) as present in lanthionine **74** and 3-methyllanthionine **75** can also be mimicked by dicarba bridges, like derivatives **76** and **77**, as shown in Scheme 19.

Lanthionines are important conformational constraints to induce structural rigidity and to conserve the three-dimensional shape and bioactive conformation of an important class of antimicrobial peptides, called lantibiotics [101–105]. This family of peptide antibiotics is ribosomally synthesized by Gram-positive bacteria to act



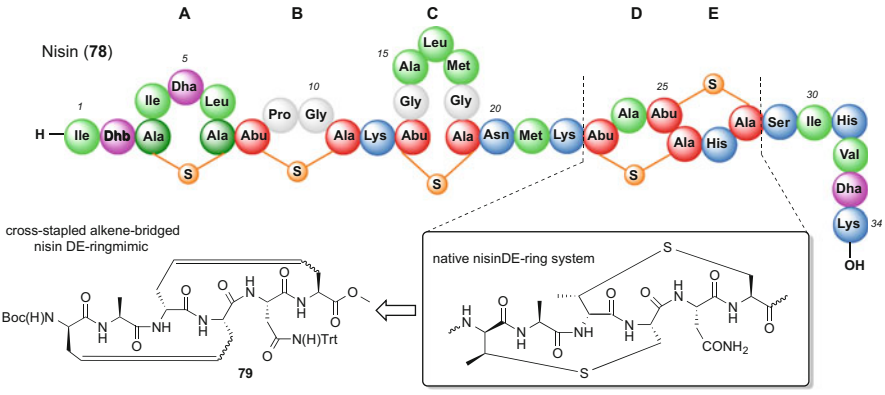
Scheme 18 Regioselective synthesis of interlocked dicarba bridges by tunable reactivity against RCM [98]



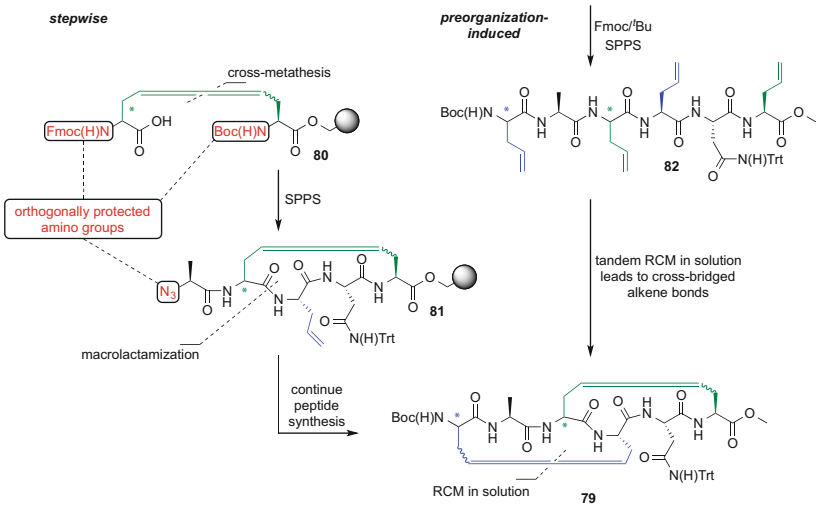
Scheme 19 Structures of the (2*S*,6*R*)-lanthionine and (2*S*,3*S*,6*R*)-3-methyllanthionine moieties and their corresponding alkene-bridged mimics formed by (*S*)-2-amino-4-pentenoic acid, (*R*)-2-amino-4-pentenoic acid, and (2*R*,3*R*)-2-amino-3-methyl-4-pentenoic acid; note: the stereochemistry of the stereogenic centers (mimic vs. lanthionine) is the same, and their *R*/*S* designations are opposite due to the CIP rules

against competing microorganisms and include nisin (**78**), subtilin, and lacticin 481. The lanthionine contains a sulfide bridge that is formed after a series of enzyme-catalyzed posttranslational modifications [106]. Although nisin is widely used as food preservative, it is unstable at basic pH and readily oxidizes and reacts with water or thiol-containing nucleophiles [107–109]. Therefore, we [110–116] and other researchers [117] initiated a program to find more redox-stable lantibiotics in which the thioether functionality is replaced by a two-carbon (dicarba) bridge, based on alkyne [113], alkene, or alkane moieties, which is conveniently introduced by ring-closing metathesis, and we successfully synthesized bioactive nisin AB [115]-, ABC [114, 116]-, and DE-fragment mimics [110–112] (**79**). The DE ring is of special interest since it contains an interlocking thioether bridge (as shown in Scheme 20) which is difficult to access synthetically [118–120].

The synthesis of bicyclic peptide **79** is shown in Scheme 21. Boc-L-Alg-OH was coupled to the solid support and subjected to a cross-metathesis reaction with Fmoc-D(or L)-Alg-OH in the presence of the second-generation Grubbs' catalyst to afford resin-bound alkene **80** [111]. Then, the Fmoc was removed and the amine was coupled to N_3-Ala-ONSu, in which the azide was used as a protecting group, orthogonally to the Boc functionality. Subsequently, the Boc group was removed by acid, followed by coupling of Fmoc-Asn(Trt)-OH and Fmoc-Alg-OH, respectively, and subsequent Fmoc removal of the allylglycine α-amino moiety. In the next step, macrolactamization between both allylglycine residues was performed in the presence of HATU/HOAt/DIPEA as coupling reagents in DMF during 16 h at room temperature. The correct side-chain to side-chain connectivity of ring E (a small amount of resin **81** was cleaved from the resin for analysis) was confirmed by NMR and mass spectrometry. After Staudinger reduction of the azide, Boc-Alg-OH was coupled, and the obtained monocyclic hexapeptide was cleaved from the resin, followed by RCM mediated by Ru-catalyst **G2** in solution. Bicyclic peptide **79** was



Scheme 20 Schematic representation of nisin (**78**); Dhb, *Z*-dehydrobutyrine; Dha, dehydroalanine; Abu, α-aminobutyric acid. The *insert* shows the structural formula of the DE ring, while **79** represents a cross-stapled alkene-bridged nisin DE-ring mimic

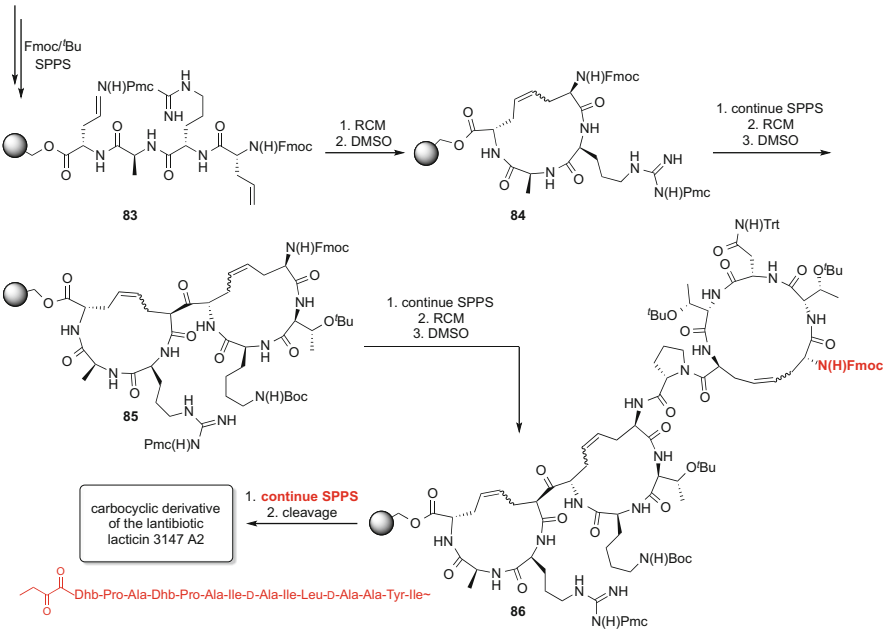


Scheme 21 Stepwise- and preorganization-induced synthesis of a crossed alkene DE-ring mimic [110–112]

obtained in 11% overall yield and characterized by NMR (^{1}H-500 MHz, COSY, TOCSY, ROESY) in combination with MS (LCES-TOF MS-MS) [111].

As an alternative synthesis route, linear hexapeptide **82** was assembled on the solid support and subsequently cleaved from the resin in its protected form. This peptide was treated with the second-generation Grubbs' catalyst in CH_2Cl_2 at 60°C for 2 h [110, 111]. The reaction mixture contained one compound, consisting as a mixture of the four geometrical isomers, which could be separated by preparative HPLC [112]. NMR and mass analyses indicated that only compounds were formed with the correct side-chain to side-chain connectivity pattern: $1 \rightarrow 4/3 \rightarrow 6$, this may hint to a certain degree of preorganization of the linear peptide. Ring-closing metathesis in the presence of the Hoveyda-Grubbs' catalyst gave an identical result [112], while treatment of peptide **82** with **G1** resulted in **79** and a monocyclic peptide with the $1 \rightarrow 4$ macrocycle in a ratio of 2:1 after 16 h of refluxing in CH_2Cl_2 [110, 111]. Dicarba AB- and ABC-ring mimics of nisin were able to recognize its natural substrate lipid II, although with a tenfold reduced affinity compared to its native counterpart [114, 115], while the DE-ring mimics were found to be devoid of membrane permeabilizing interactions [112, 116].

A multiple on-resin metathesis approach to introduce dicarba bridges as sulfide isosteres in lantibiotics was developed by Vederas and co-workers, as shown in Scheme 22 [117]. In their approach, resin-bound *bis*-allyl peptide **83** was treated with 20 mol% second-generation Grubbs' catalyst in refluxing CH_2Cl_2 for 12 h to give smoothly monocyclic peptide derivative **84**. Any ruthenium by-products were removed by a DMSO washing step. Peptide synthesis was continued, followed by a second RCM step (under the same reaction conditions as the first one) to give a single reaction product: bicyclic peptide **85**. SPPS was continued to give the



Scheme 22 Multiple on-resin ring-closing metathesis approach to form ring-expanded analogs of the lantibiotic lacticin 3147 A2 [117]

precursor to **86**; however, the last cyclization required an increase of catalyst loading to 50 mol%, and the reaction time was increased to 48 h, which ultimately gave a single reaction product. To exclude scrambling of the double bonds by ring-opening metathesis subsequently followed by RCM, a thorough LC MS-MS analysis was performed, which confirmed the absence of any crossover reaction products. The continuation of peptide synthesis by a combination of SPPS and fragment assembly in solution resulted in the successful synthesis of a carbon analog of lacticin 3147 A2. Unfortunately, the carbocyclic lacticin derivative was not active as an antimicrobial peptide at concentrations comparable to native lacticin, an indication that the mimic does not have the correct bioactive conformation to bind its target, the lacticin A1 complex, with lipid II.

2.6 RCM of Peptides in Water

Ring-closing metathesis of peptides, either in solution or on solid phase, is a well-established synthetic approach to arrive at cyclic peptides. Nevertheless, several peptide sequences are notoriously difficult to cyclize. It is assumed that a reduced solubility or aggregation phenomena on the solid support are responsible for these sluggish cyclization reactions. Semi- and unprotected peptides generally display

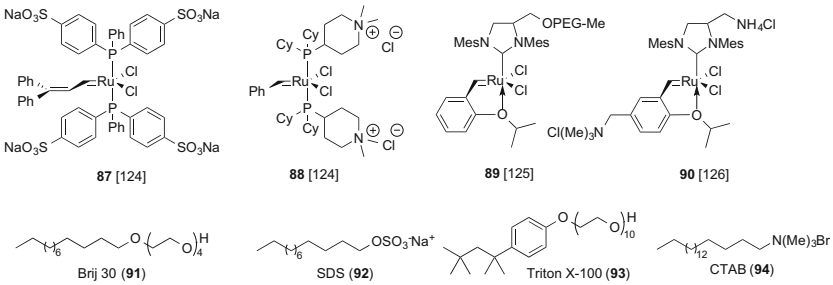


Fig. 4 Selected metathesis catalysts and surfactants used in aqueous olefin metathesis

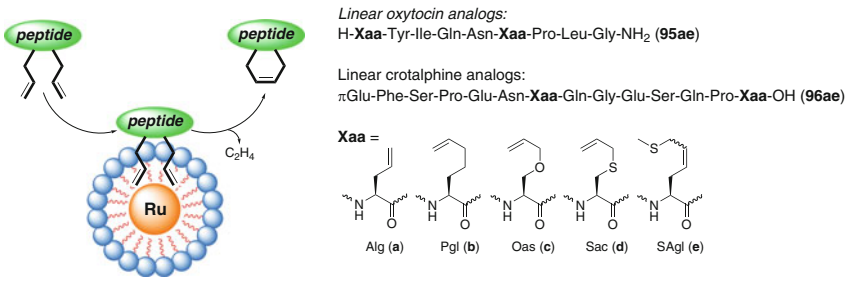
high solubility in aqueous media due to the presence of polar functional groups, like amine (Lys, N-terminus), hydroxyl (Ser, Thr), carboxylic acid (Asp, Glu, C-terminus), amide (Asn, Gln, C-terminus), imidazole (His), and guanidine (Arg) moieties. Gratifyingly, these functional groups are well tolerated by the ruthenium-based metathesis catalysts. Therefore, it is assumed that aqueous olefin metathesis [121, 122] is a promising approach, not only for peptide synthesis but also in the field of protein chemistry and bioconjugation [123].

Aqueous olefin metathesis requires the development of water-soluble (pre)catalysts, and several have already been reported in the literature [124–126] (e.g., catalysts **87**–**90**, Fig. 4), while other approaches focus on the development of micellar environments (e.g., surfactants **91**–**94**, Fig. 4) to enhance transition-metal-catalyzed carbon-carbon formation reactions [127, 128].

The latter approach was applied by Vederas and co-workers to investigate aqueous ring-closing metathesis on two peptides that were difficult to cyclize on resin [129].

In their approach (shown in Scheme 23 and Table 1), two peptide derivatives were used as model systems, oxytocin (**95ae**) and crotalphine (**96ae**) [130], a peptide hormone that controls mammary and uterine smooth muscle contraction and an orally active analgesic, isolated from the venom of the rattlesnake *Crotalus durissus terrificus*, respectively. In case of the oxytocin derivatives, all on-resin cyclizations proceeded smoothly, except in case of **96e** (containing SAlg, an analog of *S*-allyl cysteine in which the position of the sulfur and alkene moiety is switched), while all crotalphine analogs were inert toward on-resin metathesis reaction conditions. Therefore, several detergents (shown in Fig. 4), e.g., sodium dodecyl sulfate (SDS, **92**), Triton X-100 (**93**), and cetyltrimethylammonium bromide (CTAB, **94**), were applied at critical micelle concentration with unprotected oxytocin and crotalphine analogs in water with **HG2** as the solubilized RCM catalyst (Scheme 23). All these attempts failed; however, some product formation in case where Xaa was pentenylglycine (Pgl, **b**), *O*-allylserine (Oas, **c**), and *S*-allylcysteine (Sac, **d**) could be identified by mass spectrometry, while product isolation was unsuccessful (Table 1).

Finally, an aqueous solution with 30% *tert*-BuOH as cosolvent with a high catalyst loading (50 equiv **HG2**) in the presence of a large excess of $MgCl_2$ (5000 equiv), to suppress ruthenium-peptide adduct formation, at 37°C for 24 h,



Scheme 23 Aqueous RCM of oxytocin and crotalphine analogs [129]

Table 1 Ring-closing metathesis of peptides in water [129]

	Alg	Pgl	Oas	Sac	SAlg
Oxytocin					
On solid support	✔	✔	✔	✔	✗
Micellar solution	✗	–[a]	–[a]	–[a]	n.d.
Aqueous solution	✗	<[b]	<[b]	78%[c,d]	✗
Crotalphine					
On solid support	✗	✗	✗	✗	✗
Micellar solution	✗	–[a]	–[a]	–[a]	n.d.
Aqueous solution	✗	<[b]	<[b]	63%[c]	✗

[a]Although the product was formed (only in 8.2 mM SDS), it could not be isolated
[b]Isolated yield <1% in H_2O/*tert*-BuOH 7:3 v/v with $MgCl_2$ (5,000 equiv)
[c]Isolated yield in H_2O/*tert*-BuOH 7:3 v/v with $MgCl_2$ (5,000 equiv)
[d]Isolated yield only with the α-amine protected by Fmoc
n.d. not determined
This sign (✔) indicates that product is formed; while this sign (✗) indicates that product is not formed

ultimately resulted in a successful RCM of **95d** and **96d**, since their cyclic congeners were isolated in 78% and 63% yield, respectively (Table 1).

The authors conclude that "the sulfur atom within *S*-allylcysteine drastically improved the efficiency of RCM compared to pentenylglycine and *O*-allylserine analogs" and that their method provides "an alternative to the on-resin cyclization of *S*-allylcysteine, which is particularly attractive in instances where on-resin cyclization is not possible" [129].

2.7 *RCM of Peptide-Based Drug Molecules*

Peptides have unprecedented biological activities regarding selectivity, specificity, and affinity. However, due to their polar character and many functional groups, their drug-like properties are not always optimal. Therefore, peptide lead molecules

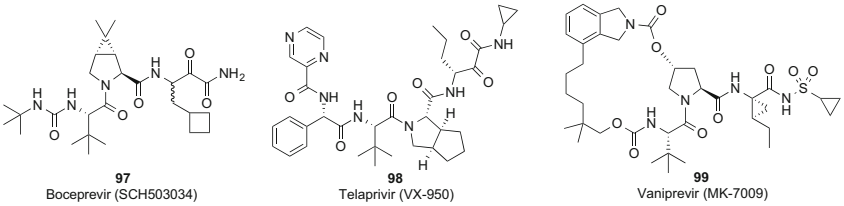


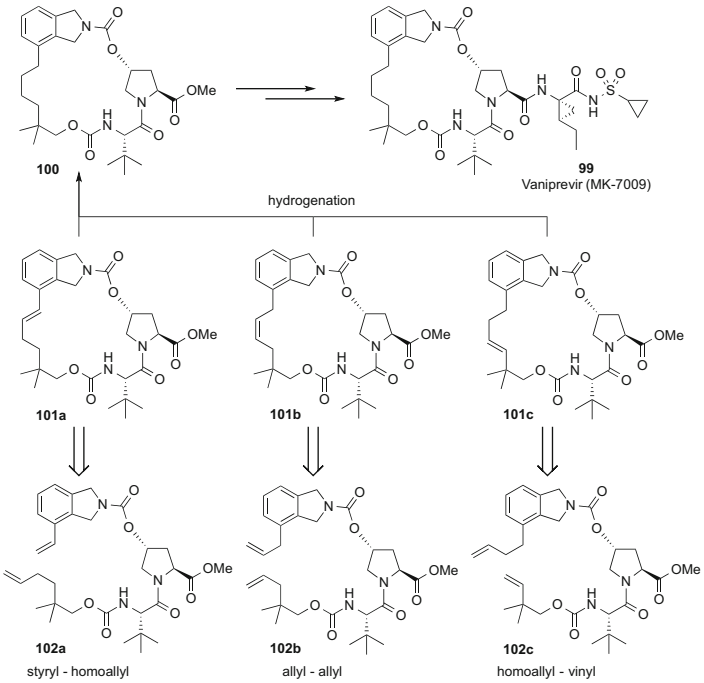
Fig. 5 HCV NS3/4a protease inhibitors

will be used to design peptidomimetics with improved properties like bioavailability and biostability. This approach has been shown to be very effective in case of the design of protease inhibitors [131]. The substrate-based inhibitor design led to the development of a series of highly active hepatitis C virus NS3/4a protease inhibitors [132, 133], like boceprevir (SCH503034, **97**) [134], telaprevir (VX-950, **98**) [135], and vaniprevir (MK-7009, **99**) [136], as shown in Fig. 5. The latter was prepared using an optimized ring-closing metathesis approach to ensure the supply of this molecule for ongoing clinical studies.

The primary challenges to develop a scalable and cost-effective synthesis for vaniprevir (MK-7009) **99** were to identify an efficient macrocyclization approach and versatile synthesis routes to the key intermediates required to assemble the cyclization precursor molecules. Macrolactamization and Heck-based couplings have been used to install the 20-membered macrocycle of vaniprevir in ten linear steps with 30% overall yield [137]. As an alternative, ring-closing metathesis was used for the highly efficient synthesis of vaniprevir in nine linear steps and 55% overall yield [138, 139]. This RCM approach employs a low catalyst loading (0.2 mol%) and a relatively high substrate concentration for ring-closing metathesis (0.13 M).

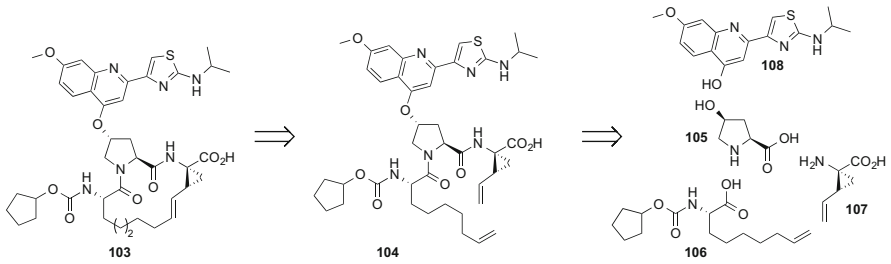
As shown in Scheme 24, there are three possible options for the construction of the macrocycle on the basis of different positions of the olefin metathesis: styryl-homoallyl (**102a**), allyl-allyl (**102b**), and homoallyl-vinyl (**102c**) diene precursors [138]. Based on an initial screening [137], ring-closing metathesis of **102a** was performed with M1 [140] (30 mol%) and gave **101a** in 91% yield, while RCM in the presence of Zhan-1B [141] (10 mol%) resulted in the isolation of **101a** in 98% yield. Despite the high yields, high catalyst loading and high dilution conditions (<3 mM) were required. When the catalyst loading was reduced or when the concentration was increased, lower yields were observed. Unfortunately, RCM of diene **102a** could not be improved by the variation of catalysts and reaction conditions. Moreover, catalyst removal after the cyclization reaction was challenging, and the catalysts' high costs and patent protection made this approach less amendable. Therefore, a more efficient ring-closing strategy was developed, and it was envisioned that the allyl-allyl diene **102b** would be a better cyclization precursor.

The first RCM reaction of diene **102b** was run by using 1 mol% of the second-generation Hoveyda-Grubbs' catalyst (**HG2**) under high dilution conditions, and



Scheme 24 Three possible options for the RCM route of the macrocyclic vaniprevir precursor **100**

macrocycle **100** was obtained in 57% yield, after hydrogenation. The yield was only marginally increased (to 67%) when the catalyst loading was increased to 5 mol%. However, the yield increased to 82% when 1 mol% of catalyst was added over 60 min at 60°C to a solution of diene **102b** in toluene (50 mL/g diene). The next step in the optimization process was to increase the concentration of the RCM reaction mixture. At 30 mL/g diene, the desired 20-membered macrocycle **101b** was obtained in only 61% yield, while a 19-membered side product was isolated in 9% yield. It was postulated that this side product was derived from a styryl isomer, which on its turn was generated from the isomerization of diene **102b**. This olefin isomerization reaction, due to the decomposition of the Ru catalyst [142], has been described in the literature, and vinyl formation can be effectively suppressed by the addition of quinone derivatives [143]. It was found that the addition of 2,6-dichloroquinone decreased the formation of the 19-membered ring to $<1\%$ without compromising the yield when the reaction was performed at 20 mL/g diene. After several optimizations, it was found that this RCM reaction gave 91% cyclization yield when the reaction was performed in toluene at 100°C at a concentration of 13.5 mL/g diene with a catalyst loading of 0.2 mol% by implementing a simultaneous slow addition of both catalyst and diene. Macrocycle **100** was conveniently obtained by hydrogenation of **101b** after crystallization from aqueous 2-propanol: 89% yield with >99% HPLC purity [138]. The third alternative route,



Scheme 25 Retrosynthesis of ciluprevir (BILN 2061) **103**, an NS3 HCV protease inhibitor [144]

the homoallyl-vinyl cyclization approach (**102c**), was not further explored by these authors, presumably by anticipating on the difficult cyclization due to the neopentylic olefin.

Another example of ring-closing metathesis to synthesize a peptide-derived drug molecule is given in Scheme 25, which describes the retrosynthesis of ciluprevir (BILN 2061) **103** [144], an NS3 protease inhibitor with proven antiviral activity in humans to treat hepatitis C infections [145].

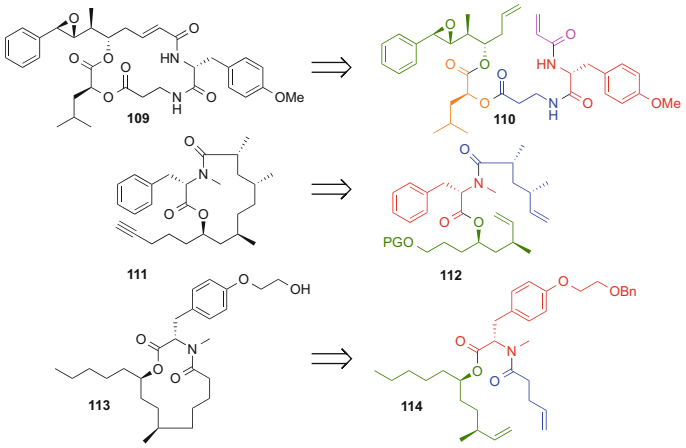
The retrosynthesis of macrocycle **103** suggests that the 15-membered ring can be obtained from its linear precursor tripeptide **104** by ring-closing metathesis. This acyclic peptide can be efficiently assembled in a convergent manner in solution-phase peptide-coupling procedures by using four building blocks, including three unnatural amino acids: *trans*-(2*S*,4*R*)-hydroxyproline (**105**), N^{α}-cyclopentyloxy-carbonyl-(*S*)-2-amino-non-8-enoic acid (**106**), and (2*R*,3*S*)-3-vinyl-2-amino-2-cyclopropyl-carboxylic acid (**107**) and a 4-quinolinol moiety (**108**). Several ruthenium catalysts were used to optimize the ring-closing metathesis reaction of **104** [146]. The initial RCM reactions were performed with the first-generation Grubbs' catalyst **G1**. It turned out that the backbone conformations of each acyclic tripeptide, and in particular the ratio of the proline *cis*/*trans* conformers, were found to be crucial to the reaction rate, yield, and diastereomeric purity of the macrocyclic tripeptide(s). The absence of any conformational preference of the peptide backbone resulted in poor RCM yields (<15%), while the bulky Boc group was responsible for a certain degree of preorganization in a β-strand-like conformation that favored macrocyclization (~80% yield). Ring size was another important determinant for macrocyclization. Dienes with the C9 linker (as in **106**) incorporated were efficiently cyclized into the 15-membered ring structure, while the more constrained 13- and 14-membered macrocyclic peptides could only be obtained with an epimerized Cβ of the vinylcyclopropane moiety. Furthermore, RCM with **G1** in CH_2Cl_2 as solvent gave the highest yield (83%); however, the amount of epimerization was relatively high (13%) under these reaction conditions and de facto unacceptable, especially on multi-kilogram production scale. Changing the catalyst to **G2** resulted in a clean conversion into the macrocyclic peptide without any noticeable amount of epimerized product; unfortunately, under these conditions, the yield was significantly lower, 52% (**G2**) versus 83% (**G1**). RCM catalyzed by the first-generation Hoveyda's catalyst [147] was slower than in case

of **G1**; however, the desired 15-membered macrocyclic peptide was obtained in 85% yield with an excellent purity of >99% as measured by chiral HPLC [146]. Besides that, Hoveyda's catalyst could be recovered from the crude reaction mixture and recycled [144].

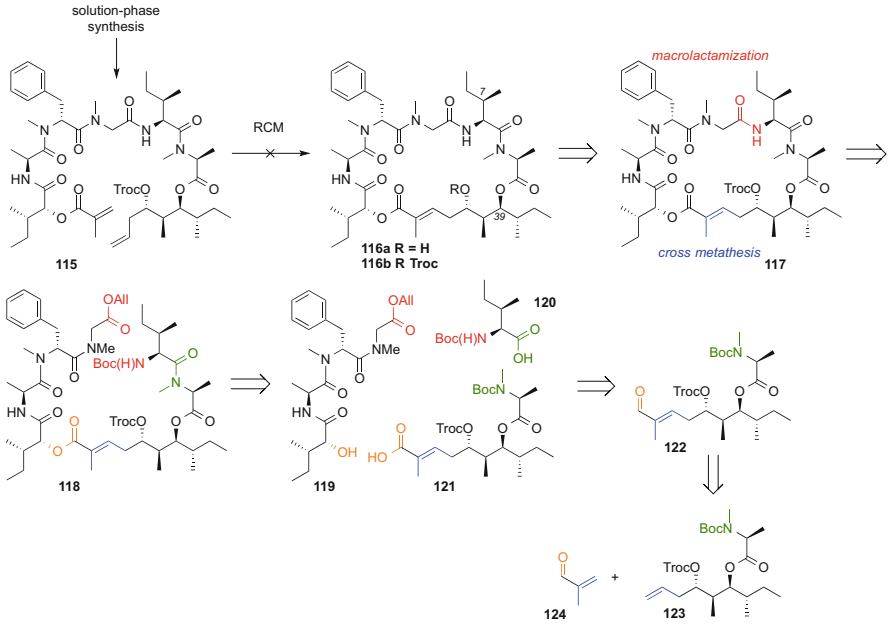
2.8 *RCM of Depsipeptides*

Cyclodepsipeptides, peptides in which one or more amide bonds have been replaced by ester moieties, are valuable compounds with a diverse spectrum of high bioactivity, like antitumor, antiproliferative, cytotoxic, antimicrobial, and antifungal behavior. This class of highly potent peptides has been isolated from marine sponges, algae, and fermentation broths. Their intriguing bioactivity in combination with their complex chemical structure make cyclodepsipeptides challenging targets for total synthesis, not only for having access to larger quantities and other congeners but also to confirm the structural assignment and absolute configuration of the stereochemical centers. In the next section, four examples of relevant metathesis-based syntheses of cyclodepsipeptides will be discussed, e.g., cryptophycin-24 (arenastatin A) **109** [148]; (−)-spongidepsin (**111**) [149]; (−)-PF1163B (**113**) [150], as shown in Scheme 26; and lagunamide A (**116a**) [151], as shown in Scheme 27.

The cryptophycins, a unique class of 16-membered macrolides with antimitotic activity, have been isolated from the cyanobacteria *Nostoc* sp. They are currently considered to be promising lead structures in antitumor therapy [152]. A structural analog, cryptophycin-24 (**109**), was isolated from the marine sponge *Dysidea arenaria* [153]. Because of their interesting biological activity and intriguing molecular structure, several total syntheses of this cyclodepsipeptide and its



Scheme 26 Retrosynthesis of cyclodepsipeptides cryptophycin-24 **109** [148], (−)-spongidepsin (**111**) [149], and (−)-PF1163B (**113**) [150]



Scheme 27 Retrosynthesis of cyclodepsipeptide lagunamide A **116a** [156]. *Note*: compound **116a** represents the 7,39-*epi*-lagunamide A which corresponds to the originally proposed structure of lagunamide A

analogs have been reported [154, 155]. The first RCM-based approach was reported by Tripathy and Georg [148] and features the introduction of the β-epoxide prior to macrolide formation. Based on the retrosynthesis (Scheme 26), cryptophycin-24 (**109**) could be synthesized from diene **110** by ring-closing metathesis in the presence of the chemically reactive styrene epoxide moiety (the other required fragments are highlighted in color). Indeed, treatment of **110** with **G1** (10 mol%) in CH_2Cl_2 under reflux for 6 h resulted in a *trans*-selective macrocyclization, and cyclodepsipeptide **109** was obtained in 70% yield, and all spectroscopic data were in agreement with the reported values [155].

A second example describes the synthesis of (−)-spongidepsin **111** [149], featuring a ring-closing metathesis reaction to construct the 13-membered macrocycle (Scheme 26). (−)-Spongidepsin is a cyclodepsipeptide, originally isolated from the Vanuatu marine sponge *Spongia* sp., with potent antitumor activity [156]. Ring-closing metathesis of diene **112** in the presence of **G2** (20 mol%) in CH_2Cl_2 at 40°C resulted in the corresponding *Z*-lactam with 93% yield. After protecting group removal, hydrogenation of the double bond, and conversion of the alcohol into the aldehyde, the terminal acetylene was installed to obtain the title compound in 60% over the four last synthesis steps. Two other syntheses of (−)-spongidepsin, both applying RCM to assemble to macrocycle, had been reported two years earlier by Forsyth [157] and Ghosh [158], respectively. Noteworthy is the difference in reaction conditions while starting with a precursor

in which PG was either 4-methoxybenzyl or *tert*-butyldiphenylsilyl, respectively. Forsyth described an efficient RCM reaction with **G2** (10 mol%) in toluene (110°C) during 20 min to obtain the macrocycle in 80% yield with a 10:1 *E/Z* ratio, while Ghosh reported an RCM reaction in the presence of **G2** (10 mol%) in CH_2Cl_2 (reflux) for 12 h affording the macrocycle as a single isomer (*Z*) in 93% yield. (−)-Spongidepsin synthesized by these approaches show identical spectroscopic data; however, different optical rotations were found. Follow-up papers that describe other RCM-based (formal) total syntheses of (−)-spongidepsin have been reported [159–162]; however, the issue of differences in the optical rotations was not further addressed.

Another example of an efficient RCM approach to obtain a 13-membered macrocycle deals with the synthesis of (−)-PF1163B (**113**) [150], a cyclodepsipeptide with antifungal properties. This class of bioactive peptides was isolated from the fermentation broth of *Penicillium* sp. [163, 164] and subsequently shown to be inhibitors of ERG25p to interfere with the ergosterol biosynthesis cascade. The 13-membered macrocycle incorporates an *O*-2-hydroxyethyl-*N*-methyl-L-tyrosine moiety, and a total synthesis starting with L-tyrosine and (*R*)-citronellol has been described in the literature [165]. This synthesis requires 13 steps, while the synthesis reported by Gesson [150] requires eight steps in which RCM of precursor **114** is the key step (Scheme 26). Ring-closing metathesis was attempted with different amounts of **G1** as the catalytic species. Unfortunately, only a limited degree of cyclization was found under these conditions. However, the use of **G2** led to better results: RCM of **114** (at a concentration of 2.1 to 3 mol/L) in refluxing $C_2H_4Cl_2$ in the presence of **G2** (12 mol%) gave the corresponding macrocycle in 60% yield as a mixture of (*E*)- and (*Z*)-diastereomers. Two other total syntheses of PF1163A have been described [166, 167] utilizing RCM as key reaction with a slightly increased yield of the macrocyclization (70 to 83%).

As a last example of a cyclodepsipeptide synthesis featuring a metathesis reaction, the synthesis of lagunamide A **116a** [151] will be discussed and is shown in Scheme 27.

Lagunamides are new cyclic depsipeptides isolated from the marine cyanobacterium *Lyngbya majuscula* collected from the western lagoon of Pulau Hantu Besar, Singapore [168, 169]. These compounds exhibit potent biological activities, such as antimicrobial, antimalarial, cytotoxic, and neurotoxic properties, which make these marine cyanobacterial compounds interesting lead compounds for further development as antitumor therapeutics. The originally assigned stereochemical configuration of lagunamide A (**116a**) [168] was revised by Dai et al. [170] and turned out to be the C7 and C39 epimer (Scheme 27). So far, the (*E*)-olefin in lagunamide A has been installed via the Horner-Wadsworth-Emmons olefination, while Huang et al. [151] designed a metathesis approach to introduce this double bond in its correct geometry. In first instance RCM was explored. Therefore, precursor **115** was prepared based on solution-phase peptide synthesis. Disappointingly, compound **115** did not undergo macrocyclization (to afford **116b**) in the presence of the second-generation Grubbs' catalyst **G2** (CH_2Cl_2 (1 mM) at reflux or toluene

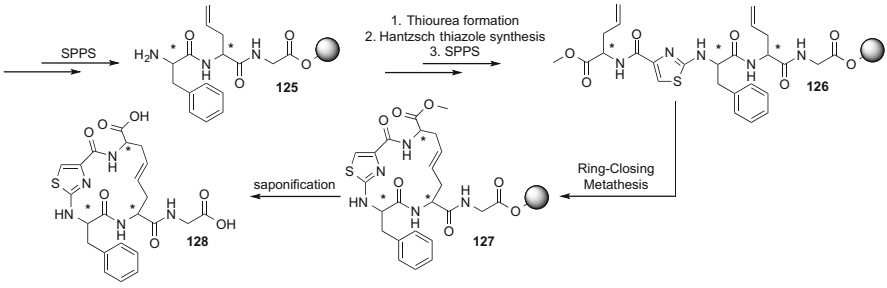
(1 mM) at 60°C). Variation of the reaction conditions by different solvents and temperatures remained unsuccessful. As an alternative, macrolactamization of precursor **118** was envisioned, while the olefin was prepared by cross metathesis (CM) of alkene **123** with methacrylaldehyde (**124**). Gratifyingly, CM between compounds **123** and **124** proceeded smoothly in the presence of second-generation Grubbs' catalyst **G2** (refluxing CH_2Cl_2), since olefin **122** was obtained in 81% yield with a *E*/*Z* ratio of >99:1. Finally, via intermediates **119**–**121**, macrolactamization precursor **118** was synthesized, and after protecting group manipulation followed by a HATU/DIPEA-mediated macrolactamization, lagunamide A derivative **116a** was obtained in good yield. Thus, although RCM did not result in the desired macrocyclization, CM was a versatile alternative approach for RCM, since a series of different homoallyl alcohol derivatives, like **123**, could be obtained for the synthesis of lagunamide A analogs.

2.9 *RCM for the Synthesis of Bioactive Peptides and Peptidomimetics*

In this section a diverse set of examples that apply ring-closing metathesis and cross metathesis for the synthesis of bioactive peptides and peptidomimetics will be discussed. Herein, these examples focus on a large structural variation of the peptide derivatives, and special attention is paid to the reaction conditions of the RCM or CM protocols and to approaches to optimize the metathesis reaction.

The first example describes the solid-phase synthesis of peptide thioureas and thiazole-containing macrocycles (represented by **128**) obtained by Ru-catalyzed ring-closing metathesis [171], as shown in Scheme 28. This class of functionalized peptides is frequently encountered in biologically active derivatives, with antibacterial, antifungal, and antiviral properties.

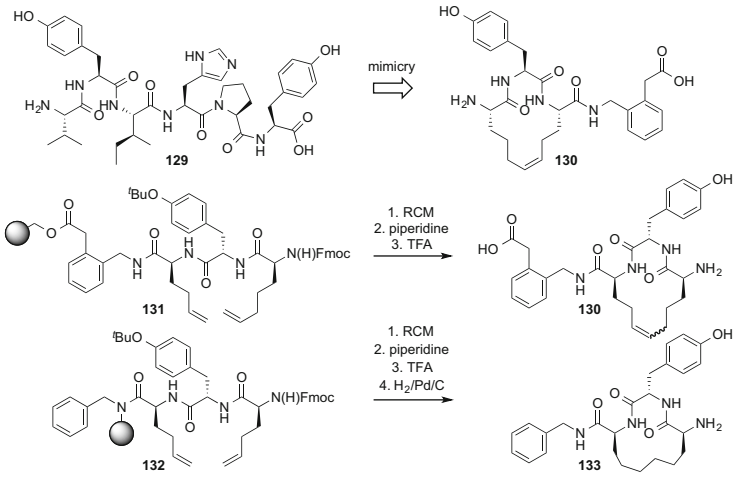
Herein, Cohrt and Nielsen describe a versatile synthesis to obtain 15–17-membered thiazole-containing peptide macrocycles in all diastereomeric forms, and their developed "build/couple/pair" approach is well suited for the generation of larger libraries of said peptidomimetics. As a typical example, solid-phase-bound



Scheme 28 Synthesis of thiazole-functionalized peptide macrocycles [171]

tripeptide H-Phe-Alg-Gly **125** was treated with *N*,*N*′-di-Boc-thiourea in the presence of Mukaiyama's reagent (2-chloro-1-methyl-pyridinium-iodide) and Et_3N as base in CH_2Cl_2 as the solvent, to afford the corresponding *N*-terminal α-thiourea intermediate in a yield >95%, after optimization of the addition/preactivation procedure. Subsequently, the Boc group could be smoothly removed by treatment of the peptide resin with 0.1 M $SnCl_4$ in CH_2Cl_2 for 2×30 min, and the free α-amine was then reacted with an α-halo ketone (e.g., $(HO_2C)COCH_2Br$, bromopyruvic acid) in EtOH/H_2O 3:1 v/v at 60°C for 16 h to effect the Hantzsch thiazole synthesis in excellent yield and purity (after deprotection a small aliquot of resin). The resulting carboxylated thiazole readily underwent amide bond formation via on-resin activation, e.g., allylglycine methyl ester, to give resin-bound cyclization precursor **126**, as shown in Scheme 28. The ring-closing metathesis was performed by exposing diene **126** to 30 mol% catalyst in dry degassed CH_2Cl_2 at room temperature for 24 h. A total of nine ruthenium-alkylidene catalysts were tested, with the **G2** and **HG2** resulting in the highest conversions (67 to 95%). Further increasing the conversion could be accomplished by repeating the RCM reaction on the solid support once. Gratifyingly, this RCM protocol afforded access to 15-, 16-, and 17-membered macrocycles with up to three stereocenters in the ring, which all underwent cyclization, in excellent product yields and purity [171].

In the second example, macrocyclic analogs of angiotensin IV (H-Val-Tyr-Ile-His-Pro-Phe-OH) **129** have been synthesized according to a replacement/cyclization SAR study, to improve their chemical and metabolic stability, as potent inhibitors of insulin-regulated aminopeptidases, as shown in Scheme 29 [172]. In their study, Hallberg and co-workers designed cyclic analogs of the hexapeptide angiotensin IV (**129**) [172], which is known to play important roles in cognition processes, like memory and learning [173]. Angiotensin IV binds with high affinity to the insulin-regulated aminopeptidase (IRAP), an enzyme localized in the brain

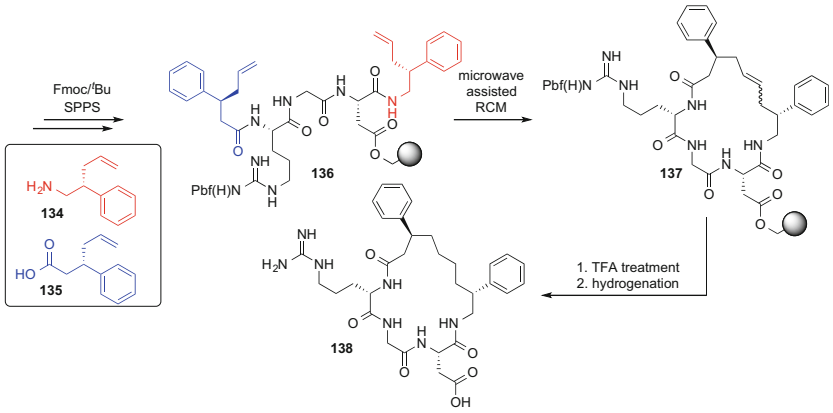


Scheme 29 Design and synthesis of angiotensin IV-derived macrocyclic peptidomimetics [172]

and is associated with the mentioned mental processes. Inhibitors of IRAP are promising novel lead compounds for the treatment of memory dysfunction, due to brain trauma, cerebral ischemia, Alzheimer's disease, and other age-related diseases.

Angiotensin IV (AngIV) is a linear hexapeptide, and to improve the drug-like character of this peptide, the group of Hallberg reported a disulfide scan which resulted in the discovery of efficient and potent cyclic AngIV analogs [174]. In the present study these disulfide peptides were converted into more stabile dicarba analogs by ring-closing metathesis. For this purpose, diene **131** was synthesized by SPPS and treated with an appropriate catalyst. For this purpose, **G2** and **HG2** (both 15 mol%) in 1,2-dichloroethane (DCE) under microwave irradiation at 150°C for 5 min were evaluated in this conversion, and after cleavage from the resin, complex mixtures of poorly separable compounds (like ring-contracted and double-bond migration products) were obtained, and only a 14-membered analog (with a shifted double bond compared to the peptide backbone) could be isolated in sufficient amounts. The addition of 1,4-dibenzoquinone (15 mol%) and a lower catalyst loading (7 mol% **HG2**) in combination with microwave heating at 120 or 140°C for 5 min in DCE resulted in the isolation of compound **130** as an *E/Z* mixture, in which the (*E*)-diastereoisomer was consistently the predominant 14-membered macrocycle (>60%). In another approach, benzylamide resin-bound diene **132** underwent RCM under the same reaction conditions as described for **131**. After Fmoc removal, cleavage from the resin and hydrogenation of the double bond, two products could be separated by HPLC, the desired 13-membered macrocycle **133** in a yield of only 2%, while the second product corresponding to a 12-membered ring-contracted derivative. Ring size was the most important factor toward the inhibitory activity against IRAP, since the following K_i values were found: AngIV 62 nM, macrocycle **130** 25 nM (14-membered ring), and macrocycle **133** 193 nM (13-membered ring) [172].

The third example describes the application of "Linked Amino Acid Mimetics" (LAAM): building blocks that provide a functional amino acid side chain in combination with a terminal olefin as a cyclization hinge via ring-closing metathesis [175]. Bornmann and co-workers synthesized two LAAM building blocks, amine **134** and carboxylic acid **135**, for the C- and N-terminal modification of a bioactive peptide sequence, respectively. In their communication, the tripeptide Arg-Gly-Asp (RGD) was chosen since it is known that cyclic RGD peptides (and especially *cyclo*[Arg-Gly-Asp-D-Phe-N^{Me}Val]) [176] exhibit high affinity toward the $\alpha_V\beta_3$ integrin [177], an important class of proteins that plays a role in angiogenesis. Precursor peptide **136** was synthesized on the solid support by Fmoc/^{t}Bu-based SPPS in which the C-terminal aspartic acid residue was anchored via its side chain to the solid support, while the α-carboxylate was coupled to amine **134** to install the C-terminal alkene moiety, as shown in Scheme 30. In turn, the α-amine of arginine was functionalized with acid **135**, and resin-bound diene **136** (0.82 mmol) was cyclized by ring-closing metathesis in the presence of **G2** (50 mol%) as the catalytic species in dichloroethane (25 mL) under microwave irradiation at 60°C for 40 h to yield resin-bound macrocycle **137**. After TFA



Scheme 30 Solid-phase synthesis of an RGD macrocycle based on Linked Amino Acid Mimetics [175]

treatment and hydrogenation, cyclic RGD peptide **138** was obtained in good yield (36%) [175].

Although the present paper does not provide any biological data regarding binding affinity of **138** toward the $\alpha_V\beta_3$ integrin, modeling suggests that the 19-membered macrocycle **138** is a representative mimic of the 15-membered *cyclo*[Arg-Gly-Asp-D-Phe-N^{Me}Val] peptide, since the RMSD was 0.8819 Å, as shown in Fig. 6.

In the fourth example, Liskamp and co-workers described the synthesis of a novel cyclic peptide MC4-ligand featuring ring-closing metathesis [178]. Based on a linear heptapeptide, Ac-Nle-Gly-Lys-D-Phe-Arg-Trp-Gly-NH_2, Ac-*Alg*-Gly-Lys (Boc)-D-Phe-Arg(Pbf)-Trp(Boc)-*Alg*-NH_2 (**139**) was synthesized by Fmoc/^{t}Bu SPPS and detached from the resin by ammonolysis to obtain the protected peptide amide (see Scheme 31).

RCM was performed in solution with a series of different solvents, reaction temperatures, and catalytic Ru species, as shown in Table 2. It has to be taken into account that precursor **139** is sparingly soluble in solvents commonly used in RCM reactions, while DMF was the only solvent in which the protected peptide reasonably dissolved (0.41 g peptide/5 mL solvent). Therefore, all cyclization reactions were carried out with 2.5 vol% DMF in the mentioned solvent (entries 1–8) at a final peptide concentration of 1.6 mM in the presence of 20 mol% catalyst under a continuous flow of dry N_2, and heating was performed in a conventional oil bath.

Remarkably, all cyclization attempts with **G2** as catalyst were unsuccessful, despite its higher activity and stability compared to **G1**. Cyclization entry 8 was performed for 1 h at 100°C, then an additional loading of 20 mol% of fresh catalyst was added, and RCM was extended for an additional 2 h at 40°C. This approach resulted in a separable mixture of linear and cyclic peptides as judged by LCES

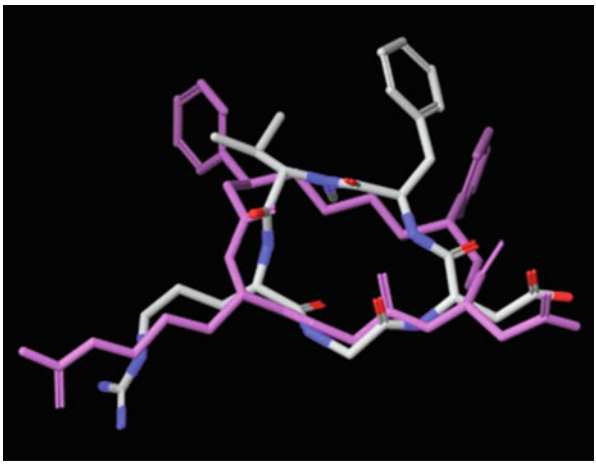
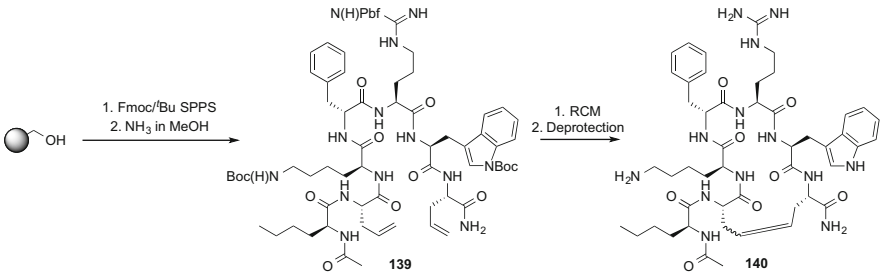


Fig. 6 Superimposition of the lowest energy structure of **138** (blue purple) with *cyclo*[Arg-Gly-Asp-D-Phe-N^{Me}Val] bound to $\alpha_V\beta_3$ integrin [177] (by-atom color). Reprinted from Tetrahedron Lett (2013) 54:5799–5801, Copyright (2013) with permission from Elsevier [175]



Scheme 31 SPPS and RCM for the synthesis of cyclic MC4-ligand **140** [178]

Table 2 Optimization of the RCM reaction of precursor **139** [178]

Entry	Solvent[a]	Catalyst	*T* (°C)	Result[b]
1	CH_2Cl_2	**G2**	40	Linear
2	DCE	**G2**	50	Linear
3	Toluene	**G2**	70	Linear
4	TCE	**G2**	70	Linear
5	TCE	**G2**	100	Linear
6	Toluene	**G1**	70	Linear
7	TCE	**G1**	100	Linear
8	TCE	**G1**	100 (1 h) → 40 (2 h)[c]	Linear/cyclic

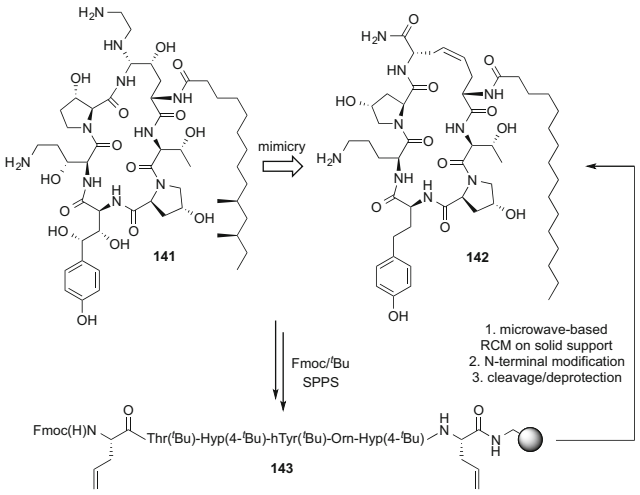
[a]Solvents: 1,2-dichloroethane (DCE), 1,1,2-trichloroethane (TCE)
[b]As determined by LCES MS
[c]Second portion of **G1** (20 mol%) was added after 1 h

MS. After protecting group removal, cyclic peptide **140** could be isolated in 63% yield as an *E*/*Z* diastereomeric mixture.

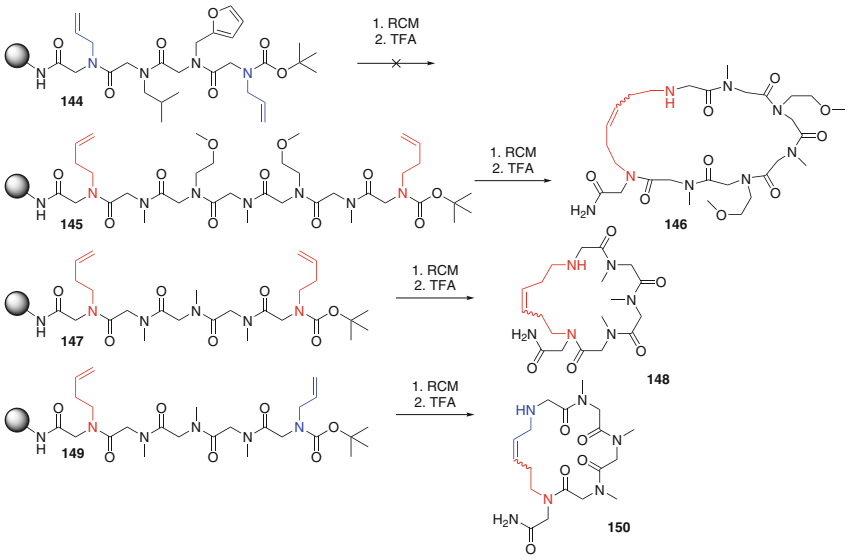
The three peptides, Ac-Nle-Gly-Lys-D-Phe-Arg-Trp-Gly-NH_2 as the control, protected linear *bis*-allyl peptide **139**, and cyclic peptide **140**, were tested in a melanocortin receptor assay. Their K_i values for the MC4 receptor were 2.4 ± 0.3, 1.9 ± 1.0, and 2.3 ± 1.0 nM, respectively. However, the measured EC_{50} values were as follows: 1 ± 0.6, 0.21 ± 0.01, and 0.34 ± 0.02 nM, respectively. These data show a clear improvement with respect to the control peptide, while cyclization was apparently of less importance. In hindsight, the absence of the "cyclization effect" might be explained by an observation made by Derksen et al., in which they found that the non-covalent hydrophobic interactions of the allyl side chains afford the correct bioactive conformation of the peptide comparable to the cyclic peptide [97].

As the fifth example [179], RCM was applied to constrain and simplify caspofungin (**141**) as an antifungal peptide that belongs to the class of echinocandins that act by inhibiting the β-(1,3)-D-glucan synthase complex [180].

The total synthesis of caspofungin is quite challenging due to the many hydroxylated unnatural amino acids, like hydroxyproline, hydroxyornithine, *bis*-hydroxyhomotyrosine, and the presence of an ornithine hemiaminal. Mulder et al. [179] designed caspofungin mimic **142** in which the alkene moiety formed an alternative constraint for the hemiaminal, since the latter is known to be unstable at $pH > 7$. Precursor peptide **143** was synthesized on the solid support following Fmoc/^{t}Bu SPPS protocols. In this peptide, the C-terminal ornithine derivative was replaced by an allylglycine residue, while the N-terminus was extended by one allylglycine (Scheme 32). Ring-closing metathesis of **143** was performed according to the procedure of Robinson et al. [99], and for this purpose, resin-bound precursor peptide **143** was treated with **G2** (15 mol%) in CH_2Cl_2 containing 10 vol % 0.4 M



Scheme 32 Caspofungin (**141**) and its mimic **142** synthesized by RCM [179]



Scheme 33 RCM for the solid-phase synthesis of cyclic peptoids [181]

LiCl in DMA under microwave irradiation at 100°C for 75 min. Finally, macrocycle **142** was isolated in 11% overall yield as a mixture of *cis*/*trans* isomers. RCM reactions that were run without microwave irradiation were not successful, since treatment of the linear precursor peptide **143** with **G2** (20 mol%) in CH_2Cl_2 containing 10 vol % 0.4 M LiCl in DMA at 50°C for 24 h resulted in only trace amounts of cyclic peptide **142**. Unfortunately, these alkene-/alkane-bridged caspofungin mimics did not show any antifungal activity, up to 100 μg/mL (96 μM), mainly due to the increased ring size by two atoms to a 23-membered macrocycle.

The sixth example describes a solid-phase approach to synthesize cyclic peptoids via ring-closing metathesis to develop a tool for the easy access of cyclic peptoid libraries and other cyclic peptide-peptoid chimeras, as shown in Scheme 33 [181].

Peptoid tetramer **144** (containing allylamines) was subjected to RCM in the presence of **G1**, **G2**, or **HG2** (2 mol%) under various reaction conditions in common metathesis solvents as dichloromethane, 1,2-dichloroethane, and 1,2-dichlorobenzene. Ring-closing metathesis failed to give the cyclic 14-membered peptoid. However, the hexamer as well as the octamer underwent macrocyclization (to give a 20- and 23-membered macrocycle, respectively) only in the presence of **HG2** under microwave irradiation (300 W, 2 min) in 1,2-dichlorobenzene albeit in very low yield (10–20%). Since it is known that RCM efficiency strongly depends on the length of the olefinic amine [29, 182], heptamer **145** was synthesized containing 3-buten-1-amine moieties. This heptamer reacted with **G1**, **G2**, and **HG2** (2 mol%) under microwave irradiation (300 W, 2 min) in 1,2-dichlorobenzene, and after cleavage from the resin, the 25-membered macrocycle **146** was isolated in 10, 20, and 80% yield, respectively. Thus, **HG2** was selected in the macrocyclization of peptoid pentamer **147**, and under similar

reaction conditions, 19-membered cyclic peptoid **148** was obtained in 70 to 85% yield. Based on MALDI-TOF analysis, dimerization products and linear peptoids were the major side products. To suppress these side reactions, **147** was treated with **HG2** in refluxing CH_2Cl_2 for 2 h, which resulted in a very clean RCM reaction, since **148** was obtained in 95% yield with <5% dimers present. In addition to this, pentamer **149** was synthesized with a combination of 3-buten-1-amine at the C-terminus and an allylamine at the N-terminus. RCM (**HG2** in refluxing CH_2Cl_2) resulted in the formation of 18-membered cyclic peptoid **150** in a yield of 60–70%. The reduced yield emphasized the importance of the correct length of the alkenyl moiety for being an optimal RCM substrate. Finally, a series of N-/C-terminally containing 3-buten-1-amino peptoids ranging from tetramers to heptamers with functionalized side chains like lysine and aspartic acid were synthesized and cyclized to give a whole range of 16- to 25-membered macrocyclic peptoids in yields ranging from 78 to 100% [181].

Finally, in the last example, six cyclic peptides (as shown in Fig. 7) will be discussed to emphasize the applicability of RCM to illustrate structural diversity within the peptide framework in relation to a wide variety in biological activities and biophysical properties.

Bicyclic pentapeptide **151** was designed as a CDE-ring mimic of the antibiotic glycopeptide vancomycin [183]. The central 4-hydroxyphenylglycine residue was found to be a suitable building block and was *bis*-allylated via a Stille coupling on the condition that the electron-donating hydroxyl functionality was converted into an electron-withdrawing moiety by acetylation. Both serine residues were introduced as their *O*-allylated congeners, and the linear pentapeptide with four allyl

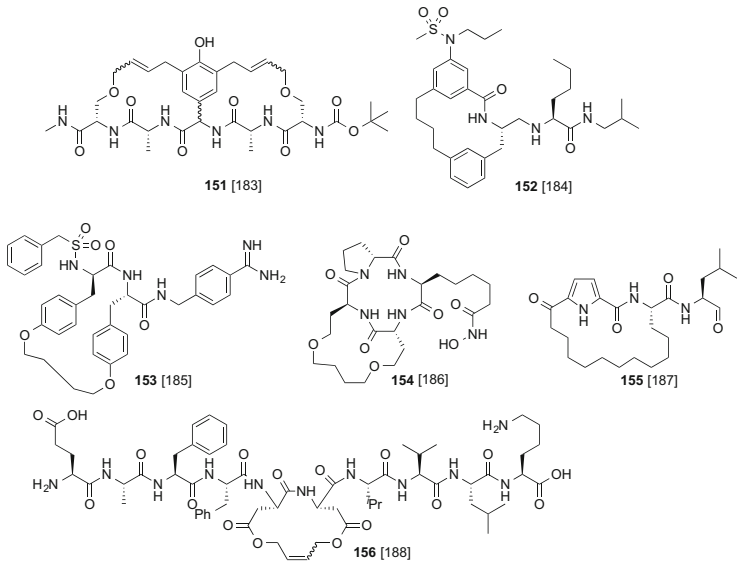


Fig. 7 Structural diversity of peptides in relation to a variety of biological activities

functionalities was treated with **G2** as the catalyst (100 mol%) to undergo tandem ring-closing metathesis in TCE/DMF 96:4 v/v (2.5 mM) at 80°C for 10 min, and bicycle **151** was obtained in 67% after column chromatography as a complex mixture of diastereomers [183].

Substituted isophthalamides emerged as early lead structures that were investigated for the inhibition of β-secretase, the enzyme responsible for releasing the neurotoxic peptide fragment $A\beta_{40–42}$ by processing the amyloid precursor protein. Targeting β-secretase is considered as an attractive therapeutic approach for the treatment and prevention of Alzheimer's disease. A macrocyclic active site-derived inhibitor of β-secretase, **152**, was designed and synthesized by covalently cross-linking the P1 and P3 side chains of the amino acid side chains that interact with the active site of the enzyme [184]. Allylation of the 3-aminobenzoic acid core was performed under Stille conditions, and ring-closing metathesis was carried out in the presence of **G2** (10 mol%) in CH_2Cl_2 (2.1 mM) at 50°C for 60 min resulting in the exclusive formation of the single (*E*)-regioisomer in high yield (84%). After hydrogenation of the double bond, macrocycle **152** displayed an IC_{50}-value of 4 nM against β-secretase with a 25% reduction of $A\beta_{40–42}$ levels in brain extracts from an in vivo mouse model [184].

A series of 20-membered macrocycles, like amidine **153**, was synthesized by Saupe and Steinmetzer to develop highly potent and selective plasmin inhibitors [185]. Herein, *O*-allyl protected tyrosine residues were ring-closed via **G1** (5 mol%) in refluxing CH_2Cl_2 for 6 h in a reasonable yield (65%). Cyclic amidine **153** was a potent plasmin inhibitor ($K_i = 1.1$ nM) with some affinity retained against plasma kallikrein ($K_i < 20$ nM), while proteases like thrombin, factor Xa, and activated protein C remained unaffected with K_i values of 510, 784, and 4,586 nM, respectively. This selectivity was explained by the so-called 99-loop residue, which is absent in plasmin, and the sterically less demanding glycine residue in case of plasma kallikrein, while bulky and sterically demanding residues (leucine, threonine, tyrosine) are present in the other proteases [185].

Histone deacetylases (HDACs) are a class of enzymes involved in the regulation of chromatin remodeling and gene transcription by deacetylation of acetylated lysine residues in the N-terminal amino acid sequences of histones. Malfunctioning of these enzymes is associated with carcinogenesis, and the development of HDAC inhibitors is a promising approach for novel anticancer drug molecules. Isoform-specific inhibitors of HDACs are often associated with less toxic side effect. To address this need, Islam et al. designed and synthesized a series of bicyclic tetrapeptides as selective HDAC inhibitors [186]. They found that bicyclic peptide **154** is almost tenfold more selective against HDAC-1 than HDAC-6. In a typical example, bicyclic peptide **154** was synthesized in solution, and the fully protected linear tripeptide was first cyclized by RCM via the *O*-allyl serine residues, followed by head-to-tail macrocyclization of the tetrapeptide with HATU/DIPEA as coupling reagents. Ring-closing metathesis in the presence of **G1** (5 mol%) in CH_2Cl_2 (7.1 mM) at room temperature during 48 h resulted in a monocyclic peptide in 52% yield, while macrolactamization was somewhat more efficient, 65% yield [186].

Protease substrates and inhibitors adopt a β-strand conformation upon binding toward the active site. Abell and co-workers [187] described a series of molecules,

like **155**, in which a planar pyrrole has been used to replace a dipeptide sequence within a peptide backbone to generate a macrocycle that adopts a β-strand conformation. Macrocyclization was performed via RCM in the presence of **G2** (10 mol %) in refluxing CH_2Cl_2 (2.5 mL/mg acyclic diene) for 18 h. The yield was determined after hydrogenation of the newly formed double bond and was in the range between 28 (18-membered ring, **155**) and 56% (24-membered ring). After RCM, the subsequent reaction steps to arrive at compound **155** were ester saponification, amino acid alcohol ((*S*)-leucinol) coupling, and oxidation to the aldehyde. Macrocyclic inhibitor **155** was assayed against ovine *m*-calpain, human cathepsin L and S (cysteine proteases), and bovine α-chymotrypsin and human leukocyte elastase (serine proteases). Inhibitor **155** was highly potent with picomolar K_i values against cathepsin L and S (0.44 and 0.92 nM, respectively) and also potent against *m*-calpain (58 nM), while it had almost no activity against α-chymotrypsin and human leukocyte elastase, since here K_i values $>10{,}000$ and $>2{,}500$ have been found, respectively. Based on an X-ray structure, it was found that this class of inhibitors exactly adopts a β-strand conformation in which the pyrrole clearly defines the required geometry for optimal binding into the active site [187].

Amyloid peptides tend to self-assemble and form well-defined supramolecular assemblies, like sheets, fibrils, vesicles, and ribbons. Such assemblies can be used as bionanomaterials as drug-carrier and controlled-release vehicles for the targeted delivery of small drug molecules or peptide therapeutics. Hamley et al. report an approach in which they use olefin metathesis to design macrocyclic amyloid peptides that self-assemble into nanotubes that comprise wrapped β-sheets [188]. They synthesized a resin-bound protected decapeptide (Fmoc-Glu(O^{t}Bu)-Ala-Phe-Phe-Asp(OAll)-Asp(OAll)-Val-Val-Leu-Lys(Boc)-O-resin) in which the vicinal aspartic acid moieties were protected as allyl esters and used for RCM in the presence of **HG2** (11 mol%) in DCE (10 mL) at 60°C for 4 days. Macrocyclization resulted in two peptides, namely, **156** (intramolecularly cyclized peptide) and its corresponding dimer (intermolecularly cyclized peptide), while also peptide dimers were formed that underwent cross metathesis. In aqueous solution (0.5 wt%), acyclic peptide H-Glu-Ala-Phe-Phe-Asp(OAll)-Asp(OAll)-Val-Val-Leu-Lys-OH self-assembles into peptide nanotubes with a radius of 25 ± 4 nm, with tube walls that comprise 10% of the tube diameter. It turned out that the molecular organization is based on β-sheet packing, since a β-strand spacing of 0.47 nm and a β-sheet stacking distance of 1.06 nm were observed. Macrocyclic peptide **156** did not self-assemble in aqueous solution at 0.5 wt%; however, a higher concentration was required (2 wt%) at which β-sheet formation, identified as macroscopic fibrils, was observed. Peptide nanotubes, however, were not formed in contrast to the linear peptide. Apparently, the conformational constraints induced by ring-closing metathesis hinder nanotube formation; instead, fibrillar nanostructures as a different supramolecular morphology were preferentially formed. This study is a nice example which shows that ring-closing metathesis can be used as a "switch" to control the self-assembly of peptides and thereby to tune the supramolecular morphology of the aggregates [188].

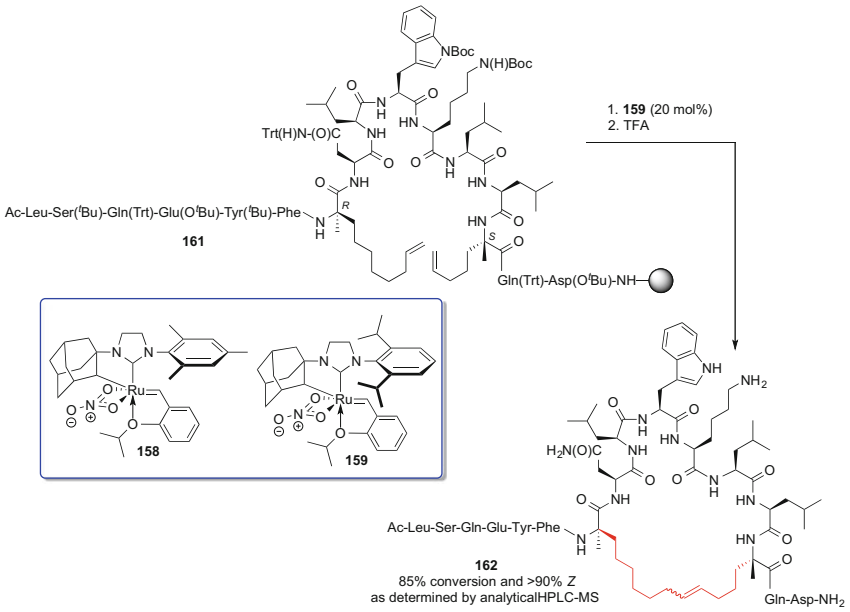
2.10 Z-Selective RCM of Peptides

Olefin metathesis revolutionized organic synthesis by providing a powerful tool to introduce the carbon-carbon double bond in many biologically active molecules. Generally, metathesis reactions result in the predominant formation of *E*-alkenes, while the synthesis of the thermodynamically less stable *Z*- or *cis*-isomer is quite challenging. Approaches toward enantioselective metathesis were pioneered by Schrock and Hoveyda, and they reported in 2009 a catalyst-controlled approach for the synthesis of *Z*-olefins [189]. These "stereogenic-at-Mo" complexes were highly efficient in *Z*-selective cross metathesis as well as ring-closing metathesis for the stereoselective synthesis of antitumor agent KRN7000 [190] and the macrocyclic natural products epothilone C and nakadomarin A [191], respectively. Shortly thereafter, chiral *N*-heterocyclic carbene ruthenium complexes were reported as being *Z*-selective olefin metathesis catalysts [192–196]. Recently, the advances in the catalytic stereoselective olefin metathesis have been extensively reviewed by Hoveyda, and this overview is recommended to the reader for more information about this topic [197].

In a recent contribution, Grubbs and co-workers addressed *Z*-selective olefin metathesis on peptides [198]. Through the combined efforts of homodimerization, cross metathesis, and ring-closing metathesis, the authors have formulated guidelines to assess the influence of amino acids and peptides on catalyst activity and selectivity, and these principles were applied to carry out *Z*-selective metathesis on challenging substrates including peptides that comprise parallel β-sheets and on the stapling of α-helical peptides.

In first instance, the *i*, *i* + 3 *Z*-selective RCM on Aib-containing peptides was studied by means of Boc-Ser(All)-Aib-Aib-Ser(All)-Aib-OMe (**157**) in which Ser (All) represents L-serine *O*-allyl residues. This peptide underwent *E*-selective RCM in the presence of **G2** (86% conversion; >20:1 *E*/*Z*, based on HPLC) and **HG2** (85% conversion; >20:1 *E*/*Z*) in DCE (5 mM) at 45°C for 3.5 h with 10 mol% catalyst loading [198] in accordance with earlier results to constrain the 3_{10}-helix in Aib-rich peptides [199]. Under similar conditions, the *Z*-selective ruthenium catalyst bearing *N*-adamantyl substituents and bidentate nitrato ligands **158** (Scheme 34) [195] did not result in macrocyclization even with a threefold increase of catalyst loading and extended reaction time. This result indicated that Aib residues at *i* + 1 and *i* + 2 control the *E*-selective macrocyclization by **G2** and **HG2**, while this conformational bias cannot be overcome by *Z*-selective catalysts **158** or **159** [198].

Z-Selective ring-closing metathesis on *i*, *i* + 4 stapled peptides was explored by resin-bound peptide **160**, an α-helical peptide known to target the BCL-2 protein family in the regulation of apoptosis [54], Ac-Glu(O^{t}Bu)-Asp(O^{t}Bu)-Ile-Ile-Arg (Pbf)-Ile-S5*-Arg(Pbf)-Leu-Leu-S5*-Glu(O^{t}Bu)-Val-Gly-Asp(O^{t}Bu)-X-resin, in which S5* denotes (*S*)-2-(4-pentenyl)alanine. RCM of **160** in DCE (at 0.05 mM) to promote α-helicity in the presence of **158** or **159** (10 mol%) at 40°C for 2 h on Wang resin (X=O) resulted in a low conversion (~20–25%), while on MBHA resin (X=NH), the conversion was increased to 55–60% with greater than 85% *Z*-selectivity.



Scheme 34 *Z*-Selective ring-closing metathesis to obtain *i*, *i* + 7 stapled peptides [198]

Prolonging reaction time to four hours and two cycles of catalyst addition afforded the macrocyclic peptide in 80% yield with more than 90% *Z*-selectivity. To investigate the generality of their method, Grubbs and co-workers used resin-bound peptide **161** with two olefinic amino acids, R8* ((*R*)-2-(7-octenyl)alanine) on position *i* and S5* on position *i* + 7 which spans two turns of an α-helix, as shown in Scheme 34.

The incorporation of the combination R8* on position *i* and S5* on position *i* + 7 was previously found to facilitate RCM [50–53]. Under the optimized reaction conditions, solid-phase-bound peptide **161** in the presence of Ru-catalyst **159** (20 mol%) was transferred (after TFA treatment) into macrocyclic peptide **162** in high conversion (85%) and an excellent *Z*-selectivity greater than 90%. Thus, these results clearly showed that Ru catalysts can promote on-resin *Z*-selective ring-closing metathesis for the synthesis of stapled peptides [198].

3 Conclusion and Outlook

Due to the development of robust ruthenium-based metathesis catalysts that allow a wide range of polar groups within the olefinic substrates and that are compatible with diverse polar solvents and higher temperatures, ring-closing metathesis and cross metathesis became undisputable tools for the synthesis of peptides and peptidomimetics. Shortly after the seminal contributions of Grubbs and Ghadiri,

who applied ring-closing metathesis for constraining dipeptides and cross metathesis to covalently cross-link self-assembled dimers of cyclic octapeptides, respectively, a plethora of metathesis reactions applied to peptide chemistry found their way in the scientific literature. The first applications of RCM in peptide chemistry dealt with alternatives for macrolactamization and disulfide bridge formation. Soon thereafter, RCM was applied to mimic and stabilize secondary structures in bioactive peptides, which culminated in the concept of peptide stapling as coined by Verdine. α-Helix stabilization and α-helix-inducing scaffolds became generally accessible, while pharmaceutically active peptides with improved conformational, chemical, and metabolic stability were realized by incorporating metathesis in their synthesis. Currently, *Z*-selective metathesis catalysts are in development, and the reported results are very promising. As discussed here, the bioactivity of a macrocyclic peptide may strongly depend on the geometry of the double bound. Tuning the *cis*/*trans* ratio of the newly double bond at will [200] will improve the efficiency of the synthesis as well as the purification of the desired peptides. In this context, ring-closing alkyne metathesis on peptides should be revitalized as an alternative approach to address this topic. Although many RCM syntheses with peptides have been described that did not require any optimization, catalyst screening, microwave irradiation, structure-inducing auxiliaries, chaotropic solvents, allyl sulfides as activated olefins, among others, have been used as a tool during optimization cycles for the efficient synthesis of peptide macrocycles. It is expected that ring-closing metathesis in aqueous solvents will usher in a new area of peptide macrocyclization, since unprotected peptides generally have higher solubility in water than (semi)protected peptides, while water-soluble ruthenium-based metathesis catalysts have been reported in the literature. To conclude this review, I will use the words of Hoveyda and Zhugralin:

> To consider catalytic olefin metathesis, or chemical synthesis, a consummated field would be akin to suggesting to Henry Ford that his model T was the be-all and end-all as far as automobiles are concerned [2].

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