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Facile and *E*-Selective Intramolecular Ring-Closing Metathesis Reactions in 3_{10}-Helical Peptides: A 3D Structural Study

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The intramolecular ring-closing metathesis reaction (RCM) is a useful method for altering the conformational and metabolic stability of α-helical peptides.[1−8] Prior RCM investigations have utilized tethers spanning *i* and *i* + 4 or *i* + 7 amino acid residues, a linkage that encompasses approximately one or two turns of an α-helical backbone and places the reactive side chains on the same side of the helix (Figure 1). This strategy has built upon earlier work with α-helices containing tethers employing salt bridges,[9] lactams,[10] disulfide bridges,[11] hydrophobic effects,[12] and metal ligation.[13]

Herein, we report the development of a minimal RCM constraint for the 3_{10}-helix, which is a relatively common structural motif in proteins and peptides containing C^{α}-tetrasubstituted α-amino acids.[14−16] The stereochemistry of the 3_{10}-helix[17] suggests that its regularity can be affected by *i*, *i* + 3 cross-links (Figure 1). This aspect has been investigated for the case of salt[18] and lactam[19] side-chain bridges. A recent theoretical study suggested that a minimal RCM constraint for a 3_{10}-helix would require two five-atom *i*, *i* + 3 olefinic side chains, thus producing an 18-atom macrocycle upon ring closure.[20]

To study this proposition in greater detail, an octapeptide with the sequence Boc-Aib-Aib-Aib-L-Ser(Al)-Aib-Aib-L-Ser(Al)-Aib-OMe (Boc, *tert*-butoxy; Aib, α-aminoisobutyric acid; Al, allyl; OMe, methoxy) (**1**) was prepared using solution-phase methods.[21] We chose this sequence because short oligopeptides containing Aib residues largely populate 3_{10}-helices.[14,16,22] When treated with the second-generation ruthenium catalyst **4** (7 mol % of **4**, 5 mM in **1**, 40 °C, 30 min), diene **1** underwent a rapid and *E*-selective (>20:1) ring-closing reaction to yield an 18-membered macrocycle in 93% yield (Scheme 1). This result is interesting because *E*/*Z* mixtures are normally observed in RCM reactions between side chains in helical peptides.[1−3] The olefin moiety in peptide **2** was reduced (cat. 10% Pd−C, 1 atm H_2, EtOH, 25 °C, 6 h) to provide the saturated macrocycle **3** in excellent yield.

An X-ray crystallographic analysis[23] (Figure 2) of peptides **1**−**3** provided a structural comparison at each stage of the modification. Each of the three peptides adopts a well-developed right-handed 3_{10}-helical structure. Peptide **1** is 3_{10}-helical for residues 1−6 and contains a type-I β-turn at the C-terminal residues 6 and 7 (a 3_{10}-helix consists of repeat type-III β-turns). This C-terminal turn behavior is also seen in peptides **2** and **3**, where the regularity of the helix is slightly disturbed at residues 4 and 5, with a deviation greater for alkene **2** than for the saturated macrocycle **3**. Despite these small differences, the structures are quite similar to one another, with rms deviations for backbone atoms of 0.996 Å between peptides **1** and **2** and 0.624 Å between peptides **1** and **3**. With the exception of the C-terminal residue 8, which is helical in **1** and **2** while semi-extended in **3**, most of the backbone ϕ,ψ torsion angle values of corresponding residues in **2** and **3** do not differ by more than 10° if compared to **1**. For **2**, the largest ϕ,ψ deviations are observed at Ser(4) and Ser(7) [$|\Delta\phi|,|\Delta\psi| = 22°$, 39° and 14°, 16°, respectively]. For **3**, deviations within 10−16° are found for ψ_2, ψ_5, ϕ_6, and ψ_6. As commonly found,[14,16,22] all internal Aib residues exhibit ϕ,ψ torsion angles typical of helical residues. In alkene **2**, the 3_{10}-helical H-bonding pattern is interrupted by the lack of the intramolecular H-bond between N6 and O3, as each of these two atoms is intermolecularly H-bonded to a co-crystallized solvent molecule. In **3**, the N6···O3 separation, 3.573(4) Å, is only slightly above the upper limit for a C═O···H−N H-bond. To the best of our knowledge, this is the first X-ray diffraction 3D structural comparison of a helical peptide before and after installation of a side-chain cross-link, RCM-derived or otherwise.

We note that in methanol solution peptides **1**−**3** exhibited circular dichroism (CD) spectra consistent with 3_{10}-helical structures[24] (Figure 3). This helix is characterized by a strong negative maximum near 205 nm and a much weaker (60−75% less intense) negative maximum at 222−232 nm.

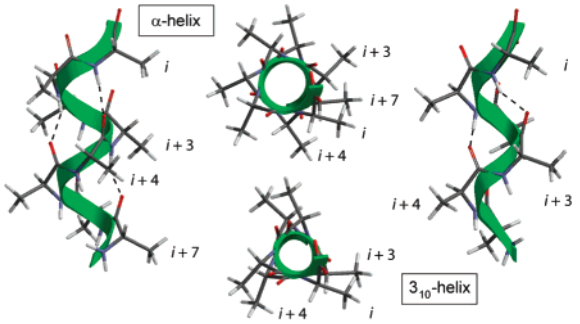
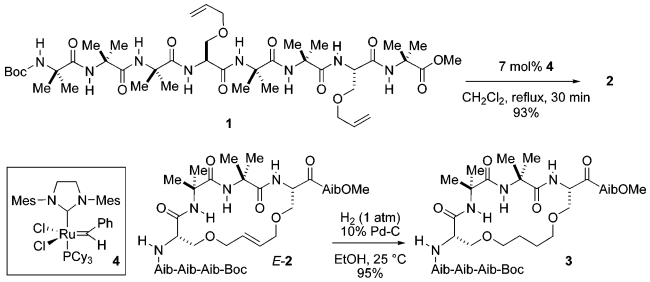


Figure 1. Molecular models for (L-Ala)$_n$ α- and 3_{10}-helices. Intramolecular hydrogen bonds are indicated with dashed lines.

Scheme 1. Synthesis of RCM Macrocyclized Peptides (*E*)-**2** and **3**



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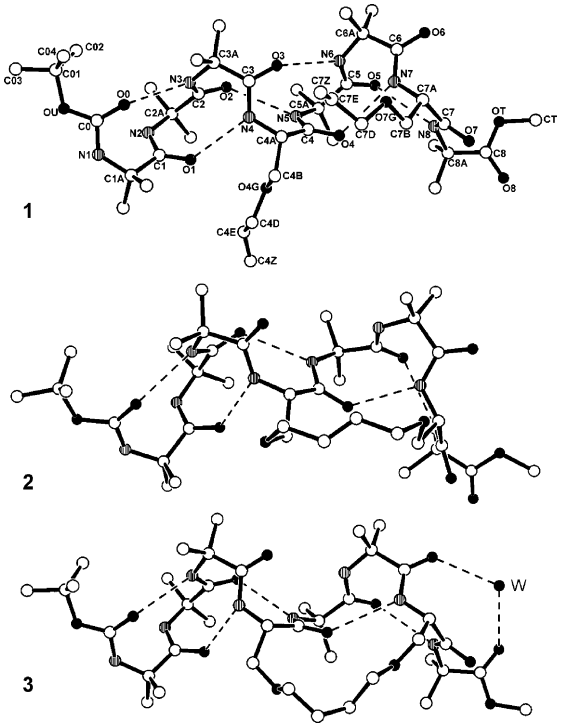

Figure 2. X-ray crystal structures of octapeptides **1**−**3**. Hydrogen atoms have been omitted for clarity. Dashed lines represent intramolecular N−H···O═C hydrogen bonds. In **3**, the co-crystallized water molecule (W) is also shown.

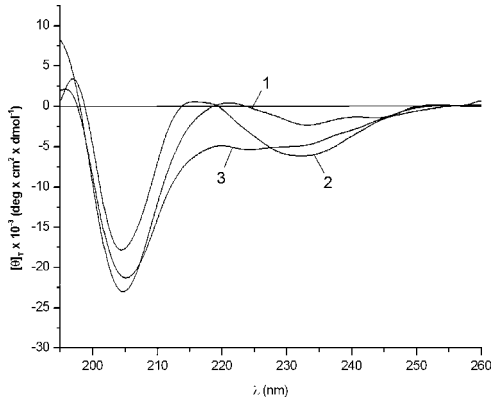

Figure 3. CD spectra of peptides **1**−**3** (1 mM in MeOH) at 25 °C.

Concerning the highly *E*-selective RCM reactivity of octapeptide diene **1**, we note that rapid RCM reactions and 12:1 *E*-selectivity are observed in a shorter sequence, the hexapeptide Boc-Aib-L-Ser(Al)-Aib-Aib-L-Ser(Al)-Aib-OMe (**5**). We have also investigated the RCM reaction in a heptapeptide with the sequence Boc-Val-Ser(Al)-Leu-Aib-Ser(Al)-Val-Leu-OMe (**6**).[25] When treated with the second-generation ruthenium catalyst **4** (10 mol % of **4**, 5 mM in **6**, 40 °C, 3 h), diene **6** formed an 18-membered macrocycle in quantitative yield with 7:1 *E*/*Z*-selectivity. The origin of the higher *E*-selectivity in the Aib-rich peptides may be due to ϕ/ψ conformational restrictions imposed by the C^{α}-tetrasubstituted α-amino residues. CD curves in 2,2,2-trifluoroethanol solution comparable to those of Figure 3 have been also obtained for the RCM macrocyclic products derived from both hexapeptide **5** and heptapeptide **6** (spectra not shown).

In conclusion, we have shown that an RCM-derived 18-membered macrocycle can be used to cross-link the side chains of *i* and $i + 3$ amino acids in short 3_{10}-helical peptide sequences. The intramolecular RCM reactions are efficient and highly *E*-selective, especially in peptides with high Aib content. In an Aib-rich octapeptide, this macrocyclization does not significantly disturb 3_{10}-helicity, as judged by an X-ray diffraction study of acyclic diene **1**, *E*-olefin RCM product **2**, and its hydrogenated derivative **3**. While other sequences (also including C^{α}-tetrasubstituted α-amino acids with allyl side chains) and tether lengths remain to be studied, it is apparent from these studies that a minimal, RCM-derived, macrocyclic constraint can be readily incorporated into 3_{10}-helical peptides.

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Supporting Information Available: Preparative procedures and characterization data, including X-ray crystal structure coordinates and files in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Relative to the α-helix ($\phi = -63°$, $\psi = -42°$, *i*, $i + 4$ hydrogen bonds), the 3_{10}-helix ($\phi = -57°$, $\psi = -30°$, *i*, $i + 3$ hydrogen bonds) is more tightly wound, contains a different pattern of intramolecular hydrogen bonds, and possesses a triangular shape when viewed down the long axis.[15] The 3_{10}-helix is characterized by three amino acids per turn and 10 atoms in the pseudo-ring formed by an intramolecular *i*, $i + 3$ hydrogen bond (a type-III β-turn).
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(23) Peptide **1** was crystallized from ethyl acetate, peptide **2** from slow evaporation of a 2:1 dichloromethane/isopropanol solution, and peptide **3** from moist acetonitrile. The structures were solved by standard methods, and the atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre.
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