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A Highly Stable Short α-Helix Constrained by a Main-Chain Hydrogen-Bond Surrogate

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Analyses of helix-coil transition in peptides emphasize the energetically demanding organization of three consecutive amino acids into the helical orientation as the slow step in helix formation.[1] Preorganization of these amino acid residues is expected to overwhelm the intrinsic nucleation propensities and initiate helix formation.[2] In an α-helix, a hydrogen bond between the C=O of the *i*th amino acid residue and the NH of the $i + 4$th amino acid residue stabilizes and nucleates the helical structure (Figure 1a). Herein we describe a strategy for the preparation of artificial α-helices involving replacement of one of the main-chain hydrogen bonds with a covalent linkage. To mimic the C=O···H−N hydrogen bond as closely as possible, we envisioned a covalent bond of the type C=X−Y−N, where X and Y would be part of the *i* and the $i + 4$ residues, respectively. The exceptional functional group tolerance displayed by the olefin metathesis catalysts for the facile introduction of non-native carbon−carbon constraints in the preparation of peptidomimetics suggested that X and Y could be two carbon atoms connected through an olefin metathesis reaction (Figure 1b).[3] This hydrogen bond replacement approach is inspired by the work of Satterthwait and co-workers, who explored the use of a hydrazone link to stabilize α-helices.[4] The metathesis-based method affords a stable and irreversible bond as compared to the hydrazone strategy and should be applicable to a broader range of peptide sequences.

The main-chain hydrogen-bond surrogate strategy is attractive because placement of the cross-link on the inside of the helix does not block solvent-exposed molecular recognition surfaces of the molecule. Thus far, helix stabilization methods have relied predominantly on side-chain constraints,[5] capping templates,[2,6] or non-natural amino acid substitutions.[7] These methods either block solvent-exposed surfaces of the target α-helices or remove important side-chain functionalities. Our strategy allows strict preservation of the helix surfaces and affords helices with unprecedented stability.

To test the stabilization properties of the metathesis-derived internal cross-link, we synthesized an 8-mer constrained peptide **3** bearing a cross-link at the *N*-terminus (Scheme 1). This particular peptide was chosen for the initial studies because the control peptide (AcGEAAAAEA-OMe, **1**) does not display any α-helicity, allowing us to observe an increase in α-helical content due to the modification. Two glutamic acid residues were incorporated in this alanine-rich peptide at different faces of the putative helix to increase the solubility of the constrained peptide in aqueous buffers. The constrained peptide **3** was synthesized from precursor peptide **2** via a ring-closing metathesis reaction with Hoveyda−Grubbs metathesis catalyst[8] followed by the removal of the *tert*-butyl ester groups. The isolated metathesized peptide **3** contained a trans alkene as indicated by NMR spectroscopy.[9]

The structure of constrained peptide **3** was determined using circular dichroism (CD) and 2D NMR spectroscopies. CD studies on the constrained peptide **3** and the control peptide **1** were performed in 30 mM phosphate buffer, pH 7.0, to obtain a quantitative measure of the helical content (Figure 2). The CD spectrum of **3** displays a double minimum at 202 and 222 nm, characteristic of α-helices.[10] The constrained peptide **3** does not show any increase in helicity upon addition of the α-helix inducing solvent trifluoroethanol (20% TFE in 30 mM phosphate buffer, pH 7.0), as measured by molar ellipticity at 222 nm; TFE does cause a slight change in the intensity of the 202-nm band (Figure 2a). The relative percent helicity of peptides can be estimated by the mean residue ellipticity at 222 nm, although these estimates are typically not accurate for short helices.[11a] We calculate this constrained peptide to be 100% helical based on Yang's method[11b] and Baldwin's correction[11a] for short peptides (see Supporting Information for details). As expected, the unconstrained peptide **1** does not display any α-helicity in aqueous buffer (Figure 2a) and 0−100% TFE solutions (Supporting Information). The constrained peptide remains fully helical when heated from 5 to 95 °C,[12] indicating that the peptide is structurally robust (Figure 2b). To further gauge the stability of the constrained peptide, we performed

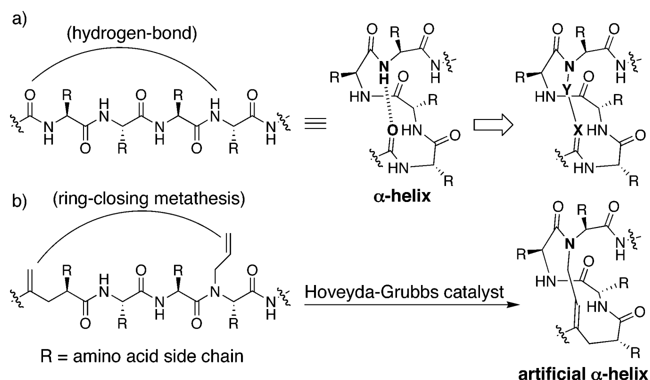
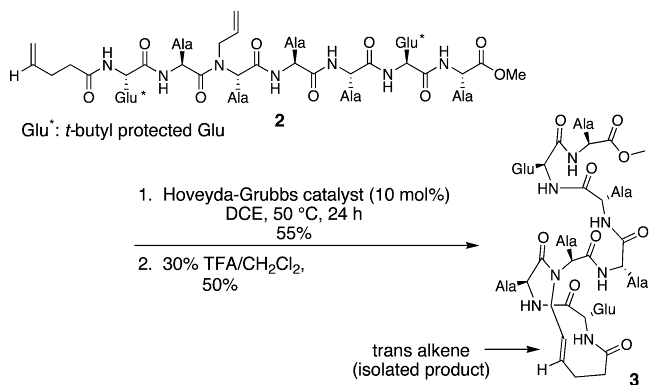


Figure 1. (a) Strategy for the stabilization of α-helices by replacement of an *i* and $i + 4$ hydrogen bond (C=O−H−N) with a covalent link (C=X−Y−N). (b) Replacement of the hydrogen bond with a carbon−carbon bond through an olefin metathesis reaction affords a stabilized α-helix.

Scheme 1. Synthesis of an Internally Constrained α-Helix



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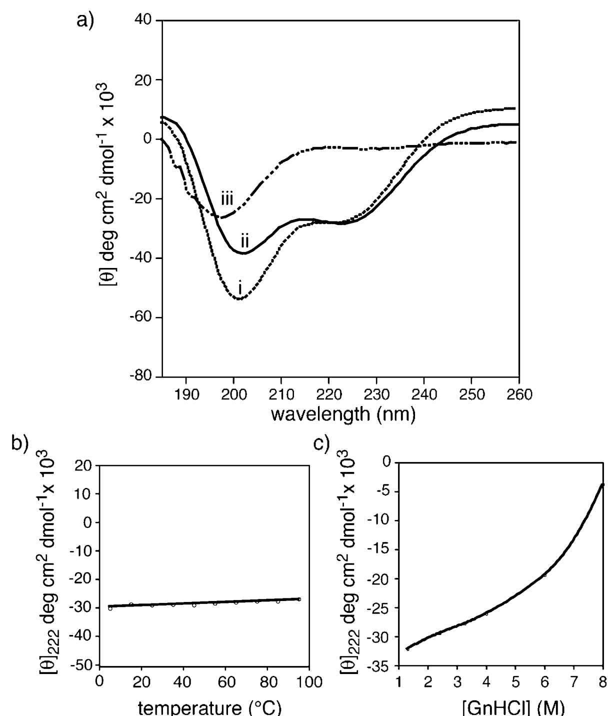

Figure 2. (a) (i) Circular dichroism spectra of α-helix **3** in 30 mM phosphate buffer (pH 7.0) and (ii) 20% TFE/phosphate buffer. (iii) Spectrum of unconstrained peptide **1** (AcGEAAAAEA) in phosphate buffer. The spectra were recorded at 25 °C. Effect of (b) temperature and (c) GnHCl on the stability of α-helix **3**.

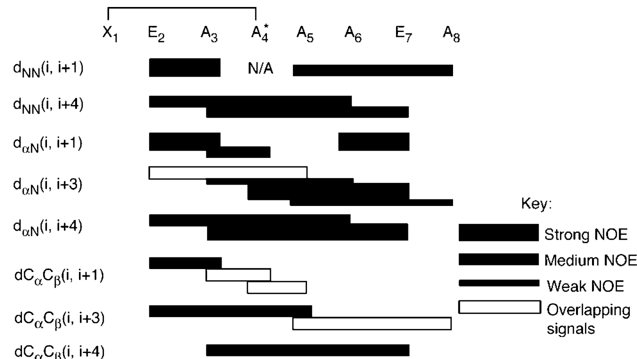

Figure 3. ROESY correlation chart for **3**. The alanine-4 residue does not contain an NH. Filled rectangles indicate relative intensity of the NOE cross-peaks. Empty rectangles indicate NOE that could not be unambiguously assigned because of overlapping signals.

a guanidinium chloride (GnHCl) titration experiment (Figure 2c). The intensity of the $[\theta]_{222}$ band for the constrained peptide shows that the peptide is still 85% helical at a concentration of 4 M GnHCl; the peptide started to unravel between 6 and 7 M GnHCl. These GnHCl titration studies illustrate that compound **3** adopts an exceedingly stable α-helical structure whose stability compares favorably to a previously reported constrained α-helix with three side-chain lactam bridges.[5d]

The α-helix structure of **3** was further confirmed by NMR spectroscopy. A combination of 2D TOCSY and ROESY spectra was used to assign ^{1}H NMR resonances for the constrained peptide. Sequential NN (i and $i + 1$) ROESY cross-peaks, which provide strong evidence for α-helical structure, were observed for the entire sequence as shown in the NOE correlation chart (Figure 3). The ROESY spectrum also reveals several medium range NOEs, for example, $d_{\alpha N}(i,i + 3)$, that provide unequivocal evidence for the helical structure. The fact that we can detect NOEs involving the last residue (alanine-8) indicates that the helix has not started fraying at the ends.

In summary, these results demonstrate that the replacement of a hydrogen bond between the i and $i + 4$ residues at the N-terminus of a short peptide with a carbon−carbon bond results in a highly stable constrained α-helix at physiological conditions as indicated by CD and NMR spectroscopies. The advantage of this strategy is that it allows access to short α-helices with strict preservation of molecular recognition surfaces required for biomolecular interactions.

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Supporting Information Available: Synthesis, ^{1}H NMR, ^{13}C NMR, and HRMS of modified amino acids. Synthesis, metathesis, and characterization of peptides. Description of ROESY, TOCSY, and circular dichroism studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Based on the alkene proton coupling constant of 16.1 Hz. Typical ring-closing metathesis reactions yield a mixture of cis and trans alkene isomers, favoring trans for large macrocycles (Furstner, A.; Langemann, K. *Synthesis* **1997**, 792−803); however, we could not detect the cis alkene isomer in the reaction mixture by HPLC.

(10) In canonical α-helices, the 202-nm band typically appears between 207 and 209 nm.

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(12) This conclusion is based on the thermal stability of the 222-nm band, which is typically used to gauge α-helicity; we observe a slight change in the intensity of the 202-nm band upon heating (Please see Supporting Information for details on this experiment.)

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