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Synthesis and 12-Helical Secondary Structure of β-Peptides Containing (2*R*,3*R*)-Aminoproline

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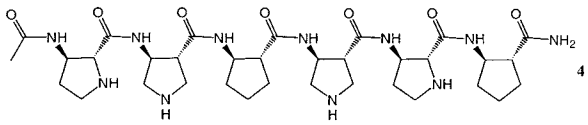
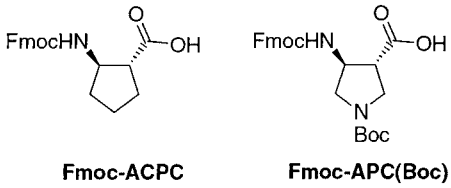
ABSTRACT



(2*R*,3*R*)-Aminoproline, a pyrrolidine-based β-amino acid, was synthesized and incorporated into hexa-β-peptide 4. This residue confers water solubility when the ring nitrogen is protonated and allows for 12-helix formation in aqueous solution. Circular dichroism spectra display the 12-helical signature, and 12-helical structure was confirmed by 2D NMR analysis.

Oligomers that are capable of taking on well-defined conformations in solution ("foldamers") have received much attention in recent years.[1] One class of foldamers, β-peptides, has been studied by several research groups.[2] Recently, β-peptides have been found to display useful biological functions.[3] Some of the biologically active β-peptides that have come from our laboratory display a 12-helical secondary structure. The 12-helix is promoted by β-amino acids that are constrained by five-membered rings, such as *trans*-aminocyclopentanecarboxylic acid (ACPC, Figure 1).[4,5] This



Fmoc-ACPC **Fmoc-APC(Boc)**

Figure 1. Protected monomers for 12-helical β-peptides.

helix is defined by 12-membered ring hydrogen bonds [C═O(*i*) → N−H(*i* + 3)] and has approximately 2.6 residues per turn. The 12-helix is well-suited for biological applica-

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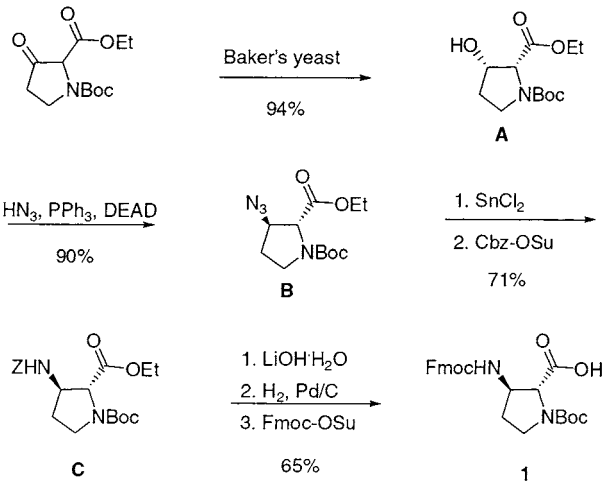
tions because its dimensions are similar to those of the α-helix found in proteins.

We have previously shown that 12-helices can form in water when a pyrrolidine monomer, *trans*-3-aminopyrrolidine-4-carboxylic acid (APC, Figure 1), is incorporated.[6] APC confers water solubility by virtue of protonation of the ring nitrogen. To have maximum flexibility in the design of *β*-peptides for biological applications, we need a pool of constrained *β*-amino acids with variety in the type or orientation of peripheral functional groups. Here we describe the synthesis of an isomer of APC, (2*R*,3*R*)-aminoproline (AP), which differs from APC in the position of the pyrrolidine nitrogen relative to the substituents but nevertheless promotes 12-helix formation.

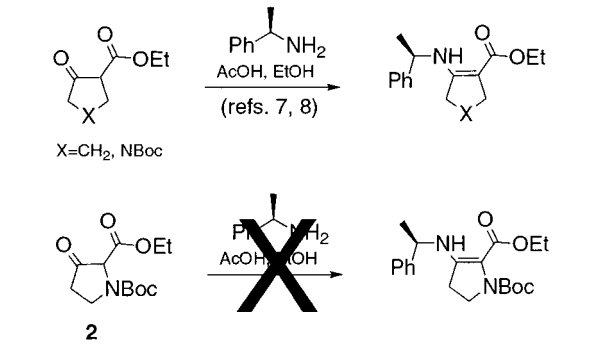
Our synthesis of enantiomerically pure AP in a protected form is outlined in Scheme 1. We were not able to synthesize

Scheme 1. Synthesis of Fmoc-AP(Boc) **1**



Fmoc-AP(Boc) **1** by a route analogous to those used for Fmoc-APC(Boc)[7] and Fmoc-ACPC;[8] the starting ketoester **2** would not react with methylbenzylamine to form the enamine necessary for the key reductive amination, even under Dean−Stark conditions (Scheme 2). The synthesis we employed for **1** is analogous to a route previously described

Scheme 2



for ACPC by Tilley et al.[9] The stereocenters are set by a nonfermenting baker's yeast reduction[10] of known ketoester **2**;[11] the reaction proceeds in approximately 89% ee (determined by chiral HPLC) and excellent yield. However, as a result of tedious workup, the yield tends to decrease upon reaction scale-up. The absolute configuration of the hydroxy-ester product has been established by Cooper et al.[11b] The alcohol can be transformed to an azide by a Mitsunobu reaction with hydrazoic acid in excellent yield. Gomez-Vidal and Silverman have reported using DPPA in the synthesis of the methyl ester version of the molecule.[12] While DPPA is a more convenient reagent than hydrazoic acid, in this case the yields are only moderate (60−75%), and an elimination byproduct inseparable from the desired azide (∼10%) is formed during the DPPA reaction. The azide is reduced to an amine with tin(II) chloride and protected with a carboxybenzyl group. The ester is hydrolyzed and the carboxybenzyl group is replaced by a fluorenylmethyoxy-carbonyl group for solid-phase synthesis.

Compound **1** was used along with Fmoc-ACPC and Fmoc-APC(Boc) in standard automated solid-phase peptide synthesis of several *β*-peptide hexamers, including **3** and **4** (Figure 2), with HBTU activation. Treatment with 95% TFA

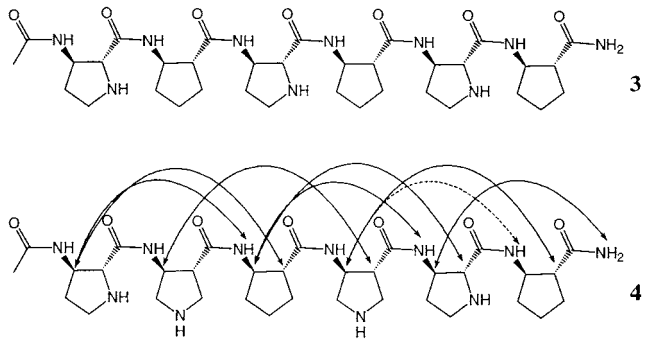


Figure 2. *β*-peptides **3** and **4**. Curved arrows superimposed on **4** indicate NOEs between residues that are not adjacent in sequence (CD_3OH). The dashed arrow indicates an NOE that is ambiguous because of resonance overlap.

cleaved the *β*-peptide from the resin and deprotected the pyrrolidine nitrogens. Although the baker's yeast reduction

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in the preparation of **1** proceeded with only 89% ee, the β-peptides described here are diastereomerically pure after HPLC purification. Initial examination of hexamer **3** in CD_3OH via NMR revealed extensive overlap among ^1H resonances, which precluded further analysis. Hexamer **4** was designed to show enhanced ^1H resonance dispersion; **4** contains three different types of β-amino acid residue, while **3** contains only two. Two-dimensional NMR (ROESY) data for **4** in CD_3OH allowed us to identify eight NOEs between residues that are not adjacent in the sequence, all of which are consistent with at least partial population of the 12-helical conformation (no NOE inconsistent with the 12-helix was detected). Two types of nonsequential NOEs were observed, $C_\beta H_i \rightarrow NH_{i+2}$ (four of five detected; one is ambiguous) and $C_\beta H_i \rightarrow C_\alpha H_{i+2}$ (all four detected). These results show that the AP residue promotes 12-helix formation in solution, since many of the NOEs involve or span the AP residues.

β-Peptide hexamers **3** and **4** were also characterized by circular dichroism for comparison with APC/ACPC β-peptides[4] and all-ACPC β-peptides.[7] Hexamer **4** showed the characteristic CD signature for the 12-helix in both methanol and aqueous buffer (10 mM Tris, pH 7.2, Figure 3).[13] Very similar data were obtained for **3** (not shown). The CD spectrum of **4** has a maximum at 204 nm and a minimum at 222 nm in methanol, and the maximum is blue-shifted to 201 nm in aqueous solution. The CD spectrum in methanol is more intense, suggesting a higher population of 12-helix in methanol than in aqueous solution. Helix stabilization by alcohols relative to water has been reported previously both for β-peptides[2a] and conventional α-peptides.[14] It is significant that hexamer **4** forms a 12-helix in aqueous solution, because α-peptides with six amino acids, as well as most short β-peptides comprised of acyclic residues,[15] do not form helices in water. We have previously shown that β-peptides consisting of ACPC, APC and related residues form the 12-helix in water with only six residues.[4,16]

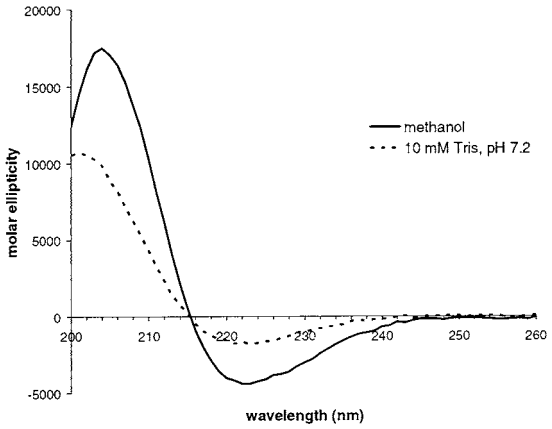

Figure 3. CD spectra of **4** (0.2 mM) in methanol and in aqueous buffer.

The AP residue reported here adds to our monomer pool for the synthesis of functionalized 12-helical β-peptides. AP has recently been incorporated into a 17-residue β-peptide that displays antimicrobial properties.[3m] We anticipate that the AP residue will be valuable in the development of additional β-peptides with useful properties.

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Supporting Information Available: Experimental procedure for compound **1**, oligomer synthesis, and 2D NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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