Design, Synthesis and Evaluation of Poly-L-Proline Type-II Peptide Mimics Based on the 3-Azabicyclo[3.1.0]hexane System

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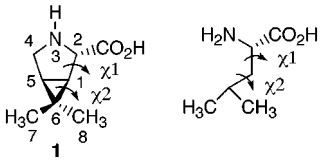
Recently, the poly-L-proline type-II (PPII) secondary structure has been shown to play a critical role in mediating several cellular signaling pathways.[1,2] Due to the potential utility of agents capable of inhibiting these signaling mechanisms, we are developing a program focused on the design and synthesis of mimics of the PPII secondary structure. Our basic strategy involves the synthesis of oligopeptides composed of proline-templated amino acids (PTAAs) that will populate the PPII conformation in solution.[3] Among the PTAAs that we desire are those that arise from the 3-azabicyclo[3.1.0]hexane system since molecular modeling studies exhibit that, for these PTAAs, the amino acid templated on the proline skeleton will possess a $\chi 1$ angle of approximately −60°.[4] These PTAAs should prove of great interest since NMR and X-ray crystal structures of receptor-bound PPII helices show that for the nonprolyl amino acids in these PPII helices a $\chi 1$ angle of approximately −60° (gauche(−) relative to the amine nitrogen) is common. An overlay of a leucine PTAA analogue with a leucine construct in which the $\chi 1$ angle is gauche(−) and the two $\chi 2$ angles are trans and gauche(−), respectively, displays the goodness of fit between these compounds (Figure 1).[5] This paper describes the synthesis of monomeric, dimeric, and trimeric leucine PTAA analogue based on the 3-azabicyclo[3.1.0]-hexane system and conformational studies of these molecules in $CDCl_3$.



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The synthesis of the oligomeric PTAAs was achieved by a modular assembly described in Scheme 1. Reaction of tricycle **2** with LAH in refluxing THF effected reduction of the lactam and oxazolidine functions to afford the *N*-benzylamino alcohol, which was debenzylated (H_2, Pd−C, 50 psi) and reprotected with Boc_2O in CH_2Cl_2 to afford the alcohol **3** (85% from **2**).[3a,6] Jones oxidation of the alcohol **3** (70%) and protection of the resulting acid (76%) gave the benzyl ester **5**. Boc deprotection and reprotection of the resultant amine afforded the trimethylacetamide **6**. Debenzylation of the ester gave the carboxylic acid **7**. The dimer **9** was obtained by diisopropylcarbodiimide (DIC)−hydroxybenzotriazole (HOBt) coupling of the acid **7** and amine **8** fragments in 75% yield. The trimer **10** was obtained by debenzylation of **9** followed by DIC−HOBt coupling with the amine fragment **8** in 72% yield.

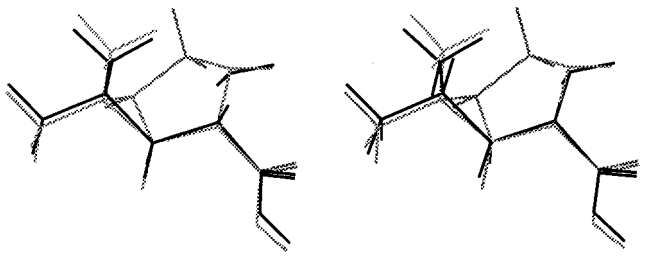
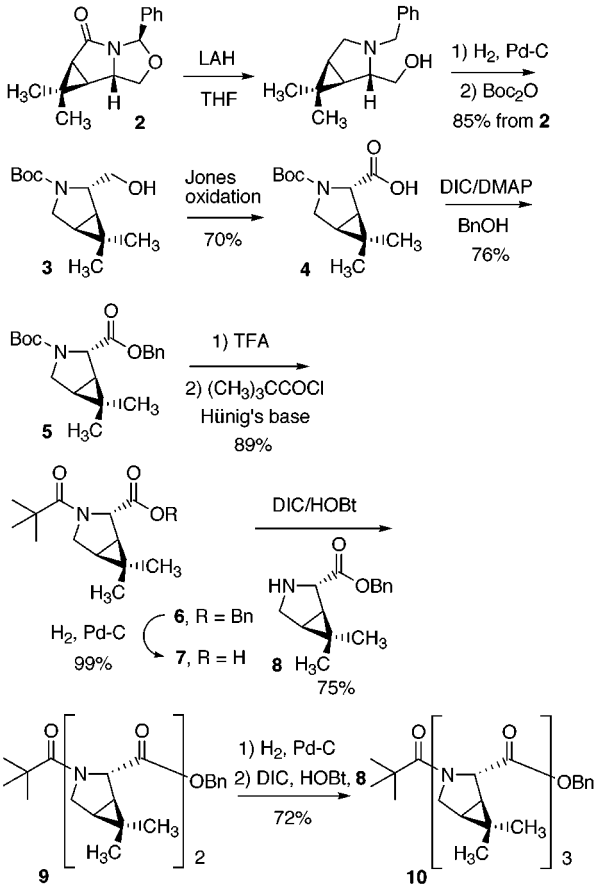


Figure 1. Stereodrawing of an overlay of **1** with leucine in which the $\chi 1$ angle is gauche(−) and the two $\chi 2$ angles are gauche(−) and trans, respectively (rms = 0.30 Å). Light bonds denote **1**. Dark bonds denote leucine.

Scheme 1



9 **10**

(1) For a recent PPII review see: Siligardi, G.; Drake, A. F. *Peptide Sci.* **1995**, *37*, 281.

(2) For recent examples of proteins that bind PPII helices see: (a) Raj, P. A.; Marcus, E.; Edgerton, M. *Biochemistry* **1996**, *35*, 4314. (b) Lee, C.-H.; Saksela, K.; Mirza, U. A.; Chait, B. T.; Kuriyan, J. *Cell* **1996**, *85*, 931. (c) Peng, S.; Kasahara, C.; Rickles, R. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12408. (d) Jardetzky, T. S.; Brown, J. H.; Gorga, J. C.; Stern, L. J.; Urban, R. G.; Strominger, J. L.; Wiley: D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 734. (e) Zeile, W. L.; Purich, D. L.; Southwick, F. S. *J. Cell Biol.* **1996**, *133*, 49.

(3) (a) Zhang, R.; Brownewell, F.; Madalengoitia, J. S. *J. Am. Chem. Soc.* **1998**, *120*, 3894. (b) Zhang, R.; Madalengoitia, J. S. *Tetrahedron Lett.* **1996**, *37*, 6235.

(4) For cyclopropane ring constraints of amino acids see: (a) Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Baker, W. R. Condon, S. L. deLara, E.; Rosenberg, S. H.; Spina, K. P.; Stein, H. H.; Cohen, J.; Kleinert, H. D. *J. Med. Chem.* **1992**, *35*, 1710. (b) Burgess, K.; Ho, K.-K.; Pettitt, B. M. *J. Am. Chem. Soc.* **1994**, *116*, 799.

(5) (a) For the leucine construct: gauche (−) = −60° and trans = 180°. Cα, Cβ, Cγ, and Cδ as well as the nitrogen and carbonyl carbon of leucine were chosen to be overlaid with the corresponding atoms on the PTAA. (b) For PPII helices in which leucine residues have a $\chi 1$ angle gauche(−), and $\chi 2$ angles, gauche(−) and ~ trans, see: Feng, S.; Chen, J. K. Yu, H.; Simon, J. A.; Schreiber, S. L. *Science* **1994**, *266*, 1241. (c) Yu, H.; Chen, J. K.; Feng, S.; Dalgarno, D. C.; Brauer, A. W.; Schreiber, S. L. *Cell* **1994**, *76*, 933.

(6) For the synthesis of **2**: Zhang, R.; Madalengoitia, J. S. *J. Org. Chem.* **1999**, *64*, 547−555.

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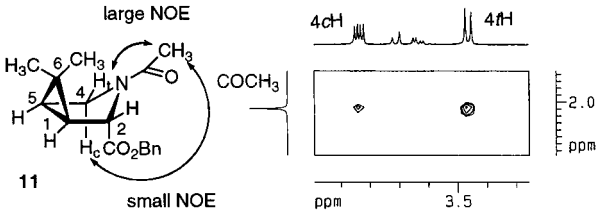

Figure 2. Selected NOEs for **11** and expanded region of NOESY spectrum showing 4*c*H and 4*t*H cross-peaks with the acetamide methyl group.

Previous studies have shown that the bicyclo[3.1.0]hexane system can adopt a flattened boat conformation.[7] Since a chair or boat pucker will result in different dihedral angles ϕ, the conformation of the pyrrolidine ring was first investigated. An NOE experiment on the model compound Ac-PTAA-OBn **11** (Figure 2) was utilized to determine the ring pucker since a populated boat or chair conformation would result in unequal distances from the acyl methyl group to 4*c*H and 4*t*H (*c* and *t* denote a cis and trans relationship relative to the ester carbonyl).[8] Indeed, a NOESY spectrum of compound **11** shows that the acyl methyl group exhibits a higher NOE intensity with 4*t*H than 4*c*H (Figure 2). This information is thus consistent with a populated boat conformation for the monomer unit **11**. The validity of this experiment is dependent on a planar geometry for the amide bond. For this reason, the trimethylacetyl-substituted monomer **6** was not used, since it might be argued that the significant sterics in this system could impart a nonplanar nature to the amide bond. However, a comparison of J_{5H-4cH} in both **11** and **6** reveals an identical value of 5.2 Hz. Since J_{5H-4cH} is a function of the pyrrolidine pucker, it may be extrapolated that structure **6** also populates a boat conformation. Molecular modeling of acetamide **11** reproduces the slight pucker and gives a value for $\phi = -63°$ well within $-75° \pm 30°$ required for the PPII conformation.[9,10]

We previously have argued that the minimization of pseudo-allylic strain between adjacent prolines can define $\psi \approx 145°$ as required in the PPII conformation.[3a] For the dimer **9**, the minimization of pseudo-allylic strain should place 4*c*H(i + 1) and 4*t*H(i + 1) in close spatial relationship to 2H(i) (Figure 3) when the PTAA−PTAA amide bond is in the trans conformation.[11] For both the dimer **9** and the trimer **10** we were able to observe only trans amide bond

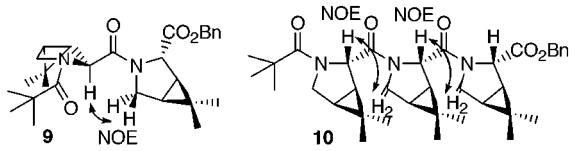

Figure 3. Selected NOEs for **9** and **10**.

rotamers. A NOESY spectrum of dimer **9** shows NOEs between 2H(i)−4*c*H(i + 1) and 2H(i)−4*t*H(i + 1). Although these NOEs are not diagnostic for the PPII conformation, there are no additional inter-PTAA NOEs of equal or greater magnitude. That the 2H(i)−4*c*H(i + 1) and 2H(i)−4*t*H(i + 1) NOEs are the strongest inter-PTAA NOEs is indicative of the preferential population of a conformation in which $\psi \approx 145°$, as required for the PPII conformation. It should be noted that the NOE between 2H(i)−4*t*H(i + 1) is greater than the NOE between 2H(i)−4*c*H(i + 1). Since both PTAAs populate the flattened boat conformation as evidenced by $J_{5H(i)-4cH(i)} = J_{5H(i+1)-4cH(i+1)} = 5.2$ Hz, the unequal NOE intensities are analogous to those found with Ac-PTAA-OBn (**11**).

The trimer **10** is an important compound for our studies since three residues define one turn of a PPII helix. A NOESY spectrum of trimer **10** also reveals the key NOEs between 2H(i)−4H(i + 1) and 2H(i + 1)−4H(i + 2). Furthermore, these NOEs are the strongest inter-PTAA NOEs, indicating a population in which $\psi \approx 145°$. A statement about the relative NOE intensities between 2H(i)−4*c*H(i + 1) and 2H(i)−4*t*H(i + 1) cannot be made in this case because 4*c*H(i + 1) and 4*t*H(i + 1) are not resolved. However, the 5H−4*c*H coupling constants are 5.0, 5.2, and 5.2 Hz for the i, i + 1, and i + 2 PTAAs, respectively, indicating that even in the trimer, the boat conformation is maintained by the individual PTAAs.

Our preliminary studies show that PTAAs based on the 3-azabicyclo[3.1.0]hexane system preferentially populate the PPII conformation in $CDCl_3$. These PTAAs will be critical for the synthesis of conformationally constrained PPII mimics that possess residues with $\chi 1 \approx -60°$ and a $\chi 2 \approx -155°$. Work is ongoing to synthesize and evaluate water-soluble PTAAs based on this framework.

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Supporting Information Available: Experimental procedures for **4** and **7** and characterization data for **3**, **5**, **6**, and **9**−**11**, ^{1}H and ^{13}C NMR spectra of **3**, **5**, **6**, and **9**−**11**, and NOESY spectra of **9**−**11** (23 pages).

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(8) Compound **11** exists as 7:3 mixture of trans/cis amide bond rotamers, respectively, while a cis rotamer is not detected in **6**.

(9) Energy minimization of the trans rotamer of **11** was performed with MMX version 6.0, Serena Software, Box 3076, Bloomington, IN 47402.

(10) The $-75° \pm 30°$ tolerance for ϕ in the PPII conformation is according to: Adzhubei, A. A.; Sternberg, M. *J. Mol. Biol.* **1993**, *229*, 472.

(11) The designation i denotes the N-terminal residue.