ORGANIC LETTERS

2010
Vol. 12, No. 23
5438−5441

Oligomers of a 5-Carboxy-methanopyrrolidine β-Amino Acid. A Search for Order

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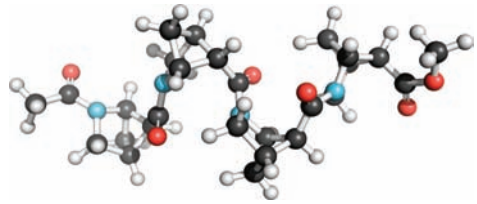
Received September 23, 2010

ABSTRACT



CD spectra for homooligomers (n = 4, 6, 8) of (1*S*,4*R*,5*R*)-5-*syn*-carboxy-2-azabicyclo[2.1.1]hexane (MPCA), a methano-bridged pyrrolidine β-carboxylic acid, suggest an ordered secondary structure. Even in the absence of internal hydrogen bonding, solution NMR, X-ray, and in silico analyses of the tetramer are indicative of conformations with *trans*-amides and C_5-amide-carbonyls oriented toward the C_4 bridgehead. This highly constrained β-amino acid could prove useful in the ongoing development of well-defined foldamers.

Among naturally occurring α-amino acids, proline **1** is the only secondary amine. Although this means that homooligomers of proline do not have *N*-H protons available to stabilize secondary structures by a hydrogen bond, polyproline still forms ordered oligomers.[1] In the course of the rational design of β-peptides related to natural peptides, Seebach[2] wondered if β-peptidic chains without backbone H-bonds might also fold into stable secondary structures. To explore this possibility, Seebach and co-workers prepared two methylene homologues of proline by formal insertion of a methylene either between the carbonyl C-atom and the C(α)-atom to give **2** or between the C(α)- and C(β)-atoms to give **3**. Oligomers (n = 3, 6, 12, 18) of (*R*)-**2** with terminal *N*-Boc and OBn groups all gave CD patterns in methanol with an intense minimum at 202 nm and a maximum at 223 that seemed indicative of order. The absolute mean-residue molar ellipticity at both 202 and 223 nm decreases with growing chain length suggesting that the secondary structure of longer peptide chains from **2** is destabilized. Similarly, the CD spectra of oligomers (n = 3, 6) of all (*S*)-**3** with terminal *N*-Boc and OEt groups in methanol also gave some

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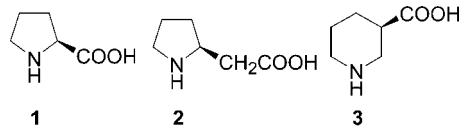
(1) (a) Kakinoki, S.; Hirano, Y.; Oka, M. *Polym. Bull.* **2005**, *53*, 109–115. (b) Cowan, P. M.; McGavin, S. *Nature* **1955**, *176*, 501–503. (c) Traub, W.; Shmueli, U. *Nature* **1963**, *198*, 1165–1166.

(2) Abele, S.; Vogtli, K.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1539–1558. It was known that peptoids form ordered secondary structures; see refs 18−21 therein.

10.1021/ol1022917
Published on Web 11/02/2010

indications of secondary structure. The mean residue molar ellipticity of the hexamer at 230 nm is nearly three times larger than the trimer indicating that in this case the

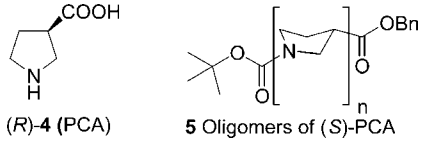


1 **2** **3**

secondary structure may be stabilized by the longer β-peptide chain. Gellman[4] independently investigated oligomers (n = 2−6) of all (*S*)-**3** with terminal *N*-Boc and OBn groups in methanol and observed a maximum ellipticity at 228 nm when $n = 3-6$. However, little evidence beyond CD spectra was provided to show that these β-amino acid oligomers adopted well-defined structures in solution.

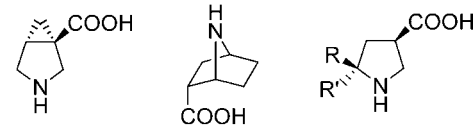
A model was proposed for the oligomeric peptides of **2**.[2] It assumed an all-*trans* amide structure with a Φ angle of −72° enforced by the pyrrolidine ring. An antiperiplanar conformation around the C(α)−C(β) bond ($\theta = 180°$) was chosen on the basis of this angle in a trifluoroacetate salt of a trimer of **2** that contains amine and benzyl ester end groups. However, it should be noted that the peptide salt crystallized with four CF_3COOH molecules and the carbonyls of the amide bonds of the salt were hydrogen bonded to acid molecules. A large angle, $\Psi = 180°$, was chosen so that the large substituents at C(α) were antiperiplanar to the large substituents at the carbonyl C-atom. The outcome of this somewhat speculative model is a right-handed 10_3 helix with three pitches to bring residue ($i + 10$) above residue *i*. The model suggests consecutive fully extended chain segments [*N*-C(β)−C(α)−CO-*N*], which are twisted by −72°. The structure of peptide oligomers of **3** was not modeled.

(*R*)-3-Carboxypyrrolidine **4** (PCA) might also be viewed as a β-amino acid proline analogue in which the acid is moved one atom away from the nitrogen. Gellman[3] has used the (*S*)-PCA enantiomer to search for order in pyrrolidine-based oligomers (n = 2−6). Normalized CD spectra in methanol for oligomers **5** terminated by *N*-Boc and OBn groups are most ordered when at the tetramer to hexamer level with a minimum ellipticity at 214 nm. A possible conformation has been modeled by calculation.[4]



(*R*)-**4** (PCA) **5** Oligomers of (*S*)-PCA

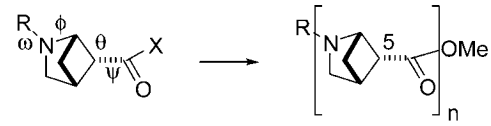
One of the difficulties in evaluating the order in homooligomers of pyrrolidine-based β-amino acids such as **2** or PCA **4** is the number of degrees of freedom in the molecules. There are no intramolecular hydrogen bonds to control geometry, and unlike *N*-acyl prolines, the amide carbonyl oxygens are not suitably oriented for $n \rightarrow \pi^*$ interactions with the β-carboxyl side chains.[5] A possible approach to solve this problem is to limit the flexibility of the five-membered ring, as in the methano-fused[6] or ethano-bridged[7] structures **6** or **7**, or to control amide conformation by α,α-disubstitution as in **8**.[8] Oligomers of **7** and **8** show evidence of order



6 **7** **8**

in their CD spectra; a possible structure for oligomers of **7** has been calculated.

An alternative conformational restriction is the introduction of a methano-bridge into an *N*-acyl-pyrrolidine ring to maintain an idealized C^β ring pucker, as in structure **9**.[9] The bridge constrains two internal angles of the pyrrolidine ($\Phi \sim -127°$ and $\theta \sim 54°$). Of the two remaining rotatable bonds, the amide bond might be biased with the carbonyl oriented either syn or anti to the bridgehead C_1, so that $\omega \sim$ 180° or 0°. Thus, the major remaining rotational freedom responsible for secondary structure would be from the external C_5−CO bond (Ψ). We believed that introducing such a conformational constraint in β-proline oligomers could provide entry into a well-defined and potentially useful foldamer secondary structure.



9 R = Boc, X = OH
10 R = Boc, X = (*S*)-NHCHMePh
11 R = Boc, X = OMe
12 R = CO-*i*-Pr, X = OMe

13a n = 2, R = Boc
13b n = 2, R = CO-*i*-Pr
14a n = 4, R = Boc
14b n = 4, R = CO-*i*-Pr
15a n = 6, R = Boc
15b n = 6, R = CO-*i*-Pr
16a n = 8, R = Boc
16b n = 8, R = CO-*i*-Pr

In preparation for homooligomer formation, racemic acid **9** was resolved as its salt using (*S*)-(−)-α-methylbenzylamine with THF as recrystallization solvent. The absolute configuration of the resolved (−) acid **9** was established as (1*S*,4*R*,5*R*) by X-ray structure determination of the condensed amide **10**.

(3) Huck, B. R.; Langenhan, J. M.; Gellman, S. H. *Org. Lett.* **1999**, *1*, 1717–1720.

(4) Sandvoss, L. M.; Carlson, H. A. *J. Am. Chem. Soc.* **2003**, *125*, 15855–15862.

(5) (a) Shoulders, M. D.; Raines, R. T. *Annu. Rev. Biochem.* **2009**, *78*, 929–958. (b) Choudhary, A.; Gandla, D.; Krow, G. R.; Raines, R. T. *J. Am. Chem. Soc.* **2009**, *131*, 7244–7246. See refs 3−6 therein.

(6) Medda, A. K.; Lee, H.-S. *Synlett* **2009**, 921–924.

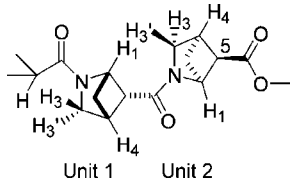
(7) Otani, Y.; Futaki, S.; Kiwada, T.; Sugiura, Y.; Muranaka, A.; Kobayashi, N.; Uchiyama, M.; Yamaguchi, K.; Ohwada, *Tetrahedron* **2006**, *62*, 11635–11644.

(8) (a) Huck, B. R.; Fisk, J. D.; Guzei, I. D.; Carlson, H. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2003**, *125*, 9035–9037. (b) Huck, B. R.; Gellman, S. H. *J. Org. Chem.* **2005**, *70*, 3353–3362.

(9) Krow, G. R.; Lin, G.; Herzon, S. B.; Thomas, A. M.; Moore, K. P.; Huang, Q.; Carroll, P. J. *J. Org. Chem.* **2003**, *68*, 7562–7564.

To test for a conformational trans/cis bias of the amide angle (ω), MPCA **9** was converted with Me_3SiCHN_2 into its methyl ester **11** and then by protecting group exchange into the isobutyryl amide **12**. NMR analysis of monomer **12** indicated two H_1 resonances in a 1:1 ratio indicating *no inherent bias* for *trans*-/*cis*-amides. The dimer **13a** was prepared by cleavage of the *N*-Boc group from ester **11** with HCl/dioxane and direct coupling of the resulting amine with (−)-acid **9** using EDC and HOBt. Multiple conformations for dimer **13a** are possible. For each of two amide $N(sp^2)-C(sp^2)$ and two $C_5(sp^3)-C(sp^2)$ bonds there are, respectively, two and three different staggered conformations. In theory, there are at least 36 potential local minima. Despite this, the ^1H NMR spectrum of dimer **13a** showed just two major downfield H_1 resonances. To facilitate the spectral analysis of these major resonances, the terminal *N*-Boc group of **13a** was replaced with an *N*-isobutyryl substituent to give **13b**. Expansion of the downfield H_1 region of dimer **13b** indicated ten H_1 resonances that individually integrate for at least 3% of the total and that together account for 95% of the H_1 peaks; however, two major H_1 resonances together represent 62% of the H_1 peaks. The key NMR resonances associated with the major conformation of dimer **13b** are shown in Table 1. NOE data show that the isopropyl C*H*Me_2

Table 1. Key Major NMR Resonances for Dimer **13b**



proton	unit 1	unit 2	carbon	unit 1	unit 2
CH	2.52[a]	-		31.9	
H_1	5.10[b,c]	4.88[d]	C_1	60.1	60.9
H_3	3.37	3.70	C_3	47.7	46.5
$H_{3'}$	3.81	3.75			
H_4	3.18	3.04	C_4	40.4	41.3

[a] NOE cross-peak with $H_{3,3'}$ of unit 1. [b] Coupled to C_3 of unit 1 (HMBC). [c] NOE cross-peak with $H_{3,3'}$ of unit 2. [d] Coupled to C_3 of unit 2.

is near the methylene $H_{3,3'}$ protons of unit 1. The C_3 carbon associated with these $H_{3,3'}$ protons (by HSQC) is coupled to H_1 of this first unit (by HMBC). Most importantly, the H_1 proton of the first unit has an NOE cross-peak with the $H_{3,3'}$ protons of the second unit, H(1,*i*)−H(3,*i* + 1). The H_4 resonances for units 1 and 2 could be identified by their W-plan coupling with H_1 protons for each unit. We designate this major dimer conformer as the T4T*4* structure. The first amide bond is trans; the carbonyl of unit 1 is directed toward H_4; and the second carbonyl is trans. To minimize dipole repulsions, the ester carbonyl is assumed to be oriented toward H_4 (vide infra).

With evidence of some solution-phase order for the dimers **13a**−**13b** in hand, the oligomers **14a**−**16a** were prepared (see Supporting Information). The CD spectra of monomer **11** and the oligomers (n = 2, 4, 6, 8) are shown in Figure 1A. The data for β-peptide concentration and number of

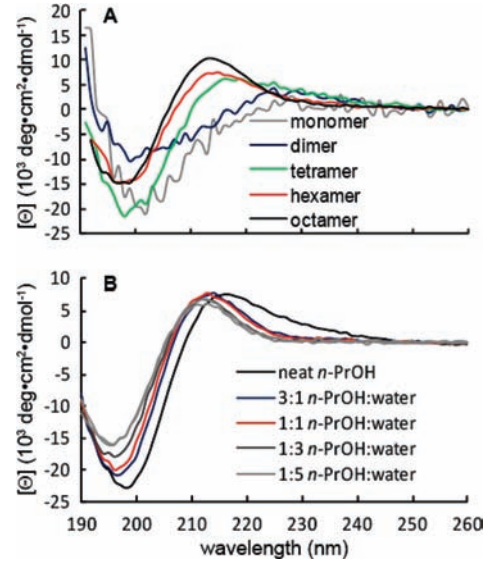


Figure 1. CD spectra for (A) 100 μM *N*-BOC-$(MPCA)_n$-OMe in methanol (25 °C). (B) 100 μM octamer **16a** in water, *n*PrOH, and mixtures of water/*n*PrOH (25 °C). Data are normalized for β-peptide concentration and number of amides.

amino acid units were normalized to facilitate comparisons among oligomers of different lengths. The CD spectra of tetramer, hexamer, and octamer have a strong negative molar ellipticity at 198 nm and a weak positive at 212−215 nm. The overlapping of spectra from monomer to octamer clearly shows that substantial order begins to develop at the tetramer level and is enhanced in longer oligomers. The positive molar elipticity increases with increasing oligomer length and shifts to slightly shorter wavelength.

CD spectra of the octamer **16a** in water, *n*PrOH, and mixed solvents of water/*n*PrOH in different ratios were collected to study the solvent effects on conformation of the oligomer (Figure 1B). From these spectra, it is clear that water has little effect on the secondary structure of octamer. Because the backbone of these peptides is tertiary amides, there are no internal hydrogen bonds for water to disrupt.

The *N*-Boc-tetramer **14a** formed a crystalline solid suitable for X-ray analysis (Figure 2). Although a water molecule attached to the C_5-carbonyl oxygen (O_2) of unit 2 alters the Ψ angles predicted by calculations (lower picture in Figure 2), the tetramer does have a T4T4T4T4 arrangement that places each carbonyl, including the terminal ester group, so that alternate dipoles point in opposite directions (see dimer **13b** above).

In solution, the *N*-Boc tetramer **14a** exhibits four major H_1 peaks that represent 58−72% of a major conformation. The NMR analysis of the *N*-isobutyryl tetramer **14b** (Table 2) shows that the major (72%) structure can be described as a T4T4T4T*4* conformation with the final ester conformation not determined by NMR (see Supporting Information).

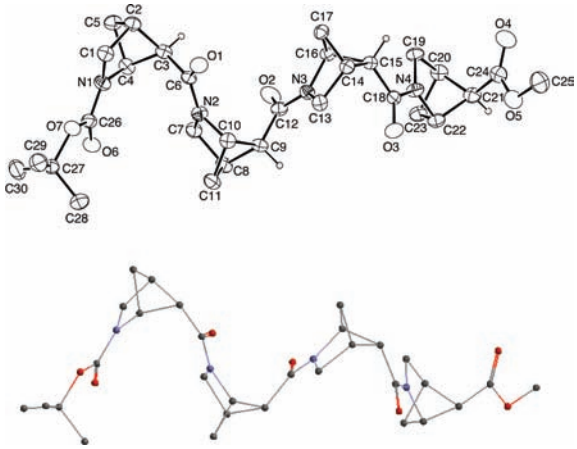
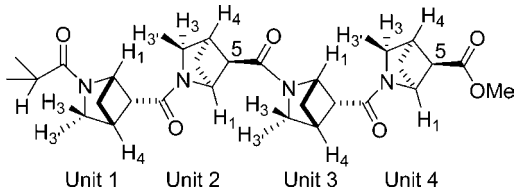


Figure 2. (Top) ORTEP drawing of tetramer **14a**. (Bottom) Spartan MMFF minimized $(T4)_n$ conformer of tetramer **14a**.

Molecular mechanics geometry optimizations of **14b**[10] and an *N*-acetyl tetramer **14c** (see Supporting Information) indicate a preference for $[T4]_n$ conformations. The calculated angles for **14b** as a $[T4]_n$ conformation are $\omega \sim 176-179°$ and $\Psi \sim 58-64.5°$. Importantly, in the favored calculated structure H_1 of the *n*th residue was in close proximity to $H_{3,3'}$ of the $(n + 1)$ residue. Orientations in which the C_5 carbonyl of unit *i* is oriented toward H_1 of unit $i + 1$, or the amide-carbonyl of any unit is oriented cis toward $H_{3,3'}$ rather than H_1, are inconsistent with ^{1}H NMR NOE correlations linking H_1 (unit *i*) with $H_{3,3'}$ (unit $i + 1$).

Table 2. Key Major NMR Resonances for Tetramer **14b**



proton	unit 1	unit 2	unit 3	unit 4
CH	2.52[a]	-		
H_1	5.08[b]	4.95[b]	4.97[b]	4.87
H_3	3.35	3.68	3.57	3.58
$H_{3'}$	3.77	3.71	3.74	3.70

[a] NOE with to $H_{3,3'}$ of unit 1. [b] NOE to $H_{3,3'}$ of the next unit.

The CD spectra for the MPCA oligomers in Figure 1 are similar to the characteristic concentration-independent CD molar elipticities at 217 nm (maximum) and 198 nm (minimum) found for oligomers of azabicycle **7** of the 2*S*-COOMe configuration. A dependence of conformational stabilization ($n = 2-8$) on chain length also was noted for oligomers of **7**. Like the present $(T4)_n$ structures for oligomers of **9**, calculations for the tetramer of **7** predict $\Psi = 65°$ to be slightly favored.[7] Calculations for oligomers **5** predict $(C1)_n$ conformations to be favored.[4] Notably, the $(C1)_4$ and $(T4)_4$ oligomers under discussion do have alternating anti orientations for their carbonyl dipoles in common.

There is indication of a major conformation in each of our oligomers, as shown by the H_1 region of each ^{1}H NMR spectrum (see Supporting Information). The crystal structure of tetramer **14a**, in silico methods, and the solution NMR analyses of oligomers **13b**−**16b** suggest $[T4]_n$ structures for all amide bonds as the major contributors. The ability to have a well-defined folding pattern in the absence of hydrogen bonds or substantial electrostatic and hydrophobic interactions should allow for the creation of foldamers with interesting functions.[11] Thus, the MPCA **9** may serve as a useful addition to the arsenal of β-amino acid building blocks.[12] We are currently preparing C_1- and C_6-substituted MetPyr acid oligomers that might adopt other interesting conformations amendable to NMR analysis. For example, although *N*-acyl-1-methyl-2-azabicyclo[2.1.1]hexanes are restricted to the conformer with the carbonyl directed to the C_1 position,[13] in oligomers of such substrates steric interference is not likely to favor the Me(1,*i*)−H(3,$i + 1$) interactions present in $(T4)_n$ conformations.

Acknowledgment. Financial support was provided by NSF (CHE 0515635 and DGE-0841377) and the NIH (AR044276). CD spectra were collected at the Biophysics Instrumentation Facility established by Grants BIR-0-9512577 (NSF) and S10 RR13790 (NIH). M.S. was supported by a Der Min Fan Fellowship, and M.D.S. was supported by a USA Department of Homeland Security Graduate Fellowship. We thank Rocky Chiang, Kevin Cannon, Ram Edupuganti (TU), and Samuel H. Gellman (UW-Madison) for helpful discussions.

Supporting Information Available: General experimental and procedures for preparation of all new compounds, X-ray diffraction analyses of amide **10** and tetramer **14a**, spectral assignments for tetramer **14b**, a table of H_1 resonances for all oligomers, a calculated $(T4)_4$ structure **14c**, and copies of reported ^{1}H NMR and ^{13}C NMR for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) (a) *PC Spartan'04*, Essential V2.0.0 Wavefunction, Inc.: Irvine, CA 92612, 2004. (b) A rise/residue of 3.5Å, 2.4 residues/turn, and a pitch of 8.4 Å can be approximated from the calculated structure.

(11) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180. (b) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4011. (c) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (d) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366–1375.

(12) (a) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399–1408. (b) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111–1239.

(13) Malpass, J. R.; Patel, A. B.; Davies, J. W.; Fulford, S. *J. Org. Chem.* **2003**, *68*, 9348–9355.