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

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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 C₅-amide-carbonyls oriented toward the C₄ bridgehead. This highly constrained β -amino acid could prove useful in the on-going 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 hydrogen bond, polypyrrolidine still forms ordered oligomers.¹ In the course of the rational design of β -peptides related to natural peptides, Seebach² wondered if β -peptidic chains without backbone H-bonds might also fold into stable secondary structures. To explore this possibility Seebach and coworkers prepared two methylene homologs 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

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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 H₁ resonances for all oligomers, a calculated (T4)₄ structure **14c**, copies of reported ¹H NMR and ¹³C NMR for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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 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 secondary structure may be stabilized by the longer β -peptide chain. Gellman⁴ 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 $C(\alpha)-C(\beta)$ bond ($\theta = 180^\circ$) 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 $\psi = 180^\circ$ was chosen so that the large substituents at $C(\alpha)$ 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 $[N-C(\beta)-C(\alpha)-CO-N]$, which are twisted by -72° . The structure of peptide oligomers of **3** were not modelled.

(*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. Gellman³ 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-fused⁶ or ethano-bridged⁷ 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 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**. The bridge constrains two internal angles of the pyrrolidine ($\Phi \sim -127^\circ$ and $\theta \sim 54^\circ$). 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 $\omega \sim 180^\circ$ 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**.

To test for a conformational trans/cis bias of the amide angle (ω), MPCA **9** was converted with $\text{Me}_3\text{SiCHN}_2$ 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 $N(\text{sp}^2)\text{--}C(\text{sp}^2)$ and two $C_5(\text{sp}^3)\text{--}C(\text{sp}^2)$ 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; but, 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 CHMe_2 is near the methylene $\text{H}_{3,3'}$ protons of

unit 1. The C₃ carbon associated with these H_{3,3'} protons (by HSQC) is coupled to H₁ of this first unit (by HMBC). Most importantly, the H₁ 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 H₄ resonances for units 1 and 2 could be identified by their W-plan coupling with H₁ protons for each unit. We designate this major dimer conformer as the T4T4 structure. The first amide bond is trans, the carbonyl of unit 1 is directed toward H₄, the second carbonyl is trans. To minimize dipole repulsions, the ester carbonyl is assumed to be oriented toward H₄ (*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 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 ellipticity 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 C₅-carbonyl oxygen (O₂) 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 H₁ 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 T4T4T4T4 conformation with the final ester conformation not determined by NMR (see: Supporting Information).

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 ω ~ 176–179° and ψ ~ 58–64.5°. Importantly, in the favored calculated structure H₁ of the *n*th residue was in close proximity to H_{3,3'} of the (*n*+1) residue. Orientations in which the C₅ carbonyl of unit *i* is oriented toward H₁ of unit *i* + 1, or the amide-carbonyl of any unit is oriented cis toward H_{3,3'} rather than H₁, are inconsistent with ¹H NMR NOE correlations linking H₁ (unit *i*) with H_{3,3'} (unit *i* + 1).

The CD spectra for the MPCA oligomers in Figure 1 are similar to the characteristic concentration-independent CD molar ellipticities at 217 nm (maximum) and 198 nm (minimum) found for oligomers of azabicyclic **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 ψ = 65° to be slightly favored.⁷ Calculations for oligomers **5** predict (C1)_n conformations to be favored.⁴ Noteably, 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 H₁ region of each ¹H 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 -aminoacid building blocks.¹² We are currently preparing C_1 - and C_6 -substituted MetPyr acid oligomers that might adopt other interesting conformations amenable 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

CD spectra for (A) $100 \mu\text{M}$ of $\text{NBOC}-(\text{MPCA})_n\text{-OMe}$ in methanol (25°C). (B) $100 \mu\text{M}$ of octamer **16a** in water, $n\text{-PrOH}$, and mixtures of water/ $n\text{-PrOH}$ (25°C). Data are normalized for β -peptide concentration and number of amides.

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

TABLE 1

Key Major NMR Resonances for Dimer 13b.

proton	Unit 1		carbon	Unit 2	
	unit 1	unit 2		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 C₃ of unit 1. (HMBC).

(^c)NOE cross-peak with H_{3,3'} of unit 2.

(^d)Coupled to C₃ of unit 2.

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 H₃,3' of unit 1.

(^b) Noe to H₃,3' of the next unit.