

## Secondary Structural Preferences of 2,2-Disubstituted Pyrrolidine-4-carboxylic Acid Oligomers: $\beta$ -Peptide Foldamers that Cannot Form Internal Hydrogen Bonds

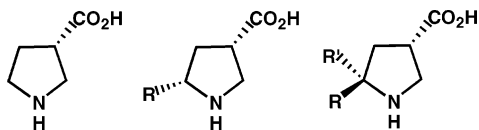
Bayard R. Huck,<sup>†</sup> John D. Fisk,<sup>‡</sup> Ilia A. Guzei,<sup>†</sup> Heather A. Carlson,<sup>\*,§</sup> and Samuel H. Gellman<sup>\*,†,‡</sup>

Department of Chemistry and Graduate Program in Biophysics, University of Wisconsin, Madison, Wisconsin 53706, and Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109-1065

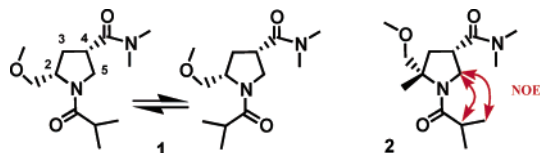
Received February 7, 2003; E-mail: gellman@chem.wisc.edu

Foldamers are oligomers that adopt well-defined conformations.<sup>1</sup> Interest in unnatural foldamers has burgeoned in recent years as it has become clear that short oligomers constructed from subunits other than  $\alpha$ -amino acids or nucleotides can adopt discrete secondary structures.<sup>2</sup> The ability to control molecular shape has allowed creation of foldamers with interesting functions.<sup>3</sup>  $\beta$ -Peptides ( $\beta$ -amino acid oligomers) are among the most widely studied unnatural foldamers.<sup>4</sup> All three types of regular secondary structure observed in proteins, helix, sheet, and reverse turn, have been documented among short  $\beta$ -peptides. To date, however, all  $\beta$ -peptide secondary structures characterized by high-resolution methods (X-ray crystallography or two-dimensional NMR spectroscopy) have contained intramolecular hydrogen bonds between backbone amide groups. Similarly, intramolecular hydrogen bonding is characteristic of the most common protein secondary structures. Non-hydrogen bonded secondary structures, for example, polyproline helices,<sup>5</sup> occur occasionally among proteins and in some short conventional peptides. Individual strands within the collagen triple helix have a polyproline II conformation, and short PPII helices play important roles in protein–protein recognition.<sup>5b</sup> The PPI helix has not yet been observed in proteins, but this secondary structure is displayed by proline oligomers in certain solvents and by some *N*-alkylglycine oligomers (“peptoids”).<sup>6</sup> Some nonpeptidic foldamers lack internal hydrogen bonding.<sup>7</sup>

We describe here a set of  $\beta$ -peptides that cannot form backbone–backbone hydrogen bonds but that nevertheless display distinct conformational preferences. These oligomers are constructed from 2,2-disubstituted-pyrrolidine-4-carboxylic acid (2,2-DPCA) residues. Because these building blocks are  $\beta$ -imino acids, the interresidue linkages are tertiary amides, as in proline and peptoid oligomers, and there are no hydrogen bond donors in the backbone.



In previous work, we examined oligomers of pyrrolidine-3-carboxylic acid (PCA) and of nipecotic acid, the six-membered ring homologue of PCA;<sup>8</sup> Seebach et al. conducted independent studies of nipecotic acid oligomers.<sup>9,10</sup> The per-residue circular dichroism (CD) spectra of these oligomers change as a function of length up to the tetramer but are independent of length for the pentamer and hexamer. Similar trends have been observed among proline



oligomers;<sup>11</sup> this precedent suggested that the PCA and nipecotic acid oligomers with four or more residues might have discrete conformational preferences. Subsequent <sup>13</sup>C NMR studies, however, revealed that even the longest oligomers in these two  $\beta$ -peptide series displayed multiple resonances for each carbonyl carbon, indicating the presence of multiple amide rotamers in slow exchange with one another.<sup>12</sup> Similar behavior has been observed for peptoids of comparable length.<sup>6</sup> The presence of multiple amide rotamers in these oligomers makes NMR structural analysis difficult and complicates the interpretation of length-dependent CD data.

In an effort to induce the backbone amide group to prefer a single conformation (*Z*), we prepared  $\beta$ -peptides from a 2-monosubstituted PCA (2-MPCA) monomer (R = methoxymethyl). However, <sup>13</sup>C NMR data for *N*-acyl 2-MPCA derivative **1** and related homooligomers indicated multiple rotameric states.<sup>12</sup> We turned to 2,2-DPCA units to impose a stronger rotamer bias.<sup>13</sup> <sup>13</sup>C NMR analysis of *N*-acyl 2,2-DPCA derivative **2** and related homooligomers (**3a–e**) in CDCl<sub>3</sub> indicated that only a single rotameric state is significantly populated in each case.<sup>12</sup> The favored rotamer of **2** was identified as *Z* by observation of NOEs from the protons on carbon-5 of the ring to the protons of the isobutyryl group, as shown in the drawing above.

A wide range of 2,2-DPCA building blocks is available via well-precedented chemistry. The key transformation is  $\alpha$ -alkylation of a protected derivative of *trans*-4-hydroxy-*L*-proline.<sup>14</sup> The alkylating agent provides one of the 2-substituents, and the proline carboxyl is transformed into the other 2-substituent. Deprotection of the 4-hydroxyl group, activation, cyanide displacement, and hydrolysis provide the carboxyl group of the desired  $\beta$ -amino acid.<sup>12</sup> The residues in dimer **4**, for example, were prepared via  $\alpha$ -alkylation with benzyl bromide. The crystal structure of **4** (Figure 1) shows that the amide group linking the two 2,2-DPCA residues adopts the expected *Z* conformation. 2,2-DPCA oligomers can be efficiently synthesized in solution with BopCl as coupling agent and Boc as the protecting group on the ring nitrogen, or on a solid support with PyBrOP as the coupling agent and Fmoc as the nitrogen protection group.<sup>12</sup>

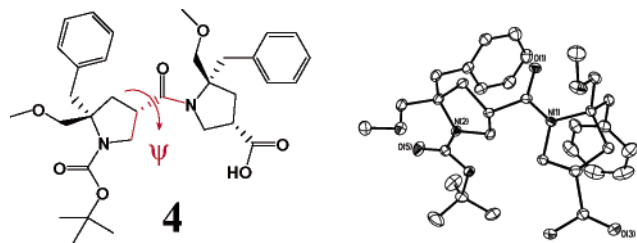
Homooligomers **3a–e** were investigated in CH<sub>3</sub>OH by CD (Figure 2). The per-residue CD signal increases up to the pentamer but changes little between pentamer and hexamer. Taken together with the <sup>13</sup>C NMR data indicating that a single amide rotamer is

\* For correspondence concerning QM calculations, contact H.A.C. (carlsonh@umich.edu); for all other concerns, contact S.H.G. (gellman@chem.wisc.edu).

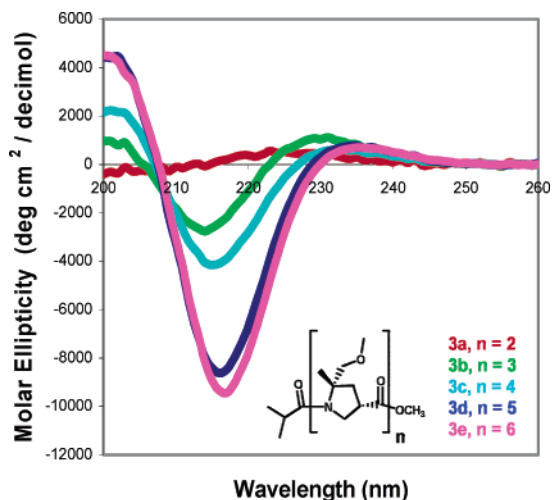
<sup>†</sup> Department of Chemistry, University of Wisconsin.

<sup>‡</sup> Graduate Program in Biophysics, University of Wisconsin.

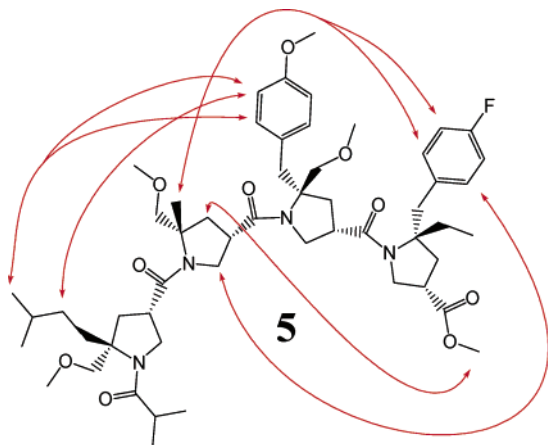
<sup>§</sup> University of Michigan.



**Figure 1.** Conformation of dimer **4** in the solid state. For clarity, hydrogen atoms are omitted. Non-carbon backbone atoms have been labeled.



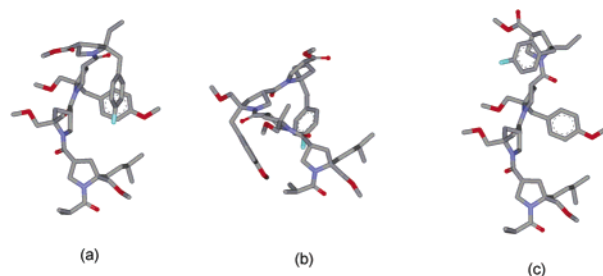
**Figure 2.** Normalized per residue CD spectra of 2,2-disubstituted PCA oligomers **3a–e** in methanol.



**Figure 3.** Selected NOEs between sequentially nonadjacent residues observed for tetramer **5** in  $\text{CD}_2\text{Cl}_2$ .

populated for each member of the series (data not shown), the CD data imply the formation of a regular structure in the longer oligomers.

Several 2,2-DPCA heterotetramers and heteropentamers were prepared as a prelude to conformational analysis via two-dimensional NMR; **5** in  $\text{CD}_2\text{Cl}_2$  (but not in  $\text{CD}_3\text{OH}$ ) displayed the best proton resonance dispersion among the heterooligomers.  $\text{C}_4\text{H}(i) \rightarrow \text{C}_5\text{H}(i+1)$  NOEs were observed between each pair of adjacent residues, which is consistent with a *Z* conformation for each of the three interresidue amide linkages ( $\text{C}_4$  is the ring carbon bearing the carbonyl substituent;  $\text{C}_5$  is the methylene carbon adjacent to the nitrogen). Figure 3 shows some of the NOEs that could be assigned from NOESY data, including all but two of the NOEs between residues that are not adjacent in sequence (the two omitted are discussed below). There were several  $i \rightarrow i+2$  NOEs involving



**Figure 4.** Computationally derived structures of **5** (non-hydrogen atoms only): (a) representative member of a family of structures derived from NOE-restrained dynamics with DYANA followed by restrained simulated annealing with SYBYL; (b) conformation with all three  $\psi$  torsion angles =  $-80^\circ$ ; (c) conformation with all three  $\psi$  torsion angles =  $-160^\circ$ . The image was generated with the program DS viewer pro.

the aryl groups in the side chains of residues 3 and 4. These NOEs suggest at least partial population of folded conformations.

Calculations were used to determine the most likely conformers of 2,2-DPCA oligomers. Gaussian 98<sup>15</sup> was used to calculate minima of a 2,2-dimethyl derivative of **2** at the RHF/6-31G\* level. Systematic searching of all conformational space yielded 22 minima (energy range 16.4 kcal/mol, half the structures within 4 kcal/mol of lowest-energy minimum). The conformers differed by combinations of *E/Z* amide bonds, equatorial/axial placement of the  $\text{C}_4$  amide (ring pucker), orientation of the *N*-acyl isobutyryl group, and the  $\psi$  torsion angle [ $\text{C}_5(i)\text{C}_4(i)-\text{C}(=\text{O})(i)\text{N}(i+1)$ ]. (The  $\psi$  torsion angle in  $\beta$ -amino acid residues is defined analogously to the  $\psi$  torsion angle in conventional  $\alpha$ -amino acid residues.) Two  $\psi$  positions were greatly preferred.  $\psi$  values of  $-76^\circ (\pm 10^\circ)$  were stabilized by favorable Coulombic interactions between the oxygen of the  $\text{C}_4$  amide and a neighboring  $\text{C}_3$  hydrogen, and  $\psi$  values of  $-165^\circ (\pm 8^\circ)$  were favored by a similar interaction between the oxygen and a  $\text{C}_5$  hydrogen. Almost no difference in energy was found between equatorial/axial conformers of the  $\text{C}_4$  amide (ring pucker conformers). The second lowest-energy minimum (0.5 kcal/mol) for the 2,2-dimethyl analogue of **2** was in excellent agreement with the crystal structure of **4**. Both exhibit similar  $\psi$  angles, a *Z* amide rotamer, and an equatorial position for the linking amide. The calculated global minimum was consistent with the NMR results discussed below.

Molecular mechanics are more appropriate than quantum mechanics to model efficiently the conformations of tetramer **5**. The NOEs summarized in Figure 3 were used as restraints for simulated annealing. Initial structures were generated using torsion angle dynamics with the program DYANA.<sup>16</sup> These structures were then used as starting points for restrained simulated annealing with the program SYBYL.<sup>17,18</sup> The simulations yielded a family of closely related conformations (Figure 4a;  $0.61 \pm 0.23 \text{ \AA}$  rmsd among the best eight structures, backbone heavy atoms only). We were surprised to observe that these conformations were not regular, that is, that the pattern of torsion angles was not consistent along the backbone. Closer inspection revealed that the  $\psi$  torsion angle was the source of the variation among the residues.  $\psi$  is approximately  $-165^\circ$  for residues 1 and 2 of tetramer **5** in the NOE-derived structures, while  $\psi$  is approximately  $-80^\circ$  for residue 3 of **5**. The  $\psi$  torsion observed for the first two residues approximates the values seen in the lowest-energy structure from the quantum mechanical calculations. The value of  $\psi$  for the third residue is in agreement with the second lowest energy quantum mechanically calculated structure and with the  $\psi$  angle observed in the solid state conformation of dimer **4** (Figure 1). The backbone  $\phi$  and  $\theta$  torsion angles, that is, the five-membered ring conformations, were reasonably consistent throughout the simulations of tetramer **5** and

matched those observed in the crystal structure of dimer **4**. Figure 4b and c shows conformations of tetramer **5** in which all three of the internal  $\psi$  torsion angles are identical. Torsion angles near  $-160^\circ$  (Figure 4c) yield a more extended conformation, and torsion angles near  $-80^\circ$  (Figure 4b) yield a more compact conformation. We suspect that the set of structures in Figure 4 represents the range of conformational options that would be sampled by 2,2-DPCA tetramers in solution. Consistent with this hypothesis is the observation of two very weak NOEs involving sequentially nonadjacent residues that are not shown in Figure 3. These weak NOEs for **5**, between the methyl protons of the N-terminal isobutyryl group and the aromatic protons of the methoxyphenyl group of residue 3, are not consistent with the conformation shown for **5** in Figure 4a and suggest instead partial population of alternative conformations with  $-80^\circ$  for the first and/or second  $\psi$  torsion.

Any short oligomer that contains at least one flexible torsion angle per residue (like the  $\psi$  torsion in 2,2-DPCA oligomers) can populate multiple conformations in solution. Interchange among these conformations is likely to be rapid on the NMR time scale, and observed NMR parameters will therefore reflect population-weighted averaging. Thus, for example, even when a set of NOEs between sequentially nonadjacent residues is entirely consistent with a particular helical conformation for a conventional peptide or a  $\beta$ -peptide, it is very unlikely that the molecule is conformationally homogeneous under the NMR conditions. Instead, the folded conformation is probably in equilibrium with an unfolded state, itself conformationally heterogeneous, that does not give rise to NOEs between sequentially nonadjacent residues. States that are partially folded and partially unfolded may also be populated. Despite such conformational heterogeneity, the observation of NOEs between protons on sequentially nonadjacent residues indicates that the oligomer under scrutiny has a distinct folding propensity (or propensities) in solution, and this tendency to adopt specific shapes can be used to create analogues with specific functions.<sup>3</sup>

The data reported here indicate that short oligomers constructed from 2,2-DPCA residues have a limited range of conformational options. 2D NMR data for **5** suggest that this tetramer has distinct conformational preferences, and the CD data in Figure 2 hint that this preference would be enhanced for longer oligomers, despite the absence of internal hydrogen bonds. The lack of secondary amides in the 2,2-DPCA backbone should render this class of  $\beta$ -peptides intrinsically less hydrophilic than other  $\beta$ -peptides that adopt specific conformations, which could endow 2,2-DPCA-based foldamers with favorable pharmacokinetic properties.<sup>19</sup> Binding of any low-energy 2,2-DPCA oligomer conformation to a complementary surface on a target molecule should involve relatively little loss of conformational entropy, because the backbone is intrinsically preorganized. This combination of physical properties suggests that 2,2-DPCA oligomers will be a fruitful source of biologically active molecules.

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**Supporting Information Available:** Crystal structure parameters for molecule **4** and NMR experimental and structure calculations for

molecule **5** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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