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Conformational Behavior of β-Proline Oligomers

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Abstract: Conformations of the monomer, dimer, and hexamer of β-proline ((*S*) pyrrolidine-3-carboxylic acid) were determined using ab initio molecular orbital calculations at the RHF/6-31G* level of theory. The calculated minima are in good agreement with experimental data for the system and imply that the conformations could be controlled through chemical modification at Cα, Cγ, or Cδ. The monomer and dimer are small and flexible with many low-energy minima. In the hexamer, two forms of regular secondary structure are preferred: left-handed helices with *cis*-peptide bonds and right-handed helices with *trans*-peptide bonds. This is similar to the behavior of α-proline helices, except that the relationship between the peptide rotamer and the handedness of the helix is reversed. Therefore, helices of the enantiomer of β-proline ((*R*)-pyrrolidine-3-carboxylic acid) should exhibit the same behavior as α-proline helices. Through understanding the conformational behavior of β-proline in various environments, it may be possible to use these protein mimics to inhibit various protein−protein recognition events. To estimate these effects, SCRF energies for the conformers were determined in dielectrics corresponding to water, methanol, and chloroform. It appears that the cis helices are more favorably solvated than the trans helices, but the cause is not clear.

Introduction

Typically, α-peptides make poor drugs due to low bioavailability as the body readily breaks down ingested proteins. Biomimetic polymers hold promise for new biomaterials and therapeutics.[1−3] We have turned our attention to β-peptides, which are oligomers similar to α-peptides.[4−6] β-peptides are composed of amino acids with the carboxylic acid functionality at Cβ rather than Cα. The difference in the chiral center allows β-peptides to resist hydrolysis by proteases even though they are amide-linked oligomers with side chains similar to those in dietary proteins.[7,8] β-peptides have the potential to be used as inhibitors with unique ADME properties.

The application of β-peptides as novel biomimetics is a blossoming field. There are numerous examples of α-peptides modified in key positions to contain a β-amino acid residue.[9] More impressive are the examples of new biomimetic compounds based solely on β-peptides. Gellman and co-workers have developed a β-peptide that mimics naturally occurring antibiotics that disrupt bacterial cell walls.[8,10] Seebach and co-workers have synthesized a small, cyclic β-peptide that mimics the hormone somatostatin.[11] Amphiphilic β-peptide helices have been used as successful inhibitors of cholesterol absorption.[12] Several inhibitors of platelet aggregation have also been developed from β-peptides.[9]

Originally, it was proposed that β-peptides would be more flexible than α-peptides because they contain an additional CH_2 between the amine and carboxylic acid groups.[4] This provides an additional "rotatable" bond in the backbone. Surprisingly, β-peptides have exhibited greater conformational stability than α-peptides. β-peptides can form stable helices with only four to six residues, whereas an α-peptide of that length would be disordered.[5,13] By understanding the conformational behavior of these interesting molecules, we may develop a means of controlling their structure. This would allow us to use β-peptides as building blocks in new therapeutics to target almost any protein recognition event (proteolysis, protein−protein association, phosphorylation in signaling pathways, ribosomal translation, etc.).

In our efforts to understand β-peptides, we have completed an extensive series of RHF/6-31G* calculations to determine the conformational behavior of β-proline. Other quantum mechanics (QM) calculations of β-peptides have only focused

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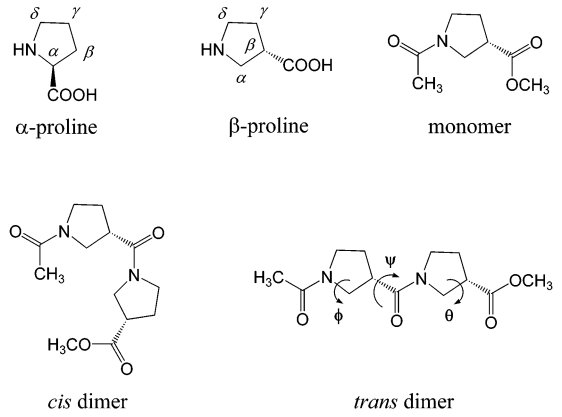

Figure 1. Comparison of L-α-proline ((*S*) pyrrolidine-2-carboxylic acid) and *β*-proline ((*S*) pyrrolidine-3-carboxylic acid) is given. The monomer, dimer, and hexamer were modeled with an N-terminal acetyl group and a C-terminal ester to be consistent with experiments and other calculations. The difference between the cis and trans peptide rotamer is highlighted using the dimer.

on systems that are capable of internal hydrogen bonding.[14−18] The conformational behavior of most *β*-peptides is, in fact, defined through the types of hydrogen-bonding patterns that they exhibit.[4−6] Some homooligomers composed of cyclic amino acids, like α-proline and *β*-proline, are incapable of internal hydrogen bonding and their flexibility is reduced by the constraint of the rings. Therefore, their conformational behavior is inherently different. Though there have been detailed molecular orbital calculations of monomers of α-proline,[19−24] there have been no similar studies of longer oligomers of α-proline or *β*-proline. Conformations of the monomer, dimer, and tetramer of *β*-proline have recently been studied by molecular mechanics (MM) calculations.[13,25] This work bridges between the QM studies of small, cyclic amino acids and the MM studies of larger oligomers.

Figure 1 compares α-proline to *β*-proline and shows the monomer and dimer of *β*-proline used in this study. We have also studied the hexamer of *β*-proline, using the same N- and C-terminal functionalities as in the dimer. The definitions we use in this study are consistent with peptide chemistry and parallel the definitions used in proteins.[6] In Figure 1, the ϕ torsion is defined as [$C_{(i-1)}-N_i-C\alpha_i-C\beta_i$], the θ torsion is defined as [$N_i-C\alpha_i-C\beta_i-C_i$], and the ψ torsion is defined as [$C\alpha_i-C\beta_i-C_i-N_{(i+1)}$]. The peptide rotamer is also defined in Figure 1. We should note that a cis peptide bond [$C\beta_i-C_i-N_{(i+1)}-C\alpha_{(i+1)}$] in protein chemistry corresponds to an *E* or trans conformation [$O_i=C_i-N_{(i+1)}-C\alpha_{(i+1)}$] in organic chemistry nomenclature. Studies in the literature use both notations, and it can be confusing to see the same conformation labeled cis or trans depending on the definitions used. In the discussions to follow, we summarize the findings of other research groups using our definitions so that it is easier for the reader to make comparisons.

On the basis of CD spectra, it was originally reported that oligomers of *β*-proline form regular secondary structure for tetramers and longer oligomers.[26] In a more recent study, Gellman and co-workers have found that NMR spectra indicate a mix of peptide rotameric states for unsubstituted *β*-proline oligomers.[13] However, they have shown that uniform peptide rotamers are possible if the oligomers are doubly substituted at Cδ. In our collaborative effort with Gellman,[13] NMR spectra, crystallography, QM calculations (RHF/6-31G*), and MM calculations (simulated annealing with the Merck molecular mechanics force field) were used to show that the doubly substituted *β*-proline oligomers only adopt conformations with *cis*-peptide bonds.

There are no NMR or crystallographic data to provide the conformations of unsubstituted *β*-proline. To provide insight into this system, we present RHF/6-31G* calculations of the monomer, dimer, and hexamer of unsubstituted *β*-proline. We have determined that two major forms of secondary structure are possible, and both would most likely be populated at room temperature. We have found that the conformational behavior about the ψ torsion is very similar to our earlier collaborative calculations. Furthermore, our findings suggest that the inherent conformation can be manipulated through chemical modification at Cα, Cγ, or Cδ.

Methods

Gaussian 98[27] was used to scan the potential surface and optimize various conformers of the monomer, dimer, and hexamer at the RHF/6-31G* level of theory. In all cases, the default convergence criteria were used. All reported minima were obtained through full optimizations, and the minima were confirmed through frequency calculations. The normal modes were visualized using XChemEdit,[28] and particular attention was given to evaluating the softest modes.

Complete, systematic conformational searching was used to identify the minima of the monomer. Those calculations revealed four general ring conformations based on ring pucker and peptide rotamer. The conformations were used to initiate the calculations of the dimer. Our interest is in regular secondary structure, so we completed full conformational sampling for "symmetric dimers," meaning that both rings have the same ring pucker and peptide rotamer. The potential energy surface of the symmetric dimers was scanned with respect to the ψ torsion at 10° increments for a full 360°.

The ϕ, θ, and ψ torsions of the dimer minima were used to create initial conformations of hexamers with regular secondary structure.

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Table 1. Energies and Characteristics of the Minima for the Monomer of β-proline

conformer	characteristics[a]	energy (H)	ΔE (kcal/mol)
1	*cis*-Eq(−176)	−589.586321	**0.00**
2	*trans*-Ax(−72)	−589.586216	**0.07**
3	*trans*-Eq(−62)	−589.585881	**0.28**
4	*cis*-Eq(−57)	−589.585436	**0.56**
5	*trans*-Eq(−171)	−589.585231	**0.68**
6	*cis*-Ax(−179)	−589.585017	**0.82**
7	*cis*-Ax(−63)	−589.584687	**1.03**
8	*trans*-Ax(−161)	−589.584534	**1.12**
9	*trans*-Eq(58)	−589.584396	**1.21**
10	*cis*-Eq(55)	−589.584322	**1.25**
11	*trans*-Ax(56)	−589.583482	**1.78**
12	*cis*-Ax(45)	−589.582773	**2.23**

[a] The characteristics note the peptide rotamer, ring pucker, and ester torsion [Cα−Cβ−C−O(CH_3)].

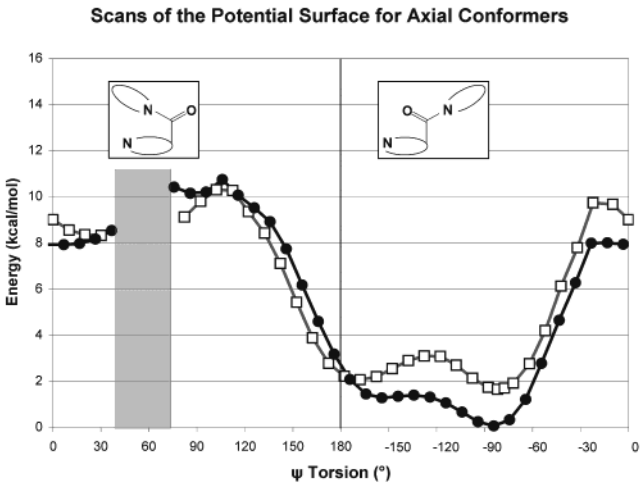

Figure 2. Potential surface was scanned with respect to the ψ torsion for the *cis*-Ax dimer (□) and the *trans*-Ax dimer (●). Energies are relative to the global minimum, *cis*-Eq(−150). Three minima for *cis*-Ax are located at ψ values of 27°, −168°, and −82°. Four minima for *trans*-Ax are located at ψ values of 7°, 89°, −154°, and −85°. The schemes show the large degree of steric clash for the Ax conformers as ψ varies. The shaded bar above ψ = 60° marks the region where the scans could not locate stable minima with the same characteristics for both rings.

These hexamers were then fully optimized with no additional constraints. To evaluate the preference for the conformers in different condensed-phase environments, single-point energy calculations were performed for each hexamer using self-consistent reaction field (SCRF) theory with the isodensity surface polarized continuum model (IPCM),[29] also at the RHF/6-31G* level. The solvent dielectric values used were 78.39 for water, 32.63 for methanol, and 4.90 for chloroform. The SCRF calculations would not converge for some of the hexamers using the default of 10 phi and 5 theta points (parameters for the radial grid employed in IPCM).[29] However, the combination of 44 phi points and 22 theta points was found to be appropriate.

Results

Monomers. Systematic searching of all conformational space for the β-proline monomer yielded 12 conformers. The energies and conformational characteristics are given in Table 1. The conformers can be described by the rotamer of the peptide bond (cis or trans), the ester torsion (ca. ±60°, 180°), and the ring pucker which places the functional group at Cβ in a pseudoaxial (Ax) or pseudoequatorial (Eq) position. Technically, a more complicated analysis could be used for the pucker of five-membered rings,[20] but it is common to simplify the description of the cyclic amino acids to endo (our Ax) or exo (Eq).[19]

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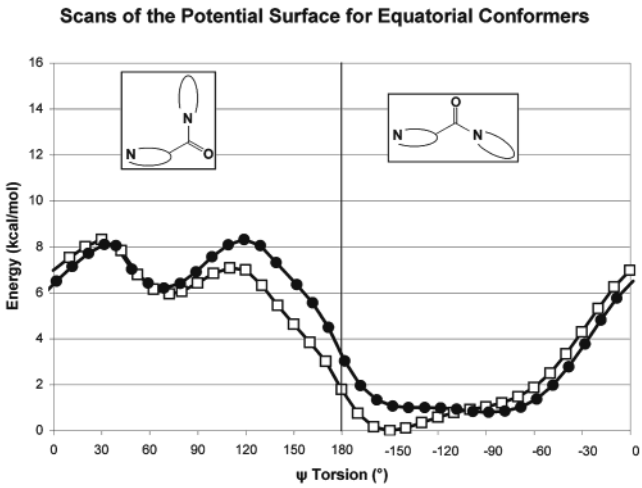

Figure 3. Potential surface was scanned with respect to the ψ torsion for the *cis*-Eq dimer (□) and the *trans*-Eq dimer (●). Energies are relative to the global minimum, *cis*-Eq(−150). Minima for *cis*-Eq are located at ψ values of 72° and −150°. Minima for *trans*-Eq are located at ψ values of 69° and −88°. Schemes are included to show the lesser degree of steric clash for the Eq conformers as ψ varies.

Table 2. Energies and Characteristics of the Minima for the Dimer of β-Proline

conformer	characteristics[a]	energy (H)	ΔE (kcal/mol)
1	*cis*-Eq(−150)	−912.335283	**0.00**
2	*trans*-Ax(−85)	−912.335182	**0.06**
3	*trans*-Eq(−88)	−912.334012	**0.80**
4	*trans*-Ax(−154)	−912.333253	**1.27**
5	*cis*-Ax(−82)	−912.332646	**1.65**
6	*cis*-Ax(−168)	−912.332005	**2.06**
7	*cis*-Eq(72)	−912.325772	**5.97**
8	*trans*-Eq(69)	−912.325380	**6.21**
9	*trans*-Ax(7)	−912.322661	**7.92**
10	*cis*-Ax(27)	−912.322022	**8.32**
11	*trans*-Ax(89)	−912.319115	**10.15**

[a] The characteristics note the peptide rotamer and ring pucker of both rings and the ψ torsion.

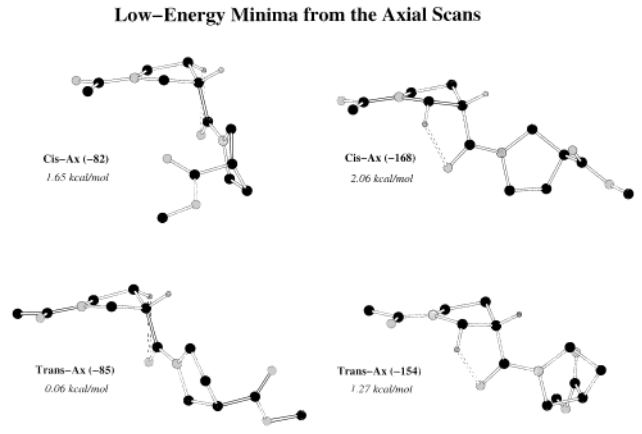

Figure 4. Conformations are provided for the four low-energy minima from the Ax dimers. The majority of hydrogen atoms are not shown for clarity. The hydrogen at Cβ is shown to better display the pseudoaxial placement of the linking peptide bond. The stabilizing Coulombic interaction between Hγ and the peptide oxygen is shown for *cis*-Ax(−82) and *trans*-Ax(−85). The interaction of the oxygen to Hα is also shown for *cis*-Ax(−168) and *trans*-Ax(−154). The dashed bond is not a hydrogen bond.

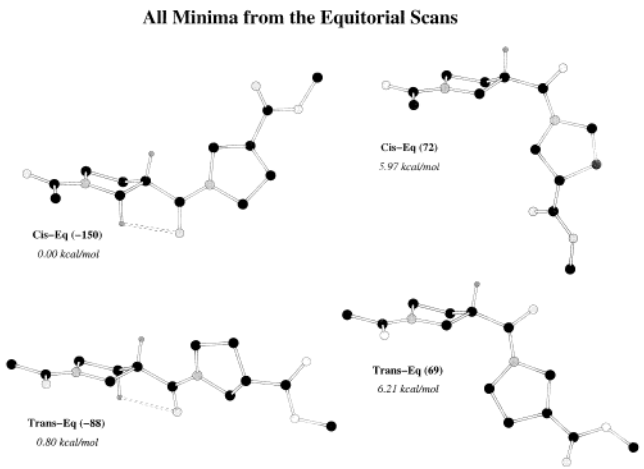

Figure 5. Conformations are provided for the four minima from the Eq dimers. Most hydrogens are not shown for clarity. The hydrogen at Cβ is shown to better display the pseudoequatorial placement of the linking peptide bond. The stabilizing Coulombic interactions to Hα and Hγ are shown for *cis*-Eq(−150) and *trans*-Eq(−88), respectively. The dashed bond is not a hydrogen bond.

Dimers. Our interest is in regular secondary structure, so our dimer conformations have the same ring pucker and peptide rotamer for both rings. Full scans of the potential surface with respect to the ψ torsion were completed for *cis*-Ax, *cis*-Eq, *trans*-Ax, and *trans*-Eq dimers (Figures 2 and 3). Though the ψ angle was obviously constrained during the scans, there were no other constraints imposed; most notably, the two ring puckers were not forced to be the same. On the basis of the energies of the monomers, the ester torsion was initially placed at the lowest energy conformation, namely 180° for the cis dimers and −60° for the trans dimers. The ester torsion was not constrained, but did remain close to the initial position during the entire scan of the potential surface. Conformations from each well in Figures 2 and 3 were subject to unrestricted, full optimization to provide the minima in Table 2 and Figures 4 and 5. Additional minima were calculated by altering the orientation of the ester. These calculations confirmed that our scans provided the lowest-energy conformers (see Table S3 in the Supporting Information).

Table 3. Energies and Characteristics of the Minima for the Hexamer of β-Proline

conformer	characteristics[a]	energy (H)	ΔE (kcal/mol)
1	*cis*-Eq(−150)	−2203.332618	**0.00**
2	*trans*-Ax(−85)	−2203.331765	**0.54**
3	*trans*-Ax(−155)	−2203.327571	**3.17**
4	*cis*-Ax(−85)	−2203.326653	**3.74**
5	*trans*-Eq(−94)	−2203.325840	**4.25**
6	*cis*-Ax(−173)	−2203.322869	**6.12**
7	*trans*-Eq(60)[b]	−2203.286521	**28.93**
8	*cis*-Eq(76)[b]	−2203.282588	**31.39**

[a] The characteristics note the peptide rotamer and ring pucker common to all six rings and the average ψ torsion. [b] The last two conformers are based on the high-energy region from the dimer scans.

Hexamers. Eight conformers of the hexamer were pursued in this study. Six were based on the low-energy minima of the dimer: *cis*-Eq(−150), *trans*-Ax(−85), *trans*-Eq(−88), *trans*-Ax(−154), *cis*-Ax(−82), and *cis*-Ax(−168). Two conformers of the hexamer were based on higher-energy dimers, *cis*-Eq(72) and *trans*-Eq(69). Initial structures for the hexamers were created using the ψ values, ring puckers, and peptide rotamers from the dimer minima. Full minimizations of the eight conformers yielded the minima in Figure 6 and Tables 3 and 4.

Each of the eight conformers was subjected to SCRF calculations to estimate environmental effects in the condensed phase (using solvent dielectrics of water, methanol, and chloroform). Those energies are given in Table 5.

Discussion

Monomers. The difference in energies for the twelve minima is only 2.2 kcal/mol, which indicates that all conformers would

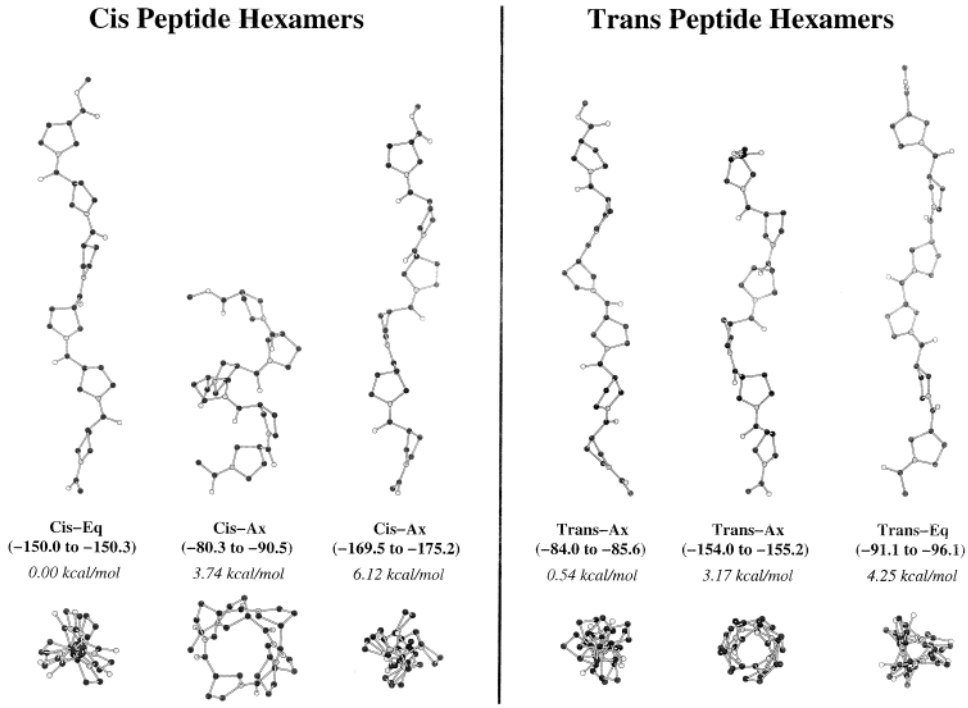

Figure 6. Side views and axial views are shown for the six low-energy minima of the hexamer. The range of ψ values in each helix is given to show how regular each structure is.

Table 4. Torsional Characteristics of the Hexamer Helices (in degrees)

		[$C_{(i-1)}$–N_i–$C\alpha_i$–$C\beta_i$]			[N_i–$C\alpha_i$–$C\beta_i$–C_i]			[$C\alpha_i$–$C\beta_i$–C_i–$N_{(i+1)}$]		
conformer	rings	min ϕ	max ϕ	ave ϕ	min θ	max θ	ave θ	min ψ	max ψ	ave ψ
1	*cis*-Eq	−166.7	−167.6	**−167.2**	−153.4	−155.1	**−153.9**	−150.0	−150.3	**−150.2**
2	*trans*-Ax	165.1	166.1	**165.5**	−89.8	−91.2	**−90.8**	−84.0	−85.6	**−84.9**
3	*trans*-Ax	156.4	169.8	**159.1**	−86.0	−95.0	**−88.9**	−154.0	−155.2	**−154.8**
4	*cis*-Ax	153.3	167.9	**158.2**	−85.4	−92.3	**−87.1**	−80.3	−90.5	**−84.9**
5	*trans*-Eq	−161.2	−166.4	**−162.7**	−153.6	−156.9	**−154.9**	−91.1	−96.1	**−94.0**
6	*cis*-Ax	145.7	160.9	**152.5**	−86.2	−91.8	**−88.4**	−169.5	−175.2	**−172.8**
7	*trans*-Eq	−175.9	176.5	**178.4**	−138.7	−150.9	**−142.6**	57.8	65.4	**59.7**
8	*cis*-Eq	−158.3	−167.6	**−160.6**	−158.7	−168.6	**−165.6**	74.0	76.7	**75.8**

Table 5. SCRF Energies of the Hexamer Minima (in kcal/mol)

conformer	characteristics	dipole (D)	gas-phase	$CHCl_3$	CH_3OH	H_2O
1	*cis-Eq*(−150)	18.3	0.00	0.00	0.00	0.00
2	*trans*-Ax(−85)	9.9	0.54	2.81	3.53	3.60
3	*trans*-Ax(−155)	22.7	3.17	5.66	6.22	6.24
4	*cis-Ax*(−85)	24.8	3.74	3.75	3.51	3.43
5	*trans*-Eq(−94)	4.0	4.25	5.39	5.76	5.80
6	*cis*-Ax(−173)	22.8	6.12	5.09	4.80	4.61
7	*trans*-Eq(60)	10.2	28.93	28.95	28.73	28.73
8	*cis*-Eq(76)	11.7	31.39	31.68	31.44	31.66

be well populated at room temperature. Boltzmann-weighted averages reveal no significant preference between cis and trans peptide or ring pucker (the dimer or hexamer also show no preference for rotamer or pucker in the gas phase). There does appear to be a small correlation between the ester torsion and the peptide rotamer. The cis conformers with ester torsions of approximately 180° are lowest in energy, whereas the trans conformers are lowest in energy with ester torsions near −60°. However, the frequency calculations reveal that the ester torsion is the softest normal mode.

These results are in excellent agreement with Chandrasekhar, Saunders, and Jorgensen's previous MM studies using BOSS and the OPLS force field.[25] They also found 12 minima with the same variation in ring pucker, peptide rotamer, and ester torsion for the (*S*) enantiomer of β-proline. Their conformers are all within 1 kcal/mol in energy. This small difference in energies between our studies is most likely due to the different computational methods employed. Both results imply that all conformers will be well populated at room temperature.

Dimers. In the MM calculations of β-proline dimers and tetramers by Chandrasekhar et al., exact values for the ψ torsion are not given, but their figures show that the most stable conformers place the oxygen of the peptide bond over the N-terminal ring.[25] This same behavior is seen in our minima from the RHF/6-31G* calculations (Figures 4 and 5). MM calculations using the Merck MMFF force field also report similar conformers.[13] Their general minima for ψ are approximately −80° and −160°, in good agreement with our low-energy conformers (Tables 2 and 3).

Günther and Hoffmann have performed torsional scans at the same level of theory for fragments of (*S*)-substituted, linear β-amino acids incapable of forming internal hydrogen bonds.[18] The ψ torsional scan was based on (*S*) $CH_3NHCO-CH(CH_3)CH_2CH_3$. They calculated a wide low-energy region between −60° and −180° and a wide high-energy region between 0° and 120°, but the barrier between the two regions was only 2 kcal/mol. Surprisingly, their evaluation of the φ torsion was an exact match to the pattern for ψ in Figures 2 and 3 (one high energy minimum at 60°, two low-energy minima at −90° and −150°, and a 6-kcal/mol barrier between the high- and low-energy regions). It could be coincidental because the φ torsion of (*S*) $CH_3CONH-CH(CH_3)CH_2CH_3$ is not necessarily related to the ψ torsion of β-proline, but the striking similarity makes us wonder if the labels for the φ and ψ torsions are reversed in Figure 1 of ref 18.

The ester torsion in the monomer was not influenced by the ring pucker, but the ψ torsion between the two rings in the dimer is very strongly coupled to the pucker. The pseudoaxial versus pseudoequatorial placement of the linking peptide bond alters the degree of steric clash between the two rings as can be seen in the schematics included in Figures 2 and 3. In the shaded region in Figure 2, it is not possible to complete the scans for the pseudoaxial systems. The conformations are high in energy for ψ near 60° due to steric clash between the rings. The short contacts force the N-terminal ring to flip to an equatorial conformation to relieve the strain. We located one shallow, high-energy minimum for *cis*-Ax at ψ of 27° and two shallow, high-energy minima for *trans*-Ax at ψ values of 7° and 89°. In the region between 180° and −60°, both Ax conformations have two minima near −80° and −160°. Though the high-energy regions are unstable, the low-energy regions reveal similar conformational behavior for both *cis*-Ax and *trans*-Ax dimers. Both Ax dimers should sample the same ψ orientations at room temperature.

The scans of the two Eq conformers are well behaved over the full range of ψ (Figure 3). In the high-energy region of ψ between 0° and 150°, the energetic profile is smooth and the conformers are stable (no inversion of the ring puckers is seen). Both *cis*-Eq and *trans*-Eq have stable minima for ψ near 70°. Though both Ax dimers have minima at −80° and −160°, the Eq dimers have only one minimum in the low-energy region between 180° and −60°. For *cis*-Eq, the minimum has a ψ value of −150° (with a shoulder in the scan around −80°). For *trans*-Eq, the minimum has a ψ torsion of −88° (shoulder near −150° in the scan). Several tighter scans and full minimizations confirmed that these shoulder regions do not contain shallow minima (data not shown).

We find the scans of the Eq conformers very interesting in light of a recent crystal structure of a dimer of β-proline.[13] The dimer in the crystal structure is doubly substituted at Cδ (structures of unsubstituted oligomers of β-proline are not available). In the crystal structure, the rings are both *cis*-Eq in agreement with our global minimum for the dimers, but the ψ torsion between the rings is −78° which corresponds to the shoulder region in the ψ scan of *cis*-Eq. Our previous RHF/6-31G* calculations for a similar doubly substituted β-proline identified several minima with ψ at −76° (±10°).[13] It is likely that our current inability to find a minimum for *cis*-Eq with ψ

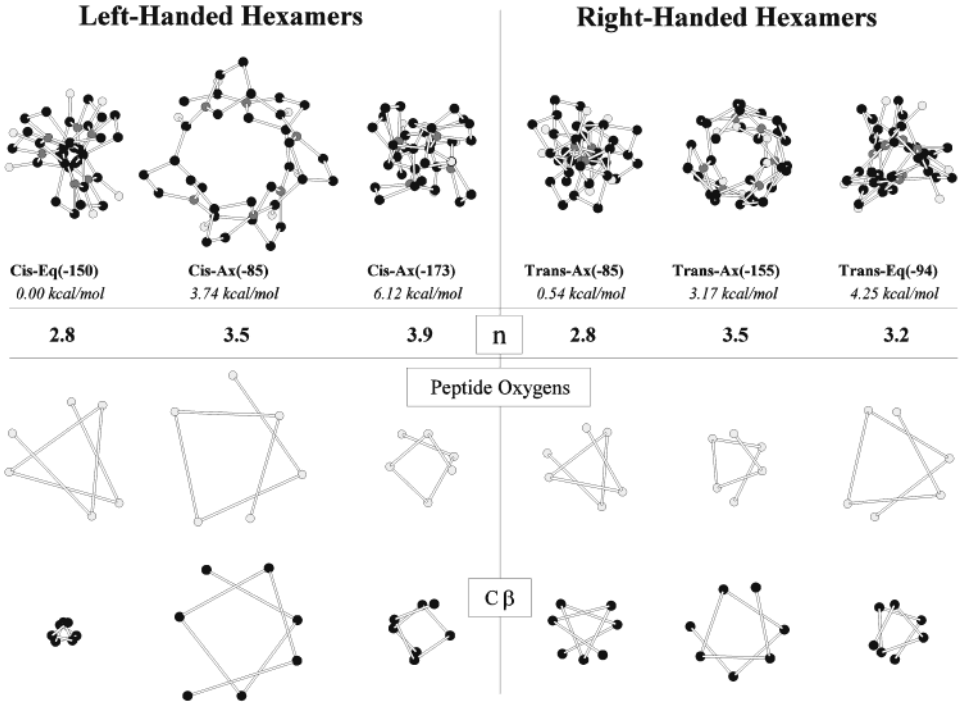

Figure 7. Enlarged axial views of the six low-energy hexamers are provided. The C-terminus of the helix is oriented toward the reader. The number of residues per turn of the helix (*n*) is noted. The patterns for the peptide oxygens and Cβ atoms are given to better show the handedness, number of residues per turn, and variation in widths of the helices. All views are scaled to the same size for accurate comparisons.

near −80° is not due to the level of theory used in the calculations. Instead, the different behavior for ψ is quite possibly due to conformational strain introduced to the system by the two bulky side chains on Cδ. *This result implies that different substitutions around the ring may create completely new conformations for β-proline* (see section on conformational control below). The frequency calculations revealed that the softest normal modes involved torsional and angular bends between the two rings. The low barriers and wide wells for the ψ torsion between 180° and −60° in Figures 2 and 3 are another indication that the orientation between the rings is easily deformed.

The eight lowest-energy conformers of the dimer are shown in Figures 4 and 5. The most important factor stabilizing the minima is the avoidance of steric overlap between the rings. However, Figures 4 and 5 show an additional stabilizing factor for some of the dimers. The oxygen of the peptide is often in close proximity to neighboring hydrogens in the N-terminal ring. Although the geometries are too strained to call these interactions C−H···O hydrogen bonds,[30−32] it does appear that there is a favorable Coulombic interaction possible. For example in *cis*-Ax(−168), the Cα_i−Cβ_i−C$_i$═O$_i$ torsional angle is nearly eclipsed (11°). In order for this conformation to be favorable, there must be some interaction stabilizing the orientation of the peptide oxygen. It appears that stabilization is provided in part by the short separation between the peptide oxygen and Hα (2.44 Å). The interactions appear most favorable for the hydrogens on Cα or Cγ.

Hexamers. Figures 6 and 7 show that both right-handed and left-handed helices were found to be stable minima for β-proline oligomers. Three of the helices have nearly 3 residues per turn (*n* values). Conformations of poly-β-alanine have helices that range from 2.6 to 3.1 residues per turn.[14] α-proline helices (polyproline I and II) have 3.3 and 3.0 residues per turn, respectively.[33−35] Typical α-helices in proteins have 3.6 residues per turn, and the other three hexamers have larger *n* values near 3.6. It appears that internal hydrogen bonding is necessary to achieve tightly wound helices with β-peptides. The different conformations of the hexamer give wide variety to the size and shape of the helices. In particular, the different orientations of the rings with respect to the helical axis are striking.

The handedness of the β-proline helices is dependent on the peptide rotamer as is the case for PPI and PPII.[33−35] However, the trend is reversed: trans-peptide bonds yield right-handed β-proline helices but left-handed α-proline helices (PPII). Of course, the minima for the enantiomer of β-proline, (*R*) pyrrolidine-3-carboxylic acid, would be the mirror images of the minima in Figures 6 and 7. Therefore, the (*R*) enantiomer should have left-handed, trans helices like naturally occurring PPII and right-handed, cis helices such as PPI.[36] In Figure 1, the (*S*) enantiomers of α-proline and β-proline place the carboxylic acid on opposite faces of the pyrrolidine ring. The (*R*) β-proline has a more similar topology to α-proline because the acid is on the same face, so the ability of the (*R*) enantiomer to exhibit more similar conformational behavior to α-proline is logical.

Experimental studies indicate that β-proline oligomers become more ordered as the chain is lengthened.[13] Chandrasekhar et al. found that lengthening β-proline induced more order in the system by creating fewer and fewer low-energy minima.[25] We

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too find that the energies separating conformers becomes greater as the oligomer is lengthened. The energies of the eight conformations of the hexamer are given in Table 3. We were surprised to find that two of the minima, *cis*-Eq(150) and *trans*-Ax(−85), are nearly isoenergetic despite having completely different peptide rotamers, ring puckers, and ψ torsions. The mix of rotameric states observed by NMR is fitting with the small energy differences between *cis*-Eq(−150) and *trans*-Ax(−85).

As one might expect, the characteristics of the six lowest-energy dimers provide the most favorable hexamer conformations. It is interesting that only the *trans*-Eq(−94) conformer changes rank in the hexamer; perhaps it is because this conformer has the greatest deviation in the ψ torsion when compared to the dimer. It appears that the inductive effects in the longer hexamer could not greatly stabilize the *trans*-Eq(60) and *cis*-Eq(76) conformations; they are so high in energy that ψ values near 70° will not be sampled at room temperature. Many attempts were made to locate *cis*-Eq(−80) and *trans*-Eq(−150) conformers of the hexamer. It was possible that the inductive effects of a longer oligomer could stabilize these "shoulder regions" from the Eq scans. However, the conformations were not stable in the hexamer. The *cis*-Eq(−80) initial structure minimized to the *cis*-Eq(−150) conformer, and the *trans*-Eq(−150) structure minimized to *trans*-Eq(−94).

Table 4 shows the variation in key internal degrees of freedom for each conformer. The greatest variation (comparing min versus max values) is seen in the ϕ torsion. In general, Ax conformers have ϕ values near 160° and θ close to −90°. The Eq conformations have ϕ torsions near −165° and θ torsions near −155°. There is little similarity between our calculated pattern for ϕ and θ torsions and the ϕ and θ torsions of related, linear β-peptide oligomers. However, there is a strong correlation in the ψ torsions. Using the same level of theory, Möhle et al. calculated conformations of monomers of β-Aib ((*S*) 3-amino-2-methylpropanoic acid).[17] In this (*S*)-substituted, linear β-peptide, conformations are controlled by internal hydrogen bonding. Half of the minima of the β-Aib monomer had ψ torsions between −75° and −156° in agreement with our low-energy region. Wu and Wang also used RHF/6-31G* calculations to examine three helical conformations of hexamers of β-Aib and β-alanine.[15] Like our calculations for β-proline, Wu and Wang found that both right-handed and left-handed helices were favorable in these β-peptide oligomers, and many of their ψ values were between −107° and −137°. Alemán and Léon surveyed the conformations of β-alanine in small molecule databases.[37] Their most relevant compounds to our discussion were cyclo(α-pro-α-pro-β-ala-β-ala) and cyclo(α-pro-β-ala-α-pro-β-ala). Three of four measured ψ values were within 8° of −150°. A ψ torsion near −150° places the peptide oxygen in close proximity of Hα for β-alanine, (*S*) β-Aib, and (*S*) β-proline.

As can be seen in Table 5, the trans helices are more poorly solvated than the cis helices. This result is surprising because trans rotamers are overwhelmingly preferred in water for α-proline helices (PPII).[35] In general, the trans helices of β-proline have smaller dipoles than the cis helices, but the trend for poor solvation does not appear to be caused by differences in the dipoles. Both the *trans*-Ax(−85) and *trans*-Ax(−155) helices are both poorly solvated despite the large difference in their dipoles (9.9 and 22.7 D, respectively). Also, *trans*-Ax(−155) is not solvated as well as *cis*-Ax(−173) even though they have nearly equal dipoles. If the effect were due to exposed surface area for interaction with the environment, then the Eq conformers would most likely be better solvated since they are slightly longer than the Ax conformers, but that is not seen. More rigorous treatment of solvation will be needed to address the cause of any differences between the conformers.

Overall, these results imply a bias toward left-handed, cis helices in solution. However, the energy differences are small enough that trans conformers will still be populated at room temperature. In fact, one could imagine that an occasional trans ring in a cis helix would cost little energy. A simple bias toward cis could explain why ^{13}C NMR would show both peptide rotamers, but the CD spectra imply stable secondary structure. The CD spectra for the most recent, doubly substituted β-proline oligomers are nearly identical to the CD spectra for unsubstituted β-proline.[13,26] Our *cis*-Eq conformer is similar to that determined for the doubly substituted β-proline. If the *cis*-Eq conformer is heavily populated in solution, that may explain why the two CD spectra are so similar. The CD spectrum for the decamer of (*R*) β-proline is completely different.[36] We predict that the (*R*) enantiomer will have a bias toward right-handed, cis helices. One would expect the opposite twist to the helix to dramatically change the CD spectra.

Conformational Control of *β*-Proline. From the frequency calculations of the hexamers, the softest normal modes were found to be broad bending motions, similar to an elastic rod. This suggests that oligomers of β-proline could easily adapt to the curvature of a binding cleft if used as a scaffold for inhibitor design.

Bulky alkyl substitutions at Cδ have been shown to strongly alter the cis/trans preference for both α-proline[35] and β-proline.[13] Obviously, oligomers of these cyclic amino acids can be manipulated into desirable conformations. Given the structures in Figures 4 and 5, we propose that the following chemical modifications to impose conformational control in β-proline oligomers. The anticipated effects of both hydrogen-bonding and nonbonding functional groups are presented.

It is straightforward to suggest that double substitutions at Cα are likely to impose a single trans rotamer for the peptide bond, much like the substitutions at Cδ restrict the peptide bond to the cis conformation.[13,35,38] Therefore, modifications at Cα and Cδ could be used to lock β-proline into right- and left-handed helices, respectively. (In the (*R*) enantiomer, the peptide rotamers would be the same, but the opposite trend would be seen in the helices. Cα substitutions would promote left-handed, trans helices, and Cδ substitutions would give right-handed, cis conformers.) Unfortunately, efforts to create derivatives of β-proline with substitutions at Cα have proven to be very challenging.[38]

Our collaborative work with Gellman and co-workers found that alkyl substitutions at Cδ do not restrict the ψ torsion to one value.[13] It might be possible for electron-withdrawing groups on Cδ to polarize Hγ. The increased Coulombic interaction between Hγ and the peptide oxygen may promote ψ values near −85°.

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We propose that substitutions at Cα and Cγ would have a stronger influence on ψ. Double alkyl substitutions at Cα would lock both the peptide rotamer and the ψ torsion, creating a very ordered oligomer. The bulk of an alkyl group in a pseudoaxial position at Cα could impose steric limitations on the peptide oxygen, forcing ψ to only take values near −85°. Halides at Cα could result in the same behavior through electrostatic repulsion, but the stability of such compounds is questionable. It is unclear whether a hydrogen-bond donor at Cα would be involved in a hydrogen bond to the N-terminal peptide oxygen, promoting the trans rotamer, or whether it would preferentially hydrogen bond with the oxygen of the C-terminal peptide and promote ψ values near −160°.

We expect that substitutions at Cγ would have little effect on the peptide rotamer. An alkyl group or halide in a pseudoaxial position at Cγ would lock ψ values near −160°. A hydrogen-bond donor in the same position would not be able to interact with the N-terminal peptide, so we suggest that it could donate a hydrogen bond to the oxygen of the C-terminal peptide, forcing ψ to take values near −85°.

It is not clear what effects these substitutions would have on the twist of the helix. They could easily shift ψ 20° beyond our general minima of −85° and −160°. That could make a significant difference in the number of residues per turn. It would be very interesting if some of the chemical modifications could promote 3 residues per turn while others promote 4 to create PPII mimics and square helices, respectively.

Conclusion

Our calculations have provided a basis for understanding the conformational behavior of β-proline. The findings suggest that the inherent behavior can be modified through chemical modification at Cα, Cγ, or Cδ. This is in excellent agreement with recent experimental findings.[13]

The helical structures of β-proline determined in this work may be useful for inhibitor design to block protein recognition events, particularly the possibility of (*R*) β-prolines to mimic PPII helices.[36] The most promising PPII mimics would use substitutions at Cα to promote the left-handed, trans helices in the (*R*) enantiomer. This substitution would also restrict the ψ torsion to provide approximately 3 residues per turn of the helix, like PPII. PPII helices have been shown to interact with SH3 and WW domains making them an important recognition factor governing cellular signaling.[33,39,40] Furthermore, proline-rich helices that incorporate nonnatural N-substituted residues ("peptoid" ligands) have been shown to bind to these domains with high affinity.[39,40]

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Supporting Information Available: Zero-point corrected energies for the monomer, dimer, and hexamer minima; energies for all 31 minima of the dimer arising from alternate ester torsions; PDB files of the six low-energy hexamer helices; PDB files of the reflections of the hexamer minima to provide the (*R*) β-proline helices. This material is available free of charge via the Internet at http://pubs.acs.org.

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