Solution Conformations of Helix-Forming β-Amino Acid Homooligomers

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Abstract: The conformational properties of β-peptides comprised of enantiomerically pure *trans*-2-aminocyclohexanecarboxylic acid (ACHC) or *trans*-2-aminocyclopentanecarboxylic acid (ACPC) units were studied by NMR spectroscopy in organic solvents. In pyridine-d_5 solution, ACPC hexamer **1** and ACPC octamer **2** displayed well-defined helical structures characterized by a series of 12-membered hydrogen-bonded rings ("12-helix"). The solution structures calculated from the NMR-derived constraints were very similar to the conformations found previously for **1** and **2** in the solid state. ACHC tetramer **3** displayed a different sort of helical conformation, characterized by a series of 14-membered hydrogen-bonded rings ("14-helix"), in methanol-d_3 solution. This solution conformation is similar to that previously found in the crystal structure of **3**.

Introduction

Unnatural oligomers that adopt specific, compact conformations ("foldamers") have a wide range of potential applications.[1] Foldamers with well-defined secondary structural preferences (i.e., helices, sheets, or turns) could, for example, be used to create new types of tertiary structures, which in turn might lead to the creation of macromolecules with functional properties akin to those of the compactly folded biopolymers, proteins and RNA. Foldamers that display specific conformations at short lengths (<10 residues) may have medicinal applications, e.g., for disruption of specific protein−protein interactions. Several types of unnatural oligomers have recently been shown to adopt well-defined secondary structures in solution.[2−5]

Short β-amino acid oligomers ("β-peptides") are among the most well-studied unnatural oligomers with discrete folding propensities.[2,3,6,7] Our group has provided crystallographic evidence that cycloalkane-based β-amino acid residues can induce formation of two different helical conformations, with helix shape determined by cycloalkane ring size. In the solid state, a tetramer and a hexamer constructed from enantiomerically pure *trans*-2-aminocyclohexanecarboxylic acid (ACHC)

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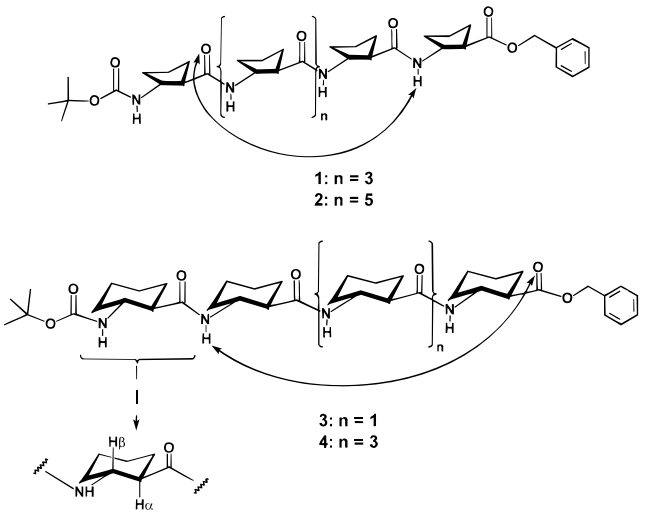

Figure 1. (A, B) Structures of compounds used in this study (**1**−**4**). Compound **4** was prepared from the (*S*,*S*)-ACHC building block and compound **3** from (*R*,*R*)-ACHC (i.e., **4** is the enantiomer of the structure shown). (C) The dashed arrow points toward an expansion of one ACHC residue and the definition of backbone protons for β-amino acids. Double sided arrows indicate the hydrogen bonding partners for a β-peptide (A) 12-helix and (B) 14-helix.

adopt the "14-helix", which is characterized by 14-membered-ring hydrogen bonds between backbone carbonyls and amide protons two residues toward the N-terminus.[3a] Seebach et al. have found 14-helical conformations for β-peptides composed of acyclic residues, via two-dimensional NMR analysis in methanol or in pyridine.[2a,b] We have shown that oligomers of *trans*-2-aminocyclopentanecarboxylic acid (ACPC) form a "12-helix", defined by 12-membered-ring hydrogen bonds between backbone carbonyls and backbone NHs three residues toward the C-terminus.[3b]

Here we present comprehensive, two-dimensional ^{1}H NMR structural data for three helix-forming β-peptides (Figure 1, compounds **1**−**3**) in organic solvents, pyridine or methanol. Two of the β-peptides, hexamer **1** and octamer **2**, are constructed from ACPC (preliminary NMR data for **2** have been previously reported[3b]); tetramer **3** is constructed from ACHC. Limited data are presented for the ACHC hexamer **4**; extensive resonance overlap precluded a complete analysis of **4** via ^{1}H NMR-based structure calculations. The availability of high-resolution solution structures for β-peptides composed of rigidified residues, in 14-helical or 12-helical forms, should facilitate the use of these foldamers for drug design and other purposes.

Experimental Section

NMR Spectroscopy. NMR spectra were collected on a Bruker AMX spectrometer operating at 500 MHz with an inverse, broadband probe. Sample concentrations varied among different experiments but were generally between 8 and 15 mM dissolved in one of the following solvents: pyridine-d_5, CD_3OD, or $CD_3OH/CDCl_3$. Spectra were collected between 4 and 25 °C under the control of a Eurotherm variable-temperature unit with an accuracy of 0.1 °C (see figures for individual conditions). Two-dimensional spectra (DQF-COSY, TOCSY, ROESY, and NOESY) were recorded employing standard pulse sequences with the number of acquisitions typically set to 64 for the NOESY,[8] ROESY,[9] and DQF-COSY[10] spectra and 32 for the TOCSY[11] spectra. Low power presaturation was used to suppress the water resonance during the relaxation delay during data collection with samples of low concentration. In general, spectra were recorded with 2K complex data points in F2 for each of 480−512 t_1 increments with a sweep width of 5050 Hz in each dimension. TOCSY spectra were recorded with an isotropic mixing time of 65 ms and trim pulses of 2.5 ms immediately before and after the spin lock period. NOESY spectra were acquired with mixing times between 100 and 500 ms. ROESY data were collected with spin lock times of 250, 300, and 350 ms. ROESY data at 298 K with a mixing time of 300 ms were used for distance restraint calculations for compounds **1** and **2** whereas a 300 ms ROESY experiment at 277 K was used for integration of peak volumes for tetramer **3**. The NOEs were classified into four groups of strong, medium, weak, and very weak with upper bounds set to 3.0, 3.5, 4.0, and 5.0 Å, respectively. The lower bound limit was set to 1.9 Å. Restraints files were also generated with "quantitative" distances, calculated by using a reference distance of an isolated geminal proton pair set to 1.8 Å with the upper and lower bounds set to 15% above or below the calculated distance (each of these sets is represented in the Supporting Information). The temperature coefficients of the amide protons were studied by collecting 1D spectra at seven different temperatures between 5 and 35 °C in 5 deg increments and are reported in −ppb/K. All spectra were processed with UXNMR on an X32 computer or XWINNMR on a Silicon Graphics O2 workstation and peak picking and volume integration were performed with NMRCOMPASS (Molecular Simulations Inc.). The data were zero-filled to 1024 points in F1 prior to Fourier transform, multiplied by a shifted sine function and baseline corrected with a polynomial of order 5.

Molecular Modeling and Dynamics. Model building and molecular dynamics simulations were performed on an SGI Indigo workstation in the context of the QUANTA molecular modeling package (v. 4.1, Molecular Simulations, Inc). The CHARMm force field[12] was used for the majority of the calculations either coupled with QUANTA or with a stand-alone version on a DEC AlphaServer 2100 (Digital Equipment Corp., Marlboro, MA). Energy minimizations were typically computed until convergence (defined as an energy gradient of 0.001 kcal·mol^{-1}), using the adopted basis Newton Raphson (ABNR) algorithm as implemented in CHARMm. For compounds **1**−**3**, molecular dynamic simulations were carried out on structures in vacuo using a distance-dependent dielectric constant to approximate solvent shielding. For ACPC hexamer **1** and octamer **2**, a shifted potential was used to a distance of 12 Å with a nonbonded cutoff of 14 Å. The nonbonded lists were updated every 25 steps of dynamics. Dynamics were carried out by first heating the system from 0 K to 1000 K over 10 ps with a time step of 0.001 ps. The system was then equilibrated for 20 ps and cooled slowly back to 300 K over 70 ps. A constant-temperature dynamic simulation was then performed for 100 ps. The simulation trajectories were recorded every 1 ps and subsequently analyzed to examine any conformational fluctuations. A SHAKE algorithm was used to constrain bonds to hydrogen to within 10^{-8} Å. Restrained MD simulations were also carried out with a protocol similar to that described as above, where the energy term for the distance restraints was added to the total potential energy of the system as an harmonic potential function. Dihedral angle restraints were added for compounds **1** and **2** based on the NH-C$_\beta$H coupling constant and were calculated from the Karplus equation given by Haasnoot et al.[13] An iterative process was applied in which additional NOEs were assigned

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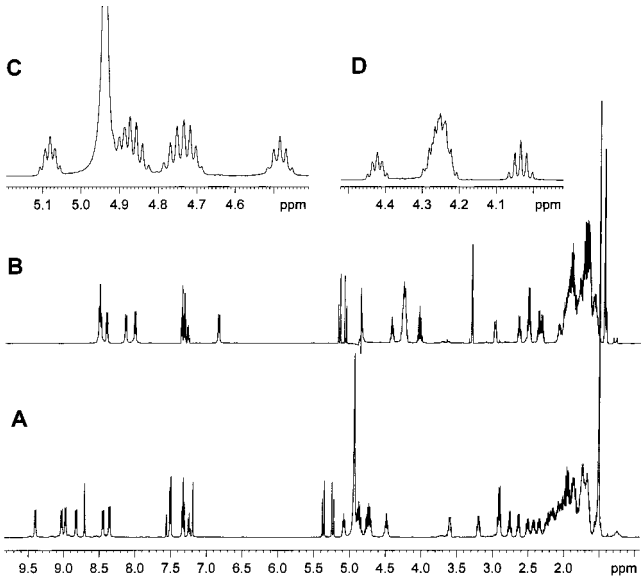

Figure 2. One-dimensional NMR spectra of **1** in (A) pyridine-d_5 and (B) CD_3OH at 298 K. (C and D) Expansions of the $C_\beta H$ region of the spectra from parts A and B, respectively.

from partially overlapped regions of the spectra based on the models generated from the simulations with unambiguous restraints. The best structures with lowest violation from this stage of the calculations were again energy minimized and subjected to restrained dynamics simulations at 277 K for 100 ps. The conformations with lowest violation and lowest energy were extracted based on analyzing the final simulation trajectories.

For ACHC tetramer **3**, simulations were performed with CHARMm in vacuo using the stochastic Langevin algorithm to provide coupling to a heat bath. A set of 20 pseudorandom conformations was generated by sampling a structure every 10 ps from a 200 ps dynamics simulation at 1000 K where the atomic charges were removed to prevent a predominance of compact structures. After minimizing these 20 structures in the neutral state, the charges were reapplied and the molecules quickly heated back to 1000 K and equilibrated for 10 ps. The distance constraints were then gradually applied over the next 24 ps by increasing the force constants above zero by 1 kcal/mol every 1 ps. The final set of structures was obtained by cooling each simulation down to 300 K over 10 ps. The resultant conformational variation was then assessed by superimposing the structures to the conformation with the lowest distance violations of the NMR constraints, by linear least-squares fitting of the backbone atoms of the four β-amino acid residues. The same fitting procedure was done to the backbone atoms in the crystal structure of the tetramer.

Results

Selection of Solvents. The syntheses of compounds **1**−**4** from enantiomerically pure ACPC[3e] or ACHC[3f] monomers have been reported. Proton NMR spectra of **1**−**4** were collected in different solvents in an effort to optimize chemical shift dispersion for NH, $C_\alpha H$, and $C_\beta H$. Interestingly, DMSO-d_6, which is often the solvent of choice for solution NMR studies of small peptides, caused severe overlap in the ^1H spectra of **1**−**4**. Pyridine-d_5 gave maximum dispersion for the ACPC hexamer **1** and octamer **2**. The critical $C_\alpha H$ and $C_\beta H$ regions were nearly first order in most spectra, and several of the aliphatic cyclopentyl protons were also well resolved. CD_3OH proved to be optimal for ACHC tetramer **3**. The one-dimensional ^1H spectra for compounds **1**−**3**, in general, revealed well-dispersed signals for the backbone protons, suggesting high populations of a single, well-defined conformation for each oligomer. A comparison of the spectra of compound **1** in CD_3OH and pyridine-d_5 is shown in Figure 2.

Table 1. ^1H Chemical Shifts of Hexamer **1** in C_5D_5N at 298 K

residue	NH	βH	αH	γH	δH	others
1	8.356	4.475	2.733	2.024, 1.698	1.634	2.153, 1.895
						CH_3 1.497
2	9.397	4.711	2.626	2.002, 1.658	1.529	2.185, 1.787
3	8.445	4.733	2.884	2.045, 1.830	1.723	2.325, 1.830
4	9.030	4.840	2.884	2.067, 1.873	1.723	2.411, 1.873
5	8.826	4.870	3.185	2.010, 1.943	1.809, 1.744	2.497, 1.943
6	8.973	5.077	3.572	2.131, 1.959	1.852, 1.637	2.217, 1.959
						OCH_2 5.356
						5.227
						(2,4)H 7.506[a]
						(3,5)H 7.328[a]
						6H 7.242[a]

[a] Refers to the chemical shifts of the aromatic protons from the C-terminal protecting group.

Aggregation Tests. For each β-peptide, we collected NMR data at different concentrations in the solvents of interest and examined the change in chemical shift of the backbone protons and the resonance line widths. There were no changes in these parameters between 0.25 and 2.5 mM. In addition, temperature coefficients for the amide protons were within 0.5 ppb/K between 2.5 and 25 mM (vide infra). These data along with concentration-dependent CD measurements performed previously[3b] suggest that all of the β-peptides we studied were monomeric under the conditions used.

Assignments. The convention used throughout this study is the accepted labeling of $C_\alpha H$ and $C_\beta H$ for β-amino acids relative to standard α-amino acids; i.e., α and β protons are those on the methine carbons bearing the carbonyl group and the nitrogen of the amide bond, respectively (Figure 1c). Residue-specific assignments were made based on a combination of DQFCOSY, TOCSY, ROESY, and NOESY spectra. Several features of the NMR data allowed us to correlate particular amide protons with their positions at either end of the oligomer. For example, the N-terminal amide proton (NH1) of hexamer **1** was the only amide proton in this molecule that did not show more than one NOE(ROE) cross-peak to a $C_\alpha H$ resonance, the sole correlation being the intraresidue NH1-$C_\alpha H1$ peak. With an assignment made for $C_\alpha H1$, inspection of the correlations to this peak offered clues to the identity of the adjacent residue. A similar line of reasoning was used for the C-terminus: the $C_\alpha H$ peak that displayed a correlation to only one NH resonance was assumed to be part of the C-terminal residue. In this manner, it was possible to perform an "NOE walk" along the backbone and assign the NH, $C_\beta H$, and $C_\alpha H$ protons for each of compounds **1**−**3** and confirm intraresidue assignments from COSY (TOCSY) data.

ACPC Hexamer 1. In a preliminary report,[3b] we outlined some of the details of the structure determination of hexamer **1**. The solution conformation of **1** is characterized by a series of 12-membered NH- -C═O hydrogen-bonded rings (12-helix). A summary of the H-bonding pattern of the 12-helix is shown in Figure 1a. The NMR data for ACPC oligomers **1** and **2** suggest that the helical conformation populated in solution is very similar to that observed in the solid state.[3b,f] A list of chemical shifts of **1** in pyridine-d_5 is shown in Table 1. Comparison of the NH-$C_\beta H$ and NH-$C_\alpha H$ regions of ROESY and TOCSY spectra of **1** in pyridine-d_5 at 298 K is shown in Figure 3. All six protons in the $C_\beta H$ region are discernible although slight overlap is observed for the $C_\beta H$ protons of residues 4 and 5 and residues 2 and 3 (*y*-axis). Protons of residues 3 and 4 show overlap in the $C_\alpha H$ region. Residue assignments were evident from the ROESY data (vide supra).

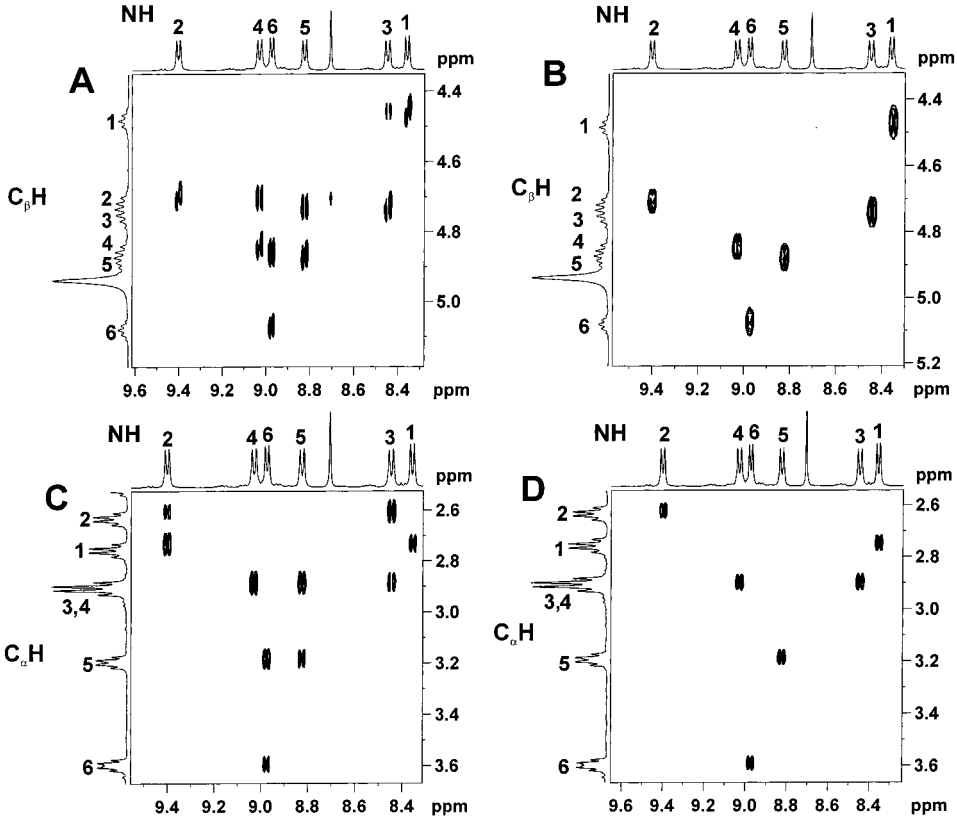

Figure 3. ROESY (A, C) and TOCSY (B, D) spectra of **1** in pyridine-d_5 at 298 K. Parts A and B represent the ROESY and TOCSY spectra for the NH-C$_\beta$H region and parts C and D illustrate the NH-C$_\alpha$H region of the spectra.

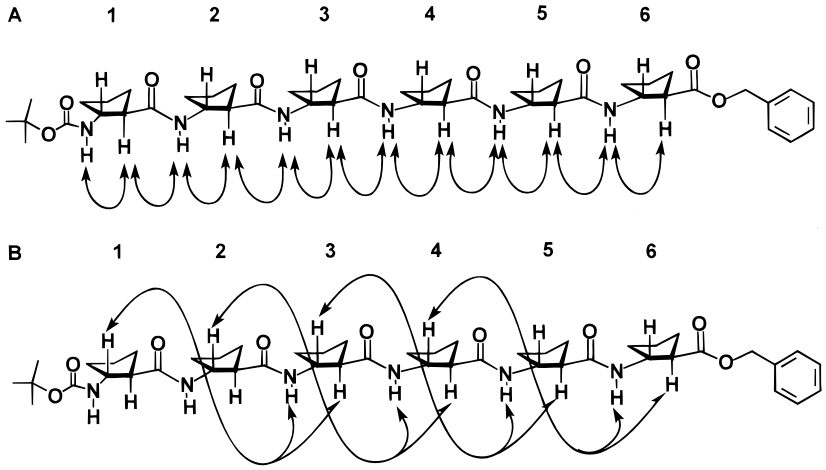

Figure 4. Typical (A) sequential and (B) medium range NOEs observed for ACPC β-peptides. Residue numbers are labeled. In part B, double sided arrows indicate C$_\beta$H(i)-NH(i+2) correlations and the corresponding half arrowheads represent C$_\beta$H(i)-C$_\alpha$H(i+2) NOEs.

For a 12-helix fold, each NH proton (except for NH1) should give through-space correlations to two C$_\alpha$H protons, since the amide proton of a particular residue is in an eclipsing arrangement with both the intraresidue C$_\alpha$H (residue i) and the C$_\alpha$H of the previous residue (i − 1). This pattern is observed for **1** in Figure 3a. A list of assignments and NOE peaks for compounds **1**−**3** is available as Supporting Information.

Medium-range NOEs reveal the backbone fold of the hexamer. We observed several long-range $d_{\beta N}(i,i+2)$, $d_{\beta\alpha}(i,i+2)$, and $d_{\beta N}(i,i+3)$ correlations that are consistent with the 12-helix fold found in the crystal structure.[3f] A diagram of the sequential and medium-range NOEs observed along the backbone of hexamer **1** is shown in Figure 4. C$_\beta$H(i)-NH(i+2) correlations were observed between all possible proton pairs that would be expected for a fully formed 12-helix, i.e., C$_\beta$H1-NH3, C$_\beta$H2-NH4, C$_\beta$H3-NH5, and C$_\beta$H4-NH6 (Figure 4b). In addition, C$_\beta$H-(i)-C$_\alpha$H(i+2) cross-peaks appeared to be present between the same residue pairs, although definitive assignments were possible only for C$_\beta$H3-C$_\alpha$H5 and C$_\beta$H4-C$_\alpha$H6. The C$_\beta$H1-C$_\alpha$-H3 and C$_\beta$H2-C$_\alpha$H4 cross-peaks were ambiguous because of overlap of the C$_\alpha$H3 and C$_\alpha$H4 proton resonances. A very weak $d_{\beta N}(i,i+3)$ cross-peak between C$_\beta$H1 and NH4 was observed providing further evidence for the 12-helix conformation in solution (not observed at the contour level depicted in Figure 3). At each terminus, we observed one correlation that is not consistent with the 12-helix-fold, the C$_\beta$H1-NH2 and C$_\beta$H5-NH6 correlations. These correlations could indicate some "fraying" of the helical structure at each terminus in solution. Similar correlations were observed for both octamer **2** and tetramer **3**. Each of these cross-peaks was very weak for

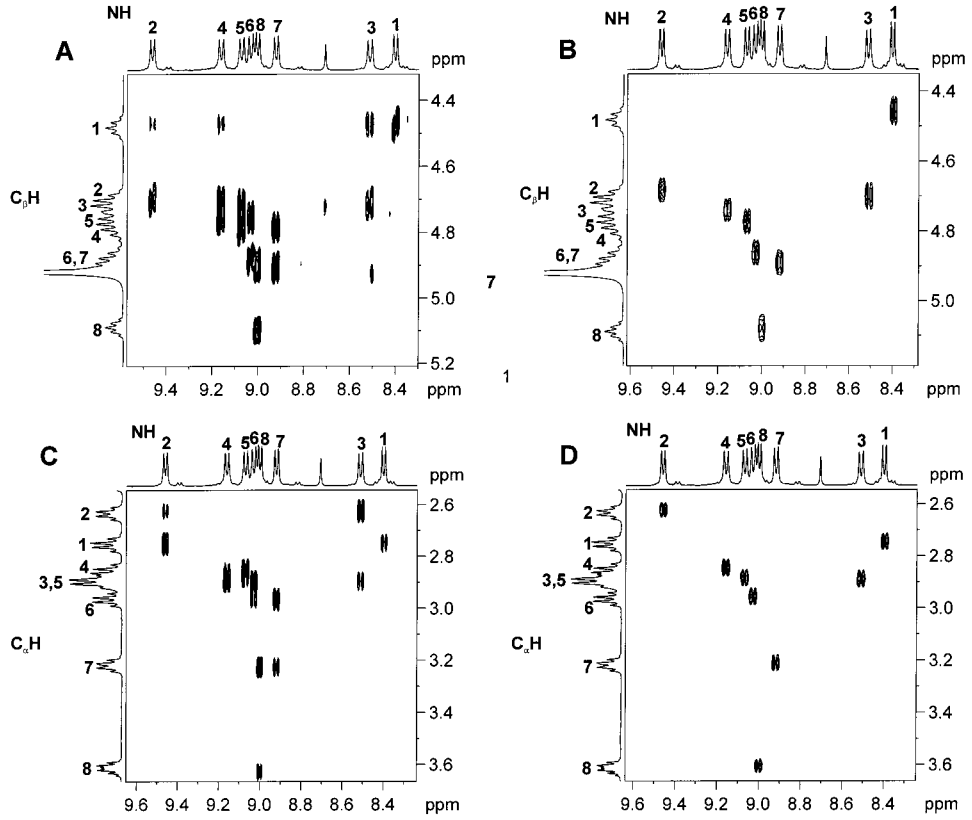

Figure 5. ROESY (A, C) and TOCSY (B, D) spectra of **2** in pyridine-d_5 at 298 K. Parts A and B represent the ROESY and TOCSY spectra for the NH-C_βH region and parts C and D illustrate the NH-C_αH region of the spectra

hexamer **1** and octamer **2** and hence did not contribute significantly to the final calculation of the low-energy structures (see below).

ACPC Octamer 2. A pattern of NOEs similar to that observed for **1** allowed us to assign a 12-helical conformation for octamer **2** in pyridine-d_5 (this β-peptide was not soluble in CD_3OH). In the C_βH region, two protons were partially buried under the residual water resonance, and four other C_αH peaks were contained within ~0.2 ppm. Even with these limitations, we could discern that the NOEs observed for **2** were similar to those seen for **1**. A set of minor peaks in the one-dimensional spectrum of octamer **2** was attributed to either an impurity or a rotamer that results from slow rotation about the N-terminal carbamate bond;[14] the presence of this minor component did not hamper the analysis of **2**. The chemical shifts of relevant protons from **2** in pyridine-d_5 are listed in Table 2. A comparison of the ROESY and the TOCSY spectra of **2** is shown in Figure 5. A strategy similar to that used for hexamer **1** corroborated the chemical shift assignments of the C_αH and C_βH protons. Medium-range correlations that paralleled those of hexamer **1** were observed for octamer **2**, i.e., $d_{\beta N}(i,i+2)$ and $d_{\alpha\beta}(i,i+2)$ correlations (a pattern of NOEs analogous to those shown in Figure 4b, over the entire length of an octamer). However, the $d_{\alpha\beta}(i,i+2)$ correlations involving protons C_αH3 and C_αH5 could not be definitively assigned because of resonance overlap (Figure 5c; see Supporting Information for a complete list of NOEs for **2**).

ACHC Tetramer 3. Tetramer **3** displays a 14-helix conformation in the solid state.[3e] Since **3** is comprised of only four residues, barely more than one turn of the 14-helix is possible. The NMR data for **3** suggest that the 14-helix is highly populated in CD_3OH solution. In contrast to **1** and **2**, the dispersion of chemical shifts for **3** in pyridine-d_5 was inferior to the dispersion in CD_3OH, where nearly all backbone resonances of **3** were well resolved. Assignments proceeded in a manner analogous to that described for **1** and **2**. A list of chemical shifts for the backbone protons of **3** is shown in Table 3. The C_αH2 peak was obscured somewhat by cyclohexane methylene resonances; however, the correlation data allowed tentative assignment of

Table 2. ^{1}H Chemical Shifts of ACPC Octamer **2** in C_5D_5N at 298 K

residue	NH	βH	αH	γH	δH	others
1	8.399	4.475	2.733	2.024, 1.693	1.648	2.153, 1.916 CH_3 1.497
2	9.459	4.690	2.626	2.002, 1.677	1.639, 1.551	2.174, 1.809
3	8.512	4.711	2.884	2.045, 1.852	1.723	2.282, 1.852
4	9.158	4.754	2.841	2.088, 1.895	1.701	2.282, 1.830
5	9.070	4.776	2.884	2.088, 1.959	1.744	2.325, 1.873
6	9.030	4.862	2.948	2.088, 1.959	1.744	2.411, 1.852
7	8.920	4.883	3.206	2.002, 1.927	1.809, 1.734	2.497, 1.927
8	8.997	5.077	3.593	2.131, 1.959	1.852, 1.648	2.239, 1.959 OCH_2 5.356 5.227 (2,4)H 7.506[a] (3,5)H 7.323[a] 6H 7.237[a]

[a] Refers to the chemical shifts of aromatic protons on the C-terminal protecting group.

Table 3. ^{1}H Chemical Shifts for the ACHC Tetramer **3**[a]

residue	NH	βH	αH
1	6.880	3.375	2.395
2	6.850	3.555	1.580
3	6.252	3.660	1.778
4	7.722	3.862	2.175

[a] At 277 K in CD_3OH.

(14) Benedetti, E.; Pedone, C.; Toniolo, C.; Nemethy, G.; Pottle, M. S.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1980**, *16*, 156.

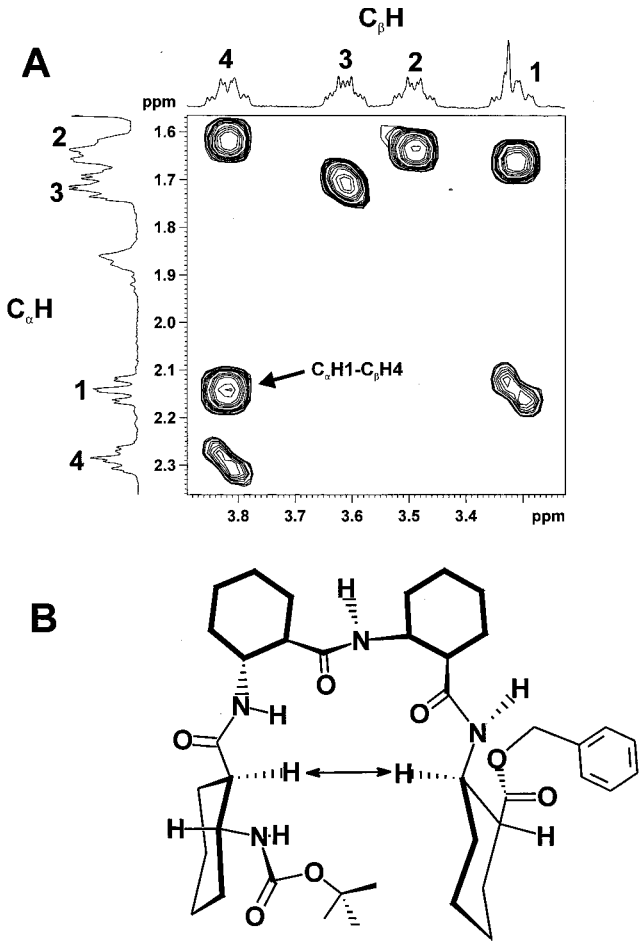

Figure 6. (A) $C_\alpha H$-$C_\beta H$ region of the ROESY spectrum of tetramer **3** in CD_3OH at 277 K. The strong, long-range $C_\beta H1$-$C_\alpha H4$ correlation is labeled. (B) Cartoon of the folded conformation of tetramer **3** indicated by $C_\beta H1$-$C_\alpha H4$ shown in part A.

this peak. We also observed $d_{N\beta}(i,i+2)$ correlations for NH1-$C_\beta H3$ and NH2-$C_\beta H4$, which are characteristic of a 14-helix.[2,3] A very strong NOE between $C_\alpha H1$ and $C_\beta H4$ is an excellent indication that the 14-helix fold is highly populated for tetramer **3** in CH_3OH (Figure 6a). This correlation is possible only if the molecule folds to bring the cyclohexyl groups of the two terminal residues very close in space (Figure 6b). In the crystal structure of **3**, these protons are within 2.59 Å of one another.[3e]

We observed additional NOEs for **3** that suggested that the amide bonds of residues 2 and 3 may be "flipping" to bring the NH proton in proximity of the β-proton of the previous residue (as discussed above, analogous NOEs were observed at the termini of **1**), viz., $C_\beta H1$-NH2 and $C_\beta H2$-NH3. An indication of some type of chemical exchange was also evident from the behavior of the NH1 proton at various temperatures. This proton was a sharp doublet between 277 and 288 K, but began to broaden at higher temperatures and virtually disappeared in the one-dimensional spectrum above 298 K. This behavior suggested that some T_2 exchange broadening is occurring on the NMR time scale at or above room temperature, possibly by C−N rotation of the N-terminal carbamate group.[14] In the NOESY spectrum of **3** at 298 K, the majority of the NOEs observed at 277 K were still present at 298 K, but some long-range correlations were weakened, suggesting that the folded structure begins to unravel as the temperature is raised.

ACHC Hexamer 4. The analysis of **4** was hampered by severe overlap of signals in the $C_\alpha H$ and $C_\beta H$ regions in all the solvents tested. However, the amide protons were well resolved

Table 4. Temperature Coefficient of Amidic Protons of β-Peptides **1**−**3** in Various Solvents[a]

residue	DMSO	CD_3OH	C_5D_5N
1	6.76	8.25 (6.72)[b]	13.9 (13.3)[c]
2	6.80	8.55 (3.84)	13.1 (12.4)
3	5.44	5.40 (4.76)	5.1 (4.7)
4	3.84	5.25 (7.00)	6.2 (5.5)
5	3.76	4.85	4.7 (4.5)
6	4.08	5.00	5.2 (4.2)
7			− (4.4)
8			− (5.0)

[a] Values are in −ppb/K. Numbers given for all three solvents are for hexamer **1**. [b] Values in parentheses are for tetramer **3** in CD_3OH. [c] Values in parentheses are for octamer **2** in C_5D_5N.

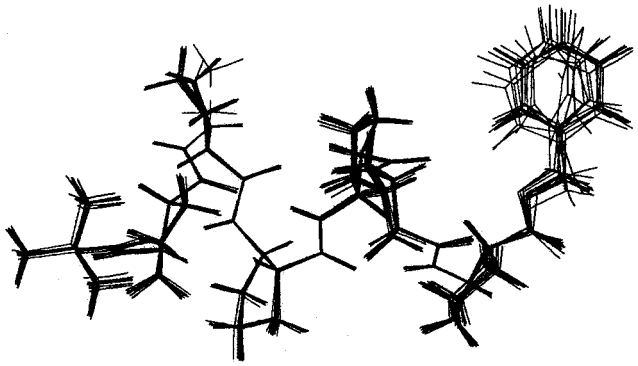

Figure 7. Overlay of the 10 best structures of the octamer **2** calculated from restrained molecular dynamics simulations.

in CD_3OH, and we tentatively assigned some of the observed NOEs. Although all peaks could not be unambiguously assigned, the pattern of correlations for this molecule was consistent with the 14-helix structure. Correlations such as intraresidue NH-$C_\alpha H$ and $d_{N\alpha}(i,i-1)$, along with long-range $d_{N\beta}(i,i+2)$ could be tentatively assigned, all of which were indications that a 14-helix is populated in solution. Only two $d_{N\beta}(i,i+2)$ correlations could be assigned unambiguously due to overlap in the $C_\beta H$ region of the spectrum. Previously reported H/D exchange data with **4** in CD_3OH suggested that this molecule adopts a stable folding pattern, which is presumably the 14-helix.[3a]

Amide Proton Temperature Coefficients. The temperature dependences of amidic proton chemical shifts ("temperature coefficients") are frequently examined in an effort to gain insight on intramolecular hydrogen-bonding patterns. In hydrogen-bonding solvents, like those employed in our study, the conventional interpretation of temperature coefficients is that small values result from intramolecularly hydrogen-bonded NH groups, while large values result from solvent exposed NH groups. These simple interpretive rules were originally derived from analysis of small cyclic peptides,[15a] which have relatively rigid conformations. For linear peptides, however, interpretation of amide temperature coefficients is, in principle, more complex. If, for example, internal hydrogen bonding is disrupted when the temperature is raised, then a large temperature coefficient will probably be observed. The uncertainties associated with temperature coefficient interpretation have been discussed in detail by Anderson et al.[15b]

We measured NH temperature coefficients for β-peptides **1**−**3** because we had independent high-resolution information on the solution conformations of these molecules, which could help

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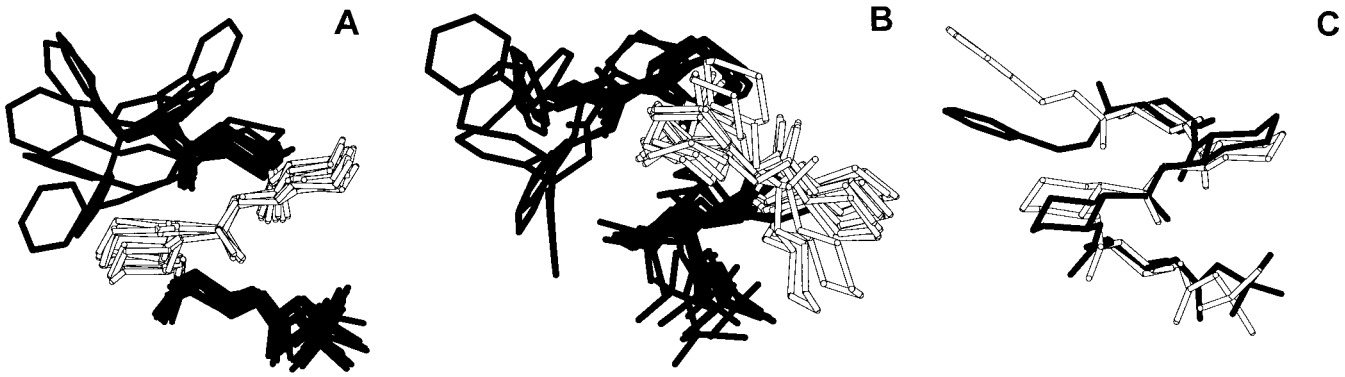

Figure 8. (A) Overlay of the β-peptide backbone of the 10 best structures of the tetramer **3** calculated from restrained molecular dynamics simulations. (B) Overlay of the β-peptide backbone of a second family of structures obtained in the restrained molecular dynamics simulations of **3** with a left-handed helical twist. The N- and C-terminal residues in parts A and B are shaded black. (C) Overlay of the crystal structure of **3** (white) and the calculated structure (black) from NOE-restrained molecular dynamics simulations that most closely matches the crystal structure coordinates.

us avoid misinterpretation. Data obtained in three solvents for ACPC hexamer **1** and data for ACPC octamer **2** in pyridine-d_5 are shown in Table 4. The data sets appear to be qualitatively consistent with 12-helix formation, because for each ACPC oligomer the two N-terminal NH groups display significantly larger temperature dependences than do the remaining NH groups. In the 12-helix conformation, only the two N-terminal NH groups would fail to form intramolecular 12-membered ring hydrogen bonds. These results hint that the 12-helical conformation is at least partially populated in all three solvents even at the highest temperature.

For ACHC tetramer **3**, NH temperature coefficients were measured only in CD_3OH (Table 4). The values (6.7, 3.8, 4.8, and 7.0 ppb/K for NH1, NH2, NH3, and NH4, respectively) also qualitatively suggest a folded structure for the tetramer **3**. The lowest coefficient was found for NH2 (3.8) which should be involved in a hydrogen bond with the carbonyl of residue 4 in a 14-helix fold. These data are consistent with the NOE data in suggesting that **3** assumes a 14-helix structure in CD_3OH solution.

Molecular Dynamics. β-Peptides **1**−**3** were studied by molecular dynamics simulation using the CHARMm force field.[12] The NOE data were used to generate qualitative distance restraints for simulated annealing[16] and minimizations. For both ACPC oligomers, **1** and **2**, the 12-helix was the predominant fold that converged during the calculations from the solution data. The calculated structures agreed well with the crystal structures of **1** and **2**. In addition, the NMR-derived structures closely reproduced the conformations that were originally predicted by a modified version[17a] of the AMBER[17b,c] force field within the molecular modeling package MACROMODEL.[18] A list of the NOE restraints used for compounds **1** and **2** is supplied with the Supporting Information. Figure 7 shows an overlay of the 10 structures of octamer **2** that had the fewest violations of the NOE restraints in the simulated annealing calculations. The RMSD values for all calculated structures were 1.2 ± 0.3 and 1.0 ± 0.2 Å for all atoms and 0.15 ± 0.05 and 0.10 ± 0.05 Å for the backbone atoms for compounds **1** and **2**, respectively, indicating that the 12-helix is highly populated for oligomers **1** and **2** in pyridine solution.

For ACHC tetramer **3**, the structures calculated from the modeling support the conclusion that a 14-helix is present in CD_3OH. As with the ACPC oligomers, starting structures of **3** were annealed with NOE restraints (a table of restraints is included in the Supporting Information). Examination of the 20 structures derived from the protocol outlined in the Experimental Section revealed that two families of conformations were populated. An overlay of the 10 structures that best fit the NOE restraints (lowest restraints energy) is shown in Figure 8a. It is evident that a right-handed, 14-helix is formed even though only a little more than one full turn of the helix is possible. There is some disorder near the C-terminal group, but the backbone atoms seem to converge well (RMSD = 0.38 ± 0.03 Å for the backbone atoms).

A second conformation of **3** was found to be also helical in nature, but the handedness was reversed (left-handed helix, Figure 8b). These structures were of much higher total energy than those with the expected right-handed helix conformation. It is also evident from Figure 8b that this family of conformations showed more structural scatter with a much higher RMSD than the structures comprised of a right-handed helix.

Figure 8c shows an overlay of the best fit structure from the 10 low-energy conformations with the crystal structure of tetramer **3**.[3e] A close fit of the NMR-derived structure and the solid state structure is evident. The average RMS difference of the 10 best structures with the crystal structure was 0.275 ± 0.027 Å. These data show that ACHC building blocks can be used to design β-peptide oligomers with well-defined and relatively rigid conformations. However, some conformational flexibility due to the limited number of helix-stabilizing interactions is evident in such a short oligomer.

Conclusion

This study shows that β-peptides prepared from enantiomerically pure ACHC or ACPC residues adopt solution conformations that mirror the well-defined helical conformations found in the solid state. Thus, the ACHC and ACPC β-peptide backbones provide two distinct molecular shapes that could serve as scaffolds for display of functional group clusters in

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predetermined arrangements, if synthetic strategies can be identified for regio- and stereospecific modification of the cycloalkane rings. Conformationally constrained scaffolds of this type should allow creation of oligomers with useful chemical or medicinal functions. It has been recently shown that functionalized β-peptides are able to populate defined conformations in aqueous solution.[19,20] These results may pave the way for the development of β-peptides as enzyme inhibitors or modulators of cellular interactions with potential therapeutic value.[2f]

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Supporting Information Available: Distance restraints derived from ROESY data for **1**−**3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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