

# Synthesis and Characterization of *trans*-2-Aminocyclohexanecarboxylic Acid Oligomers: An Unnatural Helical Secondary Structure and Implications for $\beta$ -Peptide Tertiary Structure

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**Abstract:** The preparation, crystal structures, and circular dichroism (CD) spectra of two oligomers of optically active *trans*-2-aminocyclohexanecarboxylic acid are reported. In the solid state, both the tetramer and the hexamer of this  $\beta$ -amino acid display a helical conformation that involves 14-membered-ring hydrogen bonds between a carbonyl oxygen and the amide proton of the second residue toward the N-terminus. (For comparison, the familiar  $\alpha$ -helix observed in conventional peptides is associated with a 13-membered-ring hydrogen bond between a carbonyl oxygen and the amide proton of the fourth residue toward the C-terminus.) These crystallographic data, along with CD data obtained in methanol, suggest that the 14-helix constitutes a stable secondary structure for  $\beta$ -amino acid oligomers (" $\beta$ -peptides"). In addition, the crystal packing pattern observed for the hexamer offers a blueprint for the design of  $\beta$ -peptides that might adopt a helical bundle tertiary structure.

## Introduction

Biological systems carry out a wide variety of sophisticated operations at the molecular level. Proteins are responsible for most of these complex functions, including catalysis, electron shuttling, and nucleation of inorganic phase crystallization; RNA, too, can catalyze reactions. Although proteins and RNA differ from one another considerably in terms of molecular structure, they share the ability to adopt compact and specific three-dimensional shapes, and this conformational behavior is crucial to activity. Based on the uniquely sophisticated activities displayed by proteins and RNA, one is tempted to speculate that new polymers with analogous folding properties might be engineered to display analogous activities.<sup>1</sup>

Unnatural polymers with well-defined folding propensities ("foldamers") have recently attracted a great deal of attention. For example, results from Seebach et al.<sup>2–5</sup> and from our laboratory<sup>6,7</sup> have demonstrated that oligomers of  $\beta$ -amino acids (" $\beta$ -peptides") can adopt a variety of secondary structures,

including helices, sheets, and reverse turns, depending upon residue substitution pattern, in organic solvents and in the solid state. Very recently, the conformational behavior of water-soluble  $\beta$ -peptides has been described by several groups.<sup>8</sup> In addition, Seebach et al.<sup>9</sup> and Hanessian et al.<sup>10</sup> have shown that  $\gamma$ -peptides can adopt helical conformations, Gervay et al.<sup>11</sup> have suggested helix formation by a set of  $\delta$ -peptides derived from sialic acid, Zuckermann et al.<sup>12</sup> have shown that peptoid oligomers can adopt helical conformations, Fleet et al.<sup>13</sup> have reported helix formation by  $\delta$ -peptides constructed from furanose-derived subunits, Moore et al.<sup>14</sup> have demonstrated cooperative folding in metal-binding phenylacetylene oligomers, and

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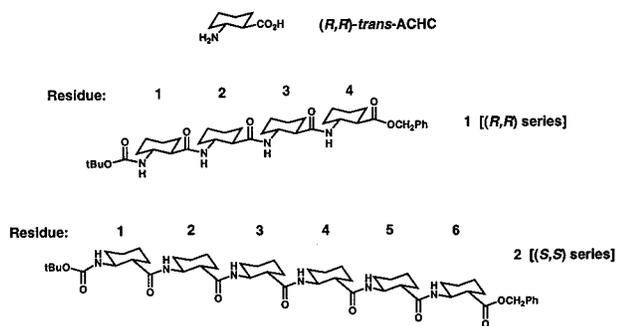
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Yang et al.<sup>15</sup> have described helix formation by  $\alpha$ -amino acid oligomers. Earlier contributions in this area have been reviewed.<sup>1</sup>

Here we describe the synthesis and structural characterization of  $\beta$ -peptide oligomers constructed from *trans*-2-aminocyclohexanecarboxylic acid (*trans*-ACHC). In the solid state, tetramer **1** and hexamer **2** both adopt helical conformations defined by the 14-membered-ring hydrogen bond that can form between a backbone carbonyl oxygen and the backbone NH two residues toward the N-terminus ("14-helix"); a preliminary description of the structure of **2** has appeared.<sup>6a</sup> Our choice of *trans*-ACHC was based on computational predictions.<sup>16</sup> Bode and Applequist independently made the same prediction.<sup>17</sup>



For both proteins and RNA, well-defined tertiary structure appears to be a minimum requirement for complex function. Although a number of foldamers that display well-defined secondary structure have been described,<sup>1-15</sup> there is no example yet of an unnatural oligomer that displays a discrete tertiary structure. The crystallographic data presented here provide insight on side-to-side packing preferences of *trans*-ACHC-based 14-helices, and this insight should guide the design of larger  $\beta$ -peptides that adopt helical bundle tertiary structures.

## Results

**Preparation of Optically Pure *trans*-ACHC.** *trans*-ACHC oligomers can adopt a 14-helical conformation only if all residues have the same absolute configuration. Our initial efforts to prepare optically pure *trans*-ACHC focused on a reported procedure for resolving *N*-benzoyl-*trans*-ACHC (**3**).<sup>18</sup> We synthesized ( $\pm$ )-**3** by following published methods,<sup>18,19</sup> with a few modifications to maximize purity (details may be found in the Supporting Information). One published procedure<sup>18</sup> for the resolution of ( $\pm$ )-**3** calls for repeated recrystallization of the quinine salt of **3**, which we designate ( $-$ , $\pm$ )-**4** (the first symbol in the parentheses refers to the absolute configuration of quinine, and the second symbol refers to the absolute configuration of acid **3**), to obtain a salt containing only quinine and ( $-$ )-(*R,R*)-*N*-benzoyl-*trans*-ACHC [( $-$ , $-$ )-**4**], with  $[\alpha]_D^{20} -112^\circ$  (*c* 2.5, ethanol). This procedure was capricious in our hands and provided only small amounts of pure ( $-$ , $-$ )-**4** and ( $-$ , $+$ )-**4** (details may be found in the Supporting Information). Structural analysis of ( $-$ , $+$ )-**4** confirmed that ( $+$ )-**3** has the (*S,S*) configuration. A modified crystallization procedure,<sup>20</sup> in which enantiopure crystals of **3** (obtained initially from quinine resolutions)

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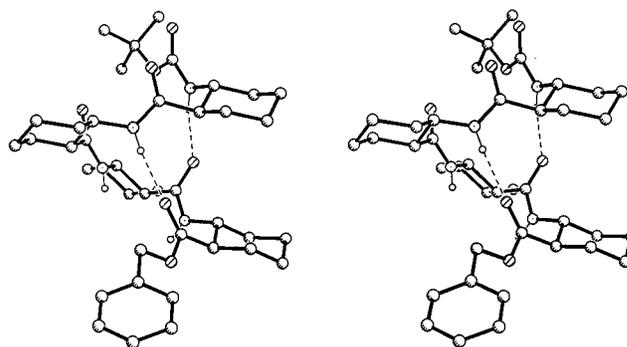
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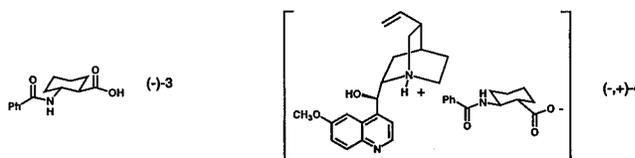
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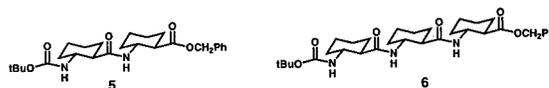


**Figure 1.** Stereoview of tetramer **1** in the solid state. Only one of the two independent molecules is shown; the two independent molecules display very similar conformations. Hydrogen atoms other than those attached to nitrogen have been omitted for clarity. Dotted lines indicate hydrogen bonds.

are used to seed a solution containing ( $\pm$ )-**3** and ( $\pm$ )-2-hydroxypropylamine, was more reliable.<sup>21</sup>



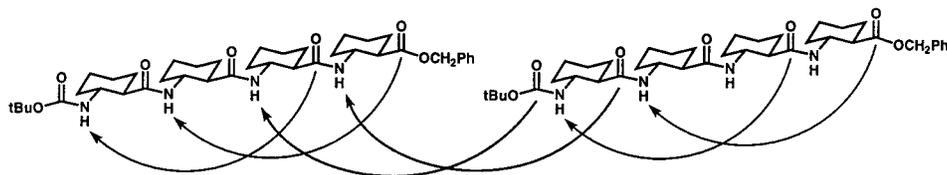
**Oligomer Synthesis.** As in conventional peptide synthesis, orthogonal amino and carboxyl protecting groups are required for  $\beta$ -peptide synthesis. We used *tert*-butoxycarbonyl (Boc) for the amino group and benzyl ester (Bn) for the carboxyl group. *trans*-ACHC dimer **5** was prepared from the monoprotected *trans*-ACHC derivatives by using the hydrochloride salt of (*N,N*-dimethylamino)propyl-3-ethylcarbodiimide (EDCI $\cdot$ HCl) in the presence of 4-(*N,N*-dimethylamino)pyridine (DMAP). Dimer **5** could be isolated in high purity after washing the crude product with aqueous 1 N HCl. Further purification was achieved via silica gel column chromatography. Trimer **6**, tetramer **1**, and hexamer **2** were prepared similarly, by coupling smaller fragments. Oligomers were constructed in both the (*R,R*) and (*S,S*) series.



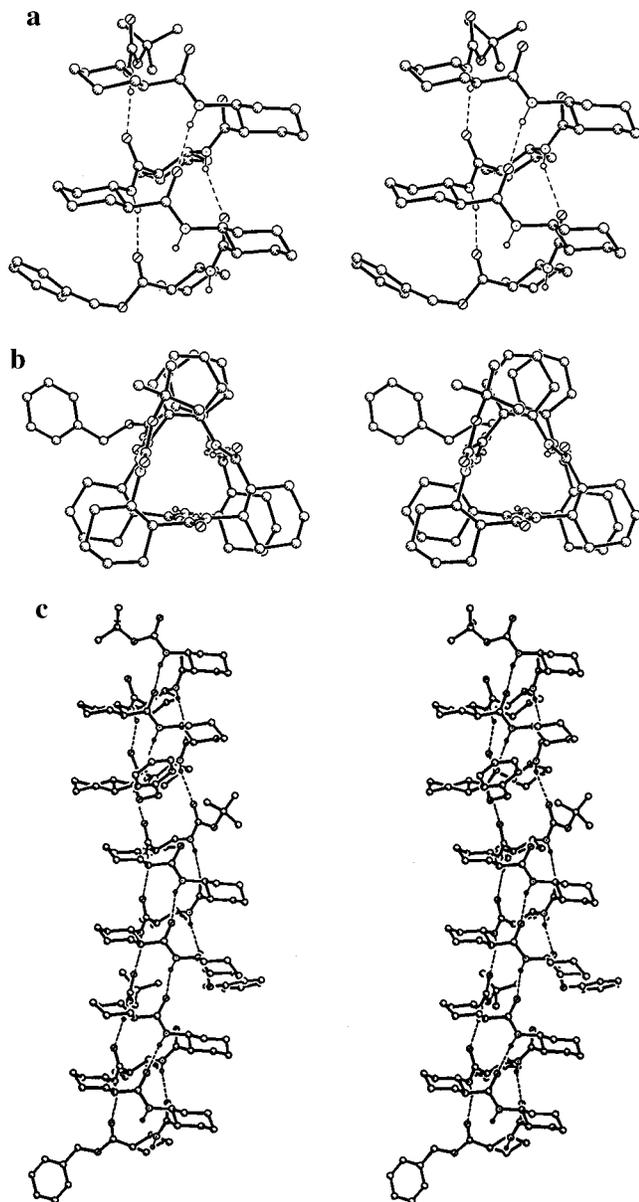
Dimer **5** is soluble in pure chloroform, but trimer **6**, tetramer **1**, and hexamer **2** require small proportions of methanol in chloroform to dissolve. All four *trans*-ACHC oligomers are soluble in methanol. Hexamer **2** was difficult to purify by chromatography, because this molecule would not elute from silica gel unless large amounts of methanol were present; therefore, **2** was purified on a silicic acid column. Replacing either protecting group with a terminal amide significantly decreased the solubility of the *trans*-ACHC oligomers in all solvents.

**Solid-State Structures.** Tetramer **1** adopts a 14-helical conformation in the solid state (Figure 1). Both of the possible 14-membered-ring hydrogen bonds form (the intramolecular and intermolecular hydrogen-bonding pattern in crystalline **1** is illustrated in Figure 2). Two molecules are present in the

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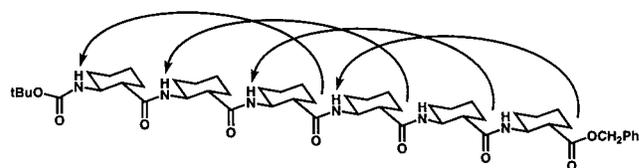


**Figure 2.** Schematic view of the intra- and intermolecular hydrogen-bonding pattern in the crystal of tetramer **1**.

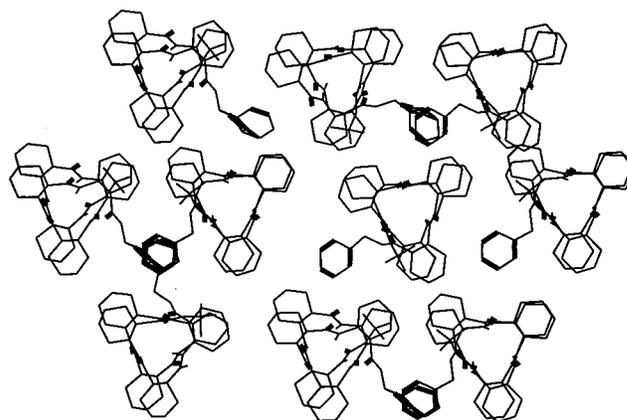


**Figure 3.** Solid-state structure of hexamer **2**. Hydrogen atoms other than those attached to nitrogen have been omitted for clarity. Dotted lines indicate hydrogen bonds. (a) Stereoview of one of the three molecules in the asymmetric unit (the three independent molecules adopt very similar conformations). (b) Stereoview of one molecule along the helix axis. (c) Stereoview of the three molecules in the asymmetric unit.

asymmetric unit of the crystal, and these two conformations are very similar. The two molecules in the asymmetric unit are aligned end-to-end such that the carbonyls of the N-terminal carbamate and residue 1 of one tetramer are hydrogen bonded to the amide protons of residues 3 and 4 of the neighbor (Figure 2). Each pair of molecules (one asymmetric unit) packs end-to-end with neighboring pairs, via the same set of intermolecular hydrogen bonds, to create infinite hydrogen-bonded columns.



**Figure 4.** Schematic view of the intramolecular hydrogen-bonding pattern in the crystal of hexamer **2**.

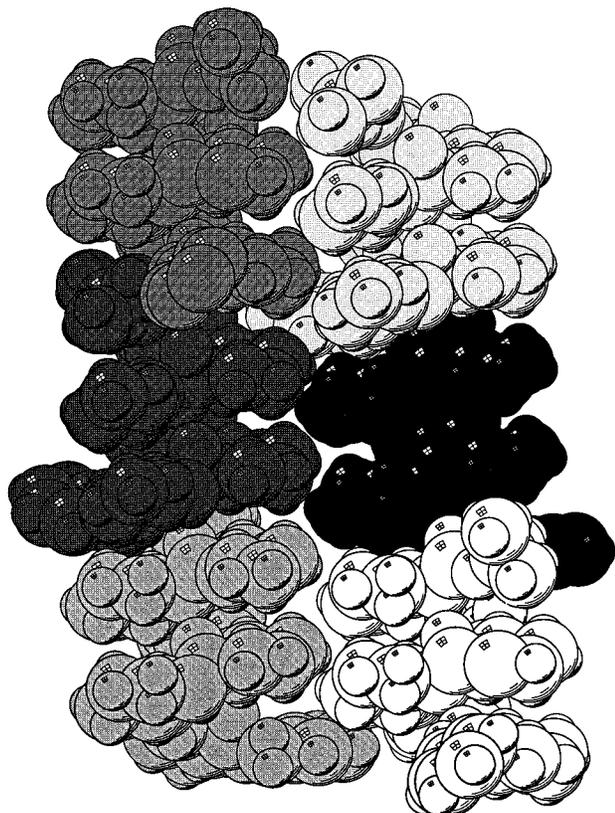


**Figure 5.** Solid-state packing pattern of hexamer **2**, viewed along the 14-helical axes. As discussed in the text, there are two types of three-way interfaces in this packing pattern, one “tight” and the other “loose”. The tight three-way interface, involving extensive cyclohexyl–cyclohexyl contacts, is displayed by the three molecules in the upper left corner of the image. The loose three-way interface, with benzyl groups and solvent at the center, is displayed by the three molecules in the lower left corner of the image.

Thus, all hydrogen-bonding sites on each  $\beta$ -peptide are satisfied either intra- or intermolecularly.  $\text{ClCH}_2\text{CH}_2\text{Cl}$  molecules from the crystallization solvent (slightly less than one per  $\beta$ -peptide) are present between these columns. There are very few side-to-side intermolecular contacts between the cyclohexyl rings in neighboring columns.

Hexamer **2** is also 14-helical in the solid state (Figure 3). Three hexamers are present in the asymmetric unit; the three independent conformations are very similar to one another (the conformation of the C-terminal benzyl group varies among these molecules, and in one case this group is disordered). All four of the possible 14-membered hydrogen-bonded rings are formed in each molecule (Figure 4), generating two turns of 14-helix.<sup>6a</sup> The three hexamers in the asymmetric unit are aligned end-to-end, with the “dangling” pair of C=O and N–H moieties of each hexamer forming hydrogen bonds to the nearest neighbor. Thus, as observed in the crystal of tetramer **1**, molecules of hexamer **2** are aligned in continuously hydrogen-bonded columns, and all hydrogen-bonding sites are satisfied either intra- or intermolecularly.

There are extensive lateral interactions between hydrogen-bonded columns of hexamer **2**, in contrast to the packing pattern observed for **1**. Figure 5 illustrates the packing pattern of **2**, as viewed along the 14-helix axis; the columns are packed in a hexagonal array. There are two types of helix interfaces in this

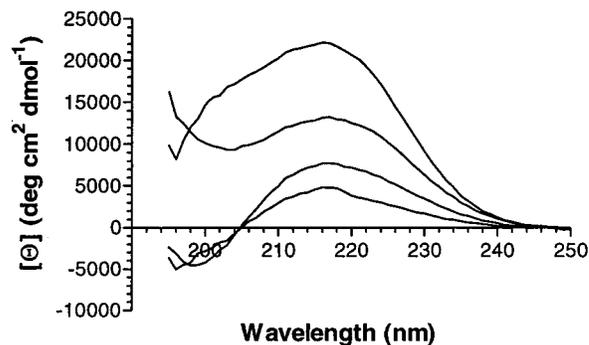


**Figure 6.** Space-filling view of six molecules of **2** involved in the tight three-way interface shown in Figure 5, viewed from a perpendicular perspective. The six molecules are distinguished by differential shading. All six molecules have the same orientation (C-terminus at the bottom); the third column of molecules involved in this tight interface (which is omitted for clarity) is antiparallel to those shown. See text for further discussion.

array, each with approximate three-fold symmetry. One interface is "tight". The center of this interface is defined by intimate interdigitation of three sets of cyclohexyl rings, each set from a different column. The interdigitation primarily involves contacts at C4 and C5 of the cyclohexane rings, and we refer to this arrangement as a "cyclohexane zipper". One of the  $\beta$ -peptide helices is antiparallel to the other two in this tight interface. The second three-fold interface is "loose". The center of this interface is filled with solvent molecules ( $\text{CH}_3\text{OH}$  and  $\text{ClCH}_2\text{CH}_2\text{Cl}$ ; both used in the crystallization protocol) and phenyl rings from the C-terminal benzyl ester.

Figure 6 offers a side-on view of the cyclohexane zipper interaction (six molecules, each with a different shade). Only two of the three columns that participate in this interaction are shown; these two columns are parallel, but the antiparallel contacts are very similar. The space-filling representation in Figure 6 highlights the intimate interweaving of cyclohexane rings from neighboring columns of **2**.

**Circular Dichroism.** Circular dichroism (CD) spectroscopy is extensively used to analyze the secondary structure of  $\alpha$ -amino acid polypeptides.<sup>22,23</sup> Each type of regular  $\alpha$ -amino acid secondary structure gives rise to a characteristic CD spectrum in the far-UV region (190–240 nm). CD in this spectral region arises from the backbone amide groups and is, therefore, comparable among peptides with different sequences.



**Figure 7.** Circular dichroism data in  $\text{CH}_3\text{OH}$  for dimer **5**, trimer **6**, tetramer **1**, and hexamer **2** (bottom to top at 215 nm). In each case, the molar ellipticity  $[\theta]$  has been normalized for oligomer concentration and the number of backbone amide groups (1, 2, 3, and 5, respectively). Hexamer **2** was prepared from (*S,S*)-*trans*-ACHC, while the shorter oligomers were prepared from (*R,R*)-*trans*-ACHC; to aid comparison, the curve shown for **2** is the mirror image of the actual curve. Data were obtained on an 62A-DS instrument at 25 °C.

At present, there is no simple way to correlate far-UV CD spectra of non- $\alpha$ -amino acid oligomers with specific conformations, but CD data for new polyamides can, nevertheless, provide useful information if correlations with other structural data are available.

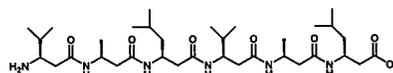
CD spectra for dimer **5**, trimer **6**, tetramer **1**, and hexamer **2**, 0.3–1.0 mM in methanol, are presented in Figure 7 (**1**, **5**, and **6** were prepared from (*R,R*)-*trans*-ACHC; **2** was prepared from (*S,S*)-*trans*-ACHC, but the data are inverted for ease of comparison). For each  $\beta$ -peptide, the spectrum has been normalized for concentration and for the number of amide chromophores, in keeping with conventional peptide practice. The monomer Boc-(*R,R*)-*trans*-ACHC-OBn, which has no amide chromophores, did not show an appreciable CD signal in the far-UV region (not shown).

Each oligomer displays a maximum at ca. 217 nm in Figure 7, and the intensity of this maximum increases with  $\beta$ -peptide length. We tentatively assign this maximum to the 14-helix, since both **1** and **2** adopt the 14-helix in the solid state. Further support for this assignment comes from previously reported amide H/D exchange data, which suggest that hexamer **2** strongly favors an intramolecularly hydrogen-bonded conformation, presumably the 14-helix, in methanol solution.<sup>6a</sup> The weak maximum at 217 nm displayed by dimer **5**, which cannot form a complete 14-helical turn, implies that the *trans*-ACHC backbone is highly preorganized for this secondary structure. The steady increase in intensity at 217 nm with additional *trans*-ACHC residues indicates that the population of the 14-helical state increases as  $\beta$ -peptide length increases. This trend suggests that 14-helix formation is cooperative. Analogous cooperativity is well-established in  $\alpha$ -helix formation by conventional peptides.<sup>24</sup> In  $\alpha$ -helical peptides, cooperativity arises because propagating an existing helix is favorable, but initiating a helix is unfavorable.

Our CD data for *trans*-ACHC oligomers are somewhat different from the CD data Seebach et al. have reported for  $\beta$ -peptides constructed from  $\beta$ -substituted residues (e.g., **7**),<sup>2–4</sup> even after accounting for the fact that our data are reported for the (*R,R*) series, while the Seebach  $\beta$ -peptides have the opposite configuration at the  $\beta$ -position. Two-dimensional NMR data

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establish that the Seebach  $\beta$ -peptides adopt the 14-helix (which Seebach et al. refer to as the  $3_1$ -helix) in organic solvents. CD data reported for **7** and related  $\beta$ -peptides show a minimum at 214 nm, a zero-point crossing at 206 nm, and a maximum at 198 nm.<sup>2-4</sup> The minimum at 214 nm is presumably related to the maximum we observe at 217 nm, but the differences at lower wavelengths are difficult to explain at present. It is likely that the Seebach  $\beta$ -peptides and our *trans*-ACHC oligomers equilibrate between the 14-helix and other conformations in solution; therefore, the CD data represent an average of the interconverting folding patterns. The variations between our CD results and those of Seebach et al. could indicate that different non-14-helical conformations are populated by the two classes of  $\beta$ -peptide.

## Discussion

### Comparison with Other $\beta$ -Peptide Structural Information.

Our crystallographic results for *trans*-ACHC oligomers **1** and **2** represent the first high-resolution evidence for helix formation by  $\beta$ -peptides. The conformations of molecules containing  $\beta$ -amino acid residues have been of interest from a variety of perspectives; we briefly summarize relevant studies below.

$\beta$ -Amino acid homopolymers are members of the nylon-3 family, and these materials have received scrutiny since the 1960s.<sup>25</sup> No high-resolution structural data are available for these polymers, but considerable effort has been devoted to deducing structural information from low-resolution data. Poly- $\beta$ -alanine,  $[\text{NHCH}_2\text{CH}_2\text{C}(=\text{O})]_n$ , has been proposed to adopt a sheet structure in the solid state but to be disordered in aqueous solution.<sup>26,27</sup> A number of homopolymers of optically active  $\beta$ -substituted  $\beta$ -amino acids have been examined. Applequist et al. examined poly- $\beta$ -L-aspartic acid in aqueous solution by a number of methods and concluded that this polymer adopts some sort of secondary structure under acidic conditions.<sup>28</sup> The secondary structure could not be identified, but these workers noted, based on physical model building, that the 14-helix appeared to be a favorable conformation. Goodman et al. proposed sheet structure for poly[(*S*)- $\beta$ -aminobutyric acid].<sup>29</sup> Poly( $\alpha$ -isobutyl-L-aspartate) has received the most attention among  $\beta$ -peptide polymers. Yuki et al. concluded that this polymer aggregates to form sheets in organic solvents and in the solid state.<sup>30</sup> Subsequent examination via X-ray diffraction of this material by Spanish workers, however, led them to conclude that the solid-state conformation of poly( $\alpha$ -isobutyl-L-aspartate) is actually helical.<sup>31-34</sup> These workers proposed a helix defined by 16-membered hydrogen-bonded rings between backbone carbonyls and amide protons ("16-helix", in our nomenclature).<sup>31,32</sup> Reexamination of this material led to a revised proposal that the  $\beta$ -peptide polymer adopts the 14-helix

in this crystalline form.<sup>33,34</sup> A second solid-state form of poly( $\alpha$ -isobutyl-L-aspartate) was obtained by varying the preparation conditions. A 20-helical conformation was originally assigned to this material,<sup>32</sup> but reexamination led the original workers subsequently to propose an 18-helix for this crystalline form of poly( $\alpha$ -isobutyl-L-aspartate).<sup>33</sup>

Several groups have examined  $\beta$ -amino acid oligomers of defined length and composition. Narita et al. have concluded that relatively short  $\beta$ -alanine oligomers adopt sheetlike packing patterns in the solid state.<sup>27</sup> Yuki et al. have concluded that the octamer and decamer of  $\alpha$ -isobutyl-L-aspartate aggregate to form sheets in organic solvents.<sup>35</sup> As discussed above, Seebach et al. have shown definitively that relatively short  $\beta$ -peptides constructed from  $\beta$ -substituted residues adopt 14-helical conformations in solution.<sup>2,3</sup> This conformation is also observed for short oligomers of  $\alpha$ -substituted residues and for oligomers containing  $\alpha,\beta$ -disubstituted residues, if the relative stereochemistry is appropriate.<sup>3</sup>

Recent work has shown that short  $\beta$ -peptide oligomers can adopt conformations other than the 14-helix if residue substitution patterns and sequence are carefully chosen. We have shown that oligomers of optically pure *trans*-2-aminocyclopentanecarboxylic acid adopt a helix defined by 12-membered-ring hydrogen bonds ("12-helix") in the solid state and organic solvents.<sup>6b</sup> Seebach et al. have discovered that short  $\beta$ -peptides with alternating  $\alpha$ - and  $\beta$ -substituted residues adopt a helix containing both 10- and 12-membered-ring hydrogen bonds in organic solvents.<sup>4</sup> The crystal structure of a tri- $\beta$ -peptide composed of  $\beta$ -substituted residues revealed an intermolecular parallel sheet-type hydrogen-bonding interaction;<sup>2a</sup> however, it is not clear that this crystal packing pattern provides insight on sheet formation in solution, since short conventional peptides are highly prone to adopt sheet packing patterns, even if the residues have low  $\beta$ -sheet propensities. We have recently shown that  $\beta$ -peptide residues containing one  $\alpha$ - and one  $\beta$ -substituent, with appropriate relative configuration, adopt antiparallel sheet secondary structure in solution and in the solid state.<sup>7</sup> A heterochiral dinipeptidic acid segment forms a reverse turn,<sup>8b</sup> the  $\beta$ -peptide equivalent of the familiar  $\beta$ -turn from conventional peptides. Seebach et al. have recently observed a reverse turn in a tri- $\beta$ -peptide crystal structure;<sup>5</sup> it will be interesting to see whether this segment displays a turn-forming propensity in solution.

There has been considerable interest in the conformational effects of placing  $\beta$ -amino acid residues, especially  $\beta$ -alanine, into peptides containing mostly  $\alpha$ -amino acid residues. Much of this work has focused on cyclic peptides. An early example is a cyclic tetramer containing two  $\alpha$ -amino acid residues and two  $\beta$ -alanine residues, for which a crystal structure was determined.<sup>36</sup> Several additional crystal structures, along with structural data from NMR, have been reported for small cyclic peptides containing one or two  $\beta$ -alanine residues.<sup>37</sup> Both Seebach et al.<sup>38</sup> and Ghadiri et al.<sup>39</sup> have described cyclic tetrapeptides containing exclusively  $\beta$ -amino acid residues. Two recent studies have focused on mimicry of standard secondary

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structures by  $\beta$ -amino acid insertions into acyclic peptides. Karle, Balam, and co-workers have shown that a  $\beta$ -alanyl- $\beta$ -aminobutyryl segment, which mimics a triglycine segment, can participate in helix formation.<sup>40</sup> Hanessian and Yang have observed that a segment containing a disubstituted  $\beta$ -amino acid residue followed by proline can induce reverse turn formation.<sup>41</sup> Gung et al. have examined the folding of small oligoamides containing  $\beta$ -amino acid residues in organic solvents.<sup>42</sup>

**Implications for  $\beta$ -Peptide Tertiary Structure.** The packing pattern displayed by hexamer **2** suggests a strategy for creating  $\beta$ -peptides that adopt a well-defined tertiary structure, because there are extensive cyclohexyl–cyclohexyl contacts between adjacent molecules. (In contrast, the packing of tetramer **1** cannot provide insight on tertiary packing of 14-helical  $\beta$ -peptides because the side-to-side contacts occur between cyclohexane rings and terminal phenyl groups or solvent.) The “tight” three-fold cyclohexane zipper interaction (Figures 5 and 6) seems likely to be a significant source of lattice stability for solid **2**, although we acknowledge that it is impossible to identify definitively the origin of any packing pattern’s stability by simple inspection. It should be possible to use this trimeric cyclohexane zipper packing motif to design  $\beta$ -peptides that adopt a three-helix bundle tertiary structure in aqueous solution, since the cyclohexane–cyclohexane contacts should be promoted by the hydrophobic effect. In crystalline **2**, there are three independent molecules, and there are three slightly different versions of the tight three-fold interaction. The amount of water-accessible surface area buried in each of these trimolecular clusters was estimated with the program MacroModel 6.0, using a spherical probe with 1.4-Å radius (approximating a water molecule). The water-accessible surfaces of isolated molecules of **2** in the solid-state conformations fall between 1103 and 1142 Å<sup>2</sup>. Assembly of the isolated molecules into the trimolecular clusters observed in crystalline **2** buries between 562 and 743 Å<sup>2</sup> (the variation arises in part from differences in benzyl group orientation).

Creating water-soluble  $\beta$ -peptides will require incorporation of hydrophilic residues to complement the hydrophobic *trans*-ACHC residues. Because the 14-helix has approximately three residues per turn,  $\beta$ -peptides that contain a *trans*-ACHC residue at every third position and hydrophilic residues at the other positions should adopt helical conformations with a hydrophobic “stripe” running along one side. Among conventional peptides ( $\alpha$ -amino acid residues), helices with well-defined hydrophilic and hydrophobic surface patches are referred to as “amphiphilic”. Relatively short amphiphilic  $\alpha$ -helices self-assemble into discrete helical bundle aggregates, and this intermolecular assembly has been parlayed into helical bundle tertiary structure formation, an intramolecular process, by connecting amphiphilic  $\alpha$ -helical segments with short loops.<sup>43–46</sup> An analogous design strategy could lead to a  $\beta$ -peptide three-helix bundle tertiary structure built around a cyclohexyl hydrophobic core.

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## Experimental Section

**General Procedures.** Melting points (mp) were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter using sodium light (D line, 589.3 nm) and are reported in degrees; concentrations (*c*) are reported in g/100 mL. Infrared spectra (IR) were obtained on a Mattson Polaris FT-IR spectrophotometer or a Nicolet 740 FT-IR spectrometer. Absorption maxima are reported in wavenumbers (cm<sup>-1</sup>) and are standardized to the 1601 cm<sup>-1</sup> reference peak of polystyrene. <sup>1</sup>H NMR spectra were recorded in deuterated solvents on a Bruker AC-300 (300 MHz) spectrometer. <sup>1</sup>H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), or quartet (q). All first-order splitting patterns are assigned on the basis of the appearance of the multiplet. Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m) or broad (br). <sup>13</sup>C NMR spectra were recorded on a Bruker AC-300 spectrometer. Carbon resonances were assigned using distortionless enhancement by polarization transfer (DEPT) spectra obtained with a phase angle of 135°: (C) not observed; (CH) positive; (CH<sub>2</sub>) negative; (CH<sub>3</sub>) positive. Mass spectra were obtained using a Kratos MS-80 mass spectrometer with a magnetic sector and direct probe when electron impact (EI, 70 eV) was the ionization method. Fast atom bombardment (FAB) mass spectra were obtained on a Micromass AutoSpec (Cs ion gun) with magnetic sector and direct probe using 3-nitrobenzyl alcohol as the ionization matrix.

Isoamyl alcohol was distilled prior to use and stored over 4-Å molecular sieves. Dimethyl formamide (DMF) was distilled from ninhydrin under vacuum at 25 °C and stored over activated 3-Å molecular sieves, under N<sub>2</sub>, at 4 °C. Benzene was distilled from sodium/benzophenone ketyl. Hexanes were distilled at atmospheric pressure. Unless otherwise noted, all other commercially available reagents and solvents were purchased from Aldrich and used without further purification, except for 4 N HCl in dioxane, which was purchased from Pierce. Analytical thin-layer chromatography (TLC) was carried out on Whatman TLC plates precoated with silica gel 60 (250 μm layer thickness). Column chromatography was performed with EM Science silica gel 60 (230–400 mesh). Solvent mixtures used for TLC and column chromatography are reported in v/v ratios.

(+)-(S,S)- and (-)-(R,R)-*N*-benzoyl-*trans*-2-aminocyclohexanecarboxylic acid ((+)-**3** and (-)-**3**) were prepared by seed-induced enantioselective crystallization, following a reported procedure.<sup>20</sup> From 4 g of (±)-*N*-benzoyl-*trans*-2-aminocyclohexanecarboxylic acid,<sup>18,19</sup> 1.63 g (41% yield) of the (-)-(R,R) enantiomer was obtained, [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -45° (*c* 0.2, EtOH), and 0.395 g (10% yield) of the (+)-(S,S) enantiomer was obtained, [ $\alpha$ ]<sub>D</sub><sup>23</sup> = +45° (*c* 0.2, EtOH).

**Boc-*trans*-2-aminocyclohexanecarboxylic acid (Boc-*trans*-ACHC-OH).** Crystals of (-)-*N*-benzoyl-*trans*-2-aminocyclohexanecarboxylic acid (0.5 g, 2.02 mmol) were suspended in 12 N HCl (55 mL). The solution was refluxed 48 h, and the crystals eventually dissolved. After the solution cooled to room temperature, the solution was cooled to 0 °C for 1 h in an ice bath, and then precipitated benzoic acid was collected by suction filtration. The filtrate was concentrated on a rotary evaporator to a few milliliters, and then 2:1 dioxane/H<sub>2</sub>O (30 mL) was added, followed by K<sub>2</sub>CO<sub>3</sub> (2.8 g, 20 mmol) and Boc<sub>2</sub>O (0.441 g, 2.02 mmol). The reaction was stirred for 5 h. The pH of the reaction was adjusted to 2 with 12 N HCl, and the resulting solution was then extracted with EtOAc (3×, 150 mL total). The organic extracts were dried over MgSO<sub>4</sub>, concentrated, and dried under vacuum to afford 0.41 g (84% yield) of the desired product as a white solid. Crystals were grown by slow evaporation of a solution of the product in 1,2-dichloroethane: mp 154–155 °C; [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -22.8 (*c* 0.325, CHCl<sub>3</sub>); IR (KBr) 3315 (N–H), 3045–2703 (broad), 2937, 2859, 1738 (C=O), 1657, 1545, 1348, 1284, 1163, 1124, 1051, 862, 690 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  10.15 (br, 1H, COOH), 4.68 (br, 1H, NH), 3.64 (br m, 1H), 2.26 (td, *J* = 11, 3 Hz, HOOCCH), 2.12–1.9 (m, 2H), 1.8–1.11 (m, 6H), 1.43 (s, 9H, CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75.4 MHz)  $\delta$  178.9 (C), 155.1 (C), 79.4 (C), 51.0 (CH), 49.6 (CH), 32.8 (CH<sub>2</sub>), 28.6 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 24.6 (CH<sub>2</sub>), 24.4 (CH<sub>2</sub>); EI-MS *m/z* (M<sup>+</sup>) calcd for C<sub>12</sub>H<sub>21</sub>NO<sub>4</sub> 243.1471, obsd 243.1475.

**Boc-*trans*-ACHC-OBn.** Boc-*trans*-ACHC-OH (0.2 g, 0.82 mmol) was dissolved in dry benzene (20 mL). Benzyl bromide (0.1 mL, 0.82 mmol) and 1,4-diazabicycloundecene (0.12 mL, 0.82 mmol) were added, and the solution was refluxed under N<sub>2</sub> for 6 h. White solid precipitated from the solution during this time. The mixture cooled to room temperature. The solution was then filtered through a fritted funnel, and the filtrate was concentrated to obtain a tan solid. The product was purified by SiO<sub>2</sub> column chromatography, eluting with 70:1 CHCl<sub>3</sub>/EtOAc (*R<sub>f</sub>* = 0.31), to afford 0.228 g (83% yield) of Boc-*trans*-ACHC-OBn as a white solid that was recrystallized from *n*-heptane: mp 103 °C; IR (KBr) 3375 (N–H), 2929, 2854, 1732 (C=O), 1684, 1527, 1369, 1315, 1271, 1176, 1014, 727 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.38–7.29 (m, 5H, ArH), 5.11 (s, 2H, ArCH<sub>2</sub>), 4.55 (br, 1H, NH), 3.69 (br td, *J* = 10.5, 9 Hz, 1H, BocHNCH), 2.30 (td, *J* = 12, 3 Hz, 1H, BnOCOCH), 2.06 (br dq, *J* = 12, 4 Hz, 1H), 1.98–1.88 (m, 1H), 1.79–1.53 (m, 3H), 1.39 (s, 9H, CH<sub>3</sub>), 1.36 (m, 1H), 1.27–1.08 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75.4 MHz) δ 173.8 (C), 154.9 (C), 136.0 (C), 128.5 (CH), 128.0 (CH), 79.2 (C), 66.3 (CH<sub>2</sub>), 51.3 (CH), 50.0 (CH), 33.0 (CH<sub>2</sub>), 28.6 (CH<sub>2</sub>), 28.3 (CH<sub>3</sub>), 24.6 (CH<sub>2</sub>), 24.4 (CH<sub>2</sub>); EI-MS *m/z* (M + H<sup>+</sup>) calcd for C<sub>19</sub>H<sub>28</sub>NO<sub>4</sub> 334.2018, obsd 334.2000.

**Boc-*(trans*-ACHC)<sub>2</sub>-OBn (5).** Boc-*trans*-ACHC-OBn (0.047 g, 0.14 mmol) was dissolved in 4 N HCl/dioxane (0.5 mL) and stirred for 1 h. The solvent was then removed under a stream of N<sub>2</sub>, and the residue was dried under vacuum. Boc-*trans*-ACHC-OH (0.034 g, 0.14 mmol) and DMAP (0.023 g, 0.19 mmol) were added to the flask, followed by DMF (1 mL). EDCI·HCl (0.059 g, 0.31 mmol) was added, and the reaction was stirred for 48 h under N<sub>2</sub>. Solvent was removed under a stream of N<sub>2</sub>, and the residue was further dried under vacuum. To this residue was added 1 N HCl (approximately 3 mL), and the solid that did not dissolve was isolated by suction filtration and washed with additional 1 N HCl. The solid was dried under vacuum to afford 0.051 g (79% yield) of Boc-*(trans*-ACHC)<sub>2</sub>-OBn as a white solid. Fluffy crystals were grown by vapor diffusion of *n*-heptane into a solution of Boc-*(trans*-ACHC)<sub>2</sub>-OBn in 1,2-dichloroethane: mp 195 °C, IR (KBr) 2773, 1744 (C=O), 1685, 1651, 1540, 1176, 1027 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.40–7.27 (m, 5H, ArH), 6.07 (br d, *J* = 6 Hz, 1H, NH), 5.11 (AB quartet, *J* = 12 Hz, 1H, ArCH<sub>2</sub>), 5.05 (AB quartet, *J* = 12 Hz, 1H, ArCH<sub>2</sub>), 4.55 (br d, *J* = 9 Hz, 1H, NH), 4.05 (tdd, *J* = 11, 9, 4 Hz, 1H, BocHNCH), 3.45 (tdd, *J* = 11, 9, 4 Hz, 1H, CONHCH), 2.36 (td, *J* = 11, 4 Hz, 1H, HNCOCH), 2.14–1.81 (m, 5H), 1.78–1.53 (m, 14H), 1.41 (s, CH<sub>3</sub>), 1.38–1.04 (m, 6H); <sup>1</sup>H NMR (CD<sub>3</sub>OH, 300 MHz) δ 7.63 (br, 1H, NH), 7.41–7.23 (m, 5H, ArH), 6.17 (br, 1H, NH), 3.94 (m, 1H, CONHCH), 3.52 (m, 1H, CONHCH), 2.44 (td, *J* = 11, 3 Hz, HNCOCH), 2.06–1.78 (m, 4H), 1.77–1.45 (m, 5H), 1.44–1.04 (m, 16H), 1.35 (s, CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75.4 MHz) δ 173.6 (C), 173.1 (C), 155.6 (C), 136.0 (C), 128.4 (CH), 128.1 (CH), 128.0 (CH), 79.3 (C), 66.2 (CH<sub>2</sub>), 52.6 (CH), 50.8 (CH), 49.6 (CH), 49.5 (CH), 33.7 (CH<sub>2</sub>), 32.2 (CH<sub>2</sub>), 30.6 (CH<sub>2</sub>), 28.3 (CH<sub>3</sub>), 25.0 (CH<sub>2</sub>), 24.4 (CH<sub>2</sub>); FAB-MS *m/z* 917.6 (2M + H<sup>+</sup>), 459.3 (M + H<sup>+</sup>), 359.2 (M + H<sup>+</sup> – Boc).

**Boc-*(trans*-ACHC)<sub>3</sub>-OBn (6), Boc-*(trans*-ACHC)<sub>4</sub>-OBn (1), and Boc-*(trans*-ACHC)<sub>6</sub>-OBn (2)** were prepared by coupling smaller fragments, using procedures analogous to those described for the synthesis of **5**. In each oligomer, all *trans*-ACHC units were of the same absolute configuration. Characterization data for these oligomers may be found in the Supporting Information.

**Crystallization and X-ray Analysis.** X-ray quality crystals of tetramer **1** and hexamer **2** were grown by vapor diffusion of a nonpolar solvent (distilled hexanes or HPLC grade heptane) into a solution of the molecule in 1,2-dichloroethane (distilled) and HPLC grade methanol. The X-ray diffraction data were measured at –140 °C using a Bruker SMART CCD area detector on a four-circle diffractometer equipped with Mo Kα radiation (λ = 0.710 73 Å). Data for both compounds were collected as a series of φ scan frames, each with a width of 0.3°/frame. The exposure times were 30 s/frame for **1** and 40 s/frame for **2**. Crystals of **1** [C<sub>40</sub>H<sub>60</sub>N<sub>4</sub>O<sub>7</sub>·0.94(C<sub>2</sub>H<sub>4</sub>Cl<sub>2</sub>), FW = 801.93] form in the orthorhombic space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with *a* = 11.6175(3) Å, *b* = 23.1923(5) Å, *c* = 33.2538(9) Å, *V* = 8959.8(4) Å<sup>3</sup>, *Z* = 8, and calculated ρ = 1.189 g/cm<sup>3</sup>. Compound **2** [C<sub>54</sub>H<sub>82</sub>N<sub>6</sub>O<sub>9</sub>·0.33(CH<sub>3</sub>OH)·0.80(C<sub>2</sub>H<sub>4</sub>Cl<sub>2</sub>), FW = 1049.24] crystallizes in the mono-

clinic space group *P*2<sub>1</sub>, with *a* = 15.3932(3) Å, *b* = 21.7643(4) Å, *c* = 27.8692(2) Å, β = 101.457(2)°, *V* = 9150.8(3) Å<sup>3</sup>, *Z* = 6, and calculated ρ = 1.142 g/cm<sup>3</sup>. Empirical absorption corrections for both compounds were based on equivalent measured intensities. For compound **1**, the 35 619 measured data out to 26.053° in θ were merged to give 15 248 unique data with *R*<sub>int</sub> = 0.0744. The 28 218 measured data out to 22.5° in θ for compound **2** were merged to give 21 585 unique data with *R*<sub>int</sub> = 0.0279.

Compound **1** was solved by direct methods. Compound **2** was solved using the PATSEE program<sup>47</sup> and a 34 atom model of the backbone from **1**. The PATSEE program first performs a three-dimensional rotational fit of the model atom vectors to a vector (Patterson) map calculated from the intensity data and then performs a three-dimensional translation search of the oriented fragment with a subset of the intensity data. The phases of the subset data for this new model are extended to other data,<sup>48</sup> and this expanded data set is used to compute an E-map<sup>49</sup> (a modified electron density map) that should show more or all of the atoms.

Both compounds were refined by full-matrix least-squares methods, minimizing Δ*F*<sup>2</sup> (ref 50). For both structures, the non-hydrogen atoms were refined with anisotropic displacement parameters. The positions for hydrogen atoms were calculated from model geometry. The 1018 variables of compound **1** were refined against 85 restraints and 15 234 data to give *wR*(*F*<sup>2</sup>) = 21.17% with a goodness-of-fit, *S*, = 1.184. For the 10 942 observed [*F* > 4σ(*F*)] data, the final *R*(*F*) was 8.49%. For compound **2**, a total of 2059 variables were refined against 638 restraints and 21 585 data to produce *wR*(*F*<sup>2</sup>) = 28.00% with *S* = 1.137, and *R*(*F*) = 10.91% for 14 406 observed [*F* > 4σ(*F*)] data. Restraints were applied only to disordered regions of the structures.

**CD Experiments.** Dry peptide samples were weighed and dissolved in an appropriate amount of HPLC grade methanol. Sample cells of 1-mm path length were typically used. Data were collected on an Aviv 62A-DS spectrometer at 25 °C. Baseline spectra were recorded with only solvent in the cell. Baseline spectra were subtracted from the raw data. Data were converted to ellipticity (deg cm<sup>2</sup> dmol<sup>-1</sup>) according to the following equation:

$$[\Theta] = \psi M_r / (100lc)$$

where ψ is the CD signal in degrees, *M<sub>r</sub>* is the molecular weight divided by the number of chromophores, *l* is the path length in decimeters, and *c* is the concentration in grams per milliliter.

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**Supporting Information Available:** Tables of crystal data, structure solution and refinement, atomic coordinates, bond lengths and angles, torsion angles, and hydrogen bond parameters for **1**, **2**, and (–,+)-**4**, and experimental protocols (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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