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Oligopeptides with Equal Amounts of L- and D-Amino Acids May Prefer a Helix Screw Sense

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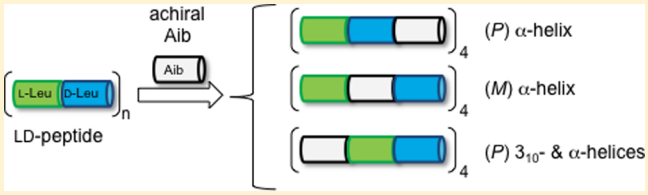
Supporting Information

ABSTRACT: We investigated the preferred conformations of two nonapeptides, Boc-(L-Leu-D-Leu-Aib)$_3$-OMe (**2**) and its enantiomer Boc-(D-Leu-L-Leu-Aib)$_3$-OMe (*ent*-**2**), four dodecapeptides, Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**3**), Boc-(L-Leu-Aib-D-Leu)$_4$-OMe (**4**), Boc-(Aib-L-Leu-D-Leu)$_4$-OMe (**5**), and Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe (**6**), and a decapeptide, Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (**7**), in solution and in the crystalline state. The nonapeptide **2** formed a right-handed (*P*) α-helix, and its enantiomer *ent*-**2** formed a left-handed (*M*) α-helix. The dodecapeptides **3** and **5** were folded into (*P*) helices, and **4** formed an (*M*) helical structure. As for **6**, roughly equivalent amounts of (*P*) and (*M*) helices were observed in solution, and two (*M*) α-helices were detected in the crystalline state. Furthermore, the decapeptide **7**, which possesses four L-Leu residues and three D-Leu residues, was folded into an (*M*) α-helix.



INTRODUCTION

The folding of oligomer and polymer molecules is intimately associated with the functions of biomolecules, including DNA and proteins. Among the folded structures found in biomolecules, helices are considered among the most important structures in a variety of fields, such as biology, chemistry, and medicinal chemistry. Therefore, various helical oligomers composed of rigid building blocks, such as α-peptides,[1] β-peptides,[2] aromatic amide oligomers,[3] and urea-type oligomers,[4] have been developed. Such helices exhibit right-handed (*P*) or left-handed (*M*) screw senses depending on the chirality of their building blocks. Natural α-helical L-peptides usually have a (*P*) screw sense because they are composed of L-amino acids (L-AA) with α-chiral centers.[5] Conversely, enantiomeric D-peptides have an (*M*) screw sense. On the other hand, it is difficult for LD-oligopeptides with alternating L-AA and D-AA residues to form one-handed α-helices,[6] and hence, they usually fold into wide-pore β- and π-helices with semiextended LD-dipeptide conformations.[7] We have recently reported that the insertion of achiral α-aminoisobutyric acid (Aib) residues into LD-peptides is useful for controlling their α-helical structures.[8] Herein, we describe the positional influence of Aib residues on the helical structures of LD-peptides. That is to say, we designed and synthesized two nonapeptides, Boc-(L-Leu-D-Leu-Aib)$_3$-OMe (**2**) and its enantiomer Boc-(D-Leu-L-Leu-Aib)$_3$-OMe (*ent*-**2**), and four dodecapeptides, Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**3**), Boc-(L-Leu-Aib-D-Leu)$_4$-OMe (**4**), Boc-(Aib-L-Leu-D-Leu)$_4$-OMe (**5**), and Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe (**6**), and studied their preferred conformations in solution and in the crystalline state. Furthermore, we also analyzed the conformation of a decapeptide, Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (**7**), containing four L-Leu and three D-Leu residues to investigate whether it forms a (*P*) helical structure (Figure 1). Conformational analyses of peptides **2**−**6** revealed that alternating dipeptide L-Leu-D-Leu segments act as single chiral inducers that control the helical screw sense of LD-peptides and Aib residues have important effects on the folding of the α-helical structures of LD-peptides. The dominant conformation of **7** was an (*M*) α-helix, despite it possessing four L-Leu residues and three D-Leu residues. This indicates that the presence of three D-Leu-L-Leu segments within a molecule, which would tend to produce an (*M*) helical screw sense, has a stronger influence on the chirality of the molecule than the presence of a single N-terminal L-Leu residue, which would tend to induce a (*P*) helical screw sense.

RESULTS

Synthesis of Peptides. Nonapeptides Boc-(L-Leu-D-Leu-Aib)$_3$-OMe (**2**) and Boc-(D-Leu-L-Leu-Aib)$_3$-OMe (*ent*-**2**), dodecapeptide Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**3**), Boc-(L-Leu-Aib-D-Leu)$_4$-OMe (**4**), Boc-(Aib-L-Leu-D-Leu)$_4$-OMe (**5**), Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe (**6**), and decapeptide Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (**7**) were synthesized by conventional

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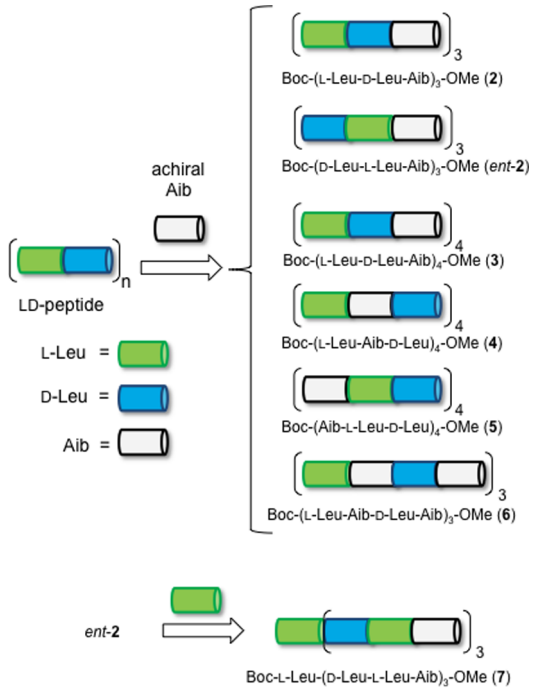


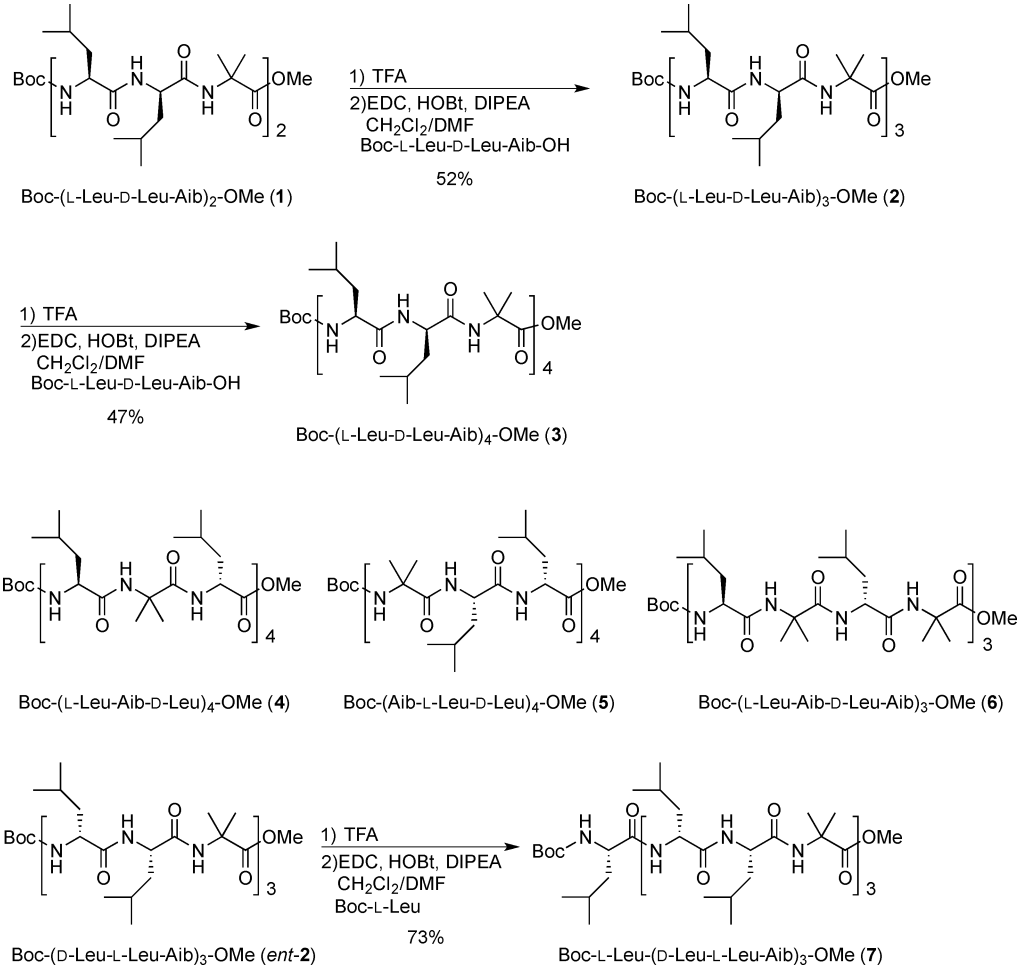
Figure 1. Structures of the peptides used in this study.

solution-phase methods according to a fragment condensation strategy using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) hydrochloride and 1-hydroxybenzotriazole (HOBt) hydrate as coupling reagents (Scheme 1).[9] After deprotection of the Boc-protecting group in the hexapeptide Boc-(L-Leu-D-Leu-Aib)$_2$-OMe (**1**),[10] the resultant N-terminal free hexapeptide was coupled with the tripeptide acid Boc-L-Leu-D-Leu-Aib-OH to give the nonapeptide **2** in 52% yield. The dodecapeptide **3** was synthesized from the nonapeptide **2** in a 47% yield. The enantiomeric nonapeptide *ent*-**2** and dodecapeptides **4**–**6** were prepared using similar methods. The decapeptide **7** was synthesized via coupling with Boc-L-Leu and the nonapeptide amine from *ent*-**2** in a 73% yield.[11]

Conformational Analysis in Solution. The Fourier transform infrared (FT-IR) spectra of peptides **3**, **6**, and **7** in the NH-stretching region (amide A 3200–3500 cm^{-1}) were measured in $CDCl_3$ solution. Representative examples are shown in Figure 2. The weak bands around the 3420 cm^{-1} region were assigned to free peptide NH groups, and the strong bands around the 3320 cm^{-1} region were assigned to peptide NH groups with N–H···O=C intramolecular hydrogen bonds. These three IR spectra were very similar to those of helical peptides in solution.[12] The other peptides also exhibited spectra that were indicative of helical peptides (data not shown).

The CD spectra of the synthetic peptides were measured in 2,2,2-trifluoroethanol (TFE) solution. The CD spectra of **2** and

Scheme 1. Synthesis of the Nonapeptides 2 and *ent*-2, the Dodecapeptides 3–6, and the Decapeptide 7



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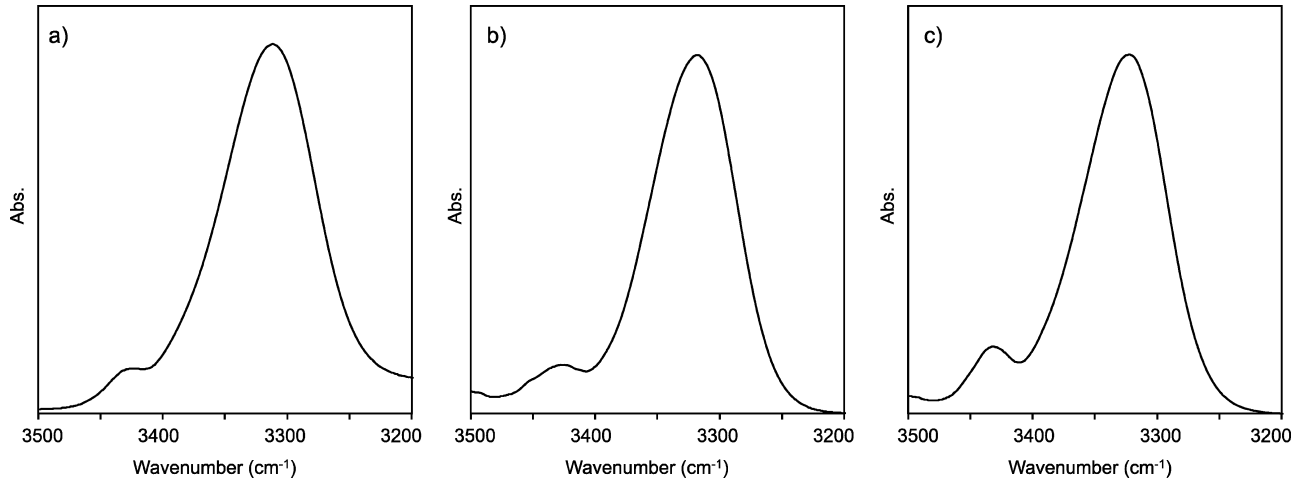

Figure 2. Representative IR spectra of (a) Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**3**), (b) Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe (**6**), and (c) Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (**7**) in $CDCl_3$ solution. Peptide concentration: 2.0 mM.

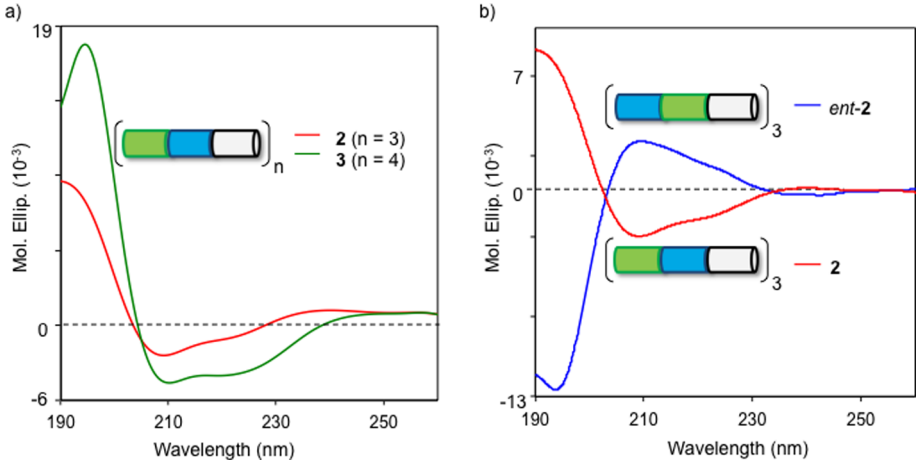

Figure 3. CD spectra in the 190−260 nm region of the nonapeptides **2** and *ent*-**2**, and the dodecapeptide **3**: (a) nonapeptide **2** (n = 3, red) and the dodecapeptide **3** (n = 4, green); (b) nonapeptide **2** (red) and its corresponding enantiomer *ent*-**2** (blue). Peptide concentration: 0.5 mM in TFE solution.

3 showed negative maxima at around 209 and 224 nm, indicating that they possessed a right handed (P) helical screw sense (Figure 3A).[13] However, both of these signals were weak, suggesting that the screw senses of these peptides were not tightly controlled. Based on the R ratios ($\theta_{224}/\theta_{209}$) of peptides **2** and **3**, it was determined that both molecules form α-helices as their dominant conformations (R = 0.7 for **2**, R = 0.8 for **3**).[14] From considering the R values of **2** and **3**, the α-helical structure of the dodecapeptide **3** is more stable than that of the nonapeptide **2**.[14,15] On the other hand, *ent*-**2** formed a left-handed (M) α-helix and displayed positive maxima at 209 and 224 nm (Figure 3B).[1d,15]

Figure 4 shows the CD spectra of **3**−**6**. The peptide **4** displayed positive maxima at around 209 and 225 nm, giving it an (M) helical screw sense. The R value of **4** was 0.9, suggesting that it forms an α-helix as its preferred secondary structure. The CD spectrum of **5** exhibited negative maxima at 206 and 223 nm (R = 0.5), which was indicative of a mixture of right-handed (P) 3_{10}- and α-helices. On the other hand, **6** did not show the characteristic maxima of a helical structure, which suggested that roughly equivalent amounts of (P) and (M) helices were present in **6**.

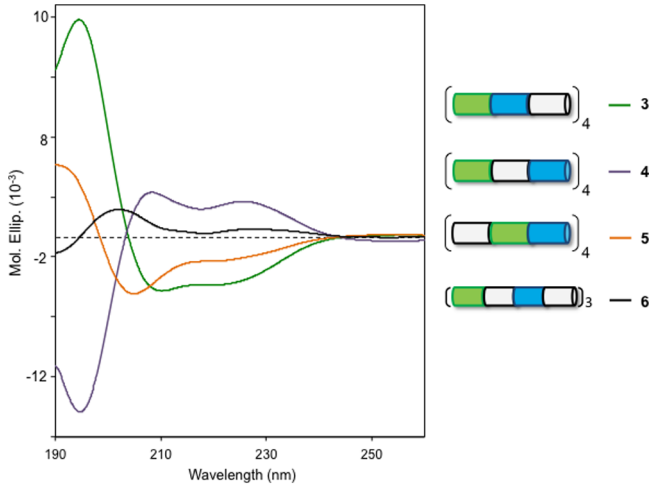

Figure 4. CD spectra of the dodecapeptides **3** (green), **4** (purple), **5** (orange), and **6** (black). Peptide concentration: 0.5 mM in TFE solution.

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The CD spectrum of **7**, which contained a single L-Leu residue at the N-terminus of its repeating D-Leu-L-Leu-Aib sequence, contained a pattern similar to that of *ent*-**2**, indicating that the helical screw sense of **7** was left handed (Figure 5). Based on the *R* value of **7** (*R* = 0.6), its dominant conformations were considered to include a mixture of (*M*) 3_{10}- and α-helices.

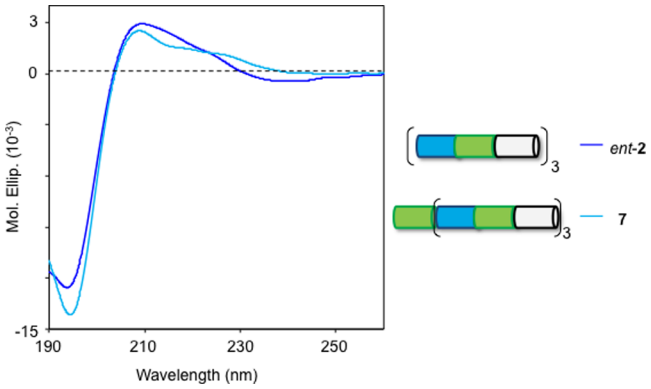

Figure 5. CD spectra of the nonapeptide *ent*-**2** (blue) and the decapeptide **7** (cyan). Peptide concentration: 0.5 mM in TFE solution.

Conformations of Peptides 2–4, 6, and 7 in the Crystalline State. The nonapeptide **2**, the dodecapeptides **3**, **4**, and **6**, and the decapeptide **7** produced suitable crystals for X-ray crystallographic analysis via the slow evaporation of the relevant solvents (1,4-dioxane/H_2O for **2** and **3**, MeOH/H_2O for **4** and **7**, and MeOH/CH_2Cl_2 for **6**) at room temperature. The structures of the peptides were solved using the SHELXS 97 direct methods[16] and expanded using the Fourier technique.[17] All non-H atoms were given anisotropic thermal parameters, some H atoms were refined isotropically, and the remaining H atoms were placed at the calculated positions.[18] The crystal and diffraction parameters of the peptides, the relevant backbone and side chain torsion angles, and intra- and intermolecular hydrogen-bond parameters are summarized in the Supporting Information.[11]

A right-handed (*P*) α-helix in which the C-terminal D-Leu (**8**) residue had been flipped was detected in the nonapeptide Boc-(L-Leu-D-Leu-Aib)$_3$-OMe (**2**) (Figure 6). This C-terminal flipping is referred to as a Schellman motif and is found in some proteins.[19] The mean ϕ and ψ torsion angles of its amino acid residues (1–6) were −59.2° and −47.2°, respectively, which are close to those of an ideal right-handed (*P*) α-helix (−60° and −45°, respectively). The flipped ϕ and ψ torsion angles of the D-Leu (**8**) residue were positive (ϕ = +64.7°, ψ = +39.4°).

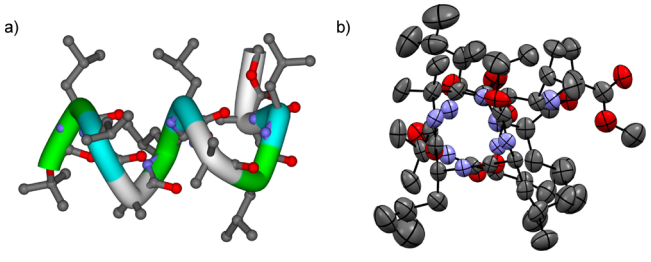

Figure 6. X-ray diffraction structure of **2** (a) as viewed perpendicular to the helical axis and (b) an ORTEP drawing as viewed along the helical axis.

One $i \leftarrow i+3$ type, three $i \leftarrow i+4$ type, and one $i \leftarrow i+5$ type hydrogen bonds were found in the α-helical molecule. Furthermore, a water molecule was captured by the hydrogen bond between H−O_w and C(7)=O(7). The helical conformers were connected by intermolecular hydrogen bonds to form head-to-tail aligned chains.[11]

The dodecapeptide Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**3**) exclusively crystallized into a (*P*) α-helical conformer containing 1,4-dioxane and water molecules (Figure 7). The mean ϕ and ψ

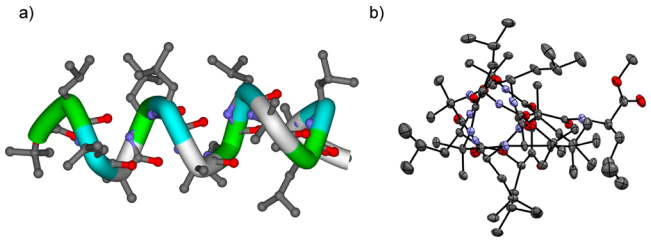

Figure 7. X-ray diffraction structure of **3** (a) as viewed perpendicular to the helical axis and (b) an ORTEP drawing as viewed along the helical axis. The 1,4-dioxane and water molecules have been omitted.

torsion angles of its amino acid residues 1–10 were −59.4° and −42.4°, respectively. The values of the flipped ϕ and ψ torsion angles of the D-Leu(11) residue were ϕ = +85.0° and ψ = +15.9°, and those of the Aib(12) residue were ϕ = +46.7° and ψ = +38.2°. In the molecule, two $i \leftarrow i+3$ type, seven $i \leftarrow i+4$ type, and one $i \leftarrow i+5$ type hydrogen bonds were detected. In the packing mode, the α-helical molecules were connected by water molecule-mediated intermolecular hydrogen bonds, forming head-to-tail aligned chains.[11]

A left-handed (*M*) α-helix and a methanol molecule were detected in the asymmetric unit of the dodecapeptide Boc-(L-Leu-Aib-D-Leu)$_4$-OMe (**4**) (Figure 8). The mean ϕ and ψ

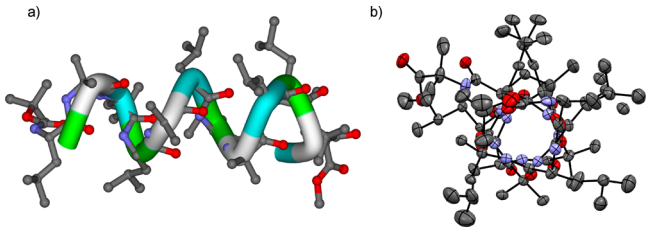

Figure 8. X-ray diffraction structure of **4** (a) as viewed perpendicular to the helical axis and (b) an ORTEP drawing as viewed along the helical axis. The methanol molecule has been omitted.

torsion angles of its residues (1–10) were +57.9° and +42.5°, respectively, which are close to those of an ideal left-handed (*M*) α-helix (+60° and +45°, respectively). A reversal of the Aib(11) torsion angles occurred; i.e., the ϕ and ψ torsion angles of the residue turned negative (−58.1° and −50.7°, respectively). Eight intramolecular hydrogen bonds were found in the α-helical molecule of **4**; i.e., two $i \leftarrow i+3$ type, six $i \leftarrow i+4$ type hydrogen bonds were present. Furthermore, a methanol molecule was held in the peptide backbone by the two hydrogen bonds between H−N(12) and O_M, and H−O_M and C(7)=O(7). In packing mode, the α-helical conformers were connected by two intermolecular hydrogen bonds, forming head-to-tail aligned chains.[11]

Two independent crystallographic molecules, *A* and *B*, were detected in the asymmetric unit of the dodecapeptide Boc-(L-

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Leu-Aib-D-Leu-Aib)$_3$-OMe (**6**). Both molecules were folded into left-handed (*M*) α-helices, in which the N- and C-terminal residues were flipped (Figures 9 and 10). The two molecules

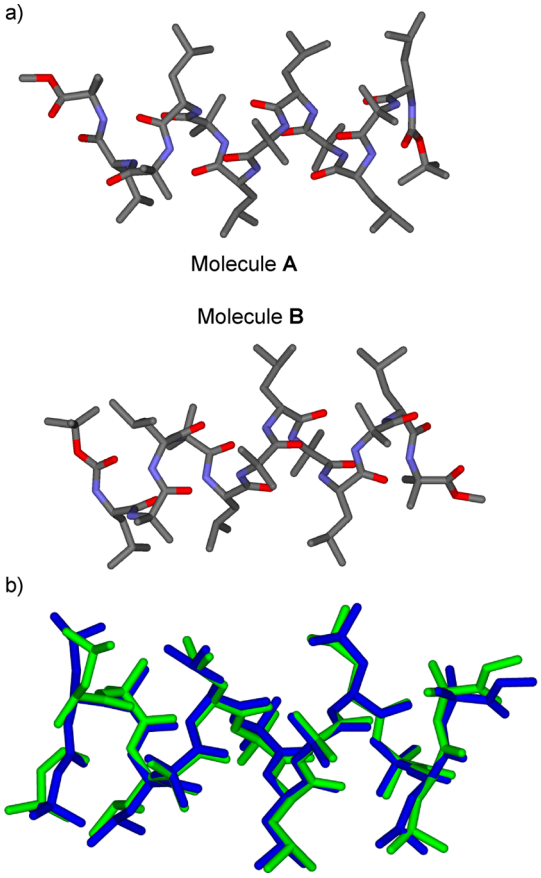

Figure 9. (a) X-ray diffraction structure of **6**. (b) Superimposed structures of molecules *A* (green) and *B* (blue).

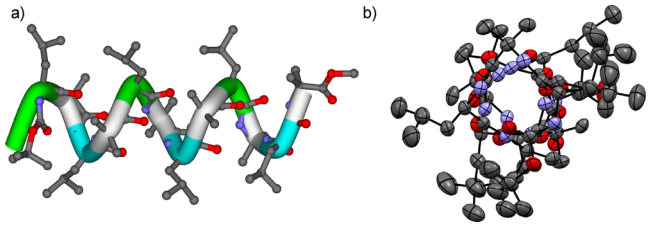

Figure 10. X-ray diffraction structure of molecule *A* (a) as viewed perpendicular to the helical axis and (b) an ORTEP drawing as viewed along the helical axis.

formed similar secondary structures but showed small differences in their side-chain conformations and N- and C-terminal peptide backbones, as shown by the overlaid structures in Figure 9b. The mean ϕ and ψ torsion angles of the α-helical residues (2−10) were +57.4° and +45.4°, respectively, for *A*, and +58.4° and +43.3°, respectively, for *B*. Regarding the intramolecular hydrogen bonds in molecule *A* and *B*, two $i \leftarrow i + 3$ type and eight $i \leftarrow i + 4$ type hydrogen bonds were observed, respectively. In packing mode, molecules *A* and *B* were alternately connected by intermolecular hydrogen bonds.[11]

The structure of the decapeptide Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (**7**), which was solved using the spacer group *P*1, was a left-handed (*M*) $\alpha/3_{10}$-helix [α-helix from D-Leu(2) to D-Leu(5); 3_{10}-helix from L-Leu(6) to D-Leu(8)] with an extended L-Leu(1) residue conformation and a flipped L-Leu(9) residue (Figure 11). The mean ϕ and ψ torsion angles of the amino

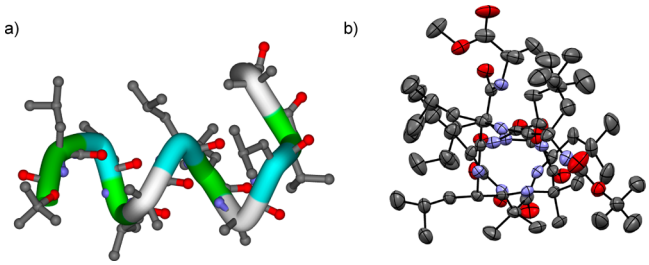

Figure 11. X-ray diffraction structure of **7** (a) as viewed perpendicular to the helical axis and (b) an ORTEP drawing as viewed along the helical axis.

acid residues (2−8) were +61.3° and +37.2°, respectively. The extended ϕ and ψtorsion angles of the D-Leu(1) residue were −160.7° and +136.7°, respectively, and the flipped ϕ and ψtorsion angles of the D-Leu (9) residue were −73.6° and −29.8°, respectively. Three different types of intramolecular hydrogen bond were observed: two $i \leftarrow i+3$ type, three $i \leftarrow i+4$ type, and an $i \leftarrow i+5$ type hydrogen bonds were detected. In packing mode, the helical molecules were connected by two intermolecular hydrogen bonds, forming head-to-tail aligned chains.[11]

DISCUSSION

The preferred conformations of the Boc-(L-Leu-D-Leu-Aib)$_n$-OMe peptides (n = 3 for **2**, n = 4 for **3**) were right-handed (*P*) α-helices both in solution and in the crystalline state, whereas the enantiomeric peptide Boc-(D-Leu-L-Leu-Aib)$_3$-OMe (*ent*-**2**) folded into a left-handed (*M*) α-helix. L-AA have a propensity to confer right-handedness on helical peptides because steric repulsion arises between the β-carbon atoms of the side chains (Cβ) and the oxygen atoms of the carbonyl groups (C═O) in α-helical L-peptides with a left-handed screw sense. Therefore, it might be difficult for LD-oligopeptides containing equal amounts of L-AA and D-AA residues to form one-handed α-helices, and hence, they usually fold into β- and π-helices. We suggest the following two reasons why the repeating L-Leu-D-Leu-Aib peptides **2** and **3** were able to form (*P*) α-helices. The first reason is that the dipeptide sequence L-Leu-D-Leu acts as a single chiral inducer that controls the screw sense of helical oligopeptides. In addition, Aib is an achiral amino acid and, thus, does not induce a bias toward a particular helical screw handedness. Therefore, the (*P*) helical screw senses of peptides **2** and **3** were controlled by the chiral centers of their L-Leu-D-Leu segments. The second reason is that Aib residues are strong promoters of helix formation and can induce LD-peptides to fold into α-helices. The dodecapeptide **4** formed an (*M*) α-helix for the same reason as the Boc-(L-Leu-D-Leu-Aib)$_n$-OMe peptides did; i.e., peptide **4** contains three D-Leu-L-Leu segments, which promote a left-handed screw sense, in its sequence. The dodecapeptide **5** formed a mixture of right-handed (*P*) 3_{10}- and α-helices in solution because it contains four (*P*) helix-inducing (L-Leu-D-Leu) segments in its sequence. On the other hand, the dodecapeptide **6**, which possesses an alternating Leu-Aib sequence rather than a consecutive L-Leu-D-Leu sequence, formed a mixture of (*P*) and (*M*) helices in

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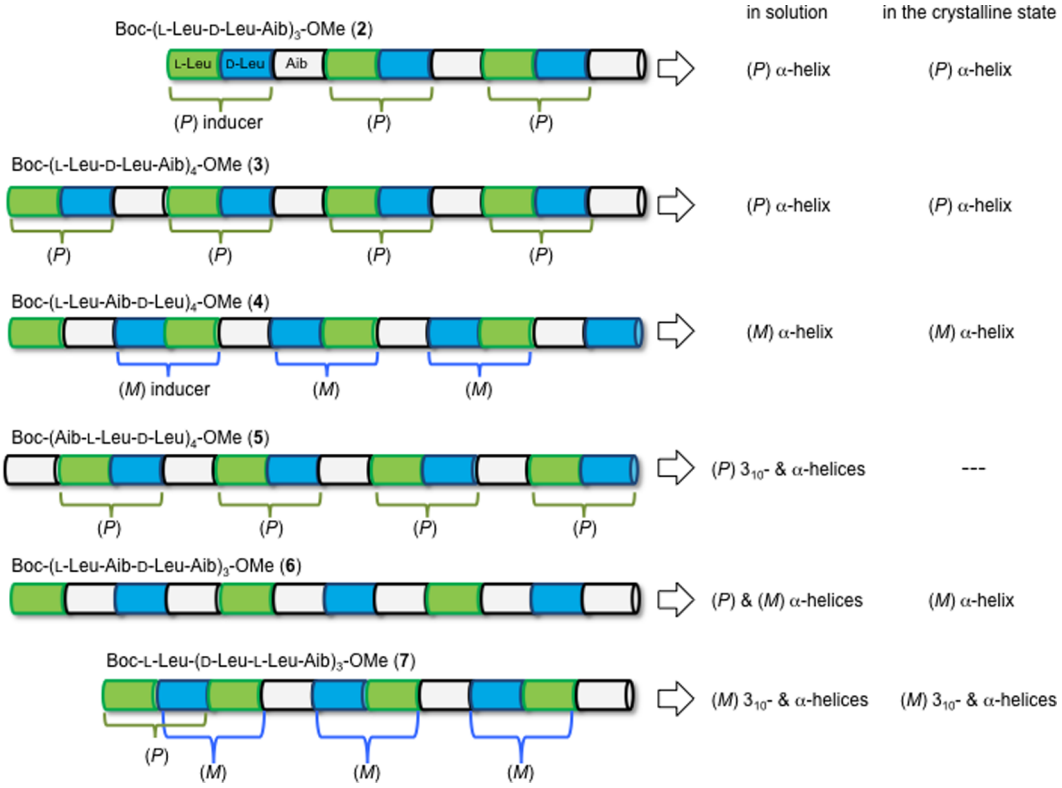

Figure 12. Cylindrical models of the sequences of the synthetic peptides.

solution. This suggests that consecutive L-Leu-D-Leu segments act as chiral inducers that control the screw sense of helical LD-peptides. In the crystalline state, two (*M*) α-helices were detected in **6**. The N-terminal L-amino acid with a type-II β-turn may in fact prefer to induce an (*M*) screw sense than a (*P*) screw sense when the next amino acid is an achiral Aib, reported by Clayden.[20] Therefore, (*M*) conformers would be preferentially packed into the crystalline conformer. The decapeptide **7**, which contains four L-Leu and three D-Leu residues within its sequence, formed an (*M*) α-helix both in solution and in the crystalline state. This probably occurred because the chirality of the three D-Leu-L-Leu segments, which promote the formation of (*M*) helices, has a stronger influence on the chirality of **7** than the chirality of the N-terminal L-Leu residue, which promotes the formation of (*P*) helices (Figure 12). Futhermore, only two L-Leu(3) and L-Leu(6) residues out of seven chiral Leu residues in **7**, adopt uncomfortable torsion angles, as L-Leu(1) is in a distorted type-II β-turn and L-Leu(9) has the correct torsion angles for an L-residue.[21] Therefore, **7** retained an (*M*) helical structure despite having four L-Leu residues and three D-Leu residues within its sequence.

CONCLUSION

In conclusion, we synthesized four dodecapeptides, Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**3**), Boc-(L-Leu-Aib-D-Leu)$_4$-OMe (**4**), Boc-(Aib-L-Leu-D-Leu)$_4$-OMe (**5**), and Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe (**6**), to investigate the positional influence of Aib residues on the secondary structures of LD-peptides. Conformational analyses of these peptides revealed that Aib residues promote the formation of α-helices in LD-peptides and alternating L-Leu-D-Leu dipeptide segments act as single chiral inducers that prefer to control the screw senses of helical peptides. Furthermore, we synthesized the decapeptide Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (**7**), which contains four L-Leu residues and three D-Leu residues within its sequence, and found that it formed an (*M*) α-helix as its dominant conformation. This result indicates that the presence of three D-Leu-L-Leu-segments, which promotes an (*M*) helical screw sense, has a stronger effect on chirality than the presence of a single N-terminal L-Leu-residue, which promotes a (*P*) helical screw sense. These results provide valuable information that will aid the design of well-defined helical LD-peptides and could also be applicable to various fields such as organic, material, bioorganic, and medicinal chemistry.

EXPERIMENTAL SECTION

Synthesis of peptides. The synthesis of peptides was carried out according to the stepwise solution-phase method using EDC and HOBT as coupling reagents. All compounds were purified by column chromatography on silica gel. The spectroscopic data of peptides **2**, **3**, and *ent*-**2** were reported in ref 8.

Boc-(L-Leu-Aib-D-Leu)$_4$-OMe (4): colorless crystals; mp 221−223 °C; $[\alpha]^{20}_D$ + 5.2 (*c* 1.0, $CHCl_3$); IR ($CDCl_3$, cm^{-1}) 3420, 3311, 2960, 2871, 1723, 1660; ^{1}H NMR (400 MHz, $CDCl_3$) δ8.16 (br s, 1H), 7.53−7.84 (m, 10H), 7.30 (br s, 1H), 4.46 (m, 1H), 3.79−4.10 (m, 7H), 3.66 (s, 3H), 1.24−1.97 (m, 57H), 0.87−0.99 (m, 48H); [HR-ESI(+)-TOF] *m*/*z* calcd for $C_{70}H_{128}N_{12}O_{15}Na$ $[M + Na]^+$ 1399.9520, found 1399.9525.

Boc-(Aib-L-Leu-D-Leu)$_4$-OMe (5): colorless crystals; mp 225−227 °C; $[\alpha]^{20}_D$ + 8.1 (*c* 1.0, $CHCl_3$); IR ($CDCl_3$, cm^{-1}) 3421, 3311, 2960, 2871, 1735, 1654; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, *J* = 6.8 Hz, 1H), 7.80 (br s, 1H), 7.75 (br s, 1H), 7.74 (br s, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.48 (br s, 1H), 7.41 (br s, 1H), 7.26−7.29 (m, 3H), 6.84 (br s, 1H), 5.80 (br s, 1H), 4.48 (m, 1H), 4.37 (m, 1H), 4.16 (m, 1H), 3.94−4.02 (m, 4H), 3.73 (s, 3H), 3.67 (m, 1H), 1.40−2.02 (m, 57H), 0.89−1.00 (m, 48H); [HR-ESI(+)-TOF] *m*/*z* calcd for $C_{70}H_{128}N_{12}O_{15}Na$ $[M + Na]^+$ 1399.9520, found 1399.9519.

Boc-(L-Leu-Aib-D-Leu-Aib)$_4$-OMe (6): colorless crystals; mp 282−283 °C; $[\alpha]^{20}_D$ − 24.7 (*c* 1.0, $CHCl_3$); IR ($CDCl_3$, cm^{-1})

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3421, 3317, 2960, 2871, 1734, 1656; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54−7.84 (m, 10H), 7.36 (br s, 1H), 5.65 (br s, 1H), 4.40 (m, 1H), 3.82−3.92 (m, 5H), 3.68 (s, 3H), 1.46−1.87 (m, 63H), 0.84−1.00 (m, 36H); [HR-ESI(+)-TOF] m/z calcd for $C_{66}H_{120}N_{12}O_{15}Na$ $[M + Na]^+$ 1343.8894, found 1343.8890.

Boc-L-Leu-(D-Leu-L-Leu-Aib)$_3$-OMe (7): colorless crystals; mp 223−225 °C; $[\alpha]^{20}_D$ − 15.4 (c 1.0, $CHCl_3$); IR ($CDCl_3$, cm^{-1}) 3430, 3325, 2960, 2871, 1733, 1661; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 5.2 Hz, 1H), 7.91 (br s, 1H), 7.64 (br s, 1H), 7.62 (br s, 1H), 7.61 (br s, 1H), 7.40 (br s, 1H), 7.39 (br s, 1H), 7.26 (br s, 1H), 7.06 (br s, 1H), 5.50 (br s, 1H), 4.26 (m, 1H), 4.09 (m, 1H), 3.83−3.95 (m, 5H), 3.67 (s, 3H), 1.38−2.01 (m, 48H), 0.79−1.00 (m, 42H); [HR-ESI(+)-TOF] m/z calcd for $C_{60}H_{110}N_{10}O_{13}Na$ $[M + Na]^+$ 1201.8152, found 1201.8161.

■ ASSOCIATED CONTENT

Supporting Information

Information about the crystallographic data and copies of the ^{1}H NMR and ^{13}C NMR spectra of the peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Crisma, M.; Formaggio, F.; Moretto, A.; Toniolo, C. *Biopolymers (Pept. Sci.)* **2006**, *84*, 3−12. (b) Royo, S.; Borggraeve, W. M. D.; Peggion, C.; Formaggio, F.; Crisma, M.; Jiménez, A. I.; Cativiela, C.; Toniolo, C. *J. Am. Chem. Soc.* **2005**, *127*, 2036−2037. (c) Demizu, Y.; Doi, M.; Kurihara, M.; Maruyama, T.; Suemune, H.; Tanaka, M. *Chem.−Eur. J.* **2012**, *18*, 2430−2439. (d) Anan, K.; Demizu, Y.; Oba, M.; Kurihara, M.; Doi, M.; Suemune, H.; Tanaka, M. *Helv. Chim. Acta* **2012**, *95*, 1694−1713.

(2) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232. (c) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015−2022. (d) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111−1239. (e) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252−262.

(3) (a) Huc, I. *Eur. J. Org. Chem.* **2004**, *2004*, 17−29. (b) Estroff, L. A.; Incarvito, C. D.; Hamilton, A. D. *J. Am. Chem. Soc.* **2004**, *126*, 2−3. (c) Gillies, E. R.; Deiss, F.; Staedel, C.; Schmitter, J. M.; Huc, I. *Angew. Chem., Int. Ed.* **2007**, *46*, 4081−4084. (d) Okamoto, I.; Nabeta, M.; Hayakawa, Y.; Morita, N.; Takeya, T.; Masu, H.; Azumaya, I.; Tamura, O. *J. Am. Chem. Soc.* **2007**, *129*, 1892−1893.

(4) (a) Fischer, L.; Guichard, G. *Org. Biomol. Chem.* **2010**, *8*, 3101−3117. (b) Kudo, M.; Katagiri, K.; Azumaya, I.; Kagechika, H.; Tanatani, A. *Tetrahedron* **2012**, *68*, 4455−4463.

(5) Branden, C.; Tooze, *Introduction to Protein Structure*; Garland: New York, 1991; pp 1−31.

(6) Examples of left- and right-handed α-helices in heterochiral peptides: (a) Shepherd, N. E.; Hoang, H. N.; Abbenante, G.; Fairlie, D. P. *J. Am. Chem. Soc.* **2009**, *131*, 15877−15886. (b) Hoang, H. N.; Abbenante, G.; Hill, T. A.; Gómez, G. R.; Fairlie, D. P. *Tetrahedron* **2012**, *68*, 4513−4516.

(7) (a) Bunkoczi, G.; Vertesy, L.; Sheldrick, G. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 1340−1342. (b) Townsley, L. E.; Tucker, A.; Sham, S.; Hinton, J. F. *Biochemistry* **2001**, *40*, 11676−11686. (c) Burkhart, B. M.; Gassman, R. M.; Langs, D. A.; Pangborn, W. A.; Duax, W. L. *Biophys. J.* **1998**, *75*, 2135−2146. (d) Lang, D. A. *Science* **1988**, *241*, 188−191. (e) Crisma, M.; Formaggio, F.; Pantano, M.; Valle, G.; Bonora, G. M.; Toniolo, C.; Schoemaker, H. E.; Kamphuis, J. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1735−1742. (f) Di Blasio, B.; Benedetti, E.; Pavone, V.; Pedone, C.; Spiniello, O.; Lorenzi, G. P. *Biopolymers* **1989**, *28*, 193−201. (g) Fick, F. G.; Semen, J.; Elias, H. G. *Makromol. Chem.* **1978**, *179*, 579−590. (h) Benedetti, E.; Di Blasio, B.; Pedone, C.; Lorenzi, G. P.; Tomasic, L.; Gramlich, V. *Nature* **1979**, *282*, 630. (i) Doi, M.; In, Y.; Ikuma, K.; Inoue, M.; Ishida, T. *Acta Crystallogr.* **1993**, *C49*, 1530−1532.

(8) Demizu, Y.; Doi, M.; Sato, Y.; Tanaka, M.; Okuda, H.; Kurihara, M. *Chem.−Eur. J.* **2011**, *17*, 11107−11109.

(9) (a) Nagano, M.; Doi, M.; Kurihara, M.; Suemune, H.; Tanaka, M. *Org. Lett.* **2010**, *12*, 3564−3566. (b) Sheehan, J.; Cruickshank, P.; Boshart, G. *J. Org. Chem.* **1961**, *26*, 2525−2528. (c) Sheehan, J.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067−1068.

(10) Demizu, Y.; Doi, M.; Sato, Y.; Tanaka, M.; Okuda, H.; Kurihara, M. *J. Org. Chem.* **2010**, *75*, 5234−5239.

(11) See the Supporting Information.

(12) (a) Crisma, M.; Bonora, G. M.; Toniolo, C.; Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C. *Int. J. Biol. Macromol.* **1988**, *10*, 300−304. (b) Benedetti, E.; Barone, V.; Bavoso, A.; Di Blasio, B.; Lelj, F.; Pavone, V.; Pedone, C.; Bonora, G. M.; Toniolo, C.; Leplawy, M. T.; Kaczmarek, K.; Redlinski, A. *Biopolymers* **1988**, *27*, 357−371. (c) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Barone, V.; Lelj, F.; Leplawy, M. T.; Kaczmarek, K.; Redlinski, A. *Biopolymers* **1988**, *27*, 373−379. (d) Demizu, Y.; Doi, M.; Kurihara, M.; Okuda, H.; Nagano, M.; Suemune, H.; Tanaka, M. *Org. Biomol. Chem.* **2011**, *9*, 3303−3312.

(13) Demizu, Y.; Tanaka, M.; Nagano, M.; Kurihara, M.; Doi, M.; Maruyama, T.; Suemune, H. *Chem. Pharm. Bull.* **2007**, *55*, 840−842.

(14) (a) Toniolo, C.; Polese, A.; Formaggio, F.; Crisma, M.; Kamphuis, J. *J. Am. Chem. Soc.* **1996**, *118*, 2744−2745. (b) Yoder, G.; Polese, A.; Silva, R. A. G. D.; Formaggio, F.; Crisma, M.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C.; Keiderling, T. A. *J. Am. Chem. Soc.* **1997**, *119*, 10278−10285. (c) Mammi, S.; Rainaldi, M.; Bellanda, M.; Schievano, E.; Peggion, E.; Broxterman, Q. B.; Formaggio, F.; Crisma, M.; Toniolo, C. *J. Am. Chem. Soc.* **2000**, *122*, 11735−11736. (d) Pengo, P.; Pasquato, L.; Moro, S.; Brigo, A.; Fogolari, F.; Broxterman, Q. B.; Kaptein, B.; Scrimin, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 3388−3392.

(15) Tanaka, M.; Demizu, Y.; Doi, M.; Kurihara, M.; Suemune, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 5360−5363.

(16) Sheldrick, G. M. *Program for Crystal Structure Refinement (SHELXL 97)*; University of Göttingen: Göttingen, 1997.

(17) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Gelder, R. de; Israel, R.; Smits, J. M. M. *The DIRDIF-99 program system*; Technical Report of the Crystallography Laboratory; University of Nijmegen: The Netherlands, 1994.

(18) CCDC-798452 for **2**, CCDC-815427 for **3**, CCDC-936415 for **4**, CCDC-936414 for **6**, and CCDC-837662 for **7** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(19) (a) Nagarajaram, H. A.; Sowdhamini, R.; Ramakrishnan, C.; Balaram, P. *FEBS Lett.* **1993**, *321*, 79−83. (b) Schellman, C. *Protein Folding*; Elsevier: North Holland, NY, 1980; pp 53−61. (c) Aravinda, S.; Shamala, N.; Bandyopadhyay, A.; Balaram, P. *J. Am. Chem. Soc.* **2003**, *125*, 15065−15075. (d) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Int. J. Pept. Protein Res.* **1993**, *42*, 401−410.

(20) (a) Brown, R. A.; Marcelli, T.; De Poli, M.; Solà, J.; Clayden, J. *Angew. Chem., Int. Ed.* **2012**, *51*, 1395−1399. (b) De Poli, M.; De

Zotti, M.; Raftery, J.; Aguilar, J. A.; Morris, G. A.; Clayden, J. *J. Org. Chem.* **2013**, *78*, 2248−2255.
(21) Benedetti, E.; De Simone, G.; Di Blasio, B.; Saviano, M.; Formaggio, F.; Polese, A.; Crisma, M.; Toniolo, C.; Kamphuis, J.; Aubry, A. *Gazz. Chim. Ital.* **1996**, *126*, 577−585.