JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Topological Study of the Structures of Heterochiral Peptides Containing Equal Amounts of L-Leu and D-Leu

Yosuke Demizu,*[,†] Hiroko Yamashita,[†,‡] Mitsunobu Doi,[§] Takashi Misawa,[†] Makoto Oba,[∥] Masakazu Tanaka,[∥] and Masaaki Kurihara*[,†,‡]

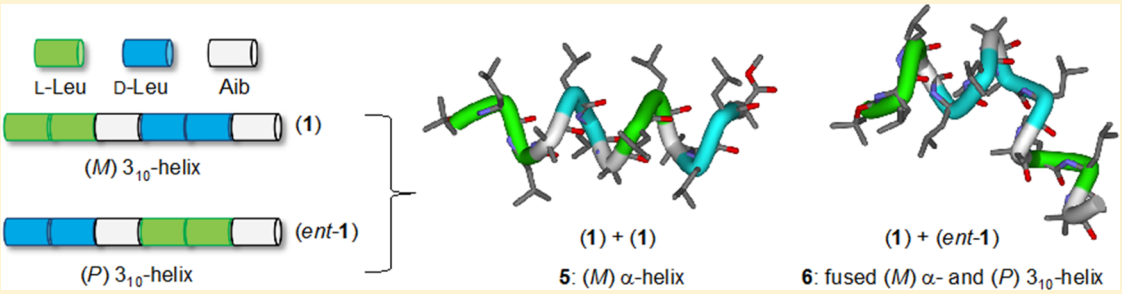
[†]Division of Organic Chemistry, National Institute of Health Sciences, Tokyo 158-8501, Japan

[‡]Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, Japan

[§]Osaka University of Pharmaceutical Sciences, Osaka 569-1094, Japan

[∥]Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8521, Japan

Supporting Information



ABSTRACT: We designed and synthesized two dodecapeptides, Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**) and Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**), that contain equal amounts of L-Leu, D-Leu, and achiral Aib residues. The conformations of peptides **5** and **6** in the crystalline state were studied using X-ray crystallographic analysis. Peptide **5** formed a left-handed (*M*) α-helical structure, whereas peptide **6** was composed of a combination of fused (*M*) α-helical and right-handed (*P*) 3$_{10}$-helical structures. In solution, roughly equivalent amounts of (*P*) and (*M*) helices were present in **5**, whereas the (*M*) α-helix was present in **6** as its dominant conformation.

INTRODUCTION

De novo peptide design is important in various fields such as biology, medicinal chemistry, and organic chemistry. It is often necessary to fold such peptides into specifically required structures. For example, helical peptides are used as protein−protein interaction inhibitors and organocatalysts.[1] Therefore, a variety of methods for controlling helical structures have been investigated, including the use of nonproteinogenic amino acids such as β-amino acids,[2] α,α-disubstituted α-amino acids (α-dAAs),[3] and cross-linked side chains.[4] In particular, α-amino-isobutyric acid (Aib), which is the simplest and most commonly used α-dAA, can stabilize the helical structures of short peptides, and the combined use of natural L-amino acids (L-AAs) and Aib is useful for enhancing peptide functionality.[5] We have performed various topological studies examining the structures of heterochiral peptides containing L-Leu, D-Leu, and achiral Aib residues. For example, we recently reported that the accurate design of hybrid peptides containing appropriate combinations of L-Leu, D-Leu, and achiral Aib residues is extremely useful for constructing short helical peptides that form specific novel conformations; i.e., we designed and synthesized three diastereomeric heterochiral hexapeptides, Boc-L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib-OMe (**1**) (Boc = *tert*-butoxycarbonyl; OMe = methyl ester), Boc-L-Leu-D-Leu-Aib-L-Leu-D-Leu-Aib-OMe (**2**), and Boc-L-Leu-D-Leu- Aib-D-Leu-L-Leu-Aib-OMe (**3**), and studied their preferred conformations in the crystalline state.[6] Peptide **1** folded into a left-handed (*M*) 3$_{10}$-helix, **2** folded into a turn structure, and **3** folded into an S-shaped turn structure. Furthermore, the dodecapeptide Boc-(L-Leu-D-Leu-Aib)$_4$-OMe (**4**), which is an elongated version of **2**, exhibited an altered conformation in which the turn structure had been replaced by a (*P*) α-helical structure (Figure 1).[6b,d] Thus, combining heterochiral peptides is another effective way to construct peptides with specific conformations.

In the present study, we aimed to develop novel peptide structures using the heterochiral hexapeptide segments **1** and *ent*-**1**. As a result, we designed and synthesized two dodecapeptides, Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**) and Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**), the latter of which consists of a combination of the enantiomeric heterochiral hexapeptides **1** and *ent*-**1** (Figure 2), and analyzed their conformations in the crystalline state and in solution.

RESULTS

Synthesis of the Peptides. The dodecapeptides Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**) and

Received: May 26, 2015
Published: August 14, 2015

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597−8603

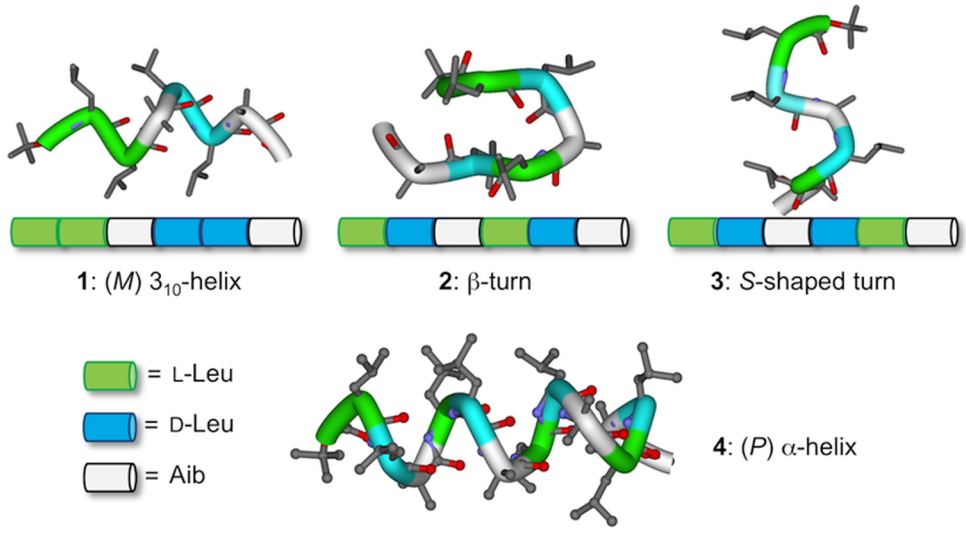


Figure 1. X-ray diffraction structures of the heterochiral peptides **1**−**4**. The structures of **1**−**3** were reprinted from ref 6a. Copyright 2010 American Chemical Society. The structure of **4** was reprinted from ref 6d. Copyright 2013 American Chemical Society.

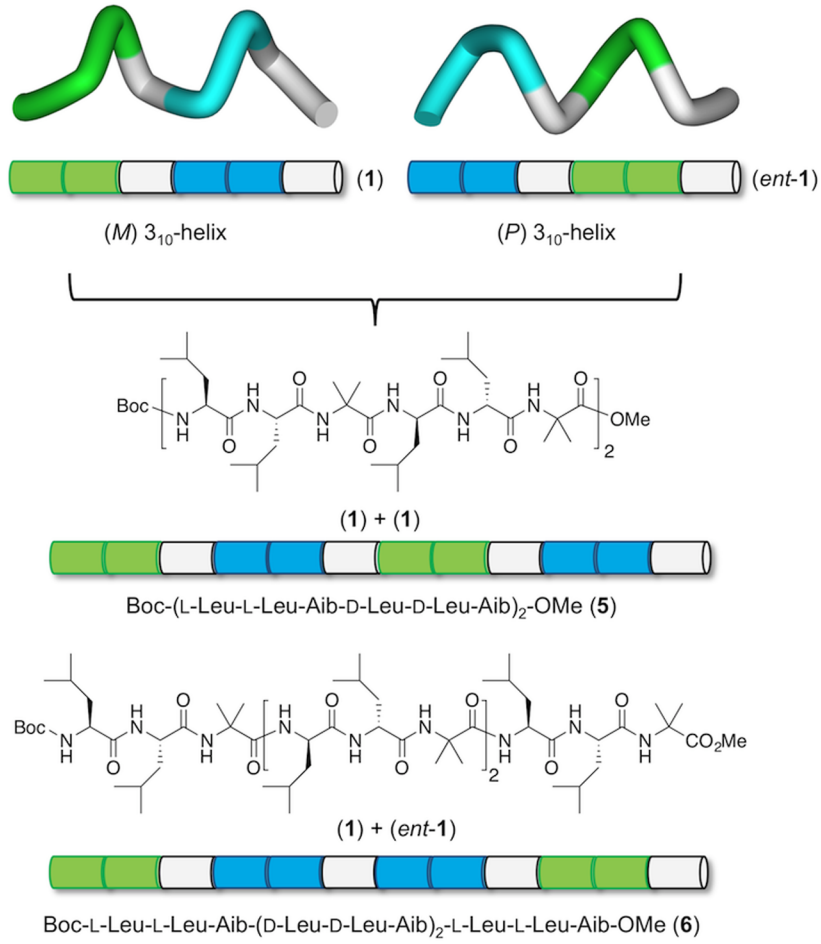


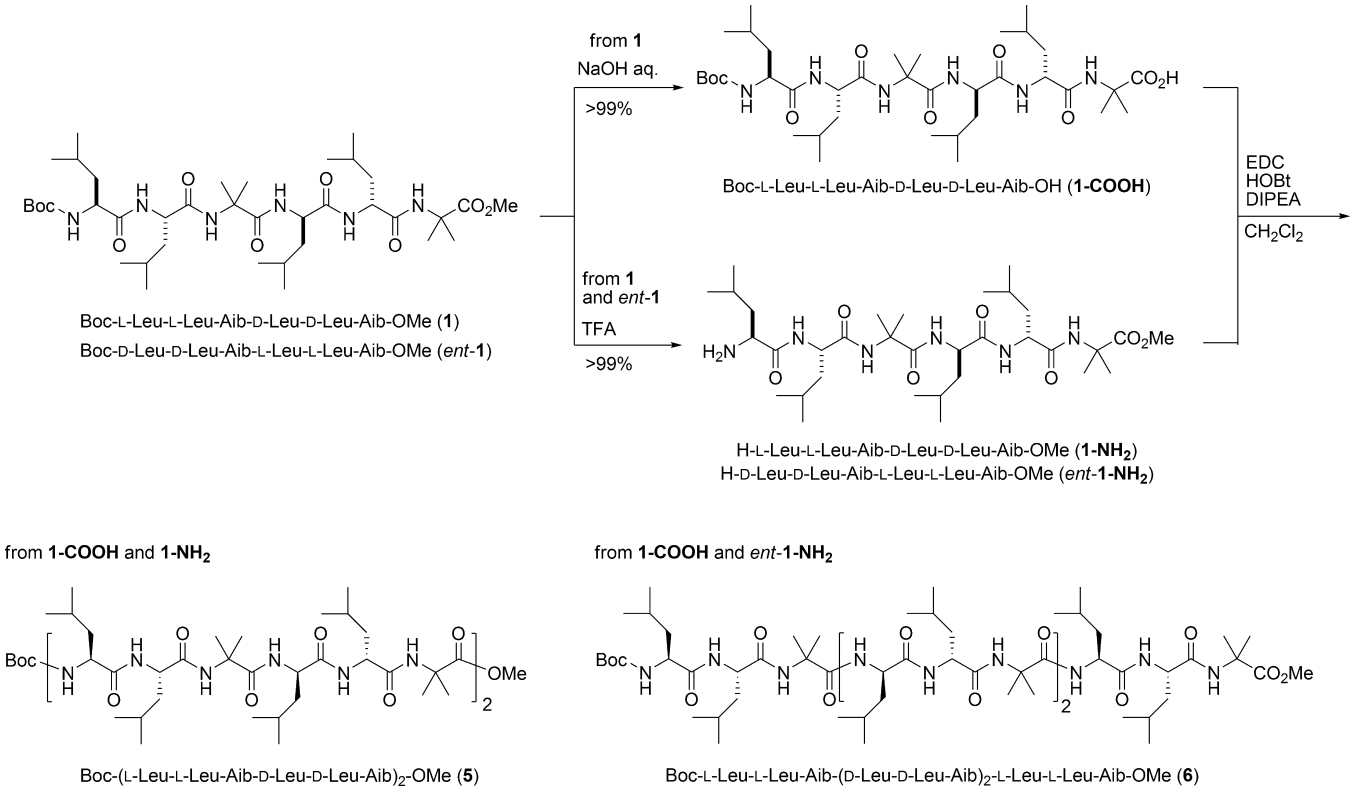
Figure 2. Chemical structures of peptides **5** and **6**.

Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**) were synthesized using conventional solution-phase methods involving a hexapeptide-fragment condensation strategy in which 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDC) hydrochloride and 1-hydroxybenzotriazole (HOBt) hydrate were employed as coupling reagents (Scheme 1). That is to say, after deprotection of the Boc protecting group in the hexapeptide **1-NH$_2$**[6a] or *ent*-**1-NH$_2$**, the resulting N-terminal-free hexapeptide was coupled with the hexapeptide acid **1-COOH** to give dodecapeptide **5** (28%) or **6** (35%), respectively.

X-ray Diffraction Analysis. Peptides **5** and **6** formed crystals suitable for X-ray crystallographic analysis after slow evaporation

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597−8603

Scheme 1. Synthesis of Dodecapeptides 5 and 6



of *N*,*N*-dimethylformamide (DMF)/H_2O (for **5**) or 2-butanone/*n*-hexane (for **6**) at room temperature. The crystallographic parameters of **5** and **6** are summarized in the Supporting Information.[7] The peptides' backbone and side chain torsion angles and intra- and intermolecular hydrogen-bond parameters are summarized in Tables 1 and 2, respectively.

A left-handed (*M*) α-helix in which the N-terminal L-Leu(1) and C-terminal Aib(12) residues had been flipped was detected in the asymmetric unit of Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**) (Figure 3). The mean ϕ and ψ torsion angles of amino acid residues 2−11 were +57.5° and +48.5°, respectively, which are close to those of an ideal (*M*) α-helix (+60° and +45°, respectively).[8] The values of the flipped ϕ and ψ torsion angles of residue L-Leu(1) were −94.7° and −24.3°, respectively, and those of residue Aib(12) were −50.5° and −45.1°, respectively. The α-helix molecule contained one $i \leftarrow i + 3$ type and eight $i \leftarrow i + 4$ type hydrogen bonds; i.e., hydrogen bonds were detected between H−N(4) and C(1)═O(1) [N(4)···O(1) = 3.11 Å], between H−N(5) and C(1)═O(1) [N(5)···O(1) = 3.06 Å], between H−N(6) and C(2)═O(2) [N(6)···O(2) = 3.01 Å], between H−N(7) and C(3)═O(3) [N(7)···O(3) = 3.09 Å], between H−N(8) and C(4)═O(4) [N(8)···O(4) = 2.83 Å], between H−N(9) and C(5)═O(5) [N(9)···O(5) = 2.92 Å], between H−N(10) and C(6)═O(6) [N(10)···O(6) = 3.15 Å], between H−N(11) and C(7)═O(7) [N(11)···O(7) = 2.91 Å], and between H−N(12) and C(8)═O(8) [N(12)···O(8) = 3.02 Å]. In packing mode, successive helical molecules were connected by intermolecular hydrogen bonds to form head-to-tail aligned chains.[7]

The dodecapeptide Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**) formed a fused helical structure containing both an (*M*) α-helical segment (L-Leu(1) to Aib(6)) and a right-handed (*P*) 3_{10}-helical segment (D-Leu(7) to L-Leu(12)) (Figure 4a). Figure 4b,c shows the two fused helices of opposite chirality that comprise **6** (the (*M*) α-helix in blue and the (*P*) 3_{10}-helix in magenta). The mean ϕ and ψ torsion angles of the (*M*) α-helical molecule, composed of amino acid residues 1−6,

Table 1. Selected Torsion Angles (ω, ϕ, ψ, and χ_1 [°]) for peptides 5 and 6 (determined by X-ray crystallographic analysis)

residue	torsion angle [deg] ϕ	ψ	ω	χ_1
Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**)				
L-Leu(1)	−94.7	−24.3	−168.4	65.7
L-Leu(2)	38.8	60.5	173.0	−157.5
Aib(3)	51.5	50.5	172.7	–
D-Leu(4)	67.8	42.3	−179.1	175.9
D-Leu(5)	62.1	45.1	−178.5	−174.2
Aib(6)	53.7	51.6	179.0	–
L-Leu(7)	57.0	51.1	177.1	−53.7
L-Leu(8)	54.1	54.6	175.8	−58.6
Aib(9)	55.2	48.0	174.7	–
D-Leu(10)	73.3	40.7	−178.3	61.7
D-Leu(11)	61.3	40.7	−174.7	175.9
Aib(12)	−50.5	−45.1	177.0	–
Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**)				
L-Leu(1)	58.2	55.5	171.2	−79.0
L-Leu(2)	55.8	50.9	177.7	−63.6
Aib(3)	53.1	51.7	172.4	–
D-Leu(4)	68.4	33.0	−178.1	67.6
D-Leu(5)	71.0	45.8	−176.4	61.2
Aib(6)	55.0	37.0	178.0	–
D-Leu(7)	87.3	−4.9	−172.2	64.5
D-Leu(8)	−60.8	−31.7	178.7	39.6
Aib(9)	−55.2	−27.3	179.2	–
L-Leu(10)	−54.0	−30.4	−178.5	−74.6
L-Leu(11)	−64.0	−20.7	−178.6	−62.8
Aib(12)	45.2	48.4	179.8	–

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597−8603

Table 2. Intra- and Intermolecular Hydrogen-Bond Parameters of Peptides 5 and 6[a]

donor	acceptor	distance [Å]	angle [deg]	symmetry operation
Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**)				
N_4–H	O_1	3.11	120	x, y, z
N_5–H	O_1	3.06	171	x, y, z
N_6–H	O_2	3.01	174	x, y, z
N_7–H	O_3	3.09	167	x, y, z
N_8–H	O_4	2.83	164	x, y, z
N_9–H	O_5	2.92	171	x, y, z
N_{10}-H	O_6	3.15	161	x, y, z
N_{11}–H	O_7	2.91	168	x, y, z
N_{12}–H	O_8	3.02	167	x, y, z
N_1–H	O_{10}'	2.83	145	$^1/_2 - x, 1 - y, ^1/_2 + z$
N_2–H	O_{11}'	2.78	121	$^1/_2 - x, 1 - y, ^1/_2 + z$
N_3–H	O_{12}'	3.19	117	$^1/_2 - x, 1 - y, ^1/_2 + z$
Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**)				
N_4–H	O_0	3.16	161	x, y, z
N_5–H	O_1	3.02	160	x, y, z
N_6–H	O_2	3.01	177	x, y, z
N_7–H	O_3	3.07	148	x, y, z
N_8–H	O_4	3.13	104	x, y, z
N_8–H	O_5	2.91	155	x, y, z
N_9–H	O_4	2.98	166	x, y, z
N_{10} – H	O_7	2.94	156	x, y, z
N_{11}–H	O_8	2.97	160	x, y, z
N_{12}–H	O_9	2.98	160	x, y, z
N_1–H	O_{12}'	3.03	143	$x, 1 + y, 1 + z$
N_2–H	O_{11}'	2.88	164	$x, 1 + y, 1 + z$
N_3–H	O_b' [b]	2.94	129	$x, y, 1 + z$

[a]The amino acid numbering begins at the N-terminus of the peptide chain. [b]O_b = the oxygen of 2-butanone.

were +60.3° and +45.7°, respectively, and those of the (*P*) 3_{10}-helical molecule, composed of amino acid resides 8–11, were −58.5° and −27.5°, respectively. The C-terminal Aib(12) residue was flipped, and its ϕ and ψ torsion angles were +45.2° and +48.4°, respectively. In regard to the intramolecular H-bonds, four $i \leftarrow i + 3$ type and five $i \leftarrow i + 4$ type hydrogen bonds along with one $i \leftarrow i + 5$ type hydrogen bond were detected in **6**; i.e., hydrogen bonds were detected between H–N(4) and C(0)═O(0) [N(4)···O(0) = 3.16 Å], between H–N(5) and C(1)═O(1) [N(5)···O(1) = 3.02 Å], between H–N(6) and C(2)═O(2) [N(6)···O(2) = 3.01 Å], between H–N(7) and C(3)═O(3) [N(7)···O(3) = 3.07 Å], between H–N(8) and C(4)═O(4) [N(8)···O(4) = 3.13 Å], between H–N(8) and C(5)═O(5) [N(8)···O(5) = 2.91 Å], between H–N(9) and C(4)═O(4) [N(9)···O(4) = 2.98 Å], between H–N(10) and C(7)═O(7) [N(10)···O(7) = 2.94 Å], between H–N(11) and C(8)═O(8) [N(11)···O(8) = 2.97 Å], and between H–N(12) and C(9)═O(9) [N(12)···O(9) = 2.98 Å]. The helical molecules were connected by two hydrogen bonds and formed chains with a head-to-tail alignment.[7]

Conformational Analysis in Solution. The infrared (IR) spectra of peptides **5** and **6** in the 3200–3500 cm^{-1} region (NH-stretching region) were measured at a peptide concentration of 5.0 mM in $CDCl_3$ solution (Figure 5). In the spectra of **5** and **6**, the weak bands at 3432 cm^{-1} for **5** and 3436 cm^{-1} for **6** were assigned to solvated free peptide NH groups, and the strong bands at 3310 cm^{-1} for **5** and 3305 cm^{-1} for **6** were assigned to peptide NH groups with N–H···O═C intramolecular hydrogen bonds. These spectra were very similar to those of the helical peptides in solution.[9]

The CD spectra of peptides **5** and **6** were measured in 2,2,2-trifluoroethanol (TFE) and in 1:1 TFE/PBS buffer solution (Figure 6). In both solutions, the spectrum of peptide **3** displayed positive maxima at around 208 and 222 nm, indicating that it possessed a left-handed (*M*) helical screw sense.[10] Furthermore, on the basis of its *R* value ($\theta_{222}/\theta_{208}$), peptide **6** formed the α-helix as its dominant conformation (R = 0.61 in TFE solution, R = 0.75 in 1:1 TFE/PBS solution).[3e] On the other hand, peptide **5** did not show the characteristic maxima of a helical structure, which suggested that roughly equivalent amounts of (*P*) and (*M*) helices were present in **5**.

DISCUSSION

The dodecapeptide Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**) folded into an (*M*) α-helix in the crystalline state, even though it contained equal amounts of L-Leu and D-Leu residues. This was probably due to flipping of the L-Leu(1) residue, which was considered to have been caused by the side-chain repulsion between the L-Leu(1) and D-Leu(10′) residues that occurs in packing mode when the torsion angles of the L-Leu(1) residue are positive. The first three amide protons from the N-terminus (N(1)–H to N(3)–H) are not usually involved in hydrogen bonds in α-helical peptides. Therefore, the N-terminal L-Leu(1) residue has a greater tendency to flip than the other amino acid residues involved in hydrogen bonds in **5** (Figure 7). Thus, the chirality of the dipeptide segment D-Leu(10)-D-Leu(11) seems to exert more control over the torsion angles of the N-terminal sequence than the chirality of the N-terminal L-Leu(1) residue, and the energetically favorable (*M*) helical conformer might be preferentially packed into the crystalline conformer. In solution, peptide **5** formed a mixture of (*P*) and (*M*) helices. We previously reported that the heterochiral dodecapeptide Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe folded into an (*M*) α-helix in the crystalline state and formed a mixture of (*P*) and (*M*) helices in solution.[6d] Thus, the insertion of achiral Aib residues between

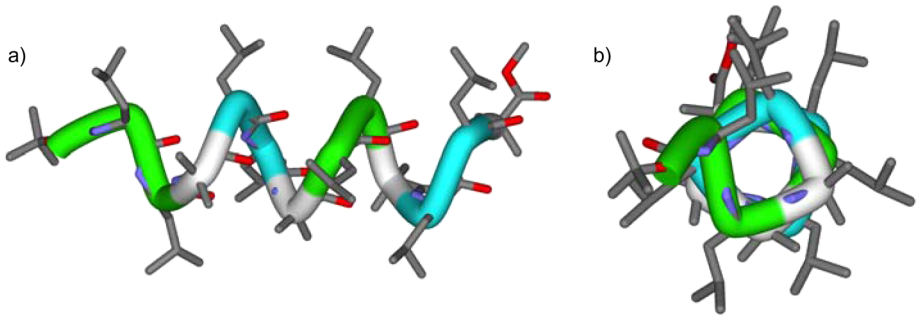


Figure 3. X-ray diffraction structure of **5** as viewed (a) perpendicular to its helical axis and (b) along its helical axis. The DMF molecule has been omitted.

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597–8603

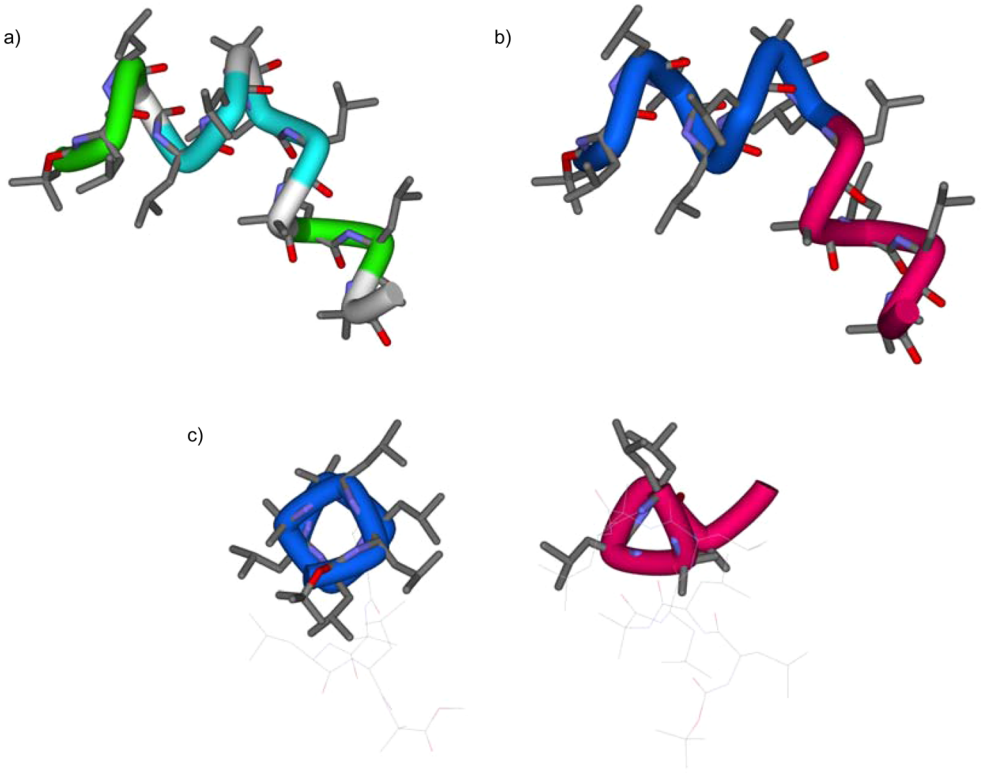


Figure 4. (a) X-ray diffraction structure of **6** as viewed perpendicular to its helical axis. The 2-butanone molecule has been omitted. (b) Color-coded image showing the two helical structures present within **6**. The (*M*) α-helical structure (L-Leu(1) to Aib(6)) is shown in blue, and the right-handed (*P*) 3_{10}-helical structure (D-Leu(7) to L-Leu(12)) is colored magenta. (c) View along the helical axes of the (*M*) α-helix and (*P*) 3_{10}-helix.

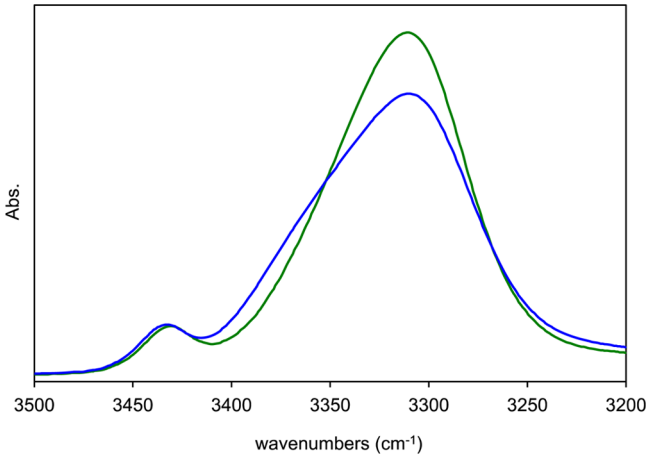


Figure 5. FTIR spectra of peptides **5** (green) and **6** (blue) in $CDCl_3$ solution. The peptide concentration was 5.0 mM.

L-Leu and D-Leu residues is capable of controlling an (*M*) α-helix in the crystalline state but cannot control the helical screw direction in solution. Therefore, peptide **5**, which has Aib residues between L-Leu-L-Leu and D-Leu-D-Leu segments, also has structural properties similar to those of the dodecapeptide Boc-(L-Leu-Aib-D-Leu-Aib)$_3$-OMe.

On the other hand, the dodecapeptide Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**) formed a fused molecule containing both (*M*) and (*P*) helical structures; i.e., the segment containing residues 1−6 (L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib) formed an (*M*) α-helix, and the segment composed of residues 7−12 (D-Leu-D-Leu-Aib-L-Leu-L-Leu-Aib) formed a (*P*) 3_{10}-helix. In both segments, the chirality of the final Leu dipeptide affected the helical screw sense of the segment; i.e., the presence of a D-Leu-D-Leu fragment in the first L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib segment induced a left-handed screw sense, whereas the presence of an L-Leu-L-Leu fragment in the D-Leu-D-Leu-Aib-L-Leu-L-Leu-Aib segment resulted in a right-handed screw sense. Thus, dodecapeptide **6**, which contained a pair of enantiomeric hexapeptide segments, was composed of a combination of fused (*M*) and (*P*) helical structures. Balaram, Karle, and co-workers previously reported that the tetradecapeptide Boc-L-Val-L-Ala-L-Leu-Aib-L-Val-L-Ala-L-Leu-D-Val-D-Ala-D-Leu-Aib-D-Val-D-Ala-D-Leu-OMe, which is composed of a combination of homochiral L- and D-heptapeptide segments, also forms a structure containing fused (*P*) and (*M*) helical segments.[5c,11] In the latter tetradecapeptide, the (*P*) helix is formed by the L-peptide segment and the (*M*) helix is formed by the D-peptide segment. The results of this study indicate that it is possible to construct helical structures that contain helices with the opposite screw sense using a combination of heterochiral peptide segments. In solution, peptide **6** formed an (*M*) α-helix as the preferred conformation, indicating that the two consecutive D-Leu-D-Leu-Aib segments strongly affected the (*M*) helical screw direction of the peptide.

CONCLUSION

We synthesized two dodecapeptides, Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (**5**) and Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (**6**), the latter of which consists of a combination of the enantiomeric heterochiral hexapeptide segments **1** and *ent*-**1**, and analyzed their conformations in the crystalline state. In the crystalline state, peptide **5** folded into an (*M*) α-helix, and peptide **6** formed a structure containing fused (*M*) and (*P*) helical segments. Thus, combining enantiomeric heterochiral peptide segments can facilitate the development of

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597−8603

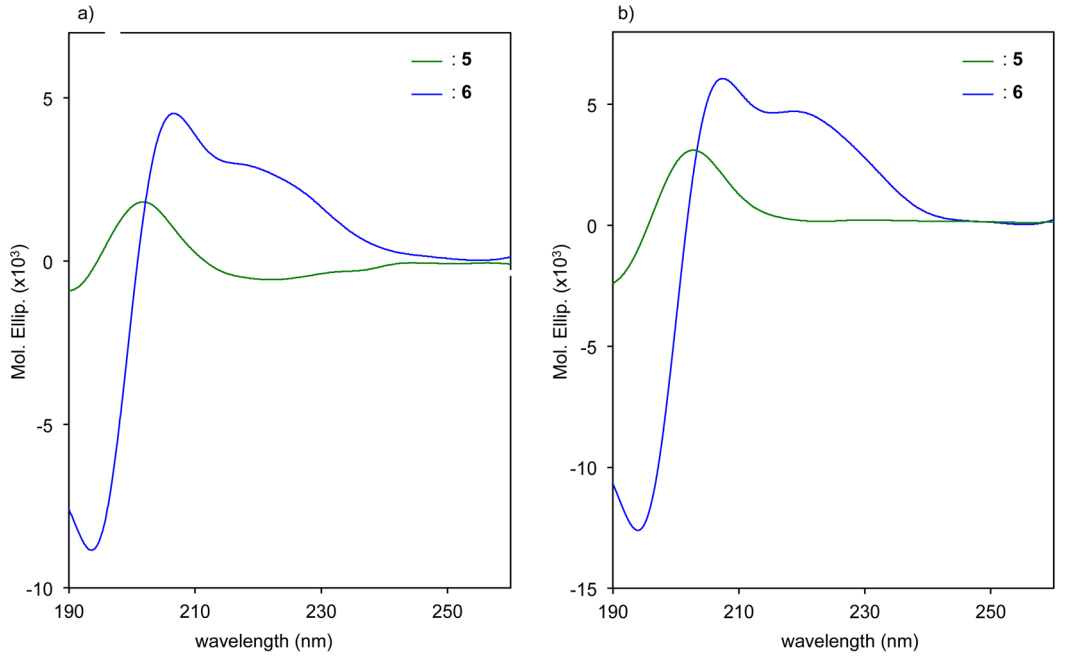


Figure 6. CD spectra of dodecapeptides **5** (green) and **6** (blue) in (a) TFE and (b) 1:1 TFE/PBS (pH 7.2) solution. The peptide concentration was 0.1 mM.

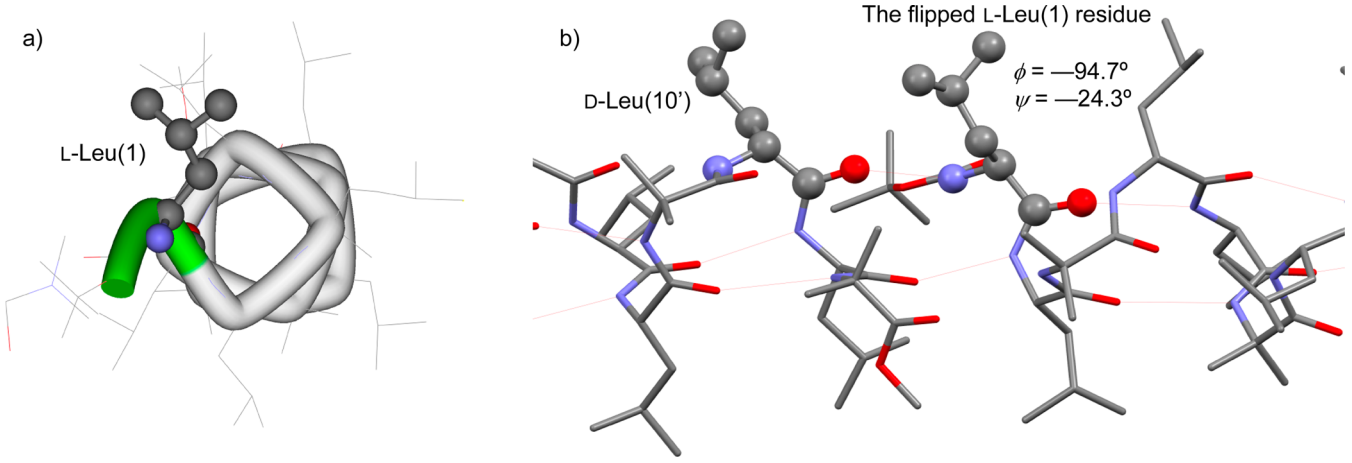


Figure 7. (a) X-ray structure of **5** as viewed along its helical axis. The flipped L-Leu(1) residue is shown as a ball-and-stick model and a green-colored tube. (b) Packing of molecules of **5** in the crystalline state. The flipped L-Leu(1) and D-Leu(10′) residues are shown as ball-and-stick models, and hydrogen bonds are indicated as red dashed lines.

specific folded structures. Our results will aid the design of peptide mimics and peptide-based crystal engineering studies.

EXPERIMENTAL SECTION

Synthesis of the Peptides. Hexapeptide **1** was synthesized in accordance with ref 6a. A solution of hexapeptide **1** (302 mg, 0.4 mmol) and 1 M aqueous NaOH (1.0 mL, 1.0 mmol) in MeOH (5 mL) was stirred at room temperature for 12 h. Then the solution was neutralized with 1 M aqueous HCl, and MeOH was evaporated. The aqueous solution was extracted with AcOEt and dried over Na_2SO_4. Removal of the solvent afforded hexapeptide carboxylic acid **1-COOH** (296 mg, >99% yield) as colorless crystals, which were used for next reaction without further purification. Trifluoroacetic acid (1 mL) was added to a solution of hexapeptide **1** or *ent*-**1** (302 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. Removal of the solvent afforded the crude N-terminal-free hexapeptide **1-NH$_2$** or *ent*-**1-NH$_2$**, which was used without further purification. A mixture of EDC (96 mg, 0.5 mmol), HOBt (67 mg, 0.5 mmol), DIPEA (174 μL, 1.0 mmol), the above C-terminal-free hexapeptide **1-COOH**, and the above N-terminal-free hexapeptide **1-NH$_2$** or *ent*-**1-NH$_2$** in CH_2Cl_2 (4 mL) was stirred at rt for 2 days, and then the solution was washed with 3% aqueous HCl, 5% aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel (*n*-hexane/AcOEt = 1:4) to give dodecapeptide **5** from **1-COOH** and **1-NH$_2$** (154 mg, 28%) and dodecapeptide **6** from **1-COOH** and *ent*-**1-NH$_2$** (193 mg, 35%).

Boc-(L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib)$_2$-OMe (5). Colorless crystals, mp 252–254 °C. $[\alpha]_D^{24}$ = −33.1 (*c* 0.5, MeOH). IR ($CDCl_3$, cm^{-1}): 3432, 3310, 2959, 2873, 1729, 1658, 1532, 1468. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16–8.04 (m, 2H), 7.97–7.86 (br s, 1H), 7.74–7.59 (m, 6H), 7.42–7.32 (m, 2H), 6.01–5.90 (br s, 1H), 4.38–4.28 (m, 1H), 4.19–3.92 (m, 6H), 3.87–3.79 (br s, 1H), 3.65 (s, 3H), 1.92–1.38 (m, 57H), 1.03–0.82 (m, 48H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.8, 176.3, 176.1, 175.1, 173.6, 173.2, 173.1, 156.1, 80.2, 57.1, 56.9, 55.8, 54.1, 53.7, 53.1, 52.5, 52.1, 40.1, 39.8, 39.5, 38.1, 28.3, 27.3, 25.1, 25.0, 24.9, 24.7, 24.6, 23.8, 23.6, 23.5, 23.4, 23.2, 21.6, 21.5, 21.3, 21.0, 20.9,

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597–8603

20.7, 20.6. [HR-ESI(+)] (m/z): calcd for $C_{70}H_{129}N_{12}O_{15}$ [M + H]$^+$, 1377.9695; found, 1377.9688.

Boc-L-Leu-L-Leu-Aib-(D-Leu-D-Leu-Aib)$_2$-L-Leu-L-Leu-Aib-OMe (6). Colorless crystals, mp 148−150 °C. $[\alpha]_D^{24} = -4.4$ (c 0.5, MeOH). IR ($CDCl_3$, cm^{-1}): 3436, 3305, 2958, 2870, 1729, 1657, 1526, 1474. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27−7.00 (m, 11H), 5.71−5.62 (br s, 1H), 4.38−3.74 (m, 8H), 3.65 (s, 3H), 1.93−1.45 (m, 48H), 1.41 (s, 9H), 1.07−0.80 (m, 48H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7, 176.5, 175.7, 175.4, 174.3, 174.1, 173.8, 173.7, 172.9, 172.8, 80.2, 56.9, 56.6, 55.9, 54.9, 53.9, 53.4, 52.5, 52.4, 52.2, 41.4, 40.2, 39.8, 39.6, 39.2, 37.8, 28.3, 27.4, 26.8, 26.5, 25.2, 25.0, 24.9, 24.8, 24.6, 24.5, 24.4, 23.7, 23.5, 23.4, 23.3, 23.2, 23.1, 22.6, 21.6, 21.4, 21.2, 21.1, 21.0, 20.8. [HR-ESI(+)] (m/z): calcd for $C_{70}H_{129}N_{12}O_{15}$ [M + H]$^+$, 1377.9695; found, 1377.9726.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01541.

Crystallographic data for peptide **5** (CIF)
Crystallographic data for peptide **6** (CIF)
Information about the crystallographic data and copies of the ^{1}H and ^{13}C NMR spectra of the peptides (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +81-3-3700-1141. Fax: +81-3-3707-6950. E-mail: demizu@nihs.go.jp.
*E-mail: masaaki@nihs.go.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported in part by JSPS KAKENHI Grant 26460169 (Y.D.) and a grant from the Research Foundation for Pharmaceutical Sciences (Y.D.).

■ REFERENCES

(1) (a) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252−262. (b) Johnson, L. M.; Gellman, S. H. *Methods Enzymol.* **2013**, *523*, 407−429. (c) Akagawa, K.; Sakai, N.; Kudo, K. *Angew. Chem., Int. Ed.* **2015**, *54*, 1822−1826. (d) Misawa, T.; Demizu, Y.; Kawamura, M.; Yamagata, N.; Kurihara, M. *Bioorg. Med. Chem.* **2015**, *23*, 1055−1061.

(2) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111−1239. (c) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399−1408. (d) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366−1375. (e) Martinek, T. A.; Fülop, F. *Chem. Soc. Rev.* **2012**, *41*, 687−702. (f) Demizu, Y.; Oba, M.; Okitsu, K.; Yamashita, H.; Misawa, T.; Tanaka, M.; Kurihara, M.; Gellman, S. H. *Org. Biomol. Chem.* **2015**, *13*, 5617−5620.

(3) (a) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747−6756. (b) Crisma, M.; Moretto, A.; Peggion, C.; Panella, L.; Kaptein, B.; Broxterman, Q. B.; Formaggio, F.; Toniolo, C. *Amino Acids* **2011**, *41*, 629−641. (c) Formaggio, F.; Crisma, M.; Ballano, G.; Peggion, C.; Venanzi, M.; Toniolo, C. *Org. Biomol. Chem.* **2012**, *10*, 2413−2421. (d) Crisma, M.; Toniolo, C. *Biopolymers* **2015**, *104*, 46−64. (e) Demizu, Y.; Doi, M.; Kurihara, M.; Maruyama, T.; Suemune, H.; Tanaka, M. *Chem. - Eur. J.* **2012**, *18*, 2430−2439. (f) Oba, M.; Takazaki, H.; Kawabe, N.; Doi, M.; Demizu, Y.; Kurihara, M.; Kawakubo, H.; Nagano, M.; Suemune, H.; Tanaka, M. *J. Org. Chem.* **2014**, *79*, 9125−9140. (g) Yamashita, H.; Demizu, Y.; Misawa, T.; Shoda, T.; Kurihara, M. *Tetrahedron* **2015**, *71*, 2241−2245.

(4) (a) Blackwell, H. E.; Sadowsky, J. D.; Howard, R. J.; Sampson, J. N.; Chao, J. A.; Steinmetz, W. E.; O'Leary, D. J.; Grubbs, R. H. *J. Org. Chem.* **2001**, *66*, 5291−5302. (b) Moellering, R. E.; Cornejo, M.; Davis, T. N.; Bianco, C. D.; Aster, J. C.; Blacklow, S. C.; Kung, A. L.; Gilliland, D. G.; Verdine, G. L.; Bradner, J. E. *Nature* **2009**, *462*, 182−188. (c) Kim, Y.-W.; Grossmann, T. N.; Verdine, G. L. *Nat. Protoc.* **2011**, *6*, 761−771. (d) Nomura, W.; Aikawa, H.; Ohashi, N.; Urano, E.; Métifiot, M.; Fujino, M.; Maddali, K.; Ozaki, T.; Nozue, A.; Narumi, T.; Hashimoto, C.; Tanaka, T.; Pommier, Y.; Yamamoto, N.; Komano, J. A.; Murakami, T.; Tamamura, H. *ACS Chem. Biol.* **2013**, *8*, 2235−2244. (e) Demizu, Y.; Yamagata, N.; Nagoya, S.; Sato, Y.; Doi, M.; Tanaka, M.; Nagasawa, K.; Okuda, H.; Kurihara, M. *Tetrahedron* **2011**, *67*, 6155−6165.

(5) (a) Paterson, Y.; Rumsey, S. M.; Benedetti, E.; Némethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 2947−2955. (b) Benedetti, E. *Biopolymers* **1996**, *40*, 3−44. (c) Karle, I. L. *Biopolymers* **2001**, *60*, 351−365. (d) Yamashita, H.; Demizu, Y.; Shoda, T.; Sato, Y.; Oba, M.; Tanaka, M.; Kurihara, M. *Bioorg. Med. Chem.* **2014**, *22*, 2403−2408.

(6) (a) Demizu, Y.; Doi, M.; Sato, Y.; Tanaka, M.; Okuda, H.; Kurihara, M. *J. Org. Chem.* **2010**, *75*, 5234−5239. (b) Demizu, Y.; Doi, M.; Sato, Y.; Tanaka, M.; Okuda, H.; Kurihara, M. *Chem. - Eur. J.* **2011**, *17*, 11107−11109. (c) Demizu, Y.; Nagoya, S.; Doi, M.; Sato, Y.; Tanaka, M.; Kurihara, M. *J. Org. Chem.* **2012**, *77*, 9361−9365. (d) Demizu, Y.; Yamashita, H.; Yamazaki, N.; Sato, Y.; Doi, M.; Tanaka, M.; Kurihara, M. *J. Org. Chem.* **2013**, *78*, 12106−12113.

(7) See the Supporting Information.

(8) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350−353.

(9) (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.

(10) (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.

(11) Banerjee, A.; Raghothama, S. R.; Karle, I. L.; Balaram, P. *Biopolymers* **1996**, *39*, 279−285.

DOI: 10.1021/acs.joc.5b01541
J. Org. Chem. 2015, 80, 8597−8603