Helical structures of L-Leu-based peptides having chiral six-membered ring amino acids

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ABSTRACT

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L-Leu-based peptides having chiral six-membered ring amino acids: Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- or (1*S*,3*R*)-Ac₆c^{3M}]}_n-OMe [*n* = 1 (**2**), 2 (**4**), 3 (**5**)], were synthesized. A conformational analysis using FT-IR **absorption** and NOESY NMR spectra revealed that these hexa- and nonapeptides having (1*R*,3*R*)-Ac₆c^{3M} or (1*S*,3*R*)-Ac₆c^{3M} formed helical structures in CDCl₃ solution, while that using CD spectra suggested that the nonapeptide **5b** having (1*S*,3*R*)-Ac₆c^{3M} become more right-handed (*P*) α -helical than the nonapeptide **5a** having (1*R*,3*R*)-Ac₆c^{3M} in TFE solution. An X-ray crystallographic analysis showed that the hexapeptide **4b** having (1*S*,3*R*)-Ac₆c^{3M} formed a partially distorted right-handed (*P*) ₃₁₀- α -helical structure in the crystal state, while the nonapeptides **5a** and **5b** having (1*R*,3*R*)-Ac₆c^{3M} or (1*S*,3*R*)-Ac₆c^{3M} formed similar right-handed (*P*) α -helical backbone structures.

1. Introduction

Cyclic α,α -disubstituted α -amino acids (cyclic dAAs) are α -amino acids in which the α -hydrogen atom of normal α -amino acids is replaced with alkyl substituents and the resultant two side chains become cyclic ring structures.^{1,2} Cyclic dAAs restrict the conformational freedom of their homo- and heteropeptides. Achiral cyclic dAA (Ac_nc; *n* = ring size)-containing oligopeptides have been reported to induce ₃₁₀/ α -helical secondary structures.³ Toniolo, Balaram, Benedetti, and co-workers studied the preferred structures of 1-aminocyclohexanecarboxylic acid (Ac₆c)-containing peptides.^{4,5} They reported that Ac₆c homopeptides formed ₃₁₀-helical structures (tripeptide: type III β -turn, tetrapeptides: ₃₁₀-helices) in the crystal state, and Ac₆c had a chair form cyclohexane conformation. In the chair form of the cyclohexane, the amino (amide) -NH- substituent showed an axial orientation and the carbonyl -CO- substituent occupied an equatorial orientation. This orientation was theoretically more stable than the opposite orientation by ~ 1.6 kcal/mol.⁶ Furthermore, the calculation of Ac-Ac₆c-NHMe suggested that the lowest energy conformation was torsion angles $\phi \pm 52^\circ$; $\psi \pm 46^\circ$ in the case of the -NH- axial position, while that with torsion angles $\phi \pm 48^\circ$; $\psi \pm 60^\circ$ ^{4b} (or $\phi \pm 40^\circ$; $\psi \pm 80^\circ$ ^{4c}) was favorable in the case of the -NH- equatorial position.

We recently reported that the axial and equatorial orientations of amino and carbonyl substituents may be controlled using chiral (1*R*,3*R*)- and (1*S*,3*R*)-1-amino-3-methylcyclohexanecarboxylic acids (Ac₆c^{3M}).⁷ Furthermore, we studied the preferred torsion angles of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} homo-chiral homopeptides. The absolute average values of the ϕ and ψ torsion angles of (1*R*,3*R*)-hexapeptide were 59.0° and 31.1°, respectively, which were close to the ideal torsion angles (60° and 30°) of the ₃₁₀-helical structure,^{8a} with the 3-methyl substituent and the -CO- group at an equatorial position and the -NH- group at an axial position. On the other hand, those of (1*S*,3*R*)-Ac₆c^{3M} hexapeptide were 55.9° and 47.1°, respectively, which were close to the standard α -helix (57° and 47°),⁸ with the 3-methyl substituent and the -NH- group at an equatorial position and the -CO- group in an axial direction. Thus, the orientation of the amino (amide) -NH- substituent of the chair form of the cyclohexane affected the secondary structure of its homo-chiral homopeptides (Fig. 1). We herein examined the effects of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} on their L-Leu-based heteropeptide conformations⁹ in solution and in the crystal state.

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Fig. 1. Structures of chiral six-membered ring α, α -disubstituted α -amino acids $\text{Ac}_6\text{c}^{3\text{M}}$ and their homopeptide secondary structures.⁷

2. Results and Discussion

2.1. Preparation of (1*R*,3*R*)- and (1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$ -containing L-Leu-based heteropeptides

We prepared Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}_{*n*}-OMe (**a**) and Boc-{L-Leu-L-Leu-[(1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}_{*n*}-OMe (**b**) (*n* = 1, 2, 3) using solution-phase methods. Tripeptide Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}-OMe (**2a**) was prepared by coupling between Boc-(L-Leu-L-Leu)-OH⁹ and (1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$ -OMe (**1a**)⁷ using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling reagents in 86% yield. The hydrolysis of the ester in **2a** under alkaline conditions (NaOH/H₂O-MeOH) gave a tripeptide acid (**3a**) in a quantitative yield, while the deprotection of the Boc-protecting group in **2a** under acidic conditions afforded a tripeptide amine in 94% yield. Coupling between the tripeptide acid (**3a**) and tripeptide amine using EDCI and HOBt gave a hexapeptide Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}₂-OMe (**4a**) in 85% yield. Similarly, coupling between the tripeptide acid (**3a**) and hexapeptide amine gave a nonapeptide Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}₃-OMe (**5a**) in 76% yield. No epimerization of peptides was observed in Boc deprotection and fragment condensation, which was confirmed by ¹H NMR spectrum measurements.¹⁰

Scheme 1. Preparation of peptides Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- & (1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}_{*n*}-OMe {*n* = 1 (**2**), 2 (**4**), 3 (**5**)}.

The (1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$ -containing heteropeptides Boc-{L-Leu-L-Leu-[(1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}_{*n*}-OMe (**2b**, **4b**, **5b**) (*n* = 1, 2, 3) were prepared in a manner similar to those of (1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$ heteropeptides, however, the hydrolysis of the C-terminal ester was performed using aqueous KOH (stronger basicity) in MeOH (Scheme 1).

2.2. Conformational analysis in solution

The dominant conformations of (1*R*,3*R*)- and (1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$ heteropeptides in solution were examined by using FT-IR absorption, ¹H NMR, and CD spectroscopies at room temperature.

The FT-IR absorption spectra of Boc-{L-Leu-L-Leu-[(1*R*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}_{*n*}-OMe [*n* = 1 (**2a**), 2 (**4a**), 3 (**5a**)] and Boc-{L-Leu-L-Leu-[(1*S*,3*R*)- $\text{Ac}_6\text{c}^{3\text{M}}$]}_{*n*}-OMe [*n* = 1 (**2b**), 2 (**4b**), 3 (**5b**)] in the 3500–3200 cm^{−1} region at a peptide concentration of 5.0 mM in CDCl₃ solution are shown in Figure 2. The FT-IR absorption spectra of helical peptides generally showed free (solvated) peptide NH groups at the 3420–3450 cm^{−1} region, and peptide NH groups with N-H...O=C intramolecular hydrogen bonds of different strengths at the 3320–3370 cm^{−1} region.⁹ The tripeptides **2a** and **2b** both showed a band at the 3440–3445 cm^{−1} region, but no band at the 3320–3370 cm^{−1} region. These results may be attributed to the tripeptide being too short for the helical structure, and the β -turn structure being relatively flexible. Also, the proteinogenic dipeptide sequence -L-Leu-L-Leu- is not expected to fold.¹¹ The FT-IR absorption spectra of the hexapeptides **4a** and **4b** and nonapeptides **5a** and **5b** showed weak bands at the 3430–3440 cm^{−1} region, as well as strong bands at the 3330–3340 cm^{−1} region. These FT-IR absorption spectra were similar to those of helical peptides.⁹

Fig. 2. FT-IR absorption spectra of a) Boc-{L-Leu-L-Leu-[(1*R*,3*R*)-Ac₆C^{3M}]}_{*n*}-OMe [*n* = 1 (**2a**), 2 (**4a**), 3 (**5a**)] and b) Boc-{L-Leu-L-Leu-[(1*S*,3*R*)-Ac₆C^{3M}]}_{*n*}-OMe [*n* = 1 (**2b**), 2 (**4b**), 3 (**5b**)].

The 2D nuclear Overhauser and exchange spectroscopies (NOESY) NMR of the nonapeptides **5a** and **5b** were measured in CDCl₃ solution (Fig. 3).

The NOESY NMR spectrum of the (1*R*,3*R*)-Ac₆C^{3M} nonapeptide **5a** showed a series of sequential NH (*i* → *i*+1) dipolar interactions from the N-terminal N(1)H to the C-terminal N(9)H, except for the NOE constrain NH (2 → 3) (Fig. 3a). On the other hand, the (1*S*,3*R*)-Ac₆C^{3M} nonapeptide **5b** showed a series of sequential NH (*i* → *i*+1) dipolar interactions from the N-terminal N(1)H to the C-terminal N(9)H, except for the NOE constrain NH (5 → 6) (Fig. 3b). These sequential NOE constraints suggested helical conformations. However, we were unable to determine whether a 3₁₀- or α-helical conformation was present because an overlap of α-proton signals occurred, and, thus, it was not possible to analyze the NOE constraints [*d*_{αN} (*i* → *i*+2)] and [*d*_{αN} (*i* → *i*+4)].¹²

Fig. 3. NOESY NMR spectra of nonapeptides. a) Boc-{L-Leu-L-Leu-[(1*R*,3*R*)-Ac₆C^{3M}]}₃-OMe (**5a**), and b) Boc-{L-Leu-L-Leu-[(1*S*,3*R*)-Ac₆C^{3M}]}₃-OMe (**5b**).

Figure 4 shows the CD spectra of the hexapeptides **4a** and **4b** and nonapeptides **5a** and **5b** in 2,2,2-trifluoroethanol (TFE) solution. The characteristics of the right-handed (*P*) helical peptide in CD spectra showed a positive maximum at 192 nm, and negative maxima at 208 nm and 222 nm.¹³ The (1*R*,3*R*)-Ac₆C^{3M} and (1*S*,3*R*)-Ac₆C^{3M} hexapeptides **4a** and **4b** both showed weak negative maxima at 204 nm (blue shift is seen) and 222 nm; however, the positive maximum at 192 nm was not clear. These negative maxima suggested the formation of a right-handed (*P*) helical structure. Right-handedness may be controlled by four L-Leu residues, and the weak intensity of maxima may be attributed to the length of the hexapeptide being insufficient to form a one-handed stable helical conformation. The

nonapeptides **5a** and **5b** showed a positive maximum at 192 nm, and negative maxima at approximately 205-207 nm and 222 nm, suggesting the formation of right-handed (*P*) helical conformations. The intensity of molar ellipticity in the (1*S*,3*R*)-Ac₆C^{3M} nonapeptide **5b** become stronger than that in **5b**, suggesting an increase in the helical content of **5b**. Based on *R* values (ratio of maxima), the (1*R*,3*R*)-Ac₆C^{3M} nonapeptide **5a** appeared to form a right-handed 3₁₀-helix ($\theta_{222}/\theta_{205}$; 0.48), while the (1*S*,3*R*)-Ac₆C^{3M} nonapeptide **5b** appeared to form a mixture of 3₁₀/α-helix ($\theta_{222}/\theta_{207}$; 0.60).

Fig. 4. CD spectra of a) hexapeptides **4a** (dash line) and **4b** (solid line) and b) nonapeptides **5a** (dash line) and **5b** (solid line) in TFE solution. Concentration: 0.05 mM.

2.3. Crystal structures of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆C^{3M}-containing L-Leu-based heteropeptides in the crystal state

The crystal and diffraction parameters of the (1*R*,3*R*)-Ac₆C^{3M} and (1*S*,3*R*)-peptides **4b**, **5a**, and **5b** are summarized in Table 1.^{14,15} No suitable crystals of **4a** were obtained for X-ray crystallographic analysis. Their molecular structures are given in Figures 5–8. Relevant backbone and side-chain torsion angles as well as intra- and intermolecular hydrogen-bond parameters are listed in Tables 2 and 3.

The structure of the (1*S*,3*R*)-Ac₆C^{3M} hexapeptide **4b** was solved in the space group *P*2₁2₁2₁. A right-handed (*P*) helical structure, along with one water molecule, existed in the asymmetric unit. The N-terminal Boc-protecting group was disordered. Figure 5 shows a right-handed (*P*) 3₁₀/α-helical structure, in which the ϕ and ψ torsion angles of the penultimate L-Leu (5) residue were distorted. The average ϕ and ψ torsion angles of residues (2–4 and 6) were -57.0° and -40.2° , respectively, were intermediate values between the ideal right-handed (*P*) 3₁₀-helical structure (-60° and -30°) and (*P*) α-helix (-57° and -47°).⁸ The conformation of side-chain cyclohexane rings at residues (3) and (6) were in the chair form, with the 3-

methyl substituent and –NH– group at an equatorial orientation and the –CO– group in an axial direction. Corresponding torsion angles (χ , χ') relating the cyclohexyl ring to the peptide chain showed nearly -180° and $+180^\circ$, which indicated that the –NH– groups occupied the equatorial positions (Table 2).^{4c}

Two intramolecular hydrogen bonds of the *i*←*i*+3 type (3₁₀-helix) were observed between H–N(4) and C(1)=O(1) [N(4)⋯O(1) = 2.90 Å] and between H–N(5) and C(2)=O(2) [N(5)⋯O(2) = 2.92 Å]. Furthermore, the intramolecular hydrogen bond of the *i*←*i*+3 type was weak between H–N(6) and C(3)=O(3) [N(6)⋯O(3) = 3.37 Å]. The intramolecular hydrogen bond of the *i*←*i*+4 type (α-helix) was also weak between H–N(5) and C(1)=O(1) [N(5)⋯O(1) = 3.32 Å]. Thus, H–N(5) was bound by bifurcated hydrogen bonds to C(1)=O(1) and C(2)=O(2). The distance between H–N(6) and C(2)=O(2) was 3.19 Å, which was within the hydrogen-bonding distance, whereas the angle for N(6)–H⋯O(2) was 106° , which was too small for the hydrogen bond. Therefore, the intramolecular hydrogen bond of the *i*←*i*+4 type may not be formed between them. In the packing mode, bifurcated intermolecular hydrogen bonds were observed between H–N(1) and H–N(2) peptide donors and the C(4')=O(4') acceptor of a symmetry-related (1/2–*x*, –*y*, 1/2+*z*) molecule. Furthermore, the H–N(3) peptide donor was hydrogen bonded to the symmetry-related (–*x*, 1/2+*y*, 1/2–*z*) O_w of water, and H–O_w was hydrogen bonded to the C(5')=O(5') acceptor.

Fig. 5. Right-handed (*P*) 3₁₀/α-helical structure of the (1*S*,3*R*)-Ac₆C^{3M} hexapeptide **4b** by an X-ray crystallographic analysis. a) View perpendicular to the helical axis and b) an ORTEP drawing as viewed along the helical axis.

The (1*R*,3*R*)-Ac₆c^{3M} nonapeptide **5a** was solved in the space group of *P*2₁ to form a right-handed (*P*) α-helical structure together with three water molecules in the asymmetric unit (Fig. 6). The average ϕ and ψ torsion angles of residues (1–7) were -60.5° and -43.6° , respectively, which were consistent with those of the ideal (*P*) α-helix (-57° and -47°);⁸ however, the ϕ and ψ torsion angles of C-terminal (1*R*,3*R*)-Ac₆c^{3M} (9) and penultimate L-Leu (8) residues were distorted, and the sign of the ϕ torsion angle of residue (9) reverted to positive. Three side-chain cyclohexane rings at residues (3), (6), and (9) formed chair-form conformations. The torsion angles (χ , χ') relating the cyclohexyl ring to the peptide chain showed nearly -60° and $+60^\circ$, which indicated that the –NH– groups occupied the axial positions.^{4c} Thus, the 3-methyl substituent and the –CO– group of cyclohexane occupied an equatorial orientation, while the –NH– group pointed in an axial direction.

Six intramolecular hydrogen bonds of the *i*←*i*+4 type (α-helix) were observed between H–N(4) and the C(0)=O(0) oxygen atom of the Boc-group [N(4)⋯O(0) = 3.14 Å], between H–N(5) and C(1)=O(1) [N(5)⋯O(1) = 2.93 Å], between H–N(6) and C(2)=O(2) [N(6)⋯O(2) = 3.07 Å], between H–N(7) and C(3)=O(3) [N(7)⋯O(3) = 3.19 Å], between H–N(8) and C(4)=O(4) [N(8)⋯O(4) = 2.88 Å], and between H–N(9) and C(5)=O(5) [N(9)⋯O(5) = 3.00 Å]. In the packing mode, an intermolecular hydrogen bond was observed between the H–N(1) peptide donor and C(8')=O(8') of a symmetry-related (1–*x*, $-1/2+y$, $-z$) molecule. Furthermore, the peptide H–N(2) donor was intermolecularly hydrogen-bonded to a water O_{w3}, and three water molecules formed a cluster. The water H–O_{w1} donor formed hydrogen bonds with C(8)=O(8) and with the C(6)=O(6) of the symmetry-related ($-1+x$, *y*, *z*) molecule. The water H–O_{w2} donor formed hydrogen bonds with the C(6)=O(6) and C(7)=O(7) acceptors of the peptide.

Fig. 6. Right-handed (*P*) α-helical structure of the (1*R*,3*R*)-Ac₆c^{3M} nonapeptide **5a** by an X-ray crystallographic analysis. a) View perpendicular to the helical axis and b) an ORTEP drawing as viewed along the helical axis.

The (1*S*,3*R*)-Ac₆c^{3M} nonapeptide **5b** was solved in the space group of *P*2₁2₁2₁ to form a right-handed (*P*) α-helical structure together with three water molecules in the asymmetric unit (Fig. 7). The average ϕ and ψ torsion angles of residues (1–7) were -62.3° and -42.9° , respectively, which corresponded to those of the ideal (*P*) α-helix (-57° and -47°),⁸ however, the ϕ and ψ torsion angles of C-terminal (1*S*,3*R*)-Ac₆c^{3M} (9) and penultimate L-Leu (8) residues were distorted. The torsion angles (χ , χ') relating the cyclohexyl rings to the peptide chain at residues (3), (6), and (9) showed near -180° and $+180^\circ$, with the –NH– groups at the equatorial positions of the chair form cyclohexane rings. Thus, the 3-methyl substituent and –NH– group of cyclohexane occupied an equatorial orientation, while the –CO– group pointed in an axial direction.

Five intramolecular hydrogen bonds of the *i*←*i*+4 type (α-helix) were observed between H–N(5) and C(1)=O(1) [N(5)⋯O(1) = 2.87 Å], between H–N(6) and C(2)=O(2) [N(6)⋯O(2) = 3.13 Å], between H–N(7) and C(3)=O(3) [N(7)⋯O(3) = 3.18 Å], between H–N(8) and C(4)=O(4) [N(8)⋯O(4) = 2.93 Å], and between H–N(9) and C(5)=O(5) [N(9)⋯O(5) = 3.17 Å]. The distance of N(4)⋯O(0) (3.47 Å) was too long for an intramolecular hydrogen bond. The peptide H–N(1), H–N(2), and H–N(3) donors were intermolecularly hydrogen-bonded to the water acceptors O_{w1}, O_{w2}, and O_{w3}, respectively. Furthermore, the water H–O_{w1} donor formed bifurcated hydrogen bonds with the C(8)=O(8) and C(6')=O(6') acceptors, and the water H–O_{w2} donor formed a hydrogen bond with the C(7)=O(7) acceptor. Three water molecules formed a cluster by intermolecular hydrogen bonds.

The superimposed structures of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} nonapeptides are shown in Figure 8. The peptide-backbone structures of **5a** and **5b** were both right-handed (*P*) α-helical structures, and fit very well; however, the C-terminal methyl esters and side-chain cyclohexane structures differed. Thus, the effects of the –NH– group orientation of the cyclohexane chair form on its L-Leu-based peptide backbone structures was weak in the crystal state.

Table 1. Crystal and diffraction parameters of (1*R*,3*R*)-Ac₆c^{3M} and (1*S*,3*R*)-Ac₆c^{3M} peptides **4b**, **5a** and **5b**.

Torsion Angle	(1 <i>S</i> ,3 <i>R</i>)-6mer 4b	(1 <i>R</i> ,3 <i>R</i>)-9mer 5a	(1 <i>S</i> ,3 <i>R</i>)-9mer 5b
empirical formula	C ₄₆ H ₈₀ N ₆ O ₉ , H ₂ O	C ₆₆ H ₁₁₇ N ₉ O ₁₂ , 3H ₂ O	C ₆₆ H ₁₁₇ N ₉ O ₁₂ , 3H ₂ O
<i>Mr</i>	879.18	1282.73	1282.73
crystal diameters [mm]	0.22 x 0.19 x 0.04	0.15 x 0.06 x 0.04	0.20 x 0.16 x 0.06
Temperature [K]	93	93	93
crystal system	orthorhombic	monoclinic	orthorhombic
lattice parameters:			
<i>a</i> , <i>b</i> , <i>c</i> [Å]	15.232, 17.878, 18.802	10.984, 29.604, 12.520	10.697, 23.277, 30.373
α , β , γ [°]	90, 90, 90	90, 109.013, 90	90, 90, 90
<i>V</i> [Å ³]	5120	3849	7563
space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>Z</i> value	4	2	4
<i>D</i> calc [g/cm ³]	1.140	1.107	1.127
μ (MoK α) [cm ⁻¹]	0.80	0.78	0.79
no. of observations (<i>I</i> > -10.0 σ (<i>I</i>))	5769	5640	8499
no. of variables	623	811	826
<i>R</i> ₁ , <i>R</i> _w	0.0723, 0.1576	0.1072, 0.1738	0.0849, 0.2026
solvent	MeOH/H ₂ O	MeOH/H ₂ O	MeOH/H ₂ O

Table 2. Selected torsion angles ω , ϕ , ψ , and χ [°] of (1*R*,3*R*)-Ac₆c^{3M} and (1*S*,3*R*)-Ac₆c^{3M} peptides **4b**, **5a** and **5b**.^a

Torsion Angle	(1 <i>S</i> ,3 <i>R</i>)-6mer 4b	(1 <i>R</i> ,3 <i>R</i>)-9mer 5a	(1 <i>S</i> ,3 <i>R</i>)-9mer 5b
$\omega 0$	---	-169.2	-178.4
$\phi 1$	---	-55.5	-65.1
$\psi 1$	-40.8	-47.5	-45.5
$\omega 1$	-179.3	-174.3	-173.1
$\phi 2$	-55.3	-63.9	-61.0
$\psi 2$	-43.5	-44.2	-43.4
$\omega 2$	176.7	178.7	176.4
$\phi 3$	-45.7	-54.0	-50.7
$\psi 3$	-49.0	-47.5	-50.8
$\omega 3$	-171.8	-179.2	-175.5
$\phi 4$	-70.8	-60.9	-66.6
$\psi 4$	-13.6	-41.1	-38.8
$\omega 4$	-176.9	180.0	178.4
$\phi 5$	-110.9	-61.0	-59.0
$\psi 5$	2.7	-47.4	-47.0
$\omega 5$	-172.6	-177.1	-177.3
$\phi 6$	-56.2	-54.3	-59.2
$\psi 6$	-54.5	-39.2	-40.1
$\omega 6$	-174.7	-179.3	-177.9
$\phi 7$	---	-73.6	-74.6
$\psi 7$	---	-38.0	-34.8
$\omega 7$	---	-169.9	-164.7
$\phi 8$	---	-91.3	-102.5
$\psi 8$	---	-13.4	-2.9
$\omega 8$	---	-176.7	169.5
$\phi 9$	---	64.4	-59.1
$\psi 9$	---	-169.8	139.0
$\omega 9$	---	-177.5	176.0

Fig. 7. Right-handed (*P*) α -helical structure of the (1*S*,3*R*)-Ac₆c^{3M} nonapeptide **5b** by an X-ray crystallographic analysis. a) View perpendicular to the helical axis and b) an ORTEP drawing as viewed along the helical axis. **Fig. 8.** Superimposed structures of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} nonapeptides **5a** (red) and **5b** (blue).

	χ^1	178.7	179.1	-178.2
	χ^2	-175.3	178.3	-59.4
	χ^3	-174.8	-67.0	-175.7
1	χ^3'	169.0	66.3	168.4
2	χ^4	-53.6	-64.4	-62.3
3	χ^5	-56.6	179.6	-60.0
4	χ^6	-175.7	-69.0	-170.3
5	χ^6'	169.0	66.8	163.5
6	χ^7	---	-65.9	-77.4
7	χ^8	---	-69.7	-62.0
8	χ^9	---	-64.9	-171.6
9	χ^9'	---	69.3	165.3

^a The number of amino acid residues begins at the *N*-terminus of the peptide chain.

^b Disordered.

Table 3. Intra- and intermolecular H-bond parameters for (1*R*,3*R*)-Ac₆C^{3M} and (1*S*,3*R*)-Ac₆C^{3M} peptides **4b**, **5a** and **5b**.

Peptide	Donor D-H	Acceptor A	Distance [Å] D...A	Angle [°] D-H...A	Symmetry operations
Boc-{L-Leu-L-Leu-[(1 <i>S</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}]} ₂ -OMe (4b)					
	N ₄ -H	O ₁	2.90	124	x,y,z
	N ₅ -H	O ₁	3.32 ^a	122	x,y,z
	N ₅ -H	O ₂	2.92	153	x,y,z
	N ₆ -H	O ₂	3.19	106 ^b	x,y,z
	N ₆ -H	O ₃	3.37 ^a	167	x,y,z
	N ₁ -H	O ₄	2.87	129	1/2-x, -y,1/2+z
	N ₂ -H	O ₄	2.98	172	1/2-x, -y,1/2+z
	N ₃ -H	O _w	2.98	151	-x,1/2+y,1/2-z
	O _w -H	O ₅	2.92	174	x,y,z
Boc-{L-Leu-L-Leu-[(1 <i>R</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}]} ₃ -OMe (5a)					
	N ₄ -H	O ₀	3.14	166	x,y,z
	N ₅ -H	O ₁	2.93	154	x,y,z
	N ₆ -H	O ₂	3.07	168	x,y,z
	N ₇ -H	O ₃	3.19	153	x,y,z
	N ₈ -H	O ₄	2.88	141	x,y,z
	N ₉ -H	O ₅	3.00	140	x,y,z
	N ₁ -H	O ₈	2.95	146	1-x, -1/2+y, -z
	N ₂ -H	O _{w3}	2.86	152	x,y,z
	O _{w1} -H	O ₈	2.81	158	x,y,z
	O _{w1} -H	O ₆	2.90	169	-1+x,y,z
	O _{w2} -H	O ₆	2.99	169	x,y,z
	O _{w2} -H	O ₇	2.77	150	x,y,z
	O _{w3} -H	O _{w1}	2.77	147	1-x, -1/2+y, -z
	O _{w3} -H	O _{w2}	2.83	153	2-x, -1/2+y, -z
Boc-{L-Leu-L-Leu-[(1 <i>S</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}]} ₃ -OMe (5b)					
	N ₄ -H	O ₀	3.47 ^a	161	x,y,z
	N ₅ -H	O ₁	2.87	159	x,y,z

N ₆ -H	O ₂	3.13	169	x,y,z
N ₇ -H	O ₃	3.18	151	x,y,z
N ₈ -H	O ₄	2.93	134	x,y,z
N ₉ -H	O ₅	3.17	141	x,y,z
N ₁ -H	O _{w1}	2.91	171	3/2-x,1-y, -1/2+z
N ₁ -H	O _{w2}	2.95	105 ^b	1/2-x,1-y, -1/2+z
N ₂ -H	O _{w2}	2.78	179	1/2-x,1-y, -1/2+z
N ₃ -H	O _{w3}	3.07	167	x,y,z
O _{w1} -H	O ₈	2.81	171	x,y,z
O _{w2} -H	O ₇	2.59	137	x,y,z
O _{w2} -H	O _{w1}	2.74	156	-1+x,y,z
O _{w1} -H	O ₆	2.82	167	-1+x,y,z
O _{w3} -H	O _{w2}	2.71	145	1/2-x,1-y, -1/2+z

^a The distance is a bit long or too long for an intramolecular hydrogen bond.

^b The angle N-H...O is too small for a hydrogen bond.

The reported theoretical calculation of Ac-Ac₆C-NHMe showed the torsion angles $\phi \pm 48^\circ$; $\psi \pm 60^\circ$ ^{4b} (or $\phi \pm 40^\circ$; $\psi \pm 80^\circ$ ^{4c}) as a minimum energy structure when the amino -NH- group of the cyclohexane amino acid occupied the equatorial position. However, the X-ray crystallographic analysis of the (1*S*,3*R*)-Ac₆C heteropeptides **4b** and **5b** did not show large ψ torsion angles, as calculated. The (1*R*,3*R*)-Ac₆C^{3M} and (1*S*,3*R*)-Ac₆C^{3M} nonapeptides **5a** and **5b** both formed almost similar right-handed (*P*) α -helical structures in the crystal state. These results may be attributed to cyclic dAA-containing L-Leu-based nonapeptides preferring to form α -helical structures over the 3_{10} -helical structures of Aib-containing peptides.⁹ We previously reported that a (1*R*,3*R*)-Ac₆C^{3M} homohexapeptide formed right-handed (*P*) and left-handed (*M*) 3_{10} -helical structures, while a (1*S*,3*R*)-Ac₆C^{3M} homohexapeptide formed (*P*) and (*M*) α -helical structures in the crystal state. In comparison, the properties of the α -helical formation of (1*S*,3*R*)-Ac₆C^{3M} were stronger than those of (1*R*,3*R*)-Ac₆C^{3M}.

In the CD spectra of the nonapeptides **5a** and **5b** in TFE solution, the intensities of the negative maxima (208 nm and 222 nm) of the (1*S*,3*R*)-Ac₆C^{3M} peptide **5b** were stronger than those of the (1*R*,3*R*)-Ac₆C^{3M} peptide **5b**. Thus, the (1*S*,3*R*)-Ac₆C^{3M} peptide **5b** may be more (right-handed, *P*) helical than the (1*R*,3*R*)-Ac₆C^{3M} peptide **5a** in TFE solution.

The results of the conformational analysis between solution and the crystal state were slightly different. These differences may have been due to the solvents used in the conformational study being different between the solution analysis (CDCl₃ or TFE) and crystal state (re-crystallized solvent: MeOH/H₂O).¹⁶ Also, the crystal packing forces and nucleation events in crystallization may affect the conformation in the crystal state.

The heteropeptides studied had the -L-Leu-L-Leu-Ac₆C^{3M}- sequence, in which the L-Leu content was 66% and Ac₆C^{3M} content was 33%. Thus, the effects of L-Leu properties on the screw sense of its peptide secondary structure were stronger than those of Ac₆C^{3M} because these peptides exclusively formed right-handed helices due to L-Leu chirality. Four stereoisomers, including a pair of enantiomers, may exist in the structure of Ac₆C^{3M}. Therefore, if other stereo-isomeric Ac₆C^{3M} were incorporated into the L-Leu sequence, the peptides may form different secondary structures due to the effects of

stereochemistry as well as the amino –NH– group equatorial or axial influence.

3. Conclusion

We prepared L-Leu-based peptides having (1*R*,3*R*)-Ac₆C^{3M} and (1*S*,3*R*)-Ac₆C^{3M}, respectively, and studied their preferred conformations in solution and in the crystal state.

In L-Leu-based peptides, the equatorial or axial orientation of the –NH– group of side-chain cyclohexane was controlled using a 3-methyl substituent of chiral cyclic Ac₆C^{3M}. Based on the CD spectra, the (1*S*,3*R*)-Ac₆C^{3M} nonapeptide **5b** may be more (right-handed, *P*) helical than the (1*R*,3*R*)-Ac₆C^{3M} nonapeptide **5a** in TFE solution; however, in the crystal state, the (1*R*,3*R*)-Ac₆C^{3M} and (1*S*,3*R*)-Ac₆C^{3M} nonapeptides **5a** and **5b** both formed almost similar right-handed (*P*) α-helical structures in the peptide backbone. The differences of conformational study between solution and the crystal state may have been due to the different solvents, and also the crystal packing forces and nucleation events in crystallization. Thus, the effects of the equatorial or axial orientation of the –NH– group of cyclic Ac₆C on the secondary structures of its peptides were not strong.

These results may be useful for designing chiral peptide catalysts for enantioselective reactions,^{17,18} which is under way in our group.¹⁹

4. Experimental Section

4.1. General

Optical rotations [α]_D were measured using a 1.0-dm cell. Circular dichroism spectra (CD) were measured using a 1.0-mm path length cell. Infrared spectra (IR) were recorded for conventional measurements (KBr), and the solution (CDCl₃) method was performed using the 0.1-mm path length of an NaCl cell. ¹H NMR spectra were determined at 400 or 500 MHz at room temperature. HRMS(ESI) spectra were measured in the ToF mode.

4.2. Synthesis of L-Leu-based peptides

4.2.1. Boc-L-Leu-L-Leu-(1*R*,3*R*)-Ac₆C^{3M}-OMe (2a). A mixture of Boc-L-Leu-L-Leu-OH (1.62 g, 4.70 mmol), amine **1a**⁷ (670 mg, 3.91 mmol), EDCI·HCl (900 mg, 4.70 mmol), and HOBT·H₂O (640 mg, 4.70 mmol) in MeCN (20 mL) was stirred at 0 °C for 30 min. After being stirred at room temperature for 17 h, the solution was evaporated, diluted with EtOAc, washed with 1M aqueous HCl, water, 5% aqueous NaHCO₃, and brine, and then dried over MgSO₄. The removal of the solvent afforded a solid, which was purified by column chromatography on silica gel. The fraction eluted with 30% EtOAc in *n*-hexane afforded the tripeptide **2a** (1.67 g, 86%) as crystals: mp 163–165 °C; [α]_D²⁵ –76.3 (*c* 1.00, CHCl₃); IR (KBr) ν 3331 (br), 2955, 1745, 1718, 1647, 1528 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 6.72 (s, 1H), 6.51 (d, *J* = 6.9 Hz, 1H), 4.91 (br d, *J* = 6.0 Hz, 1H), 4.42 (m, 1H), 4.09 (m, 1H), 3.66 (s, 3H), 2.19 (d, *J* = 12.8 Hz, 1H), 2.09 (d, *J* = 12.7 Hz, 1H), 1.79–1.46 (m, 10H), 1.45 (s, 9H), 1.41–1.27 (m, 2H), 0.99–0.82 (m, 16H); ¹³C NMR (100 MHz, CDCl₃): δ 174.5, 172.9, 170.8, 155.8, 80.4, 59.4, 53.2, 52.1, 51.5, 40.9, 40.2, 39.6, 33.9, 31.1, 28.2 (3C), 27.4, 24.8, 24.6, 22.89, 22.85, 22.3, 22.0, 21.8, 21.1; HRMS(ESI): [M+Na]⁺, found 520.3369. C₂₆H₄₇N₃O₆Na requires 520.3363.

4.2.2. Boc-L-Leu-L-Leu-(1*S*,3*R*)-Ac₆C^{3M}-OMe (2b).

Tripeptide **2b** was prepared from **1b** in a manner similar to that described for the preparation of tripeptide **2a**: 88%; crystals; mp 174–176 °C; [α]_D²⁵ –63.5 (*c* 1.00, CHCl₃); IR (KBr) ν 3309 (br), 2955, 1748, 1651, 1524 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 6.80 (s, 1H), 6.68 (br d, *J* = 7.8 Hz, 1H), 5.07 (br d, *J* = 5.3 Hz, 1H), 4.38 (m, 1H), 4.11 (m, 1H), 3.68 (s, 3H), 2.45–2.34 (m, 2H), 1.77–1.57 (m, 8H), 1.55–1.46 (m, 3H), 1.44 (s, 9H), 1.26 (m, 1H), 0.98–0.76 (m, 16H); ¹³C NMR (100 MHz, CDCl₃) δ 173.2, 172.7, 170.6, 155.7, 80.2, 59.1, 53.3, 51.9, 51.4, 42.6, 40.9, 40.2, 34.1, 34.0, 28.6, 28.2 (3C), 24.7, 24.6, 22.9, 22.8, 22.32, 22.29, 21.9 (2C); HRMS(ESI): [M+Na]⁺, found 520.3372. C₂₆H₄₇N₃O₆Na requires 520.3363.

4.2.3. Boc-L-Leu-L-Leu-(1*R*,3*R*)-Ac₆C^{3M}-OH (3a). A solution of tripeptide **2a** (600 mg, 1.20 mmol) in MeOH (200 mL) and 0.1M aqueous NaOH (600 mL) was stirred at 40 °C for 5 days. Then, the solution was acidified with 1M aqueous HCl, and MeOH was evaporated. The aqueous phase was extracted with CHCl₃, and dried over MgSO₄. The removal of the solvent afforded a crude carboxylic acid **3a** (560 mg, quantitative) as a solid, which was used for the next step without further purification.

4.2.4. Boc-[L-Leu-L-Leu-(1*R*,3*R*)-Ac₆C^{3M}]₂-OMe (4a).

Concentrated HCl (3 mL) was added to the stirred solution of **2a** (560 mg, 1.13 mmol) in EtOAc (9 mL) and MeOH (1 mL) at 0 °C, and the solution was stirred at room temperature. After being stirred for 2 h, the solution was neutralized with 5% aqueous NaHCO₃, extracted with CHCl₃, and dried over MgSO₄. The removal of the solvent afforded a crude amine (420 mg, 94%) as a solid, which was used for the next step without further purification. A solution of crude carboxylic acid **3a** (97.0 mg, 0.200 mmol), the crude amine (73.0 mg, 0.184 mmol), EDCI·HCl (38 mg, 0.20 mmol), and HOBT·H₂O (27 mg, 0.20 mmol) in MeCN (5 mL) was stirred at 0 °C for 30 min, and at room temperature for 18 h. Then, the reaction mixture was diluted with EtOAc, washed with 1M aqueous HCl, water, 5% aqueous NaHCO₃, and brine, and then dried over Na₂SO₄. The removal of the solvent afforded a solid, which was purified by column chromatography on silica gel (50% EtOAc in *n*-hexane) to give the hexapeptide **4a** (134 mg, 85%) as crystals: mp 235–237 °C; [α]_D²⁵ –29.7 (*c* 1.00, CHCl₃); IR (KBr) ν 3370 (br), 2957, 1745 (w), 1720 (w), 1649 (br), 1527 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 7.46 (d, *J* = 8.3 Hz, 1H), 7.21 (d, *J* = 5.5 Hz, 1H), 6.86 (s, 2H), 6.70 (s, 1H), 4.96 (br d, *J* = 3.0 Hz, 1H), 4.41 (m, 1H), 4.28 (m, 1H), 3.97–3.88 (m, 2H), 3.66 (s, 3H), 2.40–2.13 (m, 2H), 1.84–1.56 (m, 23H), 1.48 (s, 9H), 1.33–1.23 (m, 3H), 1.03–0.83 (m, 32H); HRMS(ESI): [M+Na]⁺, found 885.6034. C₄₆H₈₂N₆O₉Na requires 885.6041.

4.2.5. Boc-[L-Leu-L-Leu-(1*S*,3*R*)-Ac₆C^{3M}]₂-OMe (4b).

Hexapeptide **4b** was prepared from **2b** in a manner similar to that described for the preparation of tripeptide **4a**: 80%; crystals; mp 211–213 °C; [α]_D²⁶ –22.6 (*c* 1.00, CHCl₃); IR (KBr) ν 3323 (br), 2957, 1746, 1728, 1651 (br), 1526 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 7.37 (d, *J* = 8.8 Hz, 1H), 7.25 (s, 1H), 7.05 (s, 1H), 6.94 (d, *J* = 5.8 Hz, 1H), 6.52 (br d, *J* = 4.4 Hz, 1H), 4.97 (d, *J* = 2.9 Hz, 1H), 4.41 (m, 1H), 4.12 (m, 1H), 3.96–3.88 (m, 2H), 3.69 (s, 3H), 2.85 (d, *J* = 11.7 Hz, 1H), 2.42 (d, *J* = 12.7 Hz, 2H), 2.27 (m, 1H), 1.92 (d, *J* = 12.2 Hz, 1H), 1.84–1.54 (m, 20H), 1.50 (s, 9H), 1.34–1.24 (m, 3H), 1.04–0.78 (m, 32H); HRMS(ESI): [M+Na]⁺, found 885.6016. C₄₆H₈₂N₆O₉Na requires 885.6041.

4.2.6. Boc-[L-Leu-L-Leu-(1*R*,3*R*)-Ac₆C^{3M}]₃-OMe (5a).

Nonapeptide **5a** was prepared from **4a** and **3a** in a manner similar to that described for the preparation of hexapeptide **4a**: 76%; crystals; mp 222 °C (decomp.); [α]_D²⁵ –27.5 (*c* 1.00, CHCl₃); IR

(KBr) ν 3329 (br), 2955, 1744, 1654, 1524 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.78 (d, J = 4.2 Hz, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 5.4 Hz, 1H), 7.31 (d, J = 6.8 Hz, 1H), 7.03 (s, 1H), 6.98 (s, 1H), 6.96 (s, 1H), 6.92 (s, 1H), 5.43 (br s, 1H), 4.35 (m, 1H), 4.27 (m, 1H), 4.03–3.84 (m, 4H), 3.65 (s, 3H), 2.47 (d, J = 13.6 Hz, 1H), 2.33 (t, J = 12.6 Hz, 2H), 2.19 (d, J = 12.0 Hz, 1H), 2.00 (d, J = 11.7 Hz, 1H), 1.92–1.55 (m, 34H), 1.49 (s, 9H), 1.31–1.23 (m, 3H), 1.04–0.84 (m, 48H); HRMS(ESI): $[\text{M}+\text{H}]^+$, found 1228.8865. $\text{C}_{66}\text{H}_{118}\text{N}_9\text{O}_{12}$ requires 1228.8900.

4.2.7. Boc-[L-Leu-L-Leu-(1S,3R)-Ac₆C^{3M}]₃-OMe (5b).

Nonapeptide **5b** was prepared from **4b** and **3b** in a manner similar to that described for the preparation of hexapeptide **4b**: 88%; crystals; mp 237–239 °C; $[\alpha]_{\text{D}}^{26}$ –13.6 (c 1.00, CHCl_3); IR (KBr) ν 3327 (br), 2957, 1744 (w), 1719 (w), 1655 (br), 1530 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.51 (d, J = 8.3 Hz, 1H), 7.47 (d, J = 5.3 Hz, 1H), 7.39 (s, 1H), 7.22 (s, 2H), 7.01 (d, J = 6.4 Hz, 1H), 6.89 (d, J = 5.3 Hz, 1H), 6.69 (d, J = 3.2 Hz, 1H), 5.19 (s, 1H), 4.38 (m, 1H), 4.11 (m, 1H), 3.98–3.84 (m, 4H), 3.69 (s, 3H), 2.98–2.87 (m, 2H), 2.51–2.36 (m, 2H), 2.35–2.13 (m, 2H), 2.01–1.81 (m, 3H), 1.79–1.60 (m, 30H), 1.51 (s, 9H), 1.32–1.21 (m, 3H), 1.03–0.82 (m, 48H); HRMS(ESI): $[\text{M}+\text{H}]^+$, found 1228.8855. $\text{C}_{66}\text{H}_{118}\text{N}_9\text{O}_{12}$ requires 1228.8900.

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Helical structures of L-Leu-based peptides having chiral six-membered ring amino acids

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