FULL PAPER

Helical L-Leu-Based Peptides Having Chiral Five-Membered Carbocyclic Ring Amino Acids with an Ethylene Acetal Moiety

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Abstract: L-Leu-based heteropeptides having (*R*)- or (*S*)-chiral five-membered carbocyclic ring amino acids (Ac_5c^{3EG}) with an ethylene acetal moiety were prepared. A conformational analysis using FT-IR absorption, ^{1}H NMR, and CD spectra revealed that L-Leu-based hexapeptides and nonapeptides having (*R*)- or (*S*)-Ac_5c^{3EG} formed right-handed (*P*) helical structures in solution. An X-ray crystallographic analysis of nonapeptides **5a** and **5b** showed similar right-handed (*P*) α-helical structures, without an intramolecular hydrogen bond of the peptide N–H···–O– (acetal) type.

Introduction

L-Amino acid-based heteropeptides having α,α-disubstituted α-amino acids (dAAs) have been reported to form helical secondary structures.[1–6] As a dAA, α-aminoisobutyric acid (Aib) has widely been used to induce helical structures. A right-handed (*P*) 3_{10}-helix is induced in relatively shorter L-amino acid-based peptides having a high Aib content; however, a right-handed (*P*) α-helix is preferentially formed in relatively longer L-amino acid-based peptides having a low Aib content.[7] Besides Aib, we incorporated cyclic dAAs such as achiral 1-aminocyclopentanecarboxylic acid (Ac_5c),[8,9] chiral (*S*,*S*)-1-amino-3,4-(dimethoxy)cyclopentanecarboxylic acid {(*S*,*S*)-Ac_5c^{dOM}},[8–11] and (1*S*,3*S*)-1-amino-3-methoxycyclopentanecarboxylic acid {(1*S*,3*S*)-Ac_5c^{OM}}[12,13] into L-Leu-based heteropeptides –(L-Leu-L-Leu-dAA)$_n$– as model peptides. The amino acid (*S*,*S*)-Ac_5c^{dOM} has two chiral centers exclusively at the side chain without a α-chiral center, and the (1*S*,3*S*)-Ac_5c^{OM} has chiral centers both at the α-carbon and at the side chain. We reported that these cyclic amino acid-containing L-Leu-based peptides induced right-handed (*P*) α-helical structures. Furthermore, the cyclic amino acid-containing L-amino acid-based heteropeptides may be used as chiral organocatalysts[13–15] and cell-penetrating peptides.[16–19]

We previously reported the synthesis of a chiral five-membered carbocyclic ring dAA: (*R*)- or (*S*)-amino-3,3-(ethylenedioxy)cyclopentanecarboxylic acid (Ac_5c^{3EG}) with an ethylene acetal moiety, in which the α-carbon atom was a chiral center, and the helical structures of its homo-chiral homopeptides.[20] In the present study, to reveal the influence of (*S*)- or (*R*)-Ac_5c^{3EG} on its heteropeptide conformation, we prepared L-Leu-based heteropeptides having (*S*)- or (*R*)-Ac_5c^{3EG}; Cbz-[L-Leu-L-Leu-{(*S*)- or (*R*)-Ac_5c^{3EG}}]$_n$-OMe (n = 1, 2, and 3), and examined their preferred conformations in solution and in a crystal state (Figure 1).

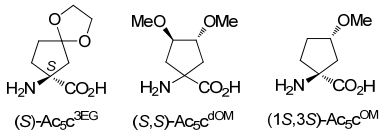

Figure 1. Chemical structures of chiral five-membered carbocyclic ring α,α-disubstituted α-amino acids: (*S*)-Ac_5c^{3EG}, (*S*,*S*)-Ac_5c^{dOM} and (1*S*,3*S*)-Ac_5c^{OM} reported by us.

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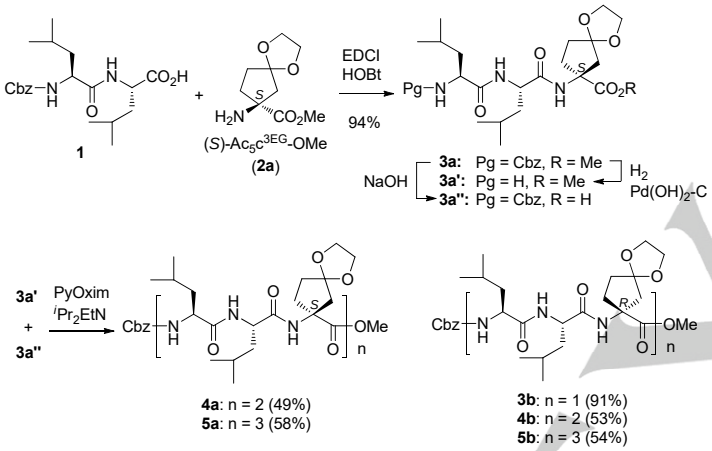
Results and Discussion

Preparation of (*S*)- and (*R*)-Ac$_5$c^{3EG}-Containing L-Leu-Based Heteropeptides

We prepared Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_n$-OMe (**a**) and Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_n$-OMe (**b**) (n = 1, 2, and 3) using the following solution-phase methods (Scheme 1). Tripeptide Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]-OMe (**3a**) was prepared by coupling between Cbz-(L-Leu-L-Leu)-OH (**1**) and (*S*)-Ac$_5$c^{3EG}-OMe (**2a**)[20] using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling reagents in 94% yield. The hydrolysis of the C-terminal methyl ester in **3a** under alkaline conditions (NaOH/H$_2$O-THF) proceeded to give a tripeptide carboxylic acid, and the N-terminal-protecting group in **3a** was removed by hydrogenolysis using H$_2$/Pd(OH)$_2$-C to produce a tripeptide amine. The coupling between them using [ethyl cyano(hydroxyimino)acetato-*O*^{2}]tri-1-pyrrolidinylphosphonium hexafluorophosphate (PyOxim)[21] and *N*,*N*-diisopropylethylamine (^{i}Pr$_2$EtN) gave the hexapeptide Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_2$-OMe (**4a**) in 49% yield. Similarly, nonapeptide (**5a**) was prepared in 58% yield from the hexapeptide amine and tripeptide carboxylic acid.

(*R*)-Ac$_5$c^{3EG}-containing L-Leu-based peptides Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_n$-OMe (**b**) {n = 1 (**3b**), 2 (**4b**), and 3 (**5b**)} were prepared in a similar manner to those of (*S*)-Ac$_5$c^{3EG}-containing peptides. The spectroscopic data of heteropeptides supported their chemical structures.



Scheme 1. Preparation of (*S*)- and (*R*)-Ac$_5$c^{3EG}-containing L-Leu-based peptides.

Conformational Analysis in Solution

Figure 2 shows the FT-IR absorption spectra of peptides Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_n$-OMe (**a**) and Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_n$-OMe (**b**) in $CDCl_3$ solution (5.0 mM). In the N-H stretching region (amide A) of peptides **4a**,**5a** and **4b**,**5b** (n = 2 and 3), strong bands were noted at 3310–3340 cm^{-1} and these bands may have been derived from the peptide N–H groups with N–H···O=C intramolecular hydrogen bonds. On the other hand, weak bands were observed at approximately 3430 cm^{-1} and these bands may have been derived from the free solvated N–H groups.[22] No band at approximately 3370–3390 cm^{-1}, which may have been derived from the intramolecular hydrogen bonds of the N–H···–O– (acetal) type, was observed.[23,24] In tripeptides **3a** and **3b**, no band or a very weak band was observed at approximately 3350 cm^{-1}. These results suggest that the β-turn structure (the peptide N–H group with N–H···O=C intramolecular hydrogen bond) was not formed or unstable in the $CDCl_3$ solution of **3**.

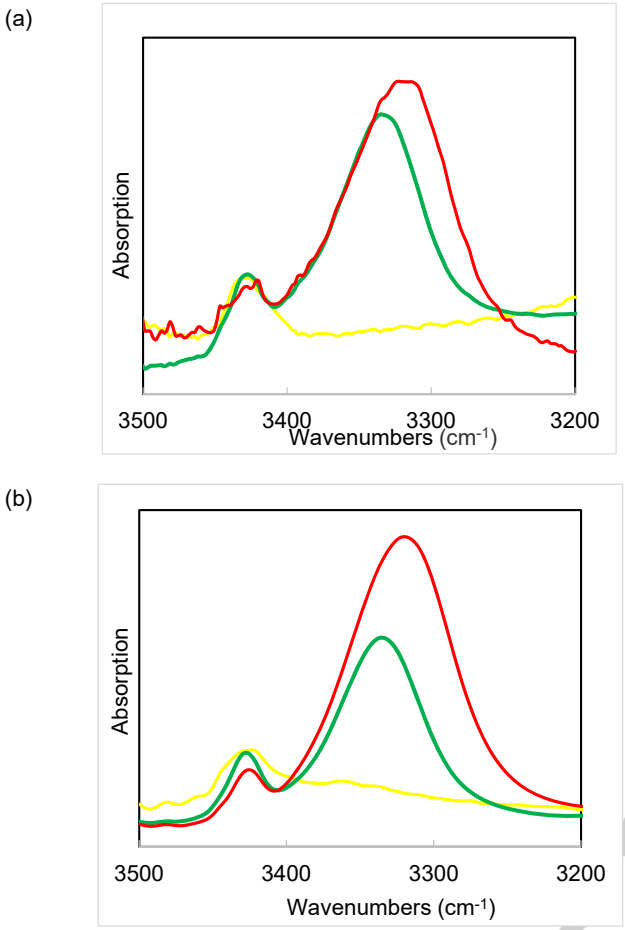


Figure 2. FT-IR absorption spectra of heteropeptides (a) Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_n$-OMe (**3a**–**5a**: n = 1–3) and (b) Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_n$-OMe (**3b**–**5b**: n = 1–3) in $CDCl_3$. Peptide concentration: 5.0 mM. Tripeptides (**3a**, **3b**): yellow, hexapeptides (**4a**, **4b**): green, and nonapeptides (**5a**, **5b**): red.

The nuclear Overhauser effect spectroscopy (NOESY) NMR spectra of hexapeptides **4a** and **4b** were measured in $CDCl_3$ solution at room temperature (Data are not shown). The spectrum of Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_2$-OMe **4a** showed partial NH ($i \rightarrow i$+1) dipolar interactions, from N(1)H to N(3)H; however, the NOE constraint NH ($i \rightarrow i$+1; i = 3, 4, 5) was not analyzed because of the overlap of signals. Unfortunately, we were unable to analyze the NOE constraints [$d_{\alpha N}$ ($i \rightarrow i$+2)] or [$d_{\alpha N}$ ($i \rightarrow i$+4)] due to signal overlaps.[25] The spectrum of Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_2$-OMe **4b** also showed only partial NH ($i \rightarrow i$+1) dipolar interactions from N(1)H to N(3)H; no other information was obtained because of signal overlaps.

The CD spectra of peptides **3a**–**5a** and **3b**–**5b** in 2,2,2-trifluoroethanol (TFE) solution are shown in Figure 3.[8,9,26,27,28] Tripeptides **3a** and **3b** show no characteristic maxima (222 nm and 208 nm) for helical structures, and these results are attributed to the peptide-main chain length not being sufficiently long to form helical secondary structures.

In contrast, the CD spectra of hexapeptides **4a**, **4b** and nonapeptides **5a**, **5b** showed negative maxima at approximately 222 nm and 208 nm, respectively, and a markedly stronger positive maximum at approximately 192 nm; however, the intensities of the maxima of **4b** were relatively weak. The chiral centers of L-Leu residues may control the helical-screw sense of peptides into right-handedness because 66% content of L-Leu exists in heteropeptides **4** and **5**, and the propensity of the helical-screw control of cyclic amino acid (*S*)-Ac_5c^{3EG} is relatively weak.[20] The intensities of maxima in (*S*)-Ac_5c^{3EG} hexapeptide **4a** were stronger than those of (*R*)-Ac_5c^{3EG} hexapeptide **4b**. This may be attributed to the chiral centers of L-Leu matching that of (*S*)-Ac_5c^{3EG} and mismatching that of (*R*)-Ac_5c^{3EG}; however, the (*S*)-Ac_5c^{3EG} homopeptides preferentially formed left-handed (*M*)-helices.[20] However, the effects of chiral (*S*)- and (*R*)-Ac_5c^{3EG} on the right-handed (*P*) helical structures of L-Leu-based peptides currently remains unclear because the CD spectra of (*S*)- and (*R*)-Ac_5c^{3EG}-containing nonapeptides **5a** and **5b** showed similar shapes.

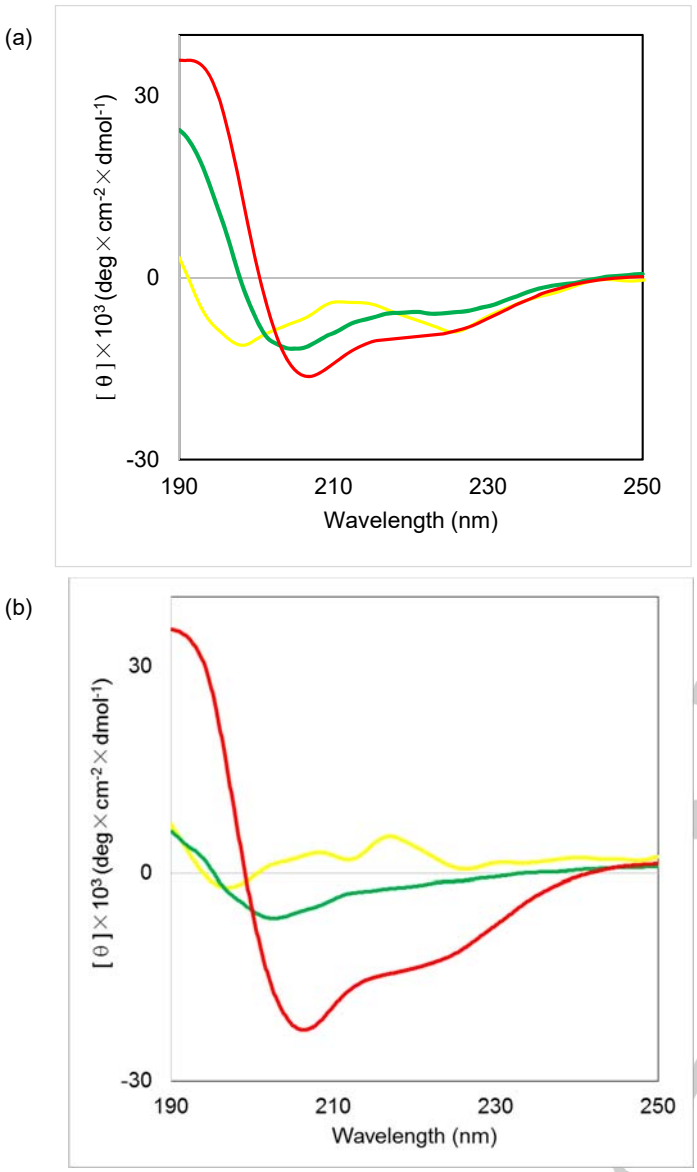


Figure 3. CD spectra of heteropeptides (a) Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_n$-OMe (**3a**–**5a**: n = 1–3) and (b) Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_n$-OMe (**3b**–**5b**: n = 1–3) in TFE solution (0.05 mM). Tripeptides (**3a**, **3b**): yellow, hexapeptides (**4a**, **4b**): green, and nonapeptides (**5a**, **5b**): red.

X-Ray Crystallographic Analysis of (*S*)- and (*R*)-Ac$_5$c^{3EG}-Containing Nonapeptides

Nonapeptides Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_3$-OMe **5a** and Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_3$-OMe **5b** provided suitable crystals for an X-ray crystallographic analysis due to the slow evaporation of ^{i}PrOH/H$_2$O (**5a**) and MeOH/H$_2$O (**5b**) at room temperature. The crystal and diffraction parameters of **5a** and **5b** are summarized in Table 1, and the relevant backbone and side-chain torsion angles as well as the intra- and intermolecular hydrogen bond parameters are listed in Tables 2 and 3. Molecular structures are shown in Figures 4 and 5.[29]

Table 1. Crystal and diffraction parameters of Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_3$-OMe **5a** and Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_3$-OMe **5b**.

	Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_3$-OMe **5a**	Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_3$-OMe **5b**
empirical formula	$C_{69}H_{109}N_9O_{18}\cdot H_2O$	$C_{69}H_{109}N_9O_{18}\cdot CH_4O\cdot H_2O$
Mr	1370.67	1402.72
crystal dimensions [mm]	0.50×0.28×0.08	0.28×0.20×0.10
crystal system	orthorhombic	monoclinic
temperature [K]	100	100
lattice parameters:		
a, *b*, *c* [Å]	11.729, 15.658, 41.277	10.697, 23.417, 15.281
α, β, γ [°]	90, 90, 90	90, 90.23, 90
V [Å^3]	7581	3827.7
space group	$P2_12_12_1$	$P2_1$
Z value	4	2
D_{calc} [g/cm^3]	1.201	1.217
μ (Cu$K\alpha$) [cm^{-1}]	0.718	0.732
no. of observations ($I > -10.0\ \sigma I$)	7092	13752
no. of variables	874	903
R_1, R_W	0.1087, 0.3792	0.0809, 0.2524
solvent	^{i}PrOH/H_2O	MeOH/H_2O

Table 2. Selected torsion angles ω, ϕ, ψ, and χ [°] of Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_3$-OMe **5a** and Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_3$-OMe **5b**.[a,b]

Torsion Angle	(*S*)-Ac_5c^{3EG} nonapeptide **5a**	(*R*)-Ac_5c^{3EG} nonapeptide **5b**
ω0	–173.3	–179.5
ϕ1	–66.4	–74.5
ψ1	–48.1	–26.1
ω1	–177.4	172.2
ϕ2	–54.1	–71.8
ψ2	–46.1	–40.2
ω2	–173.3	173.2
ϕ3	–56.6	–54.0
ψ3	–47.1	–45.3
ω3	177.7	–179.6
ϕ4	–61.6	–63.2
ψ4	–48.4	–42.9
ω4	–176.7	178.4

ϕ5	–59.7	–56.9
ψ5	–39.9	–44.9
ω5	–178.3	–177.0
ϕ6	–55.3	–58.0
ψ6	–45.3	–40.4
ω6	–176.9	178.9
ϕ7	–74.8	–67.2
ψ7	–40.9	–44.6
ω7	–177.0	–170.6
ϕ8	–64	–88.0
ψ8	–42	–38.9
ω8	–173	–177.2
ϕ9	49	36.2
ψ9	50	58.5
ω9	178	176.9
χ1	178.8	–66.6
χ2	173.7	–173.3
χ3	–88.7	86.0
χ3'	76.0	–77.3
χ4	174.7	–173.5
χ5	–58.1	–176.8
χ6	–101.9	82.9
χ6'	78.9	–77.3
χ7	–73	–74.9
χ8	–171	–67.5
χ9	–115	163.7
χ9'	90	–156.9

[a] The number of amino acid residues begins at the *N* terminus of the peptide chain. [b] χn: N-C(1)α-C(2)β-C(3)γ(acetal); χn': N-C(1)α-C(5)β'-C(4)γ' (Numbering of cyclopentane).

Table 3. Intra- and intermolecular H-bond parameters for Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_3$-OMe **5a** and Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_3$-OMe **5b**.

Peptide	Donor D–H	Acceptor A	Distance [Å] D···A	Angle [°] D–H···A	Symmetry operations
Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_3$-OMe (**5a**)					
	N_4-H	O_0	3.45[a]	168	x,y,z
	N_5-H	O_1	3.00	171	x,y,z
	N_6-H	O_2	2.86	161	x,y,z
	N_7-H	O_3	3.52[a]	159	x,y,z
	N_8-H	O_4	2.94	162	x,y,z
	N_9-H	O_5	2.89	159	x,y,z
	N_1-H	$O_{8'}$	2.85	136	x, −1+y, z

O_w-H[b]	O_7	2.86	164	x,y,z
$N_{3'}$-H	O_w	3.05	160	x, 1+y, z
O_w-H[b]	$O_{3'(acetal)}$	2.82	157	x, 1+y, z
Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_3$-OMe (**5b**)				
N_4-H	O_0	3.04	156	x,y,z
N_5-H	O_1	2.91	157	x,y,z
N_6-H	O_2	2.96	162	x,y,z
N_7-H	O_3	3.23	158	x,y,z
N_8-H	O_4	2.96	153	x,y,z
N_9-H	O_5	2.85	163	x,y,z
N_1-H	$O_{8'}$	2.82	133	x,y,1+z
$N_{3'}$-H	O_w	3.05	163	x,y,−1+z
O_M-H[b]	O_1	2.87	166	x,y,z
O_w-H[b,c]	O_7	2.86	136	x,y,z

[a] The distance is slightly long for an intramolecular hydrogen bond. [b] O_M: O atom of MeOH; O_w: O atom of water. [c] Disordered.

Nonapeptide **5a** was solved in the space group $P2_12_12_1$ to give a right-handed (*P*) α-helical structure along with one water molecule in the asymmetric unit. The average ϕ and ψ torsion angles of residues (1–8) were –61.6° and –44.7°, respectively, which were consistent with those of the ideal (*P*) α-helix (−57° and −47°).[30–33] However, the signs of the ϕ and ψ torsion angles of the (*S*)-Ac_6c^{3EG} residue (9) at the C-terminus were positive, and opposite to those of the preceding residues. The reversal of the signs of the C-terminal residue torsion angles are frequently observed in helical Aib and related peptides, and known as the helix-terminating structure.[34,35]

Four intramolecular hydrogen bonds of the *i*←*i*+4 type N–H···O=C (α-helix) were observed between H–N(*i*+4) and C(*i*)=O(*i*) (*i* = 1, 2, 4, 5), and two weak intramolecular hydrogen bonds of the *i*←*i*+4 type (α-helix) were observed between H–N(*i*+4) and C(*i*)=O(*i*) (*i* = 0, 3). In the packing mode, an intermolecular hydrogen bond was observed between the H–N(1) peptide donor and C(8')=O(8') [N(1)···O(8') = 2.85 Å] of a symmetry-related (x, −1+y, z) molecule. Furthermore, the peptide H–N(3') donor of the symmetry-related (x, 1+y, z) molecule was intermolecularly hydrogen-bonded to a water O_w, and the water H–O_w donor formed hydrogen bonds with C(7)=O(7) [O_w···O(7) = 2.86 Å] and with $O_{3'}$ of acetal oxygen [O_w···O_{acetal}(3') = 2.82 Å] of the symmetry-related (x, 1+y, z) molecule.

Diastereomeric nonapeptide **5b** crystallized in the space group $P2_1$ to form a right-handed (*P*) α-helical structure, along with one methanol and one water molecule in the asymmetric unit. In the (*P*) α-helical structure of **5b**, a reversal of the C-terminal torsion angle signs also occurred, *i.e*., the signs of the ϕ and ψ torsion angles of the C-terminal residue (9) were opposite to those of the preceding residues (1–8). The average values of the torsion angles ϕ and ψ of residues (1–8) were –66.7° and –40.4°, respectively.

Six consecutive intramolecular hydrogen bonds of the *i*←*i*+4 type N–H···O=C (α-helix) were observed between (*i*+4) and C(*i*)=O(*i*) (*i* = 0~5). In the packing mode, similar to those of **5a**, an intermolecular hydrogen bond was observed between the H–N(1) peptide donor and C(8')=O(8') [N(1)···O(8') = 2.82 Å] of a symmetry-related (x, y, 1+z) molecule. Furthermore, the peptide H–N(3') donor of the symmetry-related (x, y, –1+z) molecule was intermolecularly hydrogen-bonded to a water O_w, and the water H–O_w donor formed hydrogen bonds with C(7)=O(7) [O_w···O(7) = 2.86 Å]. An intermolecular hydrogen bond between the methanol H–O_M donor and O atom of the C(1)=O(1) acceptor was formed; however, no hydrogen bond was observed between the O atom of acetal oxygen and H–O_w of water.

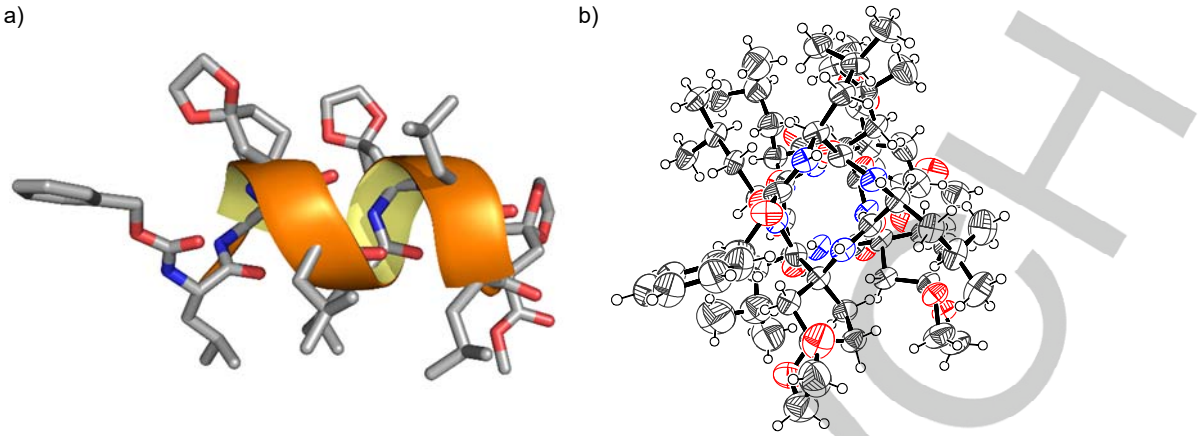


Figure 4. Right-handed (*P*) α-helical structure of Cbz-[L-Leu-L-Leu-{(*S*)-Ac_5c^{3EG}}]$_3$-OMe **5a** by an X-ray crystallographic analysis. (a) View perpendicular to the helical axis (Water omitted for clarity), and (b) an ORTEP drawing as viewed along the helical axis.

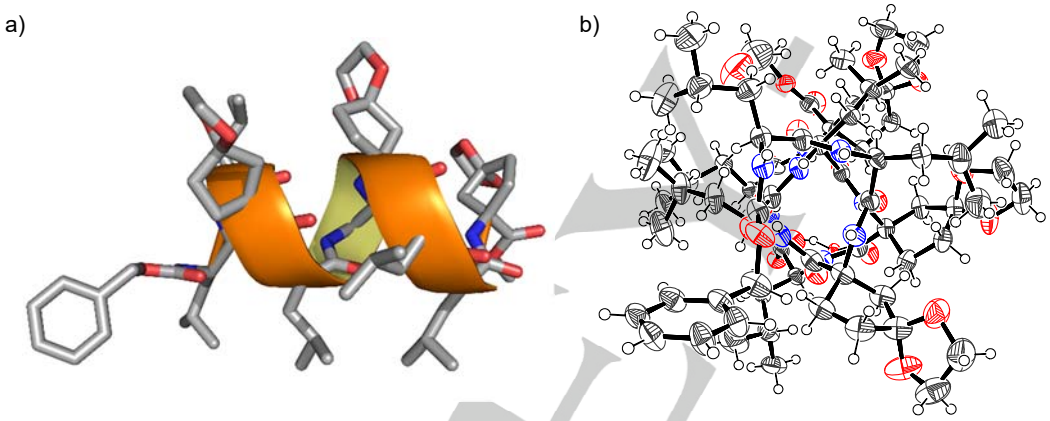


Figure 5. Right-handed (*P*) α-helical structure of Cbz-[L-Leu-L-Leu-{(*R*)-Ac_5c^{3EG}}]$_3$-OMe **5b** by an X-ray crystallographic analysis. (a) View perpendicular to the helical axis (Solvents omitted for clarity), and (b) an ORTEP drawing as viewed along the helical axis.

The superimposed structures of helices **5a** and **5b** are shown in FIGURE 6. Although the conformation of the side chain of L-Leu residues and the cyclopentane ring of the C-terminal Ac_5c^{3EG} are different, the peptide-backbone structures of **5a** and **5b** are well superimposed.

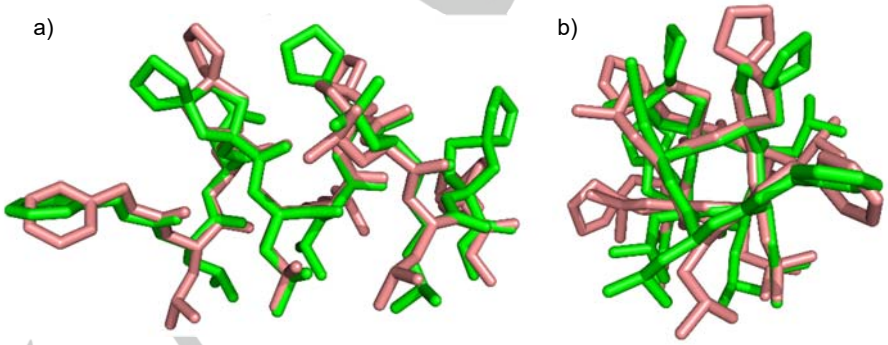


Figure 6. Superimposed structures of (*S*)-Ac_5c^{3EG}-containing nonapeptide **5a** (green) and (*R*)-Ac_5c^{3EG}-containing nonapeptide **5b** (Salmon pink).

We previously reported that short homopeptides (up to a tetrapeptide) composed of the six-membered carbocyclic ring amino acid (*R*,*R*)-Ac$_6$c^{3,5Bu} bearing two γ-acetal moieties preferentially formed helical structures with intramolecular hydrogen bonds of the N(*i*)–H···–O– (*i*, acetal) type both in solution and in the crystal state (Figure 7).[23] On the other hand, homopeptides (hepta- and octapeptides) composed of the five-membered carbocyclic ring amino acid (*R*)-Ac$_5$c^{3EG} with an acetal moiety at the γ-position showed left-handed (*M*) helical structures in solution without the N(*i*)–H···–O– (*i*, acetal)-type intramolecular hydrogen bond.[20] The carbocyclic ring size difference of dAAs may affect the distance of N(*i*)–H and –O– (*i*, acetal), and the intramolecular hydrogen bond pattern may be different.

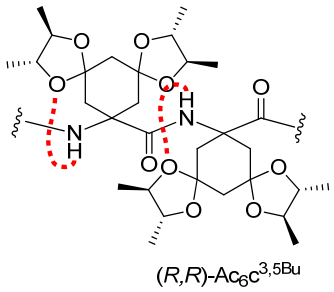


Figure 7. Hydrogen bonding pattern of (*R*,*R*)-Ac$_6$c^{3,5Bu} homopeptide.

L-Leu-based hetero-nonapeptides having three (*S*)- or (*R*)-Ac$_5$c^{3EG} both preferentially formed similar right-handed (*P*) helical structures in solution and in the crystal state. There was no intramolecular hydrogen bond of the N(*i*)–H···–O– (*i*, acetal) type, which was observed in the (*R*,*R*)-Ac$_6$c^{3,5Bu} homopeptides; however, the H–O$_w$ donor of water formed a hydrogen bond with O$_{3'}$ of acetal oxygen [O$_w$···O$_{acetal}$(3') = 2.82 Å] in the crystal state of **5a**.

Conclusions

L-Leu-based hexapeptides and nonapeptides having (*R*)- or (*S*)-Ac$_5$c^{3EG} formed right-handed (*P*) helical structures in solution. In the crystal state, L-Leu-based nonapeptides **5a** and **5b** having (*R*)- or (*S*)-Ac$_5$c^{3EG} both showed similar right-handed (*P*) α-helical structures. The -L-Leu-L-Leu- sequence worked as a determinant of direction of helix in the predominant α-helix formation. The effects of chiral five-membered carbocyclic ring amino acids (*R*)- or (*S*)-Ac$_5$c^{3EG} on the preferred structures of their L-Leu-based –(L-Leu-L-Leu-Ac$_5$c^{3EG})$_n$– peptides were very weak.

Experimental Section

General Experimental Methodology

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 absorption spectra (IR) were recorded for conventional measurements (KBr), and the solution ($CDCl_3$) method using the 0.1-mm path length of an NaCl cell. ^{1}H NMR spectra were obtained at 400 or 500 MHz. FAB-HRMS spectra were taken in the dual-focusing sector field mode, and ESI-HRMS spectra were measured in the ToF mode.

Preparation of Peptides.

(*S*)-Ac$_5$c^{3EG}-Containing Tripeptide; Cbz-L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}-OMe (3a).

N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI·HCl, 296 mg, 1.54 mmol) and 1-hydroxybenzotriazole hydrate (HOBt·H_2O, 278 mg, 1.82 mmol) were added to a solution of Cbz-(L-Leu)$_2$-OH **1** (582 mg, 1.54 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 20 min. A solution of amine (*S*)-Ac$_5$c^{3EG}-OMe **2a** (281 mg, 1.40 mmol) in CH_2Cl_2 (4 mL) was added dropwise to the reaction mixture at 0 °C. The resultant solution was gradually warmed to room temperature and stirred overnight. After the removal of CH_2Cl_2, the residue was diluted with EtOAc and washed successively with 1 M aqueous HCl, water, 5% aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (50% EtOAc in *n*-hexane) to give tripeptide **3a** (751 mg, 96%) as colorless crystals: mp 67–69 °C; $[\alpha]^{25}_D$ −38.4 (*c* 1.24, $CHCl_3$); IR (KBr) ν 3314 (br), 2955, 2874, 1744, 1701, 1651, 1539, 1261, 1238, 1042 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.37 (m, 5H), 7.01 (br s, 1H), 6.56 (br s, 1H), 5.34 (br s, 1H), 5.07–5.12 (m, 2H), 4.44 (m, 1H), 4.18 (br m, 1H), 3.85–3.92 (m, 4H), 3.68 (s, 3H), 2.53 (d, *J* = 14.5 Hz, 1H), 2.37 (m, 1H), 2.15 (d, *J* = 14.5 Hz, 1H), 1.91–2.09 (m, 3H), 1.58–1.76 (m, 4H), 1.43–1.56 (m, 2H), 0.85–0.97 (m, 12H); ESI-HRMS: *m/z* [M+H]$^+$ calcd for $C_{29}H_{44}N_3O_8$ 562.3128, found 562.3159.

(*R*)-Ac$_5$c^{3EG}-Containing Tripeptide; Cbz-L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}-OMe (3b).

Tripeptide **3b** was prepared from **1** and **2b** in a similar manner to that described for the preparation of **3a**. 91%; colorless crystals; mp 75–76 °C; $[\alpha]^{26}_D$ −51.1 (*c* 1.00, $CHCl_3$); IR (KBr) ν 3310 (br), 2955, 1744, 1701, 1651, 1535, 1261, 1238, 1042 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.38 (m, 5H), 6.88 (br s, 1H), 6.37 (br s, 1H), 5.08–5.18 (m, 3H), 4.42 (dd, *J* = 6.8, 11.5 Hz, 1H), 4.17 (br m, 1H), 3.86–3.92 (m, 4H), 3.69 (s, 3H), 2.46 (d, *J* = 14.4 Hz, 1H), 2.41 (m, 1H), 1.96–2.14 (m, 4H), 1.59–1.72 (m, 4H), 1.49–1.53 (m, 2H), 0.89–0.95 (m, 12H); ESI-HRMS: *m/z* $[M+H]^+$ calcd for $C_{29}H_{44}N_3O_8$ 562.3128, found 562.3150.

(*S*)-Ac$_5$c^{3EG}-Containing Hexapeptide; Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_2$-OMe (4a).

A suspension of tripeptide **3a** (117 mg, 0.208 mmol) and 20% $Pd(OH)_2$-C (23 mg) in THF (4 mL) was vigorously stirred for 1.5 h under a H_2 atmosphere at room temperature. The reaction mixture was filtered through a pad of Celite and the filter cake was washed with THF. Evaporation of the filtrate afforded crude amine **3a'** as a brown amorphous, which was used for the next step without purification. On the other hand, 0.2 M aqueous NaOH (1.02 mL, 0.204 mmol) was added dropwise to the stirred solution of tripeptide **3a** (57.2 mg, 0.102 mmol) in THF (1 mL) at 0 °C and the reaction mixture was gradually warmed to room temperature. After being stirred for 24 h, the reaction mixture was cooled to 0 °C, acidified with 1 M aqueous citric acid, and extracted with EtOAc. The EtOAc extracts were washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave crude carboxylic acid **3a''** in quantitative yield as a white amorphous, which was used for the next step without purification. PyOxim (132 mg, 0.250 mmol) and ^{i}Pr$_2$EtN (72.3 μL, 0.416 mmol) were added to the stirred mixture of amine **3a'** (0.208 mmol) and carboxylic acid **3a''** (114 mg, 0.208 mmol) in CH_2Cl_2 (5 mL) at 0 °C, and the reaction mixture was gradually warmed to room temperature. After being stirred for 48 h, the reaction mixture was diluted with EtOAc and washed with 5% aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (75% EtOAc in *n*-hexane containing 0.25% Et_3N) to provide hexapeptide **4a** (97.4 mg, 49% in 2 steps from **3a**) as an off-white solid: mp 209–210 °C; $[\alpha]^{25}_D$ −0.48 (*c* 1.01, $CHCl_3$); IR (KBr) ν 3310(br), 2959, 2855, 1744, 1651, 1535, 1339, 1270, 1220, 1140, 1034 cm^{-1} ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.40 (m, 5H), 7.34 (br s, 1H), 7.33 (br s, 1H), 7.29 (br s, 1H), 7.25 (br s, 1H), 6.59 (br s, 1H), 5.22 (br s, 1H), 5.22 (d, *J* = 12.0 Hz, 1H), 5.14 (d, *J* = 12.0 Hz, 1H), 4.37 (m, 1H), 4.23 (m, 1H), 4.02 (m, 1H), 3.96 (m, 1H), 3.67–3.91 (m, 8H), 3.67 (s, 3H), 2.80 (m, 1H), 2.73 (d, *J* = 14.9 Hz, 1H), 2.24–2.35 (m, 3H), 2.23 (m, 1H), 2.00–2.09 (m, 3H), 1.96 (m, 1H), 1.62–1.87 (m, 12H), 1.53–1.47 (m, 2H), 0.85–0.99 (m, 24H); FAB-HRMS: *m/z* $[M]^+$ calcd for $C_{49}H_{76}N_6O_{13}$ 956.5470, found 956.5463.

(*R*)-Ac$_5$c^{3EG}-Containing Hexapeptide; Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_2$-OMe (4b).

Hexapeptide **4b** was prepared from **3b** in a similar manner to that described for the preparation of **4a**. 53%; colorless crystals; mp 190–192 °C; $[\alpha]^{23}_D$ +2.99 (*c* 1.01, $CHCl_3$); IR (KBr) ν 3337 (br), 2959, 1740, 1713, 1667, 1528, 1258, 1215, 1034 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (br s, 1H), 7.36–7.40 (m, 5H), 7.28–7.33 (m, 3H), 6.57 (br s, 1H), 5.54 (br s, 1H), 5.24 (d, *J* = 12.0 Hz, 1H), 5.10 (d, *J* = 12.0 Hz, 1H), 4.36 (m, 1H), 4.23 (m, 1H), 3.92–3.99 (m, 2H), 3.80–3.90 (m, 8H), 3.67 (s, 3H), 3.19 (m, 1H), 2.93 (d, *J* = 14.8 Hz, 1H), 2.73 (d, *J* = 14.8 Hz, 1H), 2.25–2.39 (m, 2H), 2.16–2.24 (m, 2H), 2.04–2.13 (m, 3H), 1.95 (m, 1H), 1.58–1.82 (m, 11H), 1.54 (m, 1H), 1.44 (m, 1H), 0.82–0.99 (m, 24H); FAB-HRMS: *m/z* $[M]^+$ calcd for $C_{49}H_{76}N_6O_{13}$ 956.5470, found 956.5464.

(*S*)-Ac$_5$c^{3EG}-Containing Nonapeptide; Cbz-[L-Leu-L-Leu-{(*S*)-Ac$_5$c^{3EG}}]$_3$-OMe (5a).

Nonapeptide **5a** was prepared from **4a** in a similar manner to that described for the preparation of **4a**. 58%; colorless crystals; mp 204–206 °C; $[\alpha]^{26}_D$ +24.6 (*c* 0.87, $CHCl_3$); IR ($CDCl_3$) ν 3323 (br), 2961, 1717, 1655, 1526, 1339, 1271, 1217, 1125, 1074, 1030 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (br s, 1H), 7.69–7.72 (m, 2H), 7.49–7.58 (m, 4H), 7.35–7.43 (m, 5H), 6.98 (br s, 1H), 5.90 (br s, 1H), 5.20 (s, 2H), 4.34 (m, 1H), 4.18–4.26 (m, 4H), 4.03 (m, 1H), 3.86–3.98 (m, 12H), 3.65 (s, 3H), 2.92 (m, 1H), 2.77 (d, *J* = 14.8 Hz, 1H), 2.62 (d, *J* = 14.8 Hz, 1H), 2.42 (m, 1H), 2.21–2.28 (m, 3H), 1.48–2.16 (m, 19H), 1.22–1.47 (m, 10H), 1.19–0.83 (m, 36H); FAB-HRMS: *m/z* $[M]^+$ calcd for $C_{69}H_{109}N_9O_{18}$ 1351.7891, found 1351.7893.

(*R*)-Ac$_5$c^{3EG}-Containing Nonapeptide; Cbz-[L-Leu-L-Leu-{(*R*)-Ac$_5$c^{3EG}}]$_3$-OMe (5b).

Nonapeptide **5b** was prepared from **4b** in a similar manner to that described for the preparation of **4a**. 54%; colorless crystals; mp 224–226 °C; $[\alpha]^{24}_D$ +8.13 (*c* 1.00, $CHCl_3$); IR ($CDCl_3$) ν 3321 (br), 2959, 2361, 1709, 1659, 1531, 1339, 1261, 1215, 1034 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (br d, *J* = 8.7 Hz, 1H), 7.69 (br s, 1H), 7.57 (br s, 1H), 7.56 (br s, 1H), 7.48 (br s, 1H), 7.46 (br s, 1H), 7.37–7.42 (m, 5H), 7.35 (br s, 1H), 7.07 (br s, 1H), 6.23 (br s, 1H), 5.20 (d, *J* = 12.2 Hz, 1H), 5.13 (d, *J* = 12.2 Hz, 1H), 4.31 (m, 1H), 4.21 (m, 1H), 4.04 (m, 1H), 3.90–3.97 (m, 3H), 3.78–3.88 (m, 12H), 3.65 (s, 3H), 3.04 (d, *J* = 14.5 Hz, 1H), 2.97 (d, *J* = 14.5 Hz, 1H), 2.70 (d, *J* = 13.6 Hz, 1H), 2.45 (m, 1H), 2.24–2.40 (m, 4H), 1.98–2.22 (m, 10H), 1.59–1.96 (m, 17H), 1.52 (m, 1H), 0.78–1.04 (m, 36H); FAB-HRMS: *m/z* $[M+Na]^+$ calcd for $C_{69}H_{109}N_9O_{18}Na$ 1374.7788, found 1374.7782.

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Keywords: amino acids • helical structures • peptides •peptidomimetics • conformation analysis

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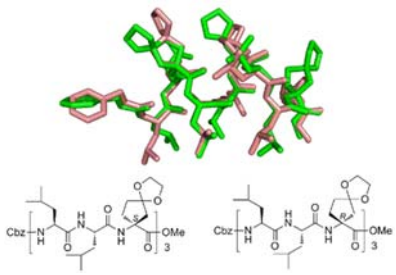
Entry for the Table of Contents



L-Leu-based nonapeptides having (*R*)- or (*S*)-chiral five-membered carbocyclic ring amino acids with an ethylene acetal moiety were prepared. An X-ray crystallographic analysis revealed the nonapeptides formed similar right-handed (*P*) α-helical structures, without an intramolecular hydrogen bond of the peptide N–H···–O– (acetal) type.