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Amino equatorial effect of a six-membered ring amino acid on its peptide 3_{10} - and α -helices

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Amino equatorial effect of a six-membered ring amino acid on its peptide 3_{10} - and α -helices

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ABSTRACT

Two diastereomeric six-membered ring α,α -disubstituted α -amino acids (1*R*,3*R*)- and (1*S*,3*R*)-1-amino-3-methylcyclohexanecarboxylic acids (Ac_6c^{3M}); side-chain restricted leucine analogs, were stereoselectively synthesized from (3*R*)-3-methylcyclohexanone by a Bucherer-Bergs or Strecker reaction. Two series of homo-chiral homo-peptides $\text{Cbz}\{-[(1*R*,3*R*)-\& (1*S*,3*R*)-\text{Ac}_6\text{c}^{3M}\}_n\text{-OMe}$, up to hexapeptides ($n = 6$), were prepared, respectively, and the preferred conformations of cyclohexane rings of amino acid residues and the peptide-backbones were studied. In solution, these peptides formed helical structures, but the helical-screw control to one handedness was not possible for the hexapeptide length. In the crystal state, all (1*R*,3*R*)- Ac_6c^{3M} residues formed cyclohexane chair-form conformations with a 3-methyl substituent at equatorial orientation and an amino group at the axial position, whereas all (1*S*,3*R*)- Ac_6c^{3M} residues assumed cyclohexane chair forms with the 3-methyl and amino groups at equatorial orientations. The preferred peptide-backbone structure of (1*R*,3*R*)- Ac_6c^{3M} hexapeptide had (*P*) and (*M*) 3_{10} -helices, and that of (1*S*,3*R*)- Ac_6c^{3M} hexapeptide had (*P*) and (*M*) α -helices in the crystal state.

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1. Introduction

α,α -Disubstituted α -amino acids restrict the conformational freedom of their oligopeptides.^{1,2} Achiral α -aminoisobutyric acid (α -methylalanine, dimethylglycine; Aib, αMeAla) is a prototype α,α -disubstituted α -amino acid, and is widely used to construct helical secondary structures.³ The Aib-containing short peptides and Aib homopeptides prefer to induce a 3_{10} -helix over an α -helix. Besides Aib, achiral cyclic α,α -disubstituted α -amino acids (Ac_nc ; n = ring size) such as 1-aminocyclopropanecarboxylic acid (Ac_3c), 1-aminocyclobutanecarboxylic acid (Ac_4c), and 1-aminocyclopentanecarboxylic acid (Ac_5c) have been synthesized, and these cyclic α,α -disubstituted α -amino acids-containing oligopeptides also induce $3_{10}/\alpha$ -helical secondary structures.⁴ Among cyclic α,α -disubstituted α -amino acids, 1-aminocyclohexanecarboxylic acid (Ac_6c)-containing peptides have also been prepared, and their preferred conformations of both the side-chain cyclohexane ring of amino acid residues and

peptide-backbone structure have been studied by Toniolo, Balaran, Benedetti and co-workers.⁵ The Ac_6c homopeptides formed 3_{10} -helical structures (tripeptide: type III β -turn, tetrapeptides: 3_{10} -helices) in the crystal state, and the Ac_6c had a chair form cyclohexane conformation. The amino (amide) $-\text{NH}-$ substituent usually showed axial orientation and the carbonyl $-\text{CO}-$ substituent occupied an equatorial orientation at the chair conformation.^{5,6} The theoretical calculations of $\text{Ac}-\text{Ac}_6\text{c}-\text{NHMe}$ revealed that the amino $-\text{NH}-$ axial minimum is more stable than the $-\text{NH}-$ equatorial minimum by ~ 1.6 kcal/mol.^{5b,7} Exceptionally, in the case of the free amino acid,^{5f,6b} an $-\text{NH}-$ substituent equatorial occurred. Furthermore, the theoretical calculation indicated that the lowest energy conformation with torsion angles $\phi \pm 52^\circ$; $\psi \pm 46^\circ$ occurred in the case of the $-\text{NH}-$ axial position, whereas that with torsion angles $\phi \pm 48^\circ$; $\psi \pm 60^\circ$ ^{5b} (or $\phi \pm 40^\circ$; $\psi \pm 80^\circ$ ^{5c}) was preferred in the case of the $-\text{NH}-$ equatorial position. These results suggest that if the $-\text{NH}-$ group of Ac_6c is controlled to an equatorial orientation (although it is unfavorable in terms of energy), the peptide-backbone torsion angles would prefer $\phi \pm 48^\circ$; $\psi \pm 60^\circ$,^{5b} which are not those of the 3_{10} -helix, but are closer to those of the α -helix (Fig. 1(a)).⁸ However, no such empirical studies on the relationship between the $-\text{NH}-$ equatorial orientation of the cyclohexane chair form or the peptide-backbone structures have been reported so far.

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Toniolo, Benedetti and co-workers reported X-ray crystallographic analysis of tetrapeptide *p*-BrBz-(Ac₆c)₄-OBu^t,^{5c} where three of the –NH– groups of four Ac₆c residues occupied an equatorial orientation, and the related ψ torsion angles became slightly larger than those of Cbz-protected Ac₆c tetrapeptides with all axial –NH– groups. However, discussions regarding the –NH– group equatorial effect on the peptide-backbone structures were limited.^{5c}

Jiménez, Cativiela and co-workers also reported the preferred conformation of heteropeptides having chiral 1-aminocyclohexanecarboxylic acids with a 2-phenyl group (c₆Phe; analogs of phenylalanine).⁹ Those c₆Phe-containing dipeptides preferentially formed β -turn structures, which were stabilized by an attractive NH to aromatic π -orbitals interaction, and the –NH– group oriented axial position, except for one crystal structure, where the –NH– and the bulky 2-phenyl groups occupied an equatorial orientation.^{9b} However, the relationship between the equatorial –NH– group and peptide-backbone structure was not discussed because of phenyl group *gauche* and π -electronic effects.

We designed two diastereomeric amino acids (1*S*,3*R*)- and (1*R*,3*R*)-1-amino-3-methylcyclohexanecarboxylic acids (Ac₆c^{3M}), which are side-chain conformational-freedom restricted leucine analogs (Fig. 1(b)). The 3-methyl substituent of side-chain cyclohexane should have an equatorial orientation because if it takes an axial position, 1,3-diaxial repulsion between the methyl group and the carbonyl (or the amino) group at the C(1)-position would arise. If the 3-methyl substituent of (1*S*,3*R*)-Ac₆c^{3M} occupies an equatorial position, the –NH– substituent assumes an equatorial direction and the –CO– group an axial direction. These orientations are opposite to those of the achiral Ac₆c, where the –NH– group occupied an axial orientation and the –CO– group occupied an equatorial one. Thus, the equatorial orientation of the –NH– group would prefer the peptide-backbone torsion angles $\phi \pm 48^\circ$; $\psi \pm 60^\circ$, by the reported calculation.^{5b}

We stereoselectively synthesized optically active (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M}, prepared two series of diastereomeric homo-chiral homopeptides, up to a hexapeptide, and studied the preferred conformation of these peptides, including the side-chain cyclohexane stereochemistry and peptide-backbone secondary structures, *i. e.*, 3₁₀- or α -helix, and also right-handed (*P*) or left-handed (*M*) helical-screw direction.

Fig. 1. Structures of achiral and chiral six-membered ring amino acids (Ac₆c and Ac₆c^{3M}), and their chair form conformations.

2. Results

2.1. Diastereoselective syntheses of two six-membered ring α,α -disubstituted α -amino acids (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M}.

Two diastereomeric cyclic amino acids (1*S*,3*R*)- and (1*R*,3*R*)-Ac₆c^{3M} were synthesized starting from an optically active (3*R*)-3-methylcyclohexanone (3*R*)-**1**, respectively.

2.1.1. Synthesis of (1*R*,3*R*)-Ac₆c^{3M} using a Bucherer-Bergs reaction.

Reaction of (3*R*)-**1** with KCN and (NH₄)₂CO₃ in 50% EtOH aqueous solution at 60°C afforded a hydantoin product **2** (Scheme 1).¹⁰ Hydantoin **2** was a diastereomeric mixture of **2a** and **2b** at a ratio of 4 to 1, and the re-crystallization of mixture **2** from hot EtOH produced a diastereomerically pure **2a** at a 67% yield. For hydrolysis of the hydantoin moiety, once the amide functions in **2a** were protected as Boc-protecting groups (92%), hydrolysis with aqueous LiOH gave a crude amino acid (1*R*,3*R*)-Ac₆c^{3M}.¹¹ The crude (1*R*,3*R*)-Ac₆c^{3M} was protected with a Cbz-group at the N-terminus and methyl ester at the C-terminus to give Cbz-[(1*R*,3*R*)-Ac₆c^{3M}]-OMe **4a** in 37% from Boc-protected hydantoin **3a**. The relative stereochemistry of **4a** was assumed by the 2D correlation (COSY) and nuclear Overhauser and exchange (NOESY) NMR spectra (Fig. S1).¹² At first, the methine proton signal at the C(3)-position was determined to have a peak at δ 1.60 based on the correlation with methyl proton signals in the COSY NMR spectrum, and then, the correlations among the methine proton, one proton (*syn* to the methine) of methylene at the C(2)-position, and the NH amide proton in the NOESY NMR spectrum suggested the stereochemistry of **4a** to be 1*R*,3*R*. Finally, the stereochemistry of **4a** was unambiguously confirmed by the X-ray crystallographic analysis of its peptides (*vide infra*).

Scheme 1. Synthesis of (1*R*,3*R*)-Ac₆c^{3M} using the Bucherer-Bergs reaction.

2.1.2. Synthesis of (1*S*,3*R*)-Ac₆c^{3M} using a Strecker reaction.

Treatment of (3*R*)-**1** with KCN and NH₄Cl in 35% EtOH aqueous solution at 65°C gave an aminonitrile **5b** at a 96% yield (Scheme 2).¹³ Acetylation of the amino function in **5b** with acetyl chloride (AcCl) afforded acetylated nitrile **6b** at a 89% yield. In the ¹H NMR spectrum, compound **6b** seemed to be diastereomerically pure with one stereoisomer. Hydrolysis of **6b** under acidic conditions, followed by esterification at C-terminus gave a methyl ester H-[(1*S*,3*R*)-Ac₆c^{3M}]-OMe **7b** at a 54% yield. Protection of the N-terminal amino group with the Cbz-group

produced N-terminal Cbz-protected amino acid Cbz-[(1*S*,3*R*)-Ac₆c^{3M}]-OMe **4b** at 91%. The ¹H NMR, ¹³C NMR, and MS spectra suggested that the amino acid **4b** was a diastereomer of **4a**. Finally, X-ray crystallographic analysis of its peptides unambiguously revealed the stereochemistry of **4b** as 1*S*,3*R* (*vide infra*).

Scheme 2. Synthesis of (1*S*,3*R*)-Ac₆c^{3M} using the Strecker reaction.

2.2. Preparation of (1*S*,3*R*)- and (1*R*,3*R*)-Ac₆c^{3M} homo peptides.

We synthesized C-terminal free amino acids **8a,b** by alkaline hydrolysis, and N-terminal free amino acids **7a,b** by hydrogenolysis using H₂/Pd-C, respectively (Scheme 1 and 2).

Scheme 3. Preparation of (1*R*,3*R*)-Ac₆c^{3M} homo peptides.

The (1*R*,3*R*)-Ac₆c^{3M} homo peptides were prepared by solution phase methods. Firstly, we prepared (1*R*,3*R*)-Ac₆c^{3M} dipeptide **9a** at a 62% yield by coupling between **7a** and **8a** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride and 1-hydroxybenzotriazole (HOBt) as coupling reagents. Next, we attempted to prepare tetrapeptide **12a** by coupling between dipeptide amine **10a** and dipeptide carboxylic acid **11a**, which were prepared from dipeptide **9a** by hydrogenolysis or hydrolysis. However, the coupling reaction proceeded only at a low yield (6%), and attempts to improve chemical yield by examining several coupling reagents such as *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium 3-oxide hexafluorophosphate (HBTU), and (1*H*-benzotriazole-1-yl)oxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) did not work well.¹⁴ Therefore, we elongated the peptide chain by stepwise one amino acid residue, up to a hexapeptide (Scheme 3). Diastereomeric (1*S*,3*R*)-Ac₆c^{3M} homo peptides were prepared in a manner similar to those of (1*R*,3*R*)-Ac₆c^{3M} peptides (Scheme 4). The spectroscopic data of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} homo peptides supported their structures.

Scheme 4. Preparation of (1*S*,3*R*)-Ac₆c^{3M} homo peptides.

2.3. Conformational analysis in solution.

The dominant conformations of two series of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} homo peptides in solution were studied by using FT-IR, ¹H NMR, and CD spectroscopies at room temperature. The FT-IR absorption spectra of Cbz-[(1*R*,3*R*)-Ac₆c^{3M}]_n-OMe [*n* = 2 (**9a**), 3 (**13a**), 4 (**12a**), 5 (**14a**), 6 (**15a**)] and Cbz-[(1*S*,3*R*)-Ac₆c^{3M}]_n-OMe [*n* = 2 (**9b**), 3 (**13b**), 4 (**12b**), 5 (**14b**), 6 (**15b**)] in the 3500–3250 cm⁻¹ region at a peptide concentration of 5.0 mM in CDCl₃ solution are shown in Figure 2. In both series of (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} homo peptides, weak bands at 3420–3440 cm⁻¹ region were assigned to free (solvated) peptide NH groups and the strong bands at the 3350–3370 cm⁻¹ region to peptide NH groups with N–H...O=C intramolecular hydrogen bonds of different strengths. With the peptide-chain length increase, the strong band shown at 3370 cm⁻¹ in tripeptides **13** shifted to slightly lower wavenumbers of 3350 cm⁻¹ in hexapeptides **15**, and the relative intensity of the bands in the 3350–3370 cm⁻¹ region gradually increased. These FT-IR spectra are very similar to those of the 3₁₀-helical peptides, such as Aib,³ Ac₄c,^{4f,h} Ac₅c,^{4a-c} and Ac₆c^{5d} peptides, but different from those of fully planar structure (*S*)-butylethylglycine,¹⁵ or diethylglycine homo peptides.¹⁶

Fig. 2. FT-IR absorption spectra in the N-H stretching region of peptides. (A) Cbz-[(1R,3R)-Ac₆c^{3M}]_n-OMe (*n* = 1, 2, 3, 4, 5 and 6) (B) Cbz-[(1S,3R)-Ac₆c^{3M}]_n-OMe (*n* = 1, 2, 3, 4, 5 and 6) in CDCl₃. Peptide concentration 5.0 mM.

The ¹H NMR spectra of (1R,3R)- and (1S,3R)-Ac₆c^{3M} hexapeptides **15a** and **15b** were measured in CDCl₃ solution. In the ¹H NMR spectra, the N(1)H signals at the N-terminus could be unambiguously determined by their high-field positions at δ 5.33 (br s, 1H) in **15a**, and δ 5.03 (br s, 1H) in **15b**, due to their urethane structure. However, the remaining five NH protons in **15a,b** could not be assigned at this stage (*vide infra*). The results of solvent perturbation experiments by the addition of the strong H-bond acceptor solvent DMSO-*d*₆ (0–10% v/v) or the paramagnetic free radical 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO; 0–5 × 10^{−2}% w/v) are shown in Figures 3 and 4. One NH chemical shift of **15a,b** was sensitive (solvent-exposed NH group), and one signal was slightly sensitive, to the addition of the perturbing reagent DMSO-*d*₆, although one NH signal of both **15a,b** could not be analyzed because of overlap with the signals of the Cbz-protecting group. Similarly, the bandwidth of two NH signals of both **15a,b** broadened with the addition of the TEMPO radical, meaning two NH protons are solvent-exposed, and suggesting two NH protons are not intramolecularly hydrogen bonded. These results are in accord with a helical structure, in which two or three N-terminal NH groups are freely solvated peptide NH groups (not intramolecularly hydrogen-bonded).

Fig. 3. Plots of NH chemical shifts in the ¹H NMR spectra of peptides (A) Cbz-[(1R,3R)-Ac₆c^{3M}]₆-OMe **15a**, (B) Cbz-[(1S,3R)-Ac₆c^{3M}]₆-OMe **15b** as a function of an increasing percentage of DMSO-*d*₆ (v/v) added to the CDCl₃ solution. Peptides concentration: 1.0 mM.

Fig. 4. Plots of bandwidth of the NH protons of peptides (A) Cbz-[(1R,3R)-Ac₆c^{3M}]₆-OMe **15a**, (B) Cbz-[(1S,3R)-Ac₆c^{3M}]₆-OMe **15b** as a function of increasing percentages of TEMPO (w/v) added to the CDCl₃ solution. Peptides concentration: 1.0 mM.

The 2D rotating frame nuclear Overhauser and exchange (ROESY) and NOESY NMR spectra of **15a** and **15b** were measured in CDCl₃ solution (Fig. S2).¹² The ROESY NMR spectrum of (1R,3R)-Ac₆c^{3M} hexapeptide **15a** showed partial NH (*i* → *i*+1) dipolar interactions, from N(2)H to the C-terminal N(6)H, but the NOE constrain NH (*i* → 2) was not observed. These partial series of sequential NH correlations suggest helical structure formation of **15a**, albeit whether a 3₁₀- or an α-helical conformation is unclear. The NOESY NMR spectrum of (1S,3R)-Ac₆c^{3M} hexapeptide **15b** showed a complete series of sequential NH (*i* → *i*+1) dipolar interactions from the N-terminal N(1)H to the C-terminal N(6)H, suggesting helical structure formation.¹⁷

We measured the ¹H NMR spectra of hexapeptides **15a** and **15b** at lower temperature. When the temperature was lowered, the NH signal lines of **15a** and **15b** broadened, and the temperature was lowered below −30 °C, the N(1)H and N(5)H signals of **15a** split with an intensity ratio of ca. 2 : 1. However, the NH signals of **15b** did not split at −40 °C (Fig. S3).¹²

The CD spectra of (1R,3R)- and (1S,3R)-Ac₆c^{3M} hexapeptides **15a,b** in 2,2,2-trifluoroethanol (TFE) solution were measured (Fig. S4).¹² However, the CD spectra did not show any characteristic maxima (222 nm, 208 nm) for a helical conformation.

2.4. Structures of (1R,3R)- and (1S,3R)-Ac₆c^{3M} homopeptides in the crystal state.

X-Ray crystallographic analyses unambiguously revealed the amino acid residue structures and the peptide-backbone secondary structures of terminally protected (1R,3R)- and (1S,3R)-Ac₆c^{3M} tetrapeptides **12a,b** and hexapeptides **15a,b** in the crystal state.^{18,19} The tetra- and hexapeptides became good crystals for X-ray crystallographic analysis by slow evaporation of the solvent at room temperature (**12a**: CH₂Cl₂/*n*-hexane, **15a**: MeOH/H₂O, **12b**: MeOH/EtOAc, **15b**: MeOH/H₂O). The crystal and diffraction parameters of **12a,b** and **15a,b** are summarized in Table S1.¹² The molecular structures are illustrated in Figs. 5–8. Relevant backbone and side-chain torsion angles and the intra- and intermolecular hydrogen-bond parameters are listed in Tables 1–4, respectively.

Table 1. Selected torsion angles of (1R,3R)-Ac₆c^{3M} homo-chiral homopeptides **12a** and **15a**.^a

Torsion Angle	(1R,3R)-tetramer 12a		(1R,3R)-hexamer 15a	
	A	B	C	D
ω0	171.1	−173.8	171.2	−175.1
φ1	63.6	−56.8	63.0	−56.0
ψ1	26.4	−29.5	31.0	−31.0
ω1	179.1	−175.8	168.8	−176.2
φ2	49.5	−49.8	56.0	−51.2
ψ2	36.7	−36.1	36.0	−37.8
ω2	170.4	−170.2	171.7	−171.9
φ3	63.6	−61.8	56.0	−55.5

ψ_3	31.0	-32.4	35.0	-38.1
ω_3	-178.9	-174.6	168.7	-171.3
ϕ_4	54.4	-56.4	59.0	-57.0
ψ_4	51.2	-54.4	27.0	-30.0
ω_4	-179.1	-177.6	172.1	-166.2
ϕ_5	---	---	68.0	-68.0
ψ_5	---	---	22.0	-23.0
ω_5	---	---	151.0	-170.8
ϕ_6	---	---	-51.0	50.0
ψ_6	---	---	-42.0	41.0
ω_6	---	---	174.0	179.0

χ_1	69.0	66.0	67.0	69.0
χ_1'	-65.1	-70.3	-68.0	-74.0
χ_2	70.3	66.9	68.0	65.1
χ_2'	-68.4	-69.7	-67.0	-68.8
χ_3	67.6	66.1	71.5	66.0
χ_3'	-63.5	-68.3	-67.0	-69.0
χ_4	68.6	64.8	70.0	68.6
χ_4'	-65.9	-69.6	-68.0	-72.8
χ_5	---	---	69.0	64.0
χ_5'	---	---	-70.0	-68.0
χ_6	---	---	46.0	71.0
χ_6'	---	---	-70.0	-76.0

^a The torsion angles of χ_n and χ_n' (n = amino acid residue numbering); χ_n : N-C¹-C²-C³, and χ_n' : N-C¹-C⁶-C⁵ (1, 2, 3, 5, and 6: cyclohexane ring numbering).

Table 2. Selected torsion angles of (1*S*,3*R*)-Ac₆C^{3M} homo-chiral homeopeptides **12b** and **15b**.^a

Torsion Angle	(1 <i>S</i> ,3 <i>R</i>)-tetramer 12b			(1 <i>S</i> ,3 <i>R</i>)-hexamer 15b	
	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>	<i>I</i>
ω_0	174.1	-180.0	-169.2	-179.9	-174.8
ϕ_1	55.6	54.9	-54.5	50.4	-51.2
ψ_1	40.3	40.2	-42.7	48.3	-51.9
ω_1	172.6	165.8	-168.1	165.0	-167.9
ϕ_2	49.8	55.8	-58.4	57.5	-58.1
ψ_2	42.8	38.6	-29.9	51.5	-49.0
ω_2	171.0	172.5	-179.4	178.7	-176.0
ϕ_3	58.1	52.8	-54.3	54.7	-60.0
ψ_3	40.6	44.7	-39.3	56.1	-48.1
ω_3	173.1	179.9	-175.4	170.5	-171.9
ϕ_4	-45.8	-51.3	42.8	58.5	-53.2
ψ_4	-51.8	-45.9	56.0	42.6	-40.1
ω_4	-177.1	179.0	-177.1	172.4	-169.8
ϕ_5	---	---	---	59.7	-55.2
ψ_5	---	---	---	42.8	-39.8
ω_5	---	---	---	177.5	-168.1
ϕ_6	---	---	---	-47.1	49.8
ψ_6	---	---	---	-52.8	53.0
ω_6	---	---	---	-174.8	178.2
χ_1	-163.9	-165.9	-172.0	-170.0	-175.1
χ_1'	168.4	171.9	166.3	172.5	168.9
χ_2	-164.5	-163.1	-168.3	-167.5	-172.2
χ_2'	170.8	169.2	163.7	171.7	167.2
χ_3	-166.6	-166.6	-169.9	-169.5	-170.7
χ_3'	172.6	171.8	162.7	175.0	165.4
χ_4	-176.5	-174.0	-170.5	-164.4	-172.5

χ_4'	171.0	167.9	173.3	168.0	167.2
χ_5	---	---	---	-166.1	-169.6
χ_5'	---	---	---	171.6	161.1
χ_6	---	---	---	-176.7	-170.5
χ_6'	---	---	---	170.3	175.4

^a The torsion angles of χ_n and χ_n' (n = amino acid residue numbering); χ_n : N-C¹-C²-C³, and χ_n' : N-C¹-C⁶-C⁵ (1, 2, 3, 5, and 6: cyclohexane ring numbering).

Table 3. Intra- and intermolecular H-bond parameters for (1*R*,3*R*)-Ac₆C^{3M} peptides **12a** and **15a**.

Peptide ^a	Donor D-H	Acceptor A	Distance [Å] D...A	Angle [°] D-H...A	Symmetry operations
Cbz-[(1 <i>R</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}] ₄ -OMe (12a)					
<i>A</i> (<i>M</i>)	N _{3a} -H	O _{0a}	3.22	165	x,y,z
	N _{4a} -H	O _{1a}	3.12	147	x,y,z
<i>B</i> (<i>P</i>)	N _{3b} -H	O _{0b}	3.11	167	x,y,z
	N _{4b} -H	O _{1b}	3.12	155	x,y,z
	N _{1b} -H	O _{3a}	2.97	165	x,y,z
	N _{1a} -H	O _{3b'}	2.99	171	x,y,z-1
Cbz-[(1 <i>R</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}] ₆ -OMe (15a) ^b					
<i>C</i> (<i>M</i>)	N _{4c} -H	O _{1c}	3.10	159	x,y,z
	N _{5c} -H	O _{2c}	3.25	164	x,y,z
	N _{6c} -H	O _{3c}	3.18	158	x,y,z
<i>D</i> (<i>P</i>)	N _{3d} -H	O _{0d}	3.12	159	x,y,z
	N _{4d} -H	O _{1d}	3.10	151	x,y,z
	N _{5d} -H	O _{2d}	3.17	162	x,y,z
	N _{6d} -H	O _{3d}	3.36 ^c	170	x,y,z
	N _{1d} -H	O _{5c}	2.85	162	x,y,z
	N _{1c'} -H	O _{5d}	2.93	165	1+x,y,-1+z

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

^b The distance N_{3c}...O_{0c} (3.42 Å) is too long for an intramolecular hydrogen bond.

^c The distance N...O is a little long for an intramolecular hydrogen bond.

Table 4. Intra- and intermolecular H-bond parameters for (1*S*,3*R*)-Ac₆C^{3M} peptides **12b** and **15b**.

Peptide ^a	Donor D-H	Acceptor A	Distance [Å] D...A	Angle [°] D-H...A	Symmetry operations
Cbz-[(1 <i>S</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}] ₄ -OMe (12b)					
<i>E</i> (<i>M</i>)	N _{3e} -H	O _{0e}	3.12	147	x,y,z
	N _{4e} -H	O _{1e}	3.01	140	x,y,z
	N _{3e} -H	O _{1e}	3.03	83 ^c	x,y,z
<i>F</i> (<i>M</i>)	N _{3f} -H	O _{0f}	3.33 ^b	146	x,y,z
	N _{4f} -H	O _{1f}	3.05	132	x,y,z
	N _{3f} -H	O _{1f}	3.05	84 ^c	x,y,z
<i>G</i> (<i>P</i>)	N _{3g} -H	O _{0g}	3.16	149	x,y,z
	N _{4g} -H	O _{1g}	2.93	148	x,y,z
	N _{3g} -H	O _{1g}	3.01	85 ^c	x,y,z
	MeO-H(<i>i</i>)	O _{2e}	2.80	175	x,y,z
	N _{2e} -H	O _{MeOH(<i>ii</i>)}	3.06	152	x,y,z
	N _{1e} -H	O _{3f}	3.13	175	x,y,z
	MeO-H(<i>ii</i>)	O _{3f}	2.91	177	x,y,z

	MeO-H(iii)	O _{2f}	2.87	160	x,y,z
	N _{1f} -H	O _{3g}	3.17	139	2-x,1/2+y,2-z
	N _{2f} -H	O _{4g}	2.88	171	2-x,1/2+y,2-z
	MeO-H(iv)	O _{2g}	2.85	173	x,y,z
	N _{1g} -H	O _{3e}	3.04	179	2-x,y-1/2,1-z
	N _{2g} -H	O _{4e}	2.97	146	2-x,y-1/2,1-z
Cbz-[(1 <i>S</i> ,3 <i>R</i>)-Ac ₆ C ^{3M}] ₆ -OMe (15b)					
<i>H</i> (<i>M</i>) ^d	N _{4h} -H	O _{0h}	3.27	172	x,y,z
	N _{5h} -H	O _{1h}	2.95	157	x,y,z
	N _{6h} -H	O _{3h}	3.20	127	x,y,z
<i>I</i> (<i>P</i>)	N _{4i} -H	O _{0i}	3.18	164	x,y,z
	N _{5i} -H	O _{1i}	3.30 ^[b]	145	x,y,z
	N _{5i} -H	O _{2i}	3.35 ^[b]	136	x,y,z
	N _{6i} -H	O _{3i}	3.16	151	x,y,z
	MeO-H(v)	O _{4h}	2.73	157	x,1+y,z
	MeO-H(vi)	O _{MeOH(v)}	2.72	173	x,y,z
	N _{1i} -H	O _{5h}	2.99	156	x,1+y,z
	N _{2i} -H	O _{6h}	2.98	170	x,1+y,z
	MeO-H(vii)	O _{4i}	2.70	162	x,y,z
	MeO-H(viii)	O _{MeOH(vii)}	2.70	172	x,y,z
	N _{1h} -H	O _{5i}	3.00	170	x,y,1+z
	N _{2h} -H	O _{6i}	3.00	156	x,y,1+z

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

^b The distance of N...O is a little long for an intramolecular hydrogen bond.

^c The D-H...A angle is too small for a hydrogen bond.

^d The distances N_{5h}...O_{2h} (3.44 Å) and N_{6h}...O_{2h} (3.41 Å) are too long for intramolecular hydrogen bonds.

In the asymmetric unit of the (1*R*,3*R*)-Ac₆C^{3M} tetrapeptide **12a**, two crystallographically independent molecules *A* and *B* occurred, along with a disordered solvent CH₂Cl₂. Molecule *A* is folded into a left-handed (*M*) 3₁₀-helical structure, and thus, displays positive signs of the ϕ and ψ torsion angles in each amino acid residue. On the other hand, molecule *B* is folded into a right-handed (*P*) 3₁₀-helical structure, showing negative signs of the ϕ and ψ torsion angles in each residue. The average ϕ and ψ torsion angles of residues (1–3) were +58.9°, 31.4° in molecule *A* and –56.1°, –32.7° in molecule *B*, although a slight distortion of ψ_4 torsion angles of residue (4) in both molecules *A* and *B* was observed. These average torsion angles are in good agreement with the ideal torsion angles (60°, 30°) of the 3₁₀-helical structure.⁸ The two molecules *A* and *B* are generally mirror-image structures of a peptide-backbone conformation, but showed small differences in the backbone structure, and have the same configuration of chiral centers (Fig. S5).¹² Two intramolecular hydrogen bonds of the *i*←*i*+3 type, which correspond to the 3₁₀-helical structure, are found in each molecule. Molecule *A* shows two intramolecular hydrogen bonds between H–N(3a) and the C(0a)=O(0a) oxygen atom of the Cbz-group (N(3a)···O(0a): 3.22 Å) and between H–N(4a) and C(1a)=O(1a) (N(4a)···O(1a): 3.12 Å). Also, molecule *B* shows two intramolecular hydrogen bonds between H–N(3b) and C(0b)=O(0b) (N(3b)···O(0b): 3.11 Å) and between H–N(4b) and C(1b)=O(1b) (N(4b)···O(1b): 3.12 Å). The side-chain cyclohexane ring of each amino acid residue in both molecules *A* and *B* forms a chair conformation, with the 3-methyl substituent and the –CO– group at an equatorial position, and with the –NH– group at an axial orientation. All torsion angles χ (N–C α –C β –C γ) and χ' (N–C α –C β' –C γ') relating the cyclohexyl ring to the peptide chain, namely N–C¹–C²–C³ (numbering of cyclohexane)

and N–C¹–C⁶–C⁵, show nearly +60°, –60°, which means the –NH– groups occupy the axial positions (Table 2).^{5c} In the packing mode, molecule *A* and molecule *B* are connected by one intermolecular hydrogen bond, to form a head-to-tail alignment of 3₁₀-helices of molecules *A* and *B*, *i. e.*, ...*A*(*M*)...*B*(*P*)...*A*(*M*)...*B*(*P*)...*A*(*M*)...*B*(*P*)... chain.

Fig. 5. 3₁₀-Helical secondary structures of Cbz-[(1*R*,3*R*)-Ac₆C^{3M}]₄-OMe (**12a**), determined by X-ray crystallographic analysis.

The (1*R*,3*R*)-Ac₆C^{3M} hexapeptide **15a** crystallized in the space group of *P*2₁ to form both left-handed (*M*) (molecule *C*) and right-handed (*P*) (molecule *D*) 3₁₀-helices in the asymmetric unit. Both in molecules *C* and *D*, reversals of the C-terminal torsion angles occur, *i. e.*, the signs of the ϕ and ψ torsion angles of the C-terminal residue (residues 6) are opposite to those of the preceding residues (1–5). These phenomena are frequently observed in 3₁₀-helical Aib and related peptides.²⁰ The average values of torsion angles ϕ and ψ of residues (1–5) were +60.4°, +30.2° in molecule *C*, and –57.5°, –32.0° in molecule *D*. Both molecules *C* and *D* are generally mirror-image structures of the peptide backbone, but show small backbone conformational differences, with the same configuration of chiral centers, and are diastereomeric conformers (Fig. S6).¹² Molecule *C* shows three intramolecular hydrogen bonds of the *i*←*i*+3 type, between H–N(4c) and C(1c)=O(1c) (N(4c)···O(1c): 3.10 Å), between H–N(5c) and C(2c)=O(2c) (N(5c)···O(2c): 3.25 Å), and between H–N(6c) and C(3c)=O(3c) (N(6c)···O(3c): 3.18 Å), although the distance of N(3c)···O(0c) (3.42 Å) was too long for an intramolecular hydrogen bond. Molecule *D* forms four consecutive intramolecular hydrogen bonds of the *i*←*i*+3 type, between H–N(3d) and C(0d)=O(0d) (N(3d)···O(0d): 3.12 Å), between H–N(4d) and C(1d)=O(1d) (N(4d)···O(1d): 3.10 Å), between H–N(5d) and C(2d)=O(2d) (N(5d)···O(2d): 3.17 Å), and between H–N(6d) and C(3d)=O(3d) (N(6d)···O(3d): 3.36 Å), respectively. Each residue in both molecules *C* and *D* assumes a chair form side-chain cyclohexane ring, with the methyl substituent and the –CO– group at the equatorial position, and with the –NH– group at an axial orientation, as the torsion angles (χ , χ') at each residue are close to +60°, –60°. Molecule *C* and molecule *D* are connected by one intermolecular hydrogen bond in the packing mode to form a head-to-tail alignment of 3₁₀-helices of molecules *C* and *D*, *i. e.*, ...*C*(*M*)...*D*(*P*)...*C*(*M*)...*D*(*P*)...*C*(*M*)...*D*(*P*)... chain.

Fig. 6. 3_{10} -Helical secondary structures of Cbz-[(1*R*,3*R*)-Ac₆c^{3M}]₆-OMe (**15a**), determined by X-ray crystallographic analysis.

Fig. 7. 3_{10} -Helical secondary structures of Cbz-[(1*S*,3*R*)-Ac₆c^{3M}]₄-OMe (**12b**) determined by X-ray crystallographic analysis.

Diastereomeric (1*S*,3*R*)-Ac₆c^{3M} tetrapeptide **12b** crystallized in the space group of $P2_1$ to adopt two left-handed (*M*) helical conformers (molecules *E* and *F*) and one right-handed (*P*) helical conformer (molecule *G*) in the asymmetric unit, along with four MeOH molecules. In each molecule, reversal of the C-terminal ϕ and ψ torsion angles to those of the preceding residues (1–3) is observed.²⁰ The average values of torsion angles ϕ and ψ of residues (1–3) are $+54.5^\circ$, $+41.2^\circ$ in molecule *E*, $+54.5^\circ$, $+41.2^\circ$ in molecule *F*, and -55.7° , -37.3° in molecule *G*. Molecules *E* and *F* are almost the same conformation both in terms of peptide-backbone and in side-chain structures, but there are small conformational differences and crystallographically-independent conformers. Molecule *G* shows a similar mirror-image peptide-backbone structure to molecules *E* and *F*, having the same 1*S*,3*R* configuration of the residues (Fig. S7).¹² Two intramolecular hydrogen bonds of the $i \leftarrow i+3$ type, which correspond to the 3_{10} -helical conformation, are shown in all three molecules *E–G*. That is to say, intramolecular hydrogen bonds between H–N(3) and C(0)=O(0) [N(3e)···O(0e): 3.12 Å; N(3f)···O(0f): 3.33 Å; N(3g)···O(0g): 3.16 Å], and between H–N(4) and C(1)=O(1) [N(4e)···O(1e): 3.01 Å; N(4f)···O(1f): 3.05 Å; N(4g)···O(1g): 2.93 Å] are observed. The distances between H–N(3) and the O(1)-atom of C(1)=O(1) in molecules *E*, *F* and *G* are N(3e)···O(1e): 3.03 Å, N(3f)···O(1f): 3.05 Å and N(3g)···O(1g): 3.01 Å, which allow for the intramolecular hydrogen bonding distance, corresponding to the $i \leftarrow i+2$ type hydrogen bonds (2.27-ribbon conformation). However, the angles for H–N(3)···O(1) are 83° (molecule *E*), 84° (molecule *F*) and 85° (molecule *G*), which are too small for intramolecular hydrogen bonding angles. Thus, the O-atom of C(1)=O(1) may not act as double-acceptor of hydrogen bonds from H–N(3) and H–N(4). The conformation of a side-chain cyclohexane ring at each residue in all three molecules *E–G* shows a chair form, with the 3-methyl substituent and the –NH– group at an equatorial orientation, and the –CO– group in an axial direction. All torsion angles (χ , χ') relating the cyclohexyl ring to the peptide chain show nearly -180° , $+180^\circ$, which means the –NH– groups occupy the equatorial positions (Table 3).^{5c} The O(2)-atom of carbonyl function C(2)=O(2) in all three molecules *E–G* forms hydrogen bonds with MeOH molecules. In the packing mode, an intermolecular hydrogen bond between H–N(1e) and C(3f)=O(3f), two intermolecular hydrogen bonds between H–N(1f) and C(3g')=O(3g'), and H–N(2f) and C(4g')=O(4g'), and also two intermolecular hydrogen bonds between H–N(1g) and C(3e')=O(3e'), and H–N(2g) and C(4e')=O(4e') are observed. Moreover, intermolecular hydrogen bonds between H–N(2e) and the O-atom of MeOH, and also between the MeO–H and the O-atom of C(3f)=O(3f) are shown. Thus, three molecules *E*, *F* and *G* form a head-to-tail alignment of $3_{10}/\alpha$ -helices $\cdots E(M) \cdots F(M) \cdots G(P) \cdots E(M) \cdots F(M) \cdots G(P) \cdots$ chain.

X-ray crystallographic analysis of (1*S*,3*R*)-Ac₆c^{3M} hexapeptide **15b** was solved in the $P1$ space group to show two helices (molecules *H* and *I*) with four MeOH molecules in the asymmetric unit. Molecule *H* has a left-handedness (*M*), and molecule *I* a right-handedness (*P*). Reversals of the C-terminal ϕ and ψ torsion angles to those of the preceding residues (1–5) are shown both in molecules *H* and *I*. The average values of torsion angles ϕ and ψ of residues (1–5) in molecule *H* are $+56.2^\circ$, $+48.3^\circ$, and those in molecule *I* are -55.5° , -45.8° , near to the ideal α -helix ($\pm 57^\circ$, $\pm 47^\circ$).^{8b} With regard to the peptide-backbone structure, the right-handed (*P*) and left-handed (*M*) helices (molecules *H* and *I*) have an almost enantiomeric relationship, but the configuration of chiral centers is the same 1*S*,3*R*, and so the relationship between molecules *H* and *I* is diastereomeric (Fig. S8).¹²

In molecule *H*, three intramolecular hydrogen bonds are observed. Two intramolecular hydrogen bonds of the $i \leftarrow i+4$ type, which correspond to the α -helical structure, are observed between H–N(4h) and C(0h)=O(0h) [N(4h)···O(0h): 3.27 Å], and between H–N(5h) and C(1h)=O(1h) [N(5h)···O(1h): 2.95 Å], and one intramolecular hydrogen bond of the $i \leftarrow i+3$ type was observed between H–N(6h) and C(3h)=O(3h) [N(5h)···O(1h): 3.20 Å]. Therefore, strictly speaking, the N-terminal amino acid residues form an α -helical structure and the C-terminal residues form a 3_{10} -helical structure. In molecule *I*, four intramolecular hydrogen bonds, *i. e.*, two $i \leftarrow i+4$ type hydrogen bonds between H–N(4i) and C(0i)=O(0i) [N(4i)···O(0i): 3.18 Å], and between H–N(5i) and C(1i)=O(1i) [N(5i)···O(1i): 3.30 Å], and two $i \leftarrow i+3$ type hydrogen bonds between H–N(5i) and C(2i)=O(2i) [N(5i)···O(2i): 3.35 Å], and between H–N(6i) and C(3i)=O(3i) [N(6i)···O(3i): 3.16 Å] are observed. Similar to molecule *H*, molecule *I* forms an α -helical structure at the N-terminal residues, and a 3_{10} -helical structure at the C-terminal residues. In both molecules *H* and *I*, the side-chain cyclohexane rings of all amino acid residues assume chair forms, with the 3-methyl and the –NH– groups in an equatorial, and the –CO– group in an axial direction as all torsion angles (χ , χ') show close to -180° , $+180^\circ$. The O(4)-atoms of carbonyl functions C(4)=O(4) in both molecules *H* and *I* form hydrogen bonds with MeOH molecules. In the packing mode, two intermolecular hydrogen bonds between H–N(1i) and C(5h')=O(5h') [N(1i)···O(5h'): 2.99 Å] and between H–N(2i) and C(6h')=O(6h') [N(2i)···O(6h'): 2.98 Å] are observed. Also, two intermolecular hydrogen bonds between H–N(1h) and C(5i')=O(5i') [N(1h)···O(5i'): 3.00 Å] and between H–N(2h) and C(6i')=O(6i') [N(2h)···O(6i'): 3.00 Å] are observed. Thus, the molecules *H* and *I* form a head-to-tail alignment of $3_{10}/\alpha$ -helices $\cdots H(M) \cdots I(P) \cdots H(M) \cdots I(P) \cdots H(M) \cdots I(P) \cdots$ chain.

Fig. 8. 3_{10} -Helical secondary structures of Cbz-[(1*S*,3*R*)-Ac₆c^{3M}]₆-OMe (**15b**) determined by X-ray crystallographic analysis.

3. Discussion

A Bucherer-Bergs reaction of **1** produced two diastereomeric hydantoin (*1R,3R*)-**2a** and (*1S,3R*)-**2b** at a ratio of 4 to 1. The Azerad²¹ and Wallace²² groups reported that the Bucherer-Bergs reaction of 3-substituted cyclopentanones gave a 1 to 1 mixture of diastereomeric products, and that of 3-carboxycyclohexanone produced (*1R**,*3R**)- and (*1R**,*3S**)-amino acid products at a ratio of 9 to 1. Therefore, our selectivity of a Bucherer-Bergs reaction of **1** is similar to those of the reported selectivities of 3-substituted cyclohexanones. In contrast, a Strecker reaction of **1** produced diastereomeric (*1S,3R*)-**5** as a sole product. The (*1S,3R*)-diastereoisomer by the Strecker reaction was different from the predominant (*1R,3R*)-product by the Bucherer-Bergs reaction. The diastereoselectivity of (3*R*)-3-(4-bromophenyl)cyclopentanone by the Strecker reaction was reported to be 3 (*1R,3R*) to 1 (*1S,3R*) by the Wallace group.²² Thus, the selectivity and predominant diastereoisomer are totally different between 3-substituted cyclopentanone and 3-methylcyclohexanone **1**. These results could be attributed to the fact that 3-substituted cyclopentanones form several envelop conformations and these conformations are flexible.

In the reactions of 3-methylcyclohexanone, thermodynamically stable various intermediates with the 3-methyl substituent at an equatorial position exist.²³ Fig. 9 shows representative intermediates, which are inter-changeable very quickly, in the Strecker and Bucherer-Bergs reactions. The Strecker reaction gives (*1S,3R*)-amino nitrile **5b** as a result of the more thermodynamically stable product than **5a**. On the other hand, the Bucherer-Bergs reaction produces (*1R,3R*)-**2a** because in the intermediate (*iii*) which leads to (*1S,3R*)-isomer **2b**, severe steric hindrance between the developing C=NH group and the 3,5-axial hydrogen atoms exists, and this path is highly disfavored, as Jitrangsi and co-author discussed the Bucherer-Bergs and Strecker reactions of 4-*tert*-butylcyclohexanone.²³

Fig. 9. Plausible reaction mechanisms for the Strecker and Bucherer-Bergs reactions, according to Jitrangsi's report.

The FT-IR and ¹H NMR spectroscopies revealed that both series of (*1R,3R*)- and (*1S,3R*)-Ac₆c^{3M} peptides preferentially formed helical secondary structures in solution. The dynamic ¹H NMR spectra of hexapeptides suggested that several conformers existed in CDCl₃ solution, and these conformers were in fast exchange at room temperature. However, discrimination between a 3_{10} -helix and an α -helix of the Ac₆c^{3M} hexapeptides by the solvent perturbation experiments was not possible because the N(3)-H of α -helical homopeptide was shielded and only two NH signals in α -helix were sensitive to the addition of DMSO-*d*₆ or TEMPO radical.^{24b} Neither the CD spectra of (*1R,3R*)- nor (*1S,3R*)-Ac₆c^{3M} homopeptides (up to hexapeptides) showed characteristic maxima for a helical structure. These results may be attributed the fact that the structure of chiral amino acid Ac₆c^{3M} does not have a strong helical-screw bias for right-handedness or left-handedness, and the length of hexapeptides was not long enough to control the helical-screw direction to one-handedness.²⁴⁻²⁶

The X-ray crystallographic analyses of (*1R,3R*)- and (*1S,3R*)-Ac₆c^{3M} tetra- and hexapeptides revealed that all peptides formed both right-handed (*P*) and left-handed (*M*) helices in the crystal state. The right-handed (*P*) and left-handed (*M*) helices are a diastereomeric relationship because the configurations of chiral centers are the same between right-handed and left-handed helices. In the asymmetric unit of (*1S,3R*)-Ac₆c^{3M} tetrapeptide **12b**, two left-handed (*M*) helical conformers and one right-handed (*P*) helical conformer formed, and a head-to-tail alignment of 3_{10} -helices $\cdots E(M) \cdots F(M) \cdots G(P) \cdots E(M) \cdots F(M) \cdots G(P) \cdots$ chain was formed. These results suggest that the left-handed (*M*) helix may be more favorable than the right-handed (*P*) helix in the case of the (*1S,3R*)-Ac₆c^{3M} peptide. However, crystal packing forces and nucleation events may also be attributed to the left-handed (*M*) helical structural preference in the crystal state of **12b** because the elongated (*1S,3R*)-Ac₆c^{3M} hexapeptide **15b** formed both right-handed (*P*) and left-handed (*M*) helices at a ratio of 1 to 1. Thus, the structure of Ac₆c^{3M} does not have a strong bias to control helical-screw direction to one-handedness,²⁴ the same as the bicyclic amino acid (*R,R*)-Ab_{5,6}=c or five-membered ring amino acid (*1S,3S*)-Ac₅c^{OM}.^{25,26}

The absolute average values of ϕ and ψ torsion angles of the (1*R*,3*R*)-Ac₆c^{3M} homopeptides are 57.5°, 32.1° (residues 1-3) in tetrapeptide **12a**, and 59.0°, 31.1° (residues 1-5) in hexapeptide **15a**, which are close to the ideal torsion angles (60°, 30°) of the 3₁₀-helical structure.⁸ Also, these two peptides showed intramolecular hydrogen bonds of the *i*←*i*+3 type, which correspond to a 3₁₀-helical structure. Thus, one property of (1*R*,3*R*)-Ac₆c^{3M} is to form the 3₁₀-helical structure, as the Aib property is to form the 3₁₀-helix. In contrast, the absolute average ϕ and ψ torsion angles of the (1*S*,3*R*)-Ac₆c^{3M} homopeptides are 54.9°, 39.9° (residues 1-3) in tetrapeptide **12b**, and 55.9°, 47.1° (residues 1-5) in hexapeptide **15b**. The absolute values of ψ torsion angles of (1*S*,3*R*)-Ac₆c^{3M} became larger than those of (1*R*,3*R*)-Ac₆c^{3M}, and the mean ϕ and ψ torsion angles of **15b** were especially close to the standard α -helix (57°, 47°).^{8b} The tetrapeptide **12b** formed two intramolecular hydrogen bonds of the *i*←*i*+3 type (3₁₀-helix), and the hexapeptide **15b** formed three or four intramolecular hydrogen bonds, *i.e.*, two *i*←*i*+4 type (α -helix) and one or two *i*←*i*+3 type (3₁₀-helix) hydrogen bonds. Crystals of helical hexapeptides **15a** (3₁₀-helix) and **15b** (α -helix) were obtained by re-crystallization from the same solvents (MeOH/H₂O). As a result, the property of (1*S*,3*R*)-Ac₆c^{3M} is more α -helical than that of (1*R*,3*R*)-Ac₆c^{3M}, although the 3₁₀- and α -helices are dependent on peptide length and re-crystallizing solvents.²⁷ These results are in good accordance with the reported theoretical calculation of Ac-Ac₆c-NHMe, where the torsion angles $\phi \pm 48^\circ$, $\psi \pm 60^\circ$ were stable for the -NH- equatorial position.

4. Conclusions

We synthesized two diastereomeric six-membered ring amino acids starting from (3*R*)-3-methylcyclohexanone. A Bucherer-Bergs reaction stereoselectively gave (1*R*,3*R*)- and (1*S*,3*R*)-Ac₆c^{3M} at a ratio of 4 to 1, and a Strecker reaction produced (1*S*,3*R*)-Ac₆c^{3M} as a sole diastereoisomer.

The X-ray crystallographic analysis of peptides **12a,b** and **15a,b** unambiguously revealed the amino acid residue conformations and peptide-backbone structures. All (1*R*,3*R*)-Ac₆c^{3M} residues formed cyclohexane chair forms 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, the (1*S*,3*R*)-Ac₆c^{3M} residues formed cyclohexane chair forms with the 3-methyl substituent and the -NH- group at an equatorial position, and the -CO- group in an axial direction. The secondary structure of (1*R*,3*R*)-Ac₆c^{3M} hexapeptide **15a** involved the (*P*) and (*M*) 3₁₀-helices, but that of (1*S*,3*R*)-Ac₆c^{3M} hexapeptide **15b** with the -NH- equatorial position was nearly the same as (*P*) and (*M*) α -helices, as predicted by the theoretical calculation.^{5b} Therefore, although the discrimination between a 3₁₀-helix and an α -helix in solution was difficult, the regulation of the -NH- equatorial and axial orientations of a six-membered ring α,α -disubstituted amino acid could be applied to control 3₁₀- and α -helical structures of peptides longer than hexamer in the crystal state.

The achiral cyclic amino acid Ac₆c and the related amino acid have been reportedly used as peptide-catalysts.^{28,29} Also, the Ac₆c are reported to be useful for structure-activity relationship studies and for the design of potent and selective analogs of bioactive peptides.^{5g,30} Therefore, the chiral Ac₆c^{3M} with a different torsion angle propensity may be useful for precise modulation of peptide-catalysts and peptide-drug candidates. Designing an α -helical-peptide catalyst based on these results is currently under investigation by our group.³¹

5. Experimental Section

5.1. General

Optical rotations $[\alpha]_D$ were measured with a Jasco P-2200 or DIP-370 polarimeter using a 1.0 dm cell. Circular dichroism spectra (CD) were measured with a Jasco J-720W spectropolarimeter using 1.0 mm path length cell. Infrared spectra (IR) were recorded on a Jasco FT/IR-420 or Shimadzu IR Affinity-1 spectrometer for conventional measurement (KBr), and the solution (CDCl₃) method using 0.1 mm path length of NaCl cell at room temperature. ¹H NMR spectra were determined at 400 or 500 MHz (Varian Unity or Joel AL) at room temperature. FAB-MS spectra were taken on Jeol JMS-SX 102, or Jeol JMS-700N spectrometer. ESI-MS spectra were measured on a Bruker microTOF II-SKF or Jeol JMS-T100TD. DART-MS spectra were recorded on a Jeol JMS-T100TD.

5.2. Synthesis of amino acids

5.2.1. (5*R*,7*R*)-7-Methyl-1,3-diazaspiro[4.5]decane-2,4-dione (2a). A mixture of (3*R*)-3-methylcyclohexanone (**1**, 30.0 g, 267 mmol), (NH₄)₂CO₃ (77.1 g, 802 mmol), and NaCN (26.2 g, 401 mmol) in 50% aqueous EtOH (300 mL) was stirred at 65 °C for 3 h. After removal of EtOH, the solution was diluted with brine, and extracted with EtOAc, and dried over MgSO₄. Removal of the solvent afforded diastereomeric mixtures (**2a** : **2b** = *ca.* 4 : 1) as a white solid. Recrystallization from EtOH gave a hydantoin **2a** (32.9 g, 67 %) as colorless crystals: mp 234–236°C; $[\alpha]_D^{22}$ -9.44 (*c* 1.00, MeOH); ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.52 (s, 1H), 8.38 (s, 1H), 1.44–1.62 (m, 7H), 1.24 (t, *J* = 12.7 Hz, 1H), 0.845 (d, *J* = 6.2 Hz, 3H), 0.80–0.95 (m, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 178.4, 156.4, 62.8, 41.2, 33.3, 32.7, 27.0, 22.2, 20.8; IR (KBr) ν 3194 (br), 3065, 2945, 1775, 1732, 1458, 1410 cm⁻¹; HRMS(ESI): [M⁺+Na], found 205.0983. C₉H₁₄N₂O₂Na requires 205.0953.

5.2.2. (5*R*,7*R*)-7-Methyl-2,4-dioxo-1,3-diazaspiro[4.5]decane-1,3-dicarboxylic Acid Di-*tert*-butyl Ester (3a). A solution of hydantoin (**2a**, 2.00 g, 10.9 mmol), 4,4-dimethylaminopyridine (4-DMAP; 0.1 g, 0.88 mmol), and di-*tert*-butyl dicarbonate [(Boc)₂O; 7.20 g, 32.9 mmol] in THF (30 mL) was stirred at room temperature under an Ar atmosphere for 2 h. After removal of THF, the solution was diluted with brine, extracted with EtOAc, and dried over MgSO₄. After removal of the solvent, the residue was purified by column chromatography on silica gel (10% EtOAc in *n*-hexane) to give **3a** (3.90 g, 92%) as colorless crystals: mp 90–92°C; $[\alpha]_D^{23}$ +3.93 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 2.14–2.21 (m, 2H), 2.06 (dd, *J* = 4.5, 14.6 Hz, 1H), 1.60–1.88 (m, 5H), 1.58 (s, 9H), 1.56 (s, 9H), 1.15 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 171.8, 149.4, 147.8, 145.4, 86.4, 84.6, 65.3, 39.7, 30.43, 30.35, 27.9, 27.7, 26.3, 21.8, 18.8; IR (KBr) ν 2982, 2931, 1813, 1782, 1736, 1369, 1142 cm⁻¹; HRMS(ESI): [M⁺+Na], found 405.2023. C₁₉H₃₀N₂O₆Na requires 405.2002.

5.2.3. (1*R*,3*R*)-1-Benzoyloxycarbonylamino-3-methylcyclohexanecarboxylic Acid Methyl Ester {Cbz-[(1*R*,3*R*)-Ac₆c^{3M}]-OMe; 4a}. A solution of **3a** (51.2 g, 134 mmol) and LiOH-H₂O (28.1 g, 669 mmol) in 50% aqueous MeOH (500 mL) was stirred at room temperature for 5 days. After neutralization with 10% aqueous HCl, MeOH was evaporated. Then, the aqueous solution was washed with CHCl₃. To the aqueous solution, K₂CO₃ (37.0 g, 268 mmol), benzyl chloroformate (CbzCl; 31 mL, 214 mmol), and acetone (200 mL) were added, and the solution was stirred overnight at room temperature. After removal of acetone, the solution was acidified with 10% aqueous HCl, extracted with CHCl₃, and dried over Na₂SO₄. Removal of

the solvent afforded a crude acid, which was dissolved in MeOH (300 mL), and SOCl₂ (31 mL, 429 mmol) was added at 0 °C. After stirring at room temperature for 24 h, the solution was diluted with 5% aqueous NaHCO₃, and then MeOH was evaporated. The aqueous solution was extracted with CHCl₃, dried over MgSO₄, and evaporated. The oily residue was purified by column chromatography on silica gel (20% EtOAc in *n*-hexane) to give cyclic amino acid **4a** (15.2 g, 37 %) as a colorless oil: $[\alpha]_D^{22}$ -7.54 (*c* 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.29–7.39 (m, 5H), 5.09 (s, 2H), 4.92 (br s, 1H), 3.67 (br s, 3H), 2.11 (br t, *J* = 15.0 Hz, 2H), 1.36–1.73 (m, 6H), 0.890 (d, *J* = 6.6 Hz, 3H), 0.85–0.95 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 175.2, 155.5, 136.6, 128.68, 128.29, 128.23, 66.9, 60.0, 52.5, 40.7, 34.2, 32.0, 27.6, 22.3, 21.3; IR (CDCl₃) ν 3445, 2953, 1734, 1502, 1456, 1265, 1239 cm⁻¹; FAB(+)MS: *m/z* 306.1 [M⁺+H]; HRMS(ESI): [M⁺+Na], found 328.1491. C₁₇H₂₃NO₄Na requires 328.1525.

5.2.4. (1S,3R)-1-Amino-3-methylcyclohexanecarbonitrile (5b). A mixture of **1** (3.50 g, 31.2 mmol), NH₄Cl (3.40 g, 62.4 mmol), and NaCN (4.10 g, 62.4 mmol) in EtOH (6 mL) and H₂O (12 mL) was heated at 60 °C for 6 h. After removal of EtOH, the aqueous residue was extracted with ether, and dried over MgSO₄. Removal of the solvent gave an oily residue, which was purified by short column chromatography on silica gel. Elution with 50% EtOAc in *n*-hexane afforded an aminonitrile **5b** (4.10 g, 96 %) as a yellowish oil: $[\alpha]_D^{22}$ -11.8 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 1.95–2.04 (m, 2H), 1.57–1.85 (m, 6H), 1.35 (dt, *J* = 3.8, 12.6 Hz, 1H), 1.10 (t, *J* = 12.6 Hz, 1H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.85 (dq, *J* = 3.5, 12.6 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 124.1, 51.8, 46.0, 37.6, 33.2, 29.4, 22.6, 21.5; IR (neat) ν 3372, 2932, 2870, 2222, 1609, 1458 cm⁻¹; FAB(+)MS: *m/z* 139.2 [M⁺+H]; HRMS(ESI): [M⁺+H], found 139.1227. C₈H₁₅N₂ requires 139.1230.

5.2.5. (1S,3R)-1-Acetoamido-3-methylcyclohexanecarbonitrile (6b). Acetyl chloride (AcCl; 0.77 mL, 11 mmol) and Et₃N (0.75 mL, 5.4 mmol) were added successively to the stirred solution of aminonitrile **5b** (0.50 g, 3.60 mmol) in CH₂Cl₂ (3 mL) at 0 °C. After stirred at room temperature for 20 h, the solution was washed with 10% aqueous HCl, 5% aqueous NaHCO₃, brine, and dried over MgSO₄. Removal of the solvent gave a white solid, which was purified by column chromatography on silica gel (50% EtOAc in *n*-hexane) to produce **6b** (0.58 g, 89 %) as colorless crystals: mp 111–113 °C; $[\alpha]_D^{24}$ -15.3 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.90 (br s, 1H), 2.52 (d, *J* = 12.6 Hz, 2H), 2.01 (s, 3H), 1.65–1.90 (m, 4H), 1.35 (m, 1H), 1.08 (t, *J* = 12.6 Hz, 1H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.89 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 170.1, 119.8, 52.1, 43.0, 34.8, 33.3, 28.9, 22.9, 22.1, 21.4; IR (KBr) ν 3298, 2963, 2943, 2870, 2237, 1663, 1535 cm⁻¹; HRMS(ESI): [M⁺+Na], found 203.1189. C₁₀H₁₆N₂ONa requires 203.1160.

5.2.6. (1S,3R)-1-Amino-3-methylcyclohexanecarboxylic Acid Methyl Ester (7b). A mixture of **6b** (7.00 g, 38.8 mmol) and concentrated aqueous HCl (50 mL) was heated at 100 °C for 24 h. After cooling to room temperature, the solution was neutralized with NaHCO₃, washed with CH₂Cl₂, and then concentrated in vacuo to leave a crude amino acid. The crude amino acid was dissolved in MeOH (100 mL), and concentrated H₂SO₄ (5 mL) was added to the stirred solution at 0 °C. After stirred at 65 °C for 48 h, the solution was diluted with 5% aqueous NaHCO₃. After removal of MeOH, the aqueous solution was extracted with CHCl₃, and dried over MgSO₄. Removal of the solvent gave an oily residue, which was purified by column chromatography on silica gel. The elution with 50% EtOAc in *n*-hexane gave an amine **7b** (3.60 g, 54 %) as a colorless oil: $[\alpha]_D^{27}$

-7.9 (*c* 1.05, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 3.71 (s, 3H), 2.10–2.20 (m, 2H), 1.65–1.72 (m, 2H), 1.46 (m, 1H), 1.34 (m, 1H), 1.19 (dt, *J* = 3.9, 12.6 Hz, 1H), 0.96 (d, *J* = 12.6 Hz, 1H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.83 (dq, *J* = 3.7, 12.6 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 176.5, 57.9, 51.6, 44.7, 35.7, 33.9, 29.4, 23.0, 22.0; IR (neat) ν 3375, 2951, 2932, 2847, 1732, 1454, 1207 cm⁻¹; FAB(+)MS: *m/z* 172.1 [M⁺+H]; HRMS(DART): [M⁺+H], found 172.1347. C₉H₁₈NO₂ requires 172.1332.

5.2.7. (1S,3R)-1-Benzylloxycarbonylamino-3-methylcyclohexanecarboxylic Acid Methyl Ester (Cbz-[(1S,3R)-Ac₆c^{3M}]-OMe; 4b). A mixture of amine **7b** (1.90 g, 11.4 mmol), K₂CO₃ (1.90 g, 13.7 mmol), and CbzCl (2.60 mL, 18.2 mmol) in acetone (20 mL) and H₂O (5 mL) was stirred at room temperature for 24 h. After removal of acetone, the aqueous solution was extracted with CHCl₃, dried over MgSO₄. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (20% EtOAc in *n*-hexane) to afford a cyclic amino acid **4b** (3.20 g, 91%) as colorless crystals: mp 54–56 °C; $[\alpha]_D^{22}$ -6.60 (*c* 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.29–7.38 (m, 5H), 5.06 (s, 2H), 4.99 (br s, 1H), 3.68 (br s, 3H), 2.41 (br t, *J* = 12.6 Hz, 2H), 1.52–1.80 (m, 4H), 1.30 (dt, *J* = 4.4, 12.6 Hz, 1H), 1.03 (t, *J* = 12.6 Hz, 1H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.84 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 173.5, 154.8, 136.3, 128.4 (2C), 128.08 (2C), 128.06, 66.6, 59.4, 52.1, 43.1, 34.6, 33.9, 28.8, 22.4, 22.3; IR (CDCl₃) ν 3442, 2954, 1744, 1732, 1717, 1506, 1456, 1226 cm⁻¹; FAB(+)MS: *m/z* 306.2 [M⁺+H]; HRMS(ESI): [M⁺+Na], found: 328.1512. C₁₇H₂₃NO₄Na requires 328.1525.

5.2.8. (1R,3R)-1-Amino-3-methylcyclohexanecarboxylic Acid Methyl Ester (7a). A mixture of amino acid **4a** (4.50 g, 14.7 mmol) and 10% Pd-C (2.2 g) in MeOH (100 mL) was rigorously stirred under a H₂ atmosphere at room temperature for 13 h. After the Pd-C catalyst was filtered off, the filtrate was evaporated in vacuo. The residue was purified by short column chromatography on silica gel to leave amine **7a** (2.50 g, quantitative) as an amorphous solid: $[\alpha]_D^{26}$ -6.6 (*c* 1.02, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 3.80 (s, 3H), 2.21–2.09 (m, 3H), 2.05 (tt, *J* = 14.2, 3.3 Hz, 1H), 1.90–1.80 (m, 2H), 1.77–1.69 (m, 1H), 1.54 (t, *J* = 14.6, 13.3 Hz, 1H), 0.99–0.88 (m, 1H), 0.93 (d, *J* = 6.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 171.7, 61.0, 52.9, 39.3, 33.2, 31.0, 25.9, 22.0, 19.8; IR (KBr) ν 3414, 2959, 2878, 1744, 1512, 1273 cm⁻¹; FAB(+)MS: *m/z* 172.1 [M⁺+H]; HRMS(DART): [M⁺+H], found 172.1358. C₉H₁₈NO₂ requires 172.1332.

5.2.9. (1R,3R)-1-Benzylloxycarbonylamino-3-methylcyclohexanecarboxylic Acid (8a). 1 M aqueous NaOH (16.0 mL, 16.0 mmol) was added to the stirred solution of amino acid **4a** (1.00 g, 3.27 mmol) in MeOH (20 mL) at 0 °C, and the solution was stirred at room temperature for 48 h. The solution was neutralized with 10% aqueous HCl, and MeOH was evaporated. The aqueous residue was extracted with CHCl₃, and dried over Na₂SO₄. Removal of the solvent afforded a crude acid **8a** (0.98 g, 98%) as colorless crystals: mp 89–91 °C; $[\alpha]_D^{21}$ -5.88 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.50 (br, 1H), 7.30–7.40 (m, 5H), 5.11 (s, 2H), 5.00 (br s, 1H), 1.96–2.29 (m, 2H), 1.40–1.80 (m, 6H), 0.83–0.98 (m, 1H), 0.91 (d, *J* = 6.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 179.5, 155.7, 136.3, 128.6 (2C), 128.2, 128.1 (2C), 66.8, 59.7, 40.2, 33.9, 31.4, 27.2, 22.1, 20.9; IR (CDCl₃) ν 3321, 2951, 1709, 1694, 1531, 1261 cm⁻¹; FAB(+)MS: *m/z* 292.1 [M⁺+H]; HRMS(DART): [M⁺+H], found 292.1551. C₁₆H₂₂NO₄ requires 292.1543.

5.2.10. (1S,3R)-1-Benzylloxycarbonylamino-3-methylcyclohexanecarboxylic Acid (8b). Compound **8b** was

prepared from **4b** in a manner similar to that described for the preparation of **8a**: >99%; colorless crystals; mp 85–86 °C; $[\alpha]_D^{22}$ –3.76 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.30–7.40 (m, 5H), 5.10 (br s, 3H), 2.46 (br t, *J* = 12.3 Hz, 2H), 1.80 (m, 1H), 1.60–1.74 (m, 3H), 1.32 (dt, *J* = 5.1, 12.6 Hz, 1H), 1.05 (t, *J* = 12.6 Hz, 1H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.85 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 177.8, 155.5, 136.2, 128.5 (2C), 128.2, 128.1 (2C), 66.9, 59.4, 42.5, 34.1, 33.8, 28.6, 22.23, 22.19; IR (KBr) ν 3387, 3090, 2955, 2928, 2839, 1724, 1701, 1531, 1254 cm^{–1}; HRMS(ESI): [M⁺+Na], found 314.1363. C₁₆H₂₁NO₄Na requires 314.1374.

5.3. Preparation of peptides

5.3.1. Cbz-[(1R,3R)-Ac₆C^{3M}]₂-OMe [(1R,3R)-dipeptide; **9a].** A solution of **8a** (4.72 g, 16.2 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC hydrochloride; 3.10 g, 16.2 mmol) and 1-hydroxybenzotriazole (HOBt; 2.20 g, 16.2 mmol) in MeCN (15 mL) was stirred at RT for 1 h. Then, a solution of **7a** (2.52 g, 14.7 mmol) in MeCN (5 mL) was added to the stirred solution, and the solution was stirred at 65 °C for 24 h. After evaporation of the solvent, the residue was diluted with EtOAc, washed with 10% aqueous HCl, 5% aqueous NaHCO₃, brine, and dried over MgSO₄. Removal of the solvent gave a white solid, which was purified by column chromatography on silica gel (20% EtOAc in *n*-hexane) to leave dipeptide **9a** (4.05 g, 62%) as colorless crystals: mp 148–150 °C; $[\alpha]_D^{21}$ –6.26 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.32–7.40 (m, 5H), 7.12 (br s, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 4.85 (br s, 1H), 3.66 (s, 3H), 2.04–2.21 (m, 4H), 1.25–1.72 (m, 12H), 0.90 (d, *J* = 6.0 Hz, 3H), 0.85 (d, *J* = 6.2 Hz, 3H), 0.80–1.00 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 174.7, 173.7, 155.6, 136.2, 128.5 (2C), 128.2, 128.0 (2C), 66.7, 60.2, 59.0, 52.0, 40.0, 39.3, 33.9, 33.7, 31.2, 31.1, 27.3, 27.2, 22.2, 22.0, 21.0, 20.9; IR (CDCl₃) ν 3432, 2953, 2928, 2868, 1733, 1669, 1507, 1456, 1244 cm^{–1}; FAB(+)MS: *m/z* 445.5 [M⁺+H]; HRMS(DART): [M⁺+H], found 445.2711. C₂₅H₃₇N₂O₅ requires 445.2697.

5.3.2. Cbz-[(1R,3R)-Ac₆C^{3M}]₂-OH (11a**).** 1 M aqueous NaOH solution (0.23 mL, 0.23 mmol) was added to a stirred solution of dipeptide **9a** (20.0 mg) in MeOH (0.23 mL) at 0 °C, and the resultant mixture was stirred for 4 days at room temperature. The reaction mixture was cooled to 0 °C and acidified to ca. pH 3 by adding 1 M aqueous HCl. After removal of MeOH, the aqueous solution was extracted with CHCl₃, and dried over MgSO₄. Removal of the solvent gave acid **11a** (19.3 mg, 99%) as a white solid: mp 208–209 °C; $[\alpha]_D^{28}$ –10.3 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.46–7.27 (m, 5H), 7.18 (br s, 1H), 5.17 (br s, 1H), 5.15–5.03 (m, 2H), 2.27–1.97 (m, 4H), 1.71–1.21 (m, 12H), 0.95–0.78 (m, 8H); IR (KBr) ν 3310, 2947, 2677, 1720, 1686, 1655, 1501 cm^{–1}; ¹³C NMR (100 MHz, CDCl₃) δ 177.3, 175.0, 155.9, 136.1, 128.6 (2C), 128.3, 128.0 (2C), 66.9, 60.3, 59.5, 39.7, 39.3, 33.8, 33.7, 31.0 (2C), 27.3 (2C), 22.2, 22.1, 20.9 (2C); HRMS(ESI): [M⁺+Na], found 453.2375. C₂₄H₃₄N₂O₅Na requires 453.2360.

5.3.3. Cbz-[(1R,3R)-Ac₆C^{3M}]₃-OMe [(1R,3R)-tripeptide; **13a].** A mixture of **9a** (4.00 g, 9.00 mmol) and 10% Pd-C (2.0 g) in MeOH (100 mL) was rigorously stirred under a H₂ atmosphere at room temperature for 4 h. After filtration, the filtrate was concentrated in vacuo to give a crude amine **10a** (2.79 g, quantitative). *H*-[(1R,3R)-Ac₆C^{3M}]₂-OMe (**10a**): mp 140–141 °C; $[\alpha]_D^{28}$ –6.30 (*c* 1.00, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 3.69 (s, 3H), 2.28–2.10 (m, 4H), 2.07–1.53 (m, 10H), 1.47 (dd, *J* = 14.3, 12.6 Hz, 1H), 1.35 (dd, *J* = 13.7, 12.2 Hz, 1H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.97–0.86 (m, 2H), 0.90 (d, *J* = 6.3 Hz, 3H); IR (KBr) ν 3279, 2989, 2681, 1721, 1655 cm^{–1}; ¹³C NMR (125

MHz, CDCl₃) δ 174.7, 170.8, 61.1, 60.5, 52.5, 40.0, 39.8, 33.9, 33.4, 31.7, 31.0, 27.5, 26.6, 22.2 (2C), 21.4, 20.3; HRMS(ESI): [M⁺+Na], found 333.2134. C₁₇H₃₀N₂O₃Na requires 333.2148. A solution of acid **8a** (2.62 g, 9.00 mmol), EDC hydrochloride (1.73 g, 9.00 mmol), 1-hydroxy-7-azabenzotriazole (HOAt; 1.23 g, 9.00 mmol), and *i*Pr₂EtN (1.6 mL, 9.0 mmol) in MeCN (10 mL) was stirred at room temperature for 1 h. Then, dipeptide amine **10a** (2.79 g, 9.00 mmol) in MeCN (20 mL) was added to the stirred solution, and the solution was stirred at 65 °C for 24 h. After evaporation of the solvent, the residue was diluted with EtOAc, washed with 10% aqueous HCl, 5% aqueous NaHCO₃, brine, and dried over MgSO₄. Removal of the solvent afforded a white solid, which was purified by column chromatography on silica gel (50% EtOAc in *n*-hexane) to give tripeptide **13a** (2.84 g, 54%) as colorless crystals: mp 94–96 °C; $[\alpha]_D^{21}$ –6.14 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.30–7.40 (m, 6H), 6.35 (br s, 1H), 5.13 (d, *J* = 12.0 Hz, 1H), 5.11 (d, *J* = 12.0 Hz, 1H), 5.06 (br s, 1H), 3.67 (s, 3H), 1.98–2.30 (m, 6H), 1.20–1.85 (m, 18H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.85 (d, *J* = 6.2 Hz, 4H), 0.80–1.00 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 175.3, 173.6, 173.4, 155.2, 136.0, 128.8 (2C), 128.6, 128.3 (2C), 67.1, 61.1, 60.6, 59.6, 52.0, 40.1, 39.7, 39.4, 34.2, 33.9, 33.6, 31.4, 31.1 (2C), 27.51, 27.46, 27.1, 22.3, 22.2, 22.1, 21.15, 21.10, 21.0; IR (CDCl₃) ν 3431, 3377, 2952, 2928, 2868, 1730, 1720, 1670, 1506, 1456, 1249 cm^{–1}; FAB(+)MS: *m/z* 584.6 [M⁺+H]; HRMS(ESI): [M⁺+Na], found 606.3520. C₃₃H₄₉N₃O₆Na requires 606.3513.

5.3.4. Cbz-[(1R,3R)-Ac₆C^{3M}]₄-OMe [(1R,3R)-tetrapeptide; **12a].** A mixture of **13a** (258 mg, 0.440 mmol) and 10% Pd-C (100 mg) in MeOH (10 mL) was rigorously stirred under a H₂ atmosphere at room temperature for 24 h. After filtration, the filtrate was concentrated in vacuo to give a crude tripeptide amine (199 mg, quantitative). A solution of acid **8a** (150 mg, 0.530 mmol), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU; 200 mg, 0.53 mmol), and HOAt (70 mg, 0.53 mmol), and *i*Pr₂EtN (0.15 mL, 0.90 mmol) in MeCN (2 mL) was stirred at room temperature for 1 h. Then, tripeptide amine (199 mg, 0.440 mmol) in MeCN (5 mL) was added, and the solution was stirred at 65 °C for 5 days. Removal of the solvent gave a residue, which was diluted with EtOAc, washed with 10% aqueous HCl, 5% aqueous NaHCO₃, brine, and dried over MgSO₄. After removal of the solvent, the white solid was purified by column chromatography on silica gel (50% EtOAc in *n*-hexane) to give tripeptide **12a** (213 mg, 66%) as colorless crystals: mp 164–166 °C; $[\alpha]_D^{21}$ –1.90 (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.32–7.40 (m, 6H), 6.80 (br s, 1H), 6.48 (br s, 1H), 5.30 (br s, 1H), 5.15 (d, *J* = 12.4 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 3.64 (s, 3H), 2.30–2.40 (m, 3H), 2.20 (m, 1H), 1.20–2.03 (m, 28H), 0.83–0.97 (m, 16H); ¹³C NMR (100 MHz, CDCl₃) δ 175.7, 174.6, 173.4, 172.8, 155.4, 136.1, 128.6 (2C), 128.4, 127.5 (2C), 66.9, 60.9, 60.5, 60.3, 59.7, 51.7, 40.0, 39.9 (2C), 39.5, 34.3, 34.0, 33.7, 33.6, 31.3, 30.7, 30.5 (2C), 27.8, 27.5, 27.4, 26.9, 22.3 (2C), 22.2, 22.1, 21.4 (2C), 21.1, 21.0; IR (CDCl₃) ν 3430, 3366, 2952, 2928, 2867, 1730, 1718, 1618, 1507, 1456, 1250 cm^{–1}; FAB(+)MS: *m/z* 723.5 [M⁺+H]; HRMS(ESI): [M⁺+Na], found 745.4505. C₄₁H₆₂N₄O₇Na requires 745.4511.

5.3.5. Cbz-[(1R,3R)-Ac₆C^{3M}]₅-OMe [(1R,3R)-pentapeptide; **14a].** Pentapeptide **14a** was prepared from **12a** and **8a** in a manner similar to that described for the preparation of **13a**: 30%; colorless crystals; mp 265–267 °C; $[\alpha]_D^{22}$ –12.4 (*c* 0.50, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.30–7.40 (m, 6H), 6.98 (br s, 1H), 6.93 (br s, 1H), 6.48 (br s, 1H), 5.30 (br s, 1H), 5.17 (d, *J* = 12.5 Hz, 1H), 5.11 (d, *J* = 12.5 Hz, 1H), 3.64 (s, 3H), 1.10–2.50

(m, 40H), 0.80–0.98 (m, 20H); ^{13}C NMR (100 MHz, CDCl_3) δ 175.9, 175.1, 173.9, 173.6, 173.5, 155.6, 136.1, 128.7 (2C), 128.5, 127.2 (2C), 66.9, 61.2, 60.8, 60.4, 59.74, 59.67, 51.8, 39.8 (5C), 34.4, 34.3, 34.0, 33.8, 33.6, 31.5 (5C), 27.9, 27.7, 27.6, 27.4, 26.8, 22.5, 22.4 (2C), 22.3, 22.1, 21.7, 21.5, 21.4, 21.2, 21.0; IR (CDCl_3) ν 3431, 3359, 2951, 2927, 2867, 1720, 1674, 1517, 1457, 1252 cm^{-1} ; HRMS(DART): $[\text{M}^+ + \text{H}]$, found 862.5719. $\text{C}_{49}\text{H}_{76}\text{N}_5\text{O}_8$ requires 862.5689.

5.3.6. Cbz-[(1*S*,3*R*)-Ac $_6$ c 3M] $_6$ -Ome [(1*S*,3*R*)-hexapeptide; **15a].** Hexapeptide **15a** was prepared from **14a** and **8a** in a manner similar to that described for the preparation of **13a**: 21%; colorless crystals; mp 234–236°C; $[\alpha]_D^{22}$ –16.2 (c 0.10, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.43–7.29 (m, 6H), 7.14 (br s, 1H), 7.05 (br s, 1H), 6.98 (br s, 1H), 6.51 (br s, 1H), 5.40 (br s, 1H), 5.25–5.05 (m, 2H), 3.65 (s, 3H), 2.50–1.13 (m, 48H), 1.07–0.62 (m, 24H); IR (CDCl_3) ν 3431, 3353, 2951, 2927, 2866, 2359, 1718, 1671, 1518, 1456, 1215 cm^{-1} ; ^{13}C NMR (100 MHz, CDCl_3) δ 176.1, 175.4, 174.8, 174.4, 173.92, 173.87, 155.6, 136.1, 128.7 (2C), 128.5, 127.1 (2C), 66.9, 61.2, 60.9, 60.5, 60.2, 59.7, 59.6, 51.8, 39.9 (6C), 34.4, 34.3, 34.1, 34.0, 33.7, 33.6, 31.4 (6C), 27.9, 27.7 (3C), 27.3, 26.8, 22.52, 22.47, 22.4, 22.3, 22.2, 22.1, 21.8, 21.6, 21.44, 21.37, 21.2, 21.0; HRMS(FAB): $[\text{M}^+ + \text{H}]$, found 1001.6663. $\text{C}_{57}\text{H}_{89}\text{N}_6\text{O}_9$ requires 1001.6686.

5.3.7. Cbz-[(1*S*,3*R*)-Ac $_6$ c 3M] $_2$ -Ome [(1*S*,3*R*)-dipeptide; **9b].** A solution of **8b** (2.03 g, 7.00 mmol), HATU (2.70 g, 7.00 mmol), HOAt (1.00 g, 7.35 mmol), and *i*Pr $_2$ EtN (2.5 ml, 14 mmol) in MeCN (10 mL) was stirred at room temperature for 1 h. Then, a solution of **7b** (1.20 g, 7.00 mmol) in MeCN (5 mL) was added to the stirred solution, and the solution was stirred at 70 °C for 24 h. After evaporation of the solvent, the residue was diluted with EtOAc, washed with 10% aqueous HCl, 5% aqueous NaHCO_3 , brine, and dried over MgSO_4 . Removal of the solvent gave a white solid, which was purified by column chromatography on silica gel (25% EtOAc in *n*-hexane) to give dipeptide **9b** (2.61 g, 84%) as colorless crystals; mp 122–124°C; $[\alpha]_D^{21}$ –14.6 (c 1.00, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.25–7.40 (m, 6H), 5.08 (s, 2H), 4.83 (br s, 1H), 3.64 (s, 3H), 2.30–2.60 (m, 4H), 1.50–1.80 (m, 8H), 1.15–1.30 (m, 2H), 0.80–1.00 (m, 10H); IR (CDCl_3) ν 3425, 2953, 2930, 1733, 1699, 1507, 1218 cm^{-1} ; ^{13}C NMR (100 MHz, CDCl_3) δ 173.6, 171.4, 155.5, 136.3, 128.6 (2C), 128.2, 128.1 (2C), 66.7, 59.2, 58.8, 51.7, 42.7, 42.6, 34.3, 34.01, 33.97, 33.89, 28.7, 28.5, 22.3, 22.24, 22.22, 22.1; HRMS(ESI): $[\text{M}^+ + \text{Na}]$, found 467.2559. $\text{C}_{25}\text{H}_{36}\text{N}_2\text{O}_5\text{Na}$ requires 467.2522.

5.3.8. Cbz-[(1*S*,3*R*)-Ac $_6$ c 3M] $_3$ -Ome [(1*S*,3*R*)-tripeptide; **13b].** Tripeptide **13b** was prepared from **9b** and **8b** in a manner similar to that described for the preparation of **9b**: 82%; colorless crystals; mp 160–162°C; $[\alpha]_D^{22}$ –13.2 (c 1.00, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.52 (br s, 1H), 7.30–7.38 (m, 5H), 6.61 (br s, 1H), 5.08 (s, 2H), 4.90 (br s, 1H), 3.66 (s, 3H), 2.38–2.52 (m, 6H), 1.51–1.77 (m, 12H), 1.20–1.30 (m, 2H), 1.10 (m, 1H), 0.79–1.02 (m, 15H); IR (CDCl_3) ν 3421, 2954, 2929, 1732, 1716, 1507, 1218 cm^{-1} ; ^{13}C NMR (100 MHz, CDCl_3) δ 173.8, 172.1, 171.9, 155.3, 136.1, 128.6 (2C), 128.4, 128.1 (2C), 66.8, 59.5 (2C), 58.7, 51.6, 43.0, 42.8, 42.5, 34.8, 34.5, 34.2, 34.0, 33.93, 33.89, 28.7, 28.5, 28.4, 22.37 (2C), 22.30, 22.25, 22.22, 22.0; HRMS(DART): $[\text{M}^+ + \text{H}]$, found 584.3712. $\text{C}_{33}\text{H}_{50}\text{N}_3\text{O}_6$ requires 584.3694.

5.3.9. Cbz-[(1*S*,3*R*)-Ac $_6$ c 3M] $_4$ -Ome [(1*S*,3*R*)-tetrapeptide; **12b].** Tetrapeptide **12b** was prepared from **13b** and **8b** in a manner similar to that described for the preparation of **9b**: 79%; colorless crystals; mp 217–219°C; $[\alpha]_D^{22}$ –21.5 (c 1.00, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.30–7.40 (m, 6H), 6.72 (br s, 1H), 6.46 (br s, 1H), 5.10 (d, J = 12.3 Hz, 1H), 5.08 (d, J = 12.3 Hz, 1H), 4.97

(br s, 1H), 3.66 (s, 3H), 2.10–2.52 (m, 8H), 1.50–2.10 (m, 16H), 0.70–1.40 (m, 24H); IR (CDCl_3) ν 3420, 3357, 2953, 2928, 1732, 1716, 1683, 1507, 1456, 1217 cm^{-1} ; ^{13}C NMR (125 MHz, CDCl_3) δ 174.2, 173.3, 172.5, 171.8, 155.4, 136.2, 128.6 (2C), 128.3, 127.7 (2C), 66.9, 59.3, 58.7, 58.6, 58.5, 51.5, 43.1, 43.0, 42.7, 42.3, 35.3, 35.2, 35.0, 34.3 (2C), 34.0, 33.9, 33.4, 28.8, 28.7, 28.2, 28.1, 22.7, 22.6, 22.53, 22.50 (2C), 22.2, 21.9, 21.8; FAB(+)MS: m/z 745.5 $[\text{M}^+ + \text{Na}]$, HRMS(DART): $[\text{M}^+ + \text{H}]$, found 723.4728. $\text{C}_{41}\text{H}_{63}\text{N}_4\text{O}_7$ requires 723.4691.

5.3.10. Cbz-[(1*S*,3*R*)-Ac $_6$ c 3M] $_5$ -Ome [(1*S*,3*R*)-pentapeptide; **14b].** Pentapeptide **14b** was prepared from **12b** and **8b** in a manner similar to that described for the preparation of **9b**: 27%; colorless crystals; mp 261–263°C; $[\alpha]_D^{22}$ –35.2 (c 0.20, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.32–7.38 (m, 5H), 7.23 (br s, 1H), 6.93 (br s, 1H), 6.66 (br s, 1H), 6.30 (br s, 1H), 5.18 (br s, 1H), 5.11 (d, J = 12.4 Hz, 1H), 5.08 (d, J = 12.4 Hz, 1H), 3.66 (s, 3H), 1.97–2.62 (m, 12H), 0.65–1.80 (m, 48H); IR (CDCl_3) ν 3454, 3354, 2953, 2928, 1732, 1716, 1682, 1508, 1456, 1219 cm^{-1} ; ^{13}C NMR (125 MHz, CDCl_3) δ 174.6, 174.1, 172.7 (2C), 172.5, 155.5, 136.3, 128.7 (2C), 128.3, 127.5 (2C), 66.9, 59.3, 58.7, 58.45, 58.41, 58.3, 51.5, 43.3, 43.2, 42.54, 42.50, 42.2, 35.7, 35.5, 35.4, 35.2, 34.5, 34.4, 34.3, 34.1, 34.0, 33.2, 28.8 (2C), 28.3, 28.12, 28.09, 22.8, 22.7, 22.61, 22.60 (2C), 22.3, 22.2, 21.9, 21.8 (2C); HRMS(ESI): $[\text{M}^+ + \text{Na}]$, found 884.5487. $\text{C}_{49}\text{H}_{75}\text{N}_5\text{O}_8\text{Na}$ requires 884.5513.

5.3.11. Cbz-[(1*S*,3*R*)-Ac $_6$ c 3M] $_6$ -Ome [(1*S*,3*R*)-hexapeptide; **15b].** Hexapeptide **15b** was prepared from **14b** and **8b** in a manner similar to that described for the preparation of **9b**: 12%; colorless crystals; mp 246–248°C; $[\alpha]_D^{22}$ –27.1 (c 0.10, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.32–7.40 (m, 6H), 7.05 (br s, 1H), 6.89 (br s, 1H), 6.60 (br s, 1H), 6.20 (br s, 1H), 5.13 (d, J = 12.5 Hz, 1H), 5.12 (br s, 1H), 5.09 (d, J = 12.5 Hz, 1H), 3.67 (s, 3H), 1.92–2.70 (m, 18H), 0.60–1.80 (m, 54H); IR (CDCl_3) ν 3447, 3350, 2953, 2928, 1718, 1673, 1518, 1455, 1244, 1220 cm^{-1} ; ^{13}C NMR (100 MHz, CDCl_3) δ 175.1, 174.4, 173.9, 173.4, 173.1, 172.8, 155.7, 136.7, 128.7 (2C), 128.3, 127.2 (2C), 66.7, 59.2, 58.8, 58.3 (2C), 58.2, 58.1, 51.5, 43.2 (2C), 42.6, 42.4 (2C), 42.1, 35.6, 35.4 (2C), 35.2, 35.0, 34.6, 34.43, 34.38, 34.30, 34.1, 34.0, 33.1, 28.8 (2C), 28.3, 28.15, 28.09 (2C), 22.9, 22.7 (2C), 22.6, 22.5, 22.4, 22.2 (2C), 21.9 (3C), 21.8; HRMS(ESI): $[\text{M}^+ + \text{Na}]$, found 1023.6525. $\text{C}_{57}\text{H}_{88}\text{N}_6\text{O}_9\text{Na}$ requires 1023.6510.

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Supplementary data

Supplementary data related to this article can be found at [xxxxxxxxx](#).

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