Research Article

Journal of PeptideScience

Received: 9 November 2009 Revised: 6 December 2009 Accepted: 7 January 2010 Published online in Wiley Interscience: 2 February 2010

(www.interscience.com) DOI 10.1002/psc.1213

Controlling the helical screw sense of peptides with *C*-terminal L-valine

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One chiral L-valine (L-Val) was inserted into the *C*-terminal position of achiral peptide segments constructed from α-aminoisobutyric acid (Aib) and α,β-dehydrophenylalanine (ΔZPhe) residues. The IR, ^{1}H NMR and CD spectra indicated that the dominant conformations of the pentapeptide Boc-Aib-ΔPhe-(Aib)$_2$-L-Val-NH-Bn (3) and the hexapeptide Boc-Aib-ΔPhe-(Aib)$_3$-L-Val-NH-Bn (4) in solution were both right-handed (*P*) 3$_{10}$-helical structures. X-ray crystallographic analyses of 3 and 4 revealed that only a right-handed (*P*) 3$_{10}$-helical structure was present in their crystalline states. The conformation of 4 was also studied by molecular-mechanics calculations. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: α-aminoisobutyric acid; α,β-dehydrophenylalanine; conformational analysis; 3$_{10}$-helix; X-ray diffraction; molecular-mechanics calculation

Introduction

Helices play a vital role in life science-related molecules; for example, the double helix is essential in DNA molecules for gene replication and transcription, and the α-helix is a common secondary structure of enzymes and drug receptors. Such helices are chiral, which results in them turning in one of two directions giving them a right-handed (*P*) or left-handed (*M*) screw sense. Attempts have been made to control the helical screw sense of peptides [1–3], and we have studied the conformation of peptides composed of α,α-disubstituted α-amino acids in order to achieve this [4–8]. In this study, we attempt to ascertain whether the attachment of a chiral amino acid is able to control the screw sense of helical peptides that do not exhibit a screw sense bias in solution and/or the solid state. As a chiral amino acid, we have selected L-valine (L-Val), and it was inserted at the *C*-terminus of helical-peptides constructed from achiral Aib and ΔZPhe residues [9–14]. That is, we have designed and synthesized peptides **1**–**4** and studied their preferred conformation (Figure 1). Furthermore, the molecular-mechanics of hexapeptide **4** were calculated with the Monte Carlo Multiple Minimum (MCMM) method and *ab initio* MO calculations.

Materials and Methods

Synthesis and Characterization of Peptides

The synthesis of peptides **1**–**4** was carried out by the stepwise solution-phase method using 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBt) as coupling reagents and an oxazolone based route [15]. All compounds were purified by column chromatography on silica gel.

Tripeptide 1

Colorless crystals; mp 169–170 °C; $[\alpha]_D^{25}$ = +16.4 (*c* = 0.92, $CHCl_3$); IR (in $CDCl_3$) 3358, 2975, 2935, 1708, 1698, 1633, 1514, 1254, 1155 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (br s, 2H), 7.19–7.39 (m, 12H), 4.88 (br s, 1H), 4.62–4.65 (m, 2H), 4.33 (dd, *J* = 5.2, 15.2 Hz, 1H), 2.60 (m, 1H), 1.45 (s, 3H), 1.41 (s, 9H), 1.34 (s, 3H), 1.01 (d, *J* = 2.8 Hz, 3H), 0.99 (d, *J* = 2.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 171.4, 164.5, 155.3, 139.0, 133.8, 129.3, 129.0, 128.8, 128.4, 128.2, 127.6, 126.7, 81.7, 58.5, 57.2, 43.2, 29.2, 28.1, 26.2, 23.4, 19.4, 17.2; ESI(+)-MS *m/z* 537 (M^+ + H).

Tetrapeptide 2

Colorless crystals; mp 111–113 °C; $[\alpha]_D^{25}$ = +13.5 (*c* = 0.89, $CHCl_3$); IR (in $CDCl_3$) 3399, 3336, 3019, 2973, 2938, 1708, 1703, 1666, 1530, 1156 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 7.79 (t, *J* = 5.6 Hz, 1H), 7.59 (br s, 1H), 7.24–7.39 (m, 8H), 7.03–7.06 (m, 3H), 6.93 (t, *J* = 7.2 Hz, 1H), 5.24 (br s, 1H), 4.56–4.66 (m, 2H), 4.23 (dd, *J* = 4.8, 14.8 Hz, 1H), 2.64 (m, 1H), 1.61 (s, 3H), 1.53 (s, 6H), 1.48 (s, 3H), 1.38 (s, 9H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 174.5, 171.7, 164.4, 155.8, 138.7, 133.5, 129.5, 129.3, 129.1, 128.7, 128.3, 127.9, 127.5, 126.4, 81.4, 58.5, 57.6, 56.9, 43.1, 29.0, 28.2, 27.8, 26.0, 23.9, 23.3, 19.5, 16.7; ESI(+)-MS *m/z* 622 (M^+ + H).

Pentapeptide 3

Colorless crystals; mp 220–222 °C; $[\alpha]_D^{25}$ = +17.1 (*c* = 1.01, $CHCl_3$); IR (in $CDCl_3$) 3331, 3019, 2984, 2935, 1708, 1703, 1626,

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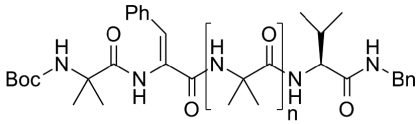

n = 0 Boc-Aib-ΔPhe-L-Val-NH-Bn (**1**)
n = 1 Boc-Aib-ΔPhe-(Aib)$_1$-L-Val-NH-Bn (**2**)
n = 2 Boc-Aib-ΔPhe-(Aib)$_2$-L-Val-NH-Bn (**3**)
n = 3 Boc-Aib-ΔPhe-(Aib)$_3$-L-Val-NH-Bn (**4**)

Figure 1. Structures of peptides.

1559, 1155 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (br s, 1H), 7.91 (t, J = 6.0 Hz, 1H), 7.78 (br s, 1H), 7.59 (br s, 1H), 7.14–7.45 (m, 12H), 5.47 (br s, 1H), 4.50–4.60 (m, 2H), 4.35 (dd, J = 6.0, 15.2 Hz, 1H), 2.57 (m, 1H), 1.40–1.56 (m, 27H), 1.00 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 175.1, 174.9, 172.1, 164.2, 155.8, 139.3, 133.5, 129.5, 129.3, 129.2, 128.8, 128.4, 128.1, 127.4, 126.4, 81.6, 58.7, 57.3, 57.1, 57.0, 43.1, 28.2, 27.7, 26.8, 26.0, 23.5, 23.4, 23.3, 19.5, 17.2; ESI(+)-MS m/z 707 ($M^+ + H$).

Hexapeptide 4

Colorless crystals; mp 230–231 °C; $[\alpha]_D^{25}$ = +13.2 (c = 0.61, $CHCl_3$); IR (in $CDCl_3$) 3323, 3019, 2983, 2937, 1765, 1703, 1665, 1534, 1156 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (br s, 1H), 7.93 (br s, 2H), 7.66 (br s, 1H), 7.65 (br s, 1H), 7.13–7.49 (m, 12H), 5.68 (br s, 1H), 4.60 (dd, J = 5.6, 15.2 Hz, 1H), 4.49 (dd, J = 4.8, 9.2 Hz, 1H), 4.30 (dd, J = 5.6, 15.2 Hz, 1H), 2.56 (m, 1H), 1.43–1.59 (m, 33H), 1.04 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 175.3, 175.2, 174.7, 172.2, 164.6, 155.9, 139.2, 133.4, 129.3, 129.2, 129.0, 128.8, 128.7, 128.0, 127.4, 126.4, 81.5, 58.8, 57.2, 57.1, 56.6, 43.0, 29.2, 28.2, 27.8, 27.0, 26.8, 25.9, 23.6, 23.3, 22.7, 19.5, 17.2; ESI(+)-MS m/z 814 ($M^+ + Na$).

FT-IR Absorption Spectra

FT-IR spectra were recorded on a *JASCO FT/IR-4100* spectrometer at 1 cm^{-1} resolution, with an average of 128 scans used for the solution ($CDCl_3$) method and a 0.1-mm path length for NaCl cells.

^{1}H NMR Absorption Spectra

^{1}H NMR spectra were recorded on a *Varian AS 400* spectrometer. Measurements were carried out in $CDCl_3$ and DMSO with tetramethylsilane as an internal standard. The TEMPO (2,2,6,6-tetramethylpiperidine-*N*-oxyl) concentration range was 1.0–5.0 × 10^{-2}% (w/v).

CD Spectra

CD spectra were recorded with a *Jasco J-720 W* spectropolarimeter using a 1.0 mm path length cell. The data were expressed in terms of $[\theta]_M$, the total molar ellipticity (deg cm^2 dmol^{-1}). 2,2,2-Trifluoroethanol (TFE) was used as a solvent.

X-Ray Diffraction

Single crystals of pentapeptide **3** and hexapeptide **4** were grown from MeOH/H_2O. Data collection was performed on Rigaku RAXIS-RAPID and Bruker AXS SMART APEX imaging plate diffractometers using graphite-monochromated Mo$K\alpha$ radiation. The crystal and collection parameters are listed in Table 1. All crystals remained stable during the X-ray data collection. The structures were solved using the SHELXS 97 direct method [16] and expanded by the Fourier technique [17]. All non-H-atoms were given anisotropic thermal parameters, some H-atoms were refined isotropically, and the remaining H-atoms at the calculated positions were given isotropic thermal parameters. The final cycle of full-matrix least-squares refinement of **3** gave an R_1 factor of 0.0448 based on 8856 [$I > 2\sigma(I)$] reflections and an *Rw* factor of 0.1270 for all data. The R_1 factor of **4** was 0.0446 based on 5505 [$I > 2\sigma(I)$] reflections and an *Rw* factor of 0.1162 for all data. All data for peptides **3** and **4** have been deposited in the Cambridge Crystallographic Data Centre (CCDC) as a supplementary publication, and their CCDC reference numbers are CCDC-751276 and -751277, respectively [18].

Table 1. Crystal and diffraction parameters of pentapeptide **3** and hexapeptide **4**

	Pentapeptide **3**	Hexapeptide **4**
empirical formula	$C_{38}H_{54}O_7N_6$	$C_{42}H_{62}O_8N_7$, $2CH_3OH$
Mr	706.88	857.07
Crystal dimensions [mm]	0.70 × 0.50 × 0.10	0.20 × 0.15 × 0.06
Crystal system	orthorhombic	monoclinic
Lattice parameters		
a, b, c [Å]	9.338, 18.380, 22.830	11.559, 14.374, 14.860
α, β, γ [°]	90, 90, 90	90, 102.550, 90
V [Å^3]	3918.6	2409.9
Space group	$P2_12_12_1$	$P2_1$
Z value	4	2
D_{calc} [g/cm^3]	1.198	1.181
μ(MoK_α)[cm^{-1}]	0.83	0.84
No. of observations	8856 ($I > 2\sigma(I)$)	5505 ($I > 2\sigma(I)$)
No. of variables	629	554
R_1, R_w	0.0448, 0.1270	0.0446, 0.1162
Solvent	MeOH/H_2O	MeOH/H_2O

Molecular-Mechanics Calculation

A conformational search calculation of hexapeptide **4** was performed using the MCMM method of *MacroModel* (version 9.1 Schrodinger, Inc.) with the AMBER* force field to obtain several local-minimum energy conformations. As an initial structure, an extended structure was used, and more than 30,000 structures were optimized. Then, the energies of their local-minimum energy conformations (the AMBER* force field), including those of the (*P*) and (*M*) 3_{10}-helices, were evaluated by *ab initio* MO calculations (3-21G level) using *Spartan '06* (Wavefunction, Inc.).

Results and Discussion

FT-IR Absorption Spectra

First, the preferred conformations of the peptides were studied in solution using IR. Figure 2 shows the IR absorption spectra of peptides **1**, **2**, **3** and **4** in the 3250–3500 cm^{-1} region at a peptide concentration of 1.0 mM in $CDCl_3$ solution. In the IR absorption spectra, the weak bands in the 3400 cm^{-1} region were assigned to free (solvated) peptide NH groups, and the strong bands at 3320–3360 cm^{-1} were assigned to peptide NH groups with N-H···O=C intramolecular hydrogen bonds of different strengths.

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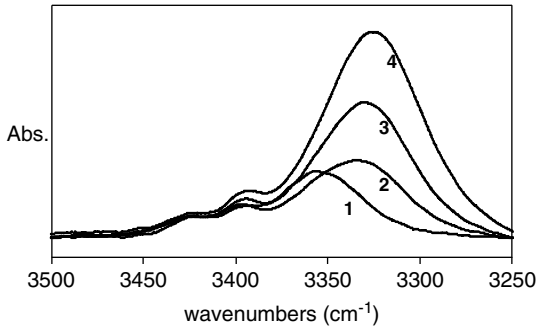

Figure 2. FT-IR absorption spectra (3250–3500 cm^{-1} region) of peptides **1**–**4** in $CDCl_3$ solution. Peptide concentration: 1.0 mM.

As the peptide-chain length increased, the strong band observed at 3360 cm^{-1} in **1** shifted to a slightly lower wavenumber (3320 cm^{-1} in **4**), and the relative intensity of the bands in the 3320–3360 cm^{-1} region gradually increased. The difference in the spectra between peptide concentrations of 1.0 and 0.1 mM was not significant (results not shown). These IR spectra are very similar to those of Aib homopeptides, which form 3_{10}-helices in solution [19].

^{1}H NMR Spectra

To obtain more detailed information on their preferred conformation, the ^{1}H NMR spectra of pentapeptide **3** and hexapeptide **4** were measured in $CDCl_3$ solution. In the ^{1}H NMR spectra of **3** and **4**, N(1)H signals at the *N*-terminus were unambiguously determined by their high-field positions at δ 4.94 (br s, 1H) in **3** and δ 4.93 (br s, 1H) in **4**, but the remaining five or six NH protons could not be assigned at this stage. Figure 3 shows solvent perturbation experiments involving the addition of the strong H-bond acceptor solvent DMSO (0–10% (*v*/*v*)) or the paramagnetic free radical TEMPO (0–5 $\times$ 10^{-2}% (*w*/*v*)). Two NH chemical shifts of **3** and **4** were sensitive (solvent-exposed NH groups) to the addition of the perturbing reagent DMSO. Also, after the addition of the TEMPO radical, the bandwidth of two NH signals broadened in the case of **3**, although one NH signal of **4** overlapped with the signals of a phenyl group. These results demonstrate that two NH protons are solvent-exposed, thus suggesting that two NH protons are not intramolecularly hydrogen-bonded. These results are in accord with a 3_{10}-helical structure, in which two NH groups at the *N*-terminus of the peptide are freely solvated (not intramolecularly hydrogen-bonded) peptide NH groups.

CD Spectra

The CD spectra of peptides **3** and **4** in 2,2,2-trifluoroethanol (TFE) solution show negative maxima at 203 and 222 nm, indicating that their helical screw sense is right-handed (*P*) (Figure 4). The ratio of R ($\theta_{222}/\theta_{203}$) suggests that the secondary structure of **4** ($R = 0.39$) is a 3_{10}-helix [20]. Furthermore, an intense positive maximum was observed at about 280 nm. This CD band indicates dipole–dipole

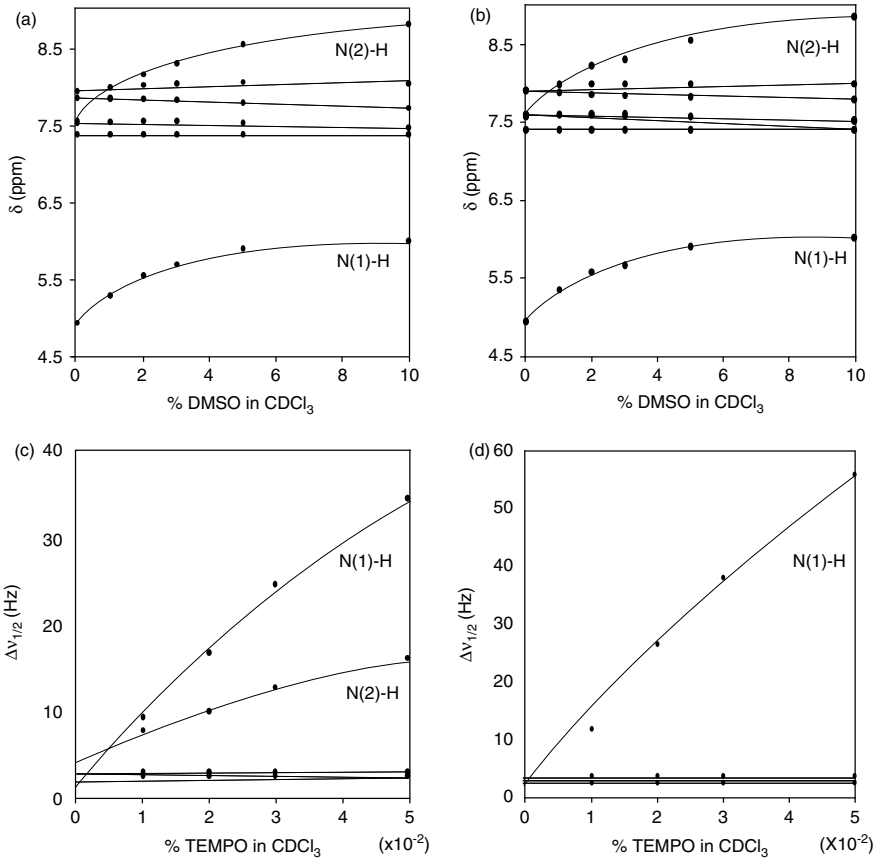

Figure 3. ^{1}H NMR experiments involving the addition of DMSO and radical TEMPO to a $CDCl_3$ solution of pentapeptide **3** and hexapeptide **4**. Plots of NH chemical shifts in the ^{1}H NMR spectra of **3** (a) and **4** (b) as a function of the percentage of DMSO (*v*/*v*) added to the $CDCl_3$ solution. Plots of the bandwidths of the NH proton in the ^{1}H NMR spectra of **3** (c) and **4** (d) as a function of the percentage of TEMPO (*w*/*v*) added to the $CDCl_3$ solution. Peptide concentration: 1.0 mM.

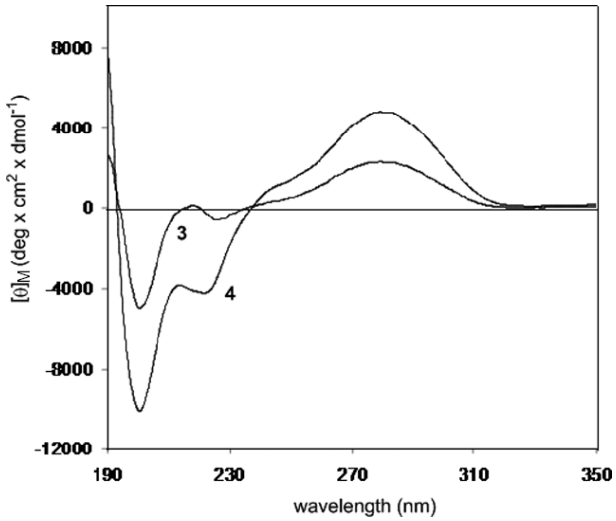

Figure 4. CD spectra in the 190–350 nm region of pentapeptide **3** and hexapeptide **4** in TFE solution. Peptide concentration: 0.5 mM.

interactions between the charge transfer electric moments of the dehydroamino acid chromophores held in a mutual, fixed disposition within the molecule [12], and this pattern is typical of a right-handed (*P*) 3_{10}-helix [13].

X-Ray Diffraction

The X-ray crystallographic analysis unambiguously revealed the molecular structural conformations of the peptides in the crystal state. The pentapeptide **3** and hexapeptide **4** formed good crystals for X-ray crystallographic analysis by slow evaporation of the solvents (MeOH-H_2O) at room temperature. The crystal and diffraction parameters of **3** and **4** are summarized in Table 1. Their molecular structures are given in Figures 5 and 6. Relevant backbone and side-chain torsion angles and the intra- and intermolecular hydrogen-bond parameters are listed in Tables 2 and 3.

In the asymmetric unit of pentapeptide **3**, only one conformer of the peptide molecule existed and it was formed into a right-handed (*P*) 3_{10}-helix. The mean values of the ϕ and ψ torsion angles of the amino acid residues (1–4) were -61.2° and -24.5°, which are close to values for an ideal right-handed (*P*) 3_{10}-helix (-60° and -30°), and the torsion angle of residue 5 was distorted ($\phi = -100.0^\circ$, $\psi = 7.4^\circ$). Figure 5 shows the X-ray structures of the (*P*) 3_{10}-helical triangle perpendicular to (a) and along (b) the helical axis.

Three intramolecular hydrogen bonds, in which each hydrogen bond forms a 10-membered (atoms) pseudo ring of the $i \leftarrow i+3$ type, exist in the 3_{10}-helical molecule of **3**. Three intramolecular hydrogen bonds are shown between the H-N(3) and C(0)═O(0) O atom of the Boc group with an N(3)···O(0) distance of 3.01 Å between the H-N(4) and C(1)═O(1) [N(4)···O(1) = 2.94 Å] and between the H-N(6) and C(3)═O(3) [N(6)···O(3) = 3.04 Å]. The distance between the H-N(5) and C(2)═O(2) [N(5)···O(2) = 3.43 Å] is too long for a hydrogen bond. In the packing mode, two intermolecular hydrogen bonds are observed between the 3_{10}-helical conformers; i.e. between the H-N(1) peptide donor and the C(4′)═O(4′) O atom of a symmetry-related molecule ($-x+2$, $y+1/2$, $-z+1/2$) [N(1)···O(4′) = 3.12 Å] and between the H-N(2) peptide donor and the C(5′)═O(5′) O atom of a symmetry-related molecule ($x-1$, y, z) [N(2)···O(5′) = 2.87 Å].

In the asymmetric unit of hexapeptide **4**, only one right-handed (*P*) 3_{10}-helix was present, together with two methanol molecules. The mean values of the ϕ and ψ torsion angles of amino acid residues (1–5) were -54.3° and -31.2°, which are close to the ideal right-handed (*P*) 3_{10}-helix values (-60° and -30°), and the torsion angle of residue 6 was distorted ($\phi = -123.0^\circ$,

Table 2. Selected torsion angles ω, ϕ, and ψ [°] for pentapeptide **3** and hexapeptide **4** as determined by X-ray crystallographic analysis

Torsion angle	**3**	**4**
ω_0	−163.4	−174.4
ϕ_1	−58.1	−55.0
ψ_1	−43.9	−34.4
ω_1	−175.2	178.7
ϕ_2	−60.2	−55.9
ψ_2	−19.4	−19.6
ω_2	−173.6	−175.2
ϕ_3	−54.0	−50.4
ψ_3	−32.9	−34.6
ω_3	−164.6	−174.0
ϕ_4	−72.6	−54.2
ψ_4	−1.8	−33.4
ω_4	−172.8	−175.9
ϕ_5	−100.0	−56.0
ψ_5	7.4	−34.1
ω_5	170.8	−169.7
ϕ_6	–	−123.0
ψ_6	–	−10.2
ω_6	–	−172.5
χ_2	171.4	176.1
χ_2	−10.3	−3.9
χ_5	61.1	–
χ_5	−65.8	–
χ_6	–	60.8
χ_6	–	−64.4

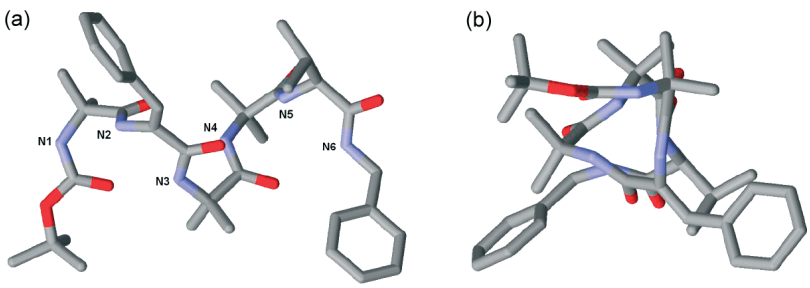

Figure 5. X-ray diffraction structures of pentapeptide **3** as viewed (a) perpendicular to and (b) along the helical axis.

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Table 3. Intra- and intermolecular H-bond parameters for pentapeptide **3** and hexapeptide **4**

Peptide[a]	Donor D—H	Acceptor A	Distance [Å] D···A	Angle [°] D—H···A	Symmetry operations
Boc-Aib-ΔPhe-$(Aib)_2$-L-Val-NH-Bn (**3**)					
	N_3-H	O_0	3.01	161.9	x, y, z
	N_4-H	O_1	2.94	157.2	x, y, z
	N_5-H[b]	O_2	3.43	167.7	x, y, z
	N_6-H	O_3	3.04	158.3	x, y, z
	N_1-H	$O_{4'}$	3.12	164.1	$-x+2, y+1/2, -z+1/2$
	N_2-H	$O_{5'}$	2.87	168.0	$x-1, y, z$
Boc-Aib-ΔPhe-$(Aib)_3$-L-Val-NH-Bn (**4**)					
	N_3-H	O_0	2.94	162.6	x, y, z
	N_4-H	O_1	2.99	165.3	x, y, z
	N_5-H	O_2	3.02	155.3	x, y, z
	N_6-H	O_3	3.01	158.0	x, y, z
	N_7-H	O_3	2.94	133.8	x, y, z
	N_2-H	O_{ma}[c]	2.84	164.4	x, y, z
	O_{mb}-H	O_4	2.74	160.7	x, y, z
	N_1-H	$O_{6'}$	2.91	152.3	$-x, y+1/2, -z$
	O_{ma}-H	$O_{6'}$	2.75	161.6	$-x, y+1/2, -z$

[a] The amino acid numbering begins at the *N*-terminus of the peptide chain.
[b] The distance is too long for a hydrogen bond.
[c] O_m: Methanol (a, b).

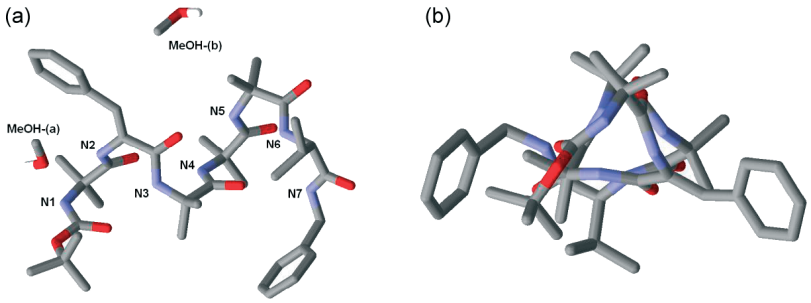

Figure 6. X-ray diffraction structures of hexapeptide **4** as viewed (a) perpendicular to and (b) along the helical axis.

$\psi = -10.2°$). Figure 6 shows the X-ray structures of the (*P*) 3_{10}-helical triangle perpendicular to (a) and along (b) the helical axis.

Five intramolecular hydrogen bonds, in which four hydrogen bonds form a 10-membered (atoms) pseudo ring of the $i \leftarrow i+3$ type and one forms a 13-membered (atoms) pseudo ring of the $i \leftarrow i+4$ type, exist in the 3_{10}-helical molecule of **4**. Five intramolecular hydrogen bonds are shown between the H-N(3) and C(0)═O(0) O atom of the Boc group with an N(3)···O(0) distance of 2.94 Å between H-N(4) and C(1)═O(1) [N(4)···O(1) = 2.99 Å], between H-N(5) and C(2)═O(2) [N(5)···O(2) = 3.02 Å], between H-N(6) and C(3)═O(3) [N(6)···O(3) = 3.01 Å], and between H-N(7) and C(3)═O(3) [N(7)···O(3) = 2.94 Å]. In the packing mode, one intermolecular hydrogen bond is observed between the 3_{10}-helical conformers; i.e. between the H-N(1) peptide donor and the C(6′)═O(6′) O atom of a symmetry-related molecule ($-x$, $y+1/2$, $-z$) [N(1)···O(4′) = 2.91 Å]. Also, intermolecular hydrogen bonds are formed between the peptides by means of a methanol molecule (a), i.e. between the H-N(2) peptide donor and the O_m(a) of the methanol (a) acceptor [N(2)···O_m(a) = 2.84 Å], and the H-O_m(a) water donor and the C(6′)═O(6′) acceptor [O_m(a)···O(6′) = 2.75 Å] of a symmetry-related molecule ($-x$, $y+1/2$, $-z$). Also, the H-O_m(b) donor of the methanol molecule (b) is hydrogen-bonded to the C(4)═O(4) acceptor [O_m(b)···O(4) = 2.74 Å].

Computational Analysis

The calculation of the structure of hexapeptide **4** produced a right-handed (*P*) 3_{10}-helix as the global minimum-energy conformation (0 kcal/mol), and the (*M*) 3_{10}-helix was obtained as a local minimum-energy conformation that exhibited an energy of +3.05 kcal/mol. The peptide main-chain structure of the (*P*) 3_{10}-helical conformer produced by the calculations was similar to that seen in the crystalline state with some differences at the *C*-terminal amide group, as shown by their superimposition in Figure 7.

Conclusions

L-Val was inserted into the *C*-termini of helical peptides constructed from achiral Aib and Δ^ZPhe residues. Conformational analysis in solution was performed by IR, ^{1}H NMR, and CD spectra, and the dominant conformations of pentapeptide **3** and hexapeptide **4** were both right-handed (*P*) 3_{10}-helical structures.

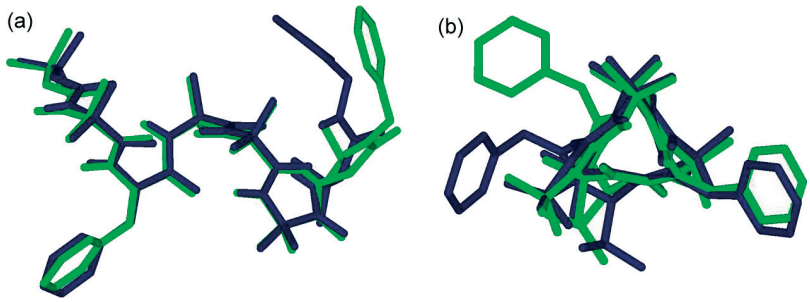

Figure 7. Superimposition of the conformation determined by X-ray analysis (blue) and the calculated minimum-energy conformation (green) of hexapeptide **4** as viewed (a) perpendicular to and (b) along the helical axis.

The conformations of **3** and **4** in the crystalline state were analyzed by X-ray crystallographic analysis, and it was revealed that both of them formed right-handed (*P*) 3_{10}-helical structures. Toniolo and coworkers described that the insertion of one chiral L-amino acid ester at the *C*-terminal of peptide induced a left-handed (M) helical structure, because unfavorable O···O interaction between the carbonyl oxygen atom of the $i-2$ amino acid from the *C*-terminus and either oxygen atom of the *C*-terminal ester (i) functionality arise if right-handed helix are formed [1]. However, this interaction does not occur in the peptides **3** and **4** with the *C*-terminal amide group, therefore both of them might form right-handed (P) helical structures. The molecular-mechanics calculations of hexapeptide **4** produced a (*P*) 3_{10}-helix as a global minimum-energy conformation, and the (*P*) 3_{10}-helical conformer produced by the calculation was similar to that in the crystalline state with some differences at the *C*-terminal amide group. These results demonstrated that the insertion of a chiral amino acid (L-Val) at the *C*-terminus can be used to control the helical screw sense of peptides (Boc-Aib-Δ^ZPhe-Aib$_n$-).

Acknowledgements

This work was supported in part by a Grant-in-Aid for Young Scientists (B) (21790018) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

1 Pengo B, Formaggio F, Crisma M, Toniolo C, Bonora GM, Broxterman QB, Kamphuis J, Saviano M, Iacovino R, Rossi F, Benedetti E. Helical screw sense of peptide molecules. *J. Chem. Soc., Perkin Trans.* 1998; **2**: 1651–1657.
2 Inai Y, Tagawa K, Takasu A, Hirabayashi T, Oshikawa T, Yamashita M. Induction of one-handed helical screw sense through the domino effect. *J. Am. Chem. Soc.* 2000; **122**: 11731–11732.
3 Inai Y, Ishida Y, Tagawa K, Takasu A, Hirabayashi T. Noncovalent domino effect on helical screw sense of chiral peptides possessing C-terminal chiral residue. *J. Am. Chem. Soc.* 2002; **124**: 2466–2473.
4 Nagano M, Tanaka M, Doi M, Demizu Y, Kurihara M, Suemune H. Helical-screw directions of diastereoisomeric cyclic α-amino acid oligomers. *Org. Lett.* 2009; **11**: 1135–1137.
5 Tanaka M, Anan K, Demizu Y, Kurihara M, Doi M, Suemune H. Side-chain chiral centers of amino acid and helical-screw handedness of its peptides. *J. Am. Chem. Soc.* 2005; **127**: 11570–11571.
6 Tanaka M, Demizu Y, Doi M, Kurihara M, Suemune H. Chiral centers in the side chains of α-amino acids control the helical screw sense of peptides. *Angew. Chem. Int. Ed.* 2004; **43**: 5360–5363.
7 Tanaka M, Nishimura S, Oba M, Demizu Y, Kurihara M, Doi M, Suemune H. C_5 conformation and a 3_{10}-helical structure of peptide composed of diverse α-ethylated α,α-disubstituted α-amino acids. *Chem. Eur. J.* 2003; **9**: 3082–3090.
8 Oba M, Tanaka M, Kurihara M, Suemune H. Conformation of peptides containing (*S*)-α-ethylleucine. *Helv. Chim. Acta* 2002; **85**: 3197–3218.
9 Prasad BVV, Balaram P. The stereochemistry of peptides containing α-aminoisobutyric acid. *CRC Crit. Rev. Biochem.* 1984; **16**: 307–348.
10 Benedetti E, Bavoso A, Di Balsio B, Pavone V, Pedone C, Crisma M, Bonora GM, Toniolo C. Conformation of homooligo (α-aminoisobutyric acids) from tripeptide to pentapeptide. *J. Am. Chem. Soc.* 1982; **104**: 2437–2444.
11 Pieroni O, Fissi A, Pratesi C, Temussi PA, Ciardelli F. Reversible screw sense inversion of the 3_{10}-helix in a dehydropeptide. *J. Am. Chem. Soc.* 1991; **113**: 6338–6340.
12 Tuzi A, Ciajolo MR, Guarino G, Temussi PA, Fissi A, Pieroni O. Solid state and solution structure of dehydropeptides. *Boipolymers* 1993; **33**: 1111–1121.
13 Pieroni O, Fissi A, Jain RM, Chauhan VS. Solution structure of dehydropeptides. a CD investigation. *Boipolymers* 1996; **38**: 97–108.
14 Padyana AK, Ramakumar S, Mathur P, Jagannathan NR, Chauhan VS. Crystal and solution structure of dehydropeptides. *J. Peptide Sci.* 2003; **9**: 54–63.
15 Ousaka N, Inai Y. Preparation of Boc-Aib-ΔPhe oxazolone. *J. Org. Chem.* 2009; **74**: 1429–1439.
16 Sheldrick GM. *SHELXL 97, Program for Crystal Structure Refinement*. University of Göttingen: G¨{o}ttingen, 1997.
17 Beurskens PT, Admiraal G, Beurskens G, Bosman WP, de Gelder R, Israel R, Smits JMM. The DIRDIF-99 program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1994.
18 CCDC-751276, and 751277 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
19 Kennedy DF, Crisma M, Toniolo C, Chapman D. Studies of peptides forming 3_{10}-helical structure in solution by FT-IR. *Biochemistry* 1991; **30**: 6541–6548.
20 Toniolo C, Polese A, Formaggio F, Crisma M, Kamphuis J. CD spectrum of a peptide 3_{10}-helix. *J. Am. Chem. Soc.* 1996; **118**: 2744–2745.

