Solid-state conformations of oligopeptides possessing an -(Aib-Δ^{Z}Phe)$_2$- segment †

Yoshihito Inai,*[a] Tatsuo Oshikawa,[b] Mitsuji Yamashita,[b] Tadamichi Hirabayashi[a] and Satoru Ashitaka[a]

[a] *Department of Environmental Technology and Urban Planning, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, 466-8555, Japan*

[b] *Department of Materials Chemistry, Faculty of Engineering, Shizuoka University, Hamamatsu, 432-8561, Japan*

Received (in Cambridge, UK) 23rd January 2001, Accepted 23rd April 2001
First published as an Advance Article on the web 11th May 2001

An X-ray crystallographic analysis was carried out for Boc-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe **1** and Boc-L-Pro-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe **2** (Aib = α-aminoisobutyric acid; Δ^{Z}Phe = α,β-dehydrophenylalanine; Boc = *tert*-butoxycarbonyl; OMe = methoxy) to provide detailed conformational data for oligopeptides possessing an -(Aib-Δ^{Z}Phe)$_2$- segment. Both peptides adopted a typical 3_{10}-helical conformation characterized by $<\varphi> = 52.8°$, $<\psi> = 29.3°$, and $<\omega> = -173.8°$ for the average values of the four residues of the -(Aib-Δ^{Z}Phe)$_2$- segment in peptide **1**, and $<\varphi> = 54°$, $<\psi> = 27°$, and $<\omega> = -175°$ for those in peptide **2**. The preference for a 3_{10}-helix in the -(Aib-Δ^{Z}Phe)$_2$- segment is ascribed to the presence of Aib and Δ^{Z}Phe residues being strong inducers for the formation of a 3_{10}-helix. In peptide **2**, the N-terminal L-Pro residue adopted a semiextended conformation, leading to a left-handed screw sense for the following achiral segment. This result was also supported by conformational energy calculation, in which the L-Pro residue leading to a left-handed 3_{10}-helical segment prefers a semiextended conformation rather than a right-handed helical conformation.

Introduction

To clarify what factors govern the helical screw sense of biopolymers is a significant and common theme in a wide range of chemical fields including biological macromolecules, synthetic polymers, and supramolecules. Recently, we have attempted to reveal which screw sense is preferred for an achiral helical host peptide possessing an -(Aib-Δ^{Z}Phe)$_n$- segment (n = 2–4; Aib = α-aminoisobutyric acid; Δ^{Z}Phe = α,β-dehydrophenylalanine)[1–4] when a chiral amino acid residue as a guest is introduced into the host peptide through the covalent bond.‡ As a result, the L-residue introduced into the N-terminal position induces a left-handed screw sense for the following achiral segment preferentially,[1] although most L-amino acid residues are well recognized to prefer a right-handed screw sense in helical segments of peptides or proteins. Such a left-handed screw sense induced by an N-terminal L-residue was observed, irrespective of the types of L-residues,[2] types of solvents,[1,2] and of chain lengths of achiral host segments.[3] Also, an L-Leu residue second from N-terminal leads to both left- and right-handed screw senses for the following achiral segment, in which the preference for a screw sense depends on the type of solvent.[4] More recently, the excess of one-handed screw sense is induced for N-deprotected nonapeptide H-(Aib-Δ^{Z}Phe)$_4$-Aib-OMe (OMe = methoxy) through the noncovalent *domino effect* based on interacting the N-terminal amino group with a chiral carboxylic acid.[5]

In our systems, sequential -(Aib-Δ^{Z}Phe)$_n$- segments have been commonly used for an achiral helical host segment, since Aib[6,7] and Δ^{Z}Phe[8–10] residues are achiral ones and strong inducers for the formation of a 3_{10}-helix.[11] Herein the Δ^{Z}Phe residue is also useful as an excellent probe for CD measurement in UV regions, due to its intense absorption band around 280 nm.[12–14] Actually, NMR and CD spectroscopy revealed that achiral peptides possessing -(Aib-Δ^{Z}Phe)$_n$- segments (n = 2–4) adopt a 3_{10}-helical conformation in solution, adopting both screw senses to the same extent.[1–4] Conformational energy calculations also supported a helix-forming tendency in oligopeptides possessing an -(Aib-Δ^{Z}Phe)$_n$- segment.[1,3] On the other hand, the detailed conformation of the -(Aib-Δ^{Z}Phe)$_n$- segment in solution, *i.e.*, torsion angles of the main chain, is still unknown. The reason is mainly based on the lack of $C^{\alpha}H$ protons, for which the NMR data can provide valuable information to more precisely specify a helical conformation.

The present paper provides the solid-sate conformations of oligopeptides possessing an -(Aib-Δ^{Z}Phe)$_2$- segment. For our purpose, X-ray crystallographic analysis was carried out for pentapeptide Boc-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe **1** (Boc = *tert*-butoxycarbonyl) and hexapeptide Boc-L-Pro-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe **2**.

We also focus on the conformation of the N-terminal L-Pro residue in peptide **2**. Peptide **2** in solution was already shown to form a left-handed 3_{10}-helix preferentially, whereas most L-residues are well recognized to induce a right-handed screw sense. Thus, the detailed conformational data of peptide **2** can enable us to understand how the N-terminal L-Pro residue induces a left-handed screw sense in the following achiral segment.

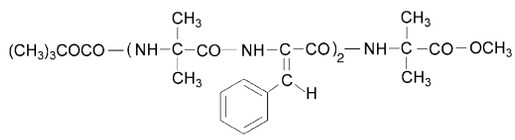
† Electronic supplementary information (ESI) available: stereoviews of the crystal structures of peptides **1** and **2**. See http://www.rsc.org/suppdata/p2/b1/b100774m/

‡ The host–guest notation is used for covalent introduction in our system. Similar usage can be seen for the system in which a residue (guest) is introduced into a given position of a longer peptide (host) through a covalent bond.

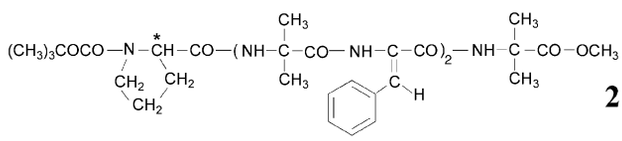
 DOI: 10.1039/b100774m

Table 1 Crystallographic details for peptides **1** and **2**

Parameter	**1**	**2**
Empirical formula	$C_{36}H_{47}N_5O_8 \cdot C_2H_5OH$	$C_{41}H_{54}N_6O_9$
Molecular weight	723.86	774.91
Crystal dimensions/mm^3	0.30 × 0.20 × 0.20	0.40 × 0.60 × 0.70
Crystal system/space group	Monoclinic/$P2_1$	Orthorhombic/$P2_12_12_1$
a/Å	10.812(2)	19.099(3)
b/Å	19.228(4)	19.789(2)
c/Å	19.324(3)	11.980(2)
α/°	90	90
β/°	101.98(1)	90
γ/°	90	90
V/Å^3	3929(1)	4527.8(9)
Z	4	4
Density calculated/g cm^{-3}	1.223	1.137
Radiation used	CuKα (λ = 1.54178 Å)	CuKα (λ = 1.54178 Å)
F(000)	1552.00	1656.00
Temperature/K	223	296
Scan type	$\omega - 2\theta$	$\omega - 2\theta$
$2\theta_{max}$/°	119.2	120.2
Observed reflections [$I > 2\sigma(I)$]	3124	2114
Variables	652	552
Refinement method	Full-matrix least squares on F^2	Full-matrix least squares on F^2
Final agreement factors	R = 0.073 (observed data) R_w = 0.232 (all data)	R = 0.118 (observed data) R_w = 0.329 (all data)



1



2

Experimental

Sample preparation

The synthesis and characterization followed ref. 1 for peptide **1**, and ref. 2 for peptide **2**. Single crystals were obtained at ambient temperature by slowly evaporating a solution of peptide **1** in absolute ethanol and of peptide **2** in acetonitrile at ambient temperature.

X-Ray structure determination

A colorless, prismatic single crystal (0.30 × 0.20 × 0.20 mm^3 for **1** and 0.40 × 0.60 × 0.70 mm^3 for **2**) was used for collecting three-dimensional X-ray data on a RIGAKU AFC7R diffractometer. The structure was solved by direct methods,[15] and expanded using Fourier techniques.[16] All the nonhydrogen atoms were refined anisotropically. Hydrogen atoms of peptide **1** were refined isotropically. The positions of hydrogen atoms of peptide **2** were not refined, but isotropic B values were refined. The final cycle of full-matrix least squares refinement[17] on F^2 was based on 3124 observed reflections [$I > 2\sigma(I)$] for peptide **1**, and 2114 reflections [$I > 2\sigma(I)$] for peptide **2**. The refinement was converged with $R = 0.073$ (for observed data) and $R_w = 0.232$ (for all data) for peptide **1**, and $R = 0.118$ (for observed data) and $R_w = 0.329$ (for all data) for peptide **2**. The crystallographic details are summarized in Table 1. The molecular graphics were illustrated using molecular modeling software.[18]

Tables of final positional parameters, equivalent thermal factors, bond lengths, bond angles, and van der Waals contacts for peptides **1** and **2** have been deposited in the Cambridge Crystallographic Data Bank as a supplementary publication.§

Conformational energy calculation

In order to estimate the energetically favored conformations of the N-terminal L-Pro residue in peptide **2**, an empirical conformational energy calculation was carried out using the structural and energy parameters based on the ECEPP system.[19] The ECEPP parameters of the Δ^ZPhe residue were determined in our previous study.[14] The program PEPCON[19–21] for obtaining a conformational energy calculation and graphics of a given peptide was modified to be applicable to β-aryldehyroalanine-containing peptides.[14,22–24] On the basis of the present result and many crystallographic data[8] for Δ^ZPhe-containing peptides, all amide groups were fixed to the *trans* conformation ($\omega = 180°$), and each Δ^ZPhe side chain was fixed to the *Z*-configuration ($\chi^1 = 0°$). Conformational energy was calculated for Ac-L-Pro-(Aib-Δ^ZPhe)$_2$-Aib-OMe (Ac = acetyl) with varying ψ_{Pro} values (−180° to +180°). Here the Boc group was replaced by an Ac group to simplify the calculation. In the ECEPP system, the *up* puckering of the Pro ring ($\varphi_{Pro} = -67.6°$)[19] was chosen on the basis of the present data of peptide **2**. The achiral segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was set to a standard left- or right-handed 3_{10}-helix: *i.e.*, (φ, ψ) = (60°, 30°) or (−60°, −30°),[25,26] respectively.

Results and discussion

Conformation of peptide 1

Bond lengths and bond angles of peptide **1** were in general agreement with previous reports for Aib[7,24] and Δ^ZPhe[8] residues. A perspective view of peptide **1** is shown in Fig. 1, and the torsion angles are summarized in Table 2. The backbone of Aib(1)-Δ^ZPhe(4) residues took a 3_{10}-helical conformation characterized by $<\varphi> = 52.8°$, $<\psi> = 29.3°$, and $<\omega> = -173.8°$ for the average values of the four residues. Here three successive intramolecular ($i + 3$)⟶i hydrogen bonds were observed for the pairs of CO(Boc)–NH[Aib(3)], CO[Aib(1)]–NH[Δ^ZPhe(4)], and CO[Δ^ZPhe(2)]–NH[Aib(5)]. Obviously, the -(Aib-Δ^ZPhe)$_2$- segment induces a 3_{10}-helical conformation predominantly. The other NH groups participated in intermolecular hydrogen bonding: *i.e.*, Aib(1) NH was hydrogen-

§ CCDC reference numbers 157037 and 157038. See http://www.rsc.org/suppdata/p2/b1/b100774m/ for crystallographic files in .cif or other electronic format.

Table 2 Selected torsion angles (°)[a]

Peptide **1** Residue	φ_i	ψ_i	ω_i	χ_i^1, $\chi_i^{1'}$	$\chi_i^{2,1}$, $\chi_i^{2,2}$
Boc(0)			172.4(4)		
Aib(1)	54.9(6)	36.3(5)	−174.0(3)		
Δ^ZPhe(2)	47.8(5)	20.7(6)	−175.3(4)	5.0(8), 178.1(4)	24.5(8) −157.3(5)
Aib(3)	53.1(6)	27.6(6)	−175.2(4)		
Δ^ZPhe(4)	55.3(6)	32.4(6)	−170.5(4)	4.4(9), −173.9(5)	−26.0(9), 152.8(6)
Aib(5)	−52.4(7)	−52.2(7)	−164.8(6)		

Peptide **2** Residue	φ_i	ψ_i	ω_i	χ_i^1, $\chi_i^{1'}$	$\chi_i^{2,1}$, $\chi_i^{2,2}$
Boc(0)			178(1)		
Pro(1)	−44(1)	131(1)	174(1)		
Aib(2)	57(1)	28(1)	−176(1)		
Δ^ZPhe(3)	50(1)	22(1)	−177(1)	6(2), 175(1)	22(2), −161(1)
Aib(4)	50(1)	34(1)	176(1)		
Δ^ZPhe(5)	58(1)	24(1)	−163(1)	6(2), −170(1)	−15(2), 169(1)
Aib(6)	−53(1)	−48(2)	173(1)		

[a] Torsion angles for *i*th residue are defined as follows: φ_i for C_{i-1}'–N_i–C_i^{α}–C_i'; ψ_i for N_i–C_i^{α}–C_i'–N_{i+1} (N_i–C_i^{α}–C_i'–O_{i+1} for C-terminal residue); ω_i for C_i^{α}–C_i'–N_{i+1}–$C_{i+1}{}^{\alpha}$ (C_i^{α}–C_i'–O_{i+1}–$C_{i+1}{}^{\alpha}$ for C-terminal residue); χ_i^1 for N_i–C_i^{α}–C_i^{β}–C_i^{γ}; $\chi_i^{1'}$ for C_i'–C_i^{α}–C_i^{β}–C_i^{γ}; $\chi_i^{2,1}$ for C_i^{α}–C_i^{β}–C_i^{γ}–C_i^{o1}; $\chi_i^{2,2}$ for C_i^{α}–C_i^{β}–C_i^{γ}–C_i^{o2}.

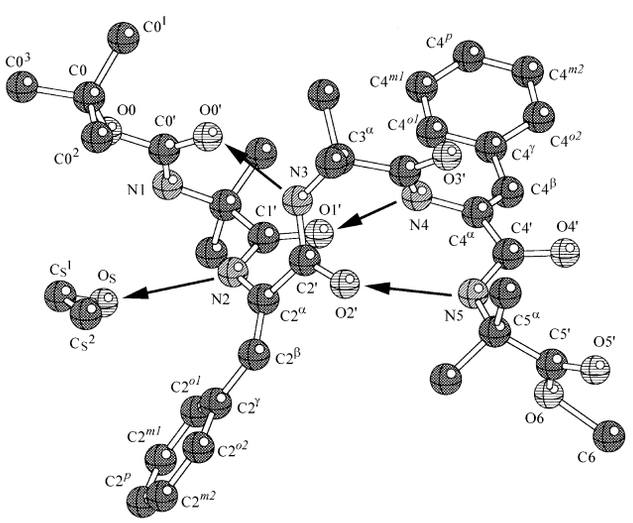


Fig. 1 Conformation of peptide **1** obtained from X-ray crystallographic analysis. The three intramolecular hydrogen bonds of $(i + 3) \longrightarrow i$ type are indicated by the arrows.

bonded with CO[Δ^ZPhe(4)] of another neighboring chain, and Δ^ZPhe(2)NH with an oxygen atom of a cocrystallized ethanol molecule. The intra- and intermolecular hydrogen-bond parameters are summarized in Table 3. The C-terminal Aib(5) residue also adopted a helical conformation, but its screw sense was opposite to that of the preceding segment. This tendency has often been found in Aib-rich 3_{10}-helical peptides.[29,30] Also in proteins, Schellman noted that many right-handed helical segments end with a residue in left-handed conformation.[31]

In general, the urethane group tends to favor a planar conformation ($\theta_0 = 180°$ and $\omega_0 = 180°$).[32] Also in the present case, the Boc group adopted a *trans*,*trans* conformation characterized by θ_0(C0–O0–C0′–N1) = 172.4(4)° and $\omega_0 = 173.1(4)°$. This conformation is required for the presence of the O0′ oxygen atom (in the Boc group) participating in the first $(i + 3) \longrightarrow i$ hydrogen bond of the 3_{10}-helix. The methyl carbon atoms of the Boc group were staggered with respect to the O0–C0′ bond [$\theta_0{}^1$($C0^1$–C0–O0–C0′) = 61.0(6)°, $\theta_0{}^2$($C0^2$–C0–O0–C0′) = −64.7(7)°, and $\theta_0{}^3$($C0^3$–C0–O0–C0′) = 179.2(7)°]. All four peptide bonds took *trans* conformations essentially. The side-chain torsion angles about the C^{α}=C^{β} double bond, χ^1 and $\chi^{1'}$, are 5.0(8)° and 178.1(4)° for Δ^ZPhe(2), and 4.4(9)° and −173.9(5)° for Δ^ZPhe(4), meaning the stereochemistry about the C^{α}=C^{β} double bond is essentially planar. On the other hand, the side-chain about the C^{β}–C^{γ} bond was nonplanar, characterized by $\chi^{2,1} = 24.5(8)°$ and $\chi^{2,2} = -157.3(5)°$ for the Δ^ZPhe(2) residue, and $\chi^{2,1} = -26.0(9)°$ and $\chi^{2,2} = 152.8(6)°$ for the Δ^ZPhe(4) residue. This nonplanarity was found in several 3_{10}-helical peptides containing Δ^ZPhe residues.[33,34]

The solution conformation of peptide **1** was investigated by ^{1}H NMR spectroscopy.[1] The variation of NH chemical shifts for peptide **1** in $CDCl_3$ with $(CD_3)_2SO$ indicated that three NHs of Aib(3)–Aib(5) residues participate in intramolecular hydrogen bonding. In addition, marked NOEs were observed for N_iH–$N_{i+1}H$ proton pairs over the peptide chain, suggesting the presence of a helical conformation. These NMR data reflect the conformation obtained in the present study, thus indicating that the 3_{10}-helical conformation in the solid state is retained in solution.

Conformation of peptide 2

The conformation of peptide **2** is shown in Fig. 2, and the torsion angles are listed in Table 2. The main-chain conformation took a 3_{10}-helical conformation characterized by $<\varphi> = 54°$, $<\psi> = 27°$, and $<\omega> = -175°$ for the average values of the Aib(2)–Δ^ZPhe(5) residues. The hydrogen-bond parameters are shown in Table 3. The four intramolecular $(i + 3) \longrightarrow i$ hydrogen-bonds were observed for CO(Boc)–NH[Δ^ZPhe(3)], CO[Pro(1)]–NH[Aib(4)], CO[Aib(2)]–NH[Δ^Z-Phe(5)], and CO[Δ^ZPhe(3)]–NH[Aib(6)] pairs. The remaining NH of the Aib(2) residue participated in an intermolecular hydrogen bonding with the carbonyl group of the Aib(6) residue in another neighboring chain. The intramolecular hydrogen-bonding patterns were consistent with the solvent dependence on NH resonances,[2] suggesting that the 3_{10}-helical conformation in the solid state is retained in solution. Therefore, peptide **2** as well as peptide **1** adopts a typical 3_{10}-helical conformation. The conformational similarity in peptides **1** and

Table 3 Intra- and intermolecular hydrogen-bond parameters for peptides **1** and **2**

Peptide	Donor D–H	Acceptor A	Distance/Å D···A	Distance/Å H···A	Angle (°) D–H···A	Symmetry[a]
1	N3–H	O0′	2.860(5)	1.85(5)	162(4)	*x*, *y*, *z*
	N4–H	O1′	2.983(3)	2.07(5)	173(4)	*x*, *y*, *z*
	N5–H	O2′	2.875(5)	1.95(4)	165(3)	*x*, *y*, *z*
	N1–H	O4′	3.081(5)	2.23(6)	154(4)	$x-1/2$, $-y+1/2$, $z+1/2$
	N2–H	OS	3.039(6)	2.07(7)	163(5)	*x*, *y*, *z*
2[b]	N3–H	O0′	2.82(1)	2.06	130	*x*, *y*, *z*
	N4–H	O1′	2.99(1)	2.01	167	*x*, *y*, *z*
	N5–H	O2′	3.00(1)	2.01	172	*x*, *y*, *z*
	N6–H	O3′	2.98(1)	2.03	160	*x*, *y*, *z*
	N2–H	O6′	3.13(1)	2.65	110	$-x$, $y+1/2$, $-z+3/2$

[a] The symmetry operations are applied to the acceptors. [b] The hydrogen positions were based on AM1 semiempirical molecular orbital calculation[27] in MOPAC97.[28]

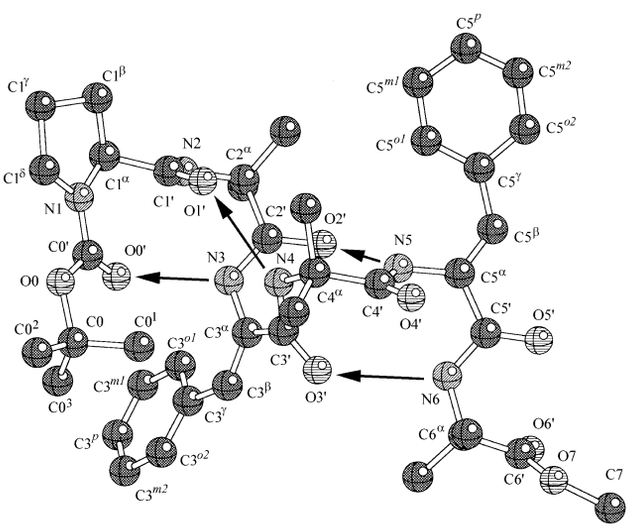


Fig. 2 Conformation of peptide **2** obtained from X-ray crystallographic analysis. The four intramolecular hydrogen bonds of $(i+3) \longrightarrow i$ type are indicated by the arrows.

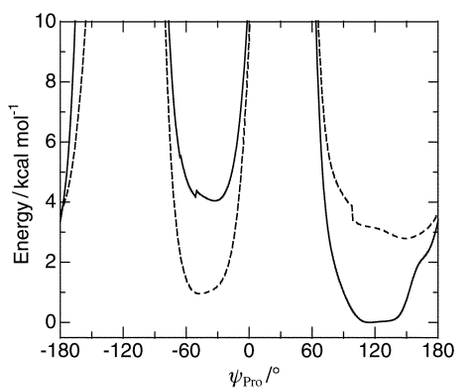


Fig. 3 Dependence of conformational energy on the ψ_{Pro} angle of peptide **2** [Ac-L-Pro-(Aib-Δ^ZPhe)$_2$-Aib-OMe] in a standard left-handed 3_{10}-helix (solid line) and right-handed one (broken line) of the -(Aib-Δ^ZPhe)$_2$-Aib-OMe segment: *i.e.*, $(\varphi, \psi) = (60°, 30°)$ or $(-60°, -30°)$, respectively. The *up* puckering of the Pro ring ($\varphi_{Pro} = -67.6°$)[19] was chosen on the basis of the present data.

2 was also observed in the following parts. The C-terminal Aib(6) residue took a helical conformation, of which the screw sense was opposite to that of the preceding segment. The Boc group adopted a *trans*,*trans* conformation, characterized by $\theta_0 = 172.4(4)°$ and $\omega_0 = -164(1)°$, essentially. Four peptide bonds for the Pro(1)–Aib(4) residues (ω_1–ω_4) took *trans* conformations, although that for Δ^ZPhe(5) residue (ω_5) deviated somewhat from 180°. The pyrrolidine ring of the L-Pro residue has symmetry close to C_2–C^γ *exo* (C^β *endo*)[35] with $\Phi_2 = 87°$ and $q_2 = -0.39$ for the ring puckering parameters.[36]

The N-terminal L-Pro residue as a chiral guest led to a left-handed screw sense for the following achiral segment -(Aib-Δ^ZPhe)$_2$-. Herein the Pro residue was not incorporated into a left-handed helical conformation, but took a semiextended conformation characterized by $\varphi_1 = -44(1)°$ and $\psi_1 = 131(1)°$. More exactly, the N-terminal segment -Pro(1)-Aib(2)- adopted a type II β-bend, which is responsible for the left-handed helix of the following achiral residues. This fact agrees well with our previous conclusion driven from conformational studies of Boc-X-(Aib-Δ^ZPhe)$_2$-Aib-OMe (X = L-amino acids) in solution.[1–3]

Also, elegant examples for clarifying the positional effect of a chiral residue on helical screw sense have been reported using an achiral homooligomer of -(Aib)$_4$-.[37–39] Peptide *p*BrBz-L-Pro-D-Ala-(Aib)$_4$-O*t*Bu (*p*BrBz = *p*-bromobenzoyl; O*t*Bu = *t*-butoxy) in the solid state took a type II β-bend for the -L-Pro-D-Ala- segment, which is followed by a left-handed 3_{10}-helix spanning the helix.[37] Conversely, *p*BrBz-D-Pro-D-Ala-(Aib)$_4$-O*t*Bu adopted a distorted type II′ β-bend for the -D-Pro-D-Ala- segment, which induces a right-handed 3_{10}-helix in the following -D-Ala-(Aib)$_3$-segment.[37] Interestingly, peptide **2** resembles the former case very well in the mechanism for inducing a left-handed screw sense of achiral host segment by an N-terminal L-residue.

To obtain further information about the preferred conformation of L-Pro, Fig. 3 shows how the conformational energy of peptide **2** depends on the conformation of the L-Pro residue (ψ_{Pro}). Here the following achiral segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was set to a left- or right-handed standard 3_{10}-helix. For each screw sense, there are mainly two stable regions, *i.e.*, $\psi_{Pro} = -60°$ to $-30°$ (type A), and 100° to 140° (type B). Type A corresponds to a right-handed 3_{10}-α-helical conformation, and type B to a semiextended conformation. Thus, the N-terminal Pro residue tends to adopt both helical and semiextended conformations energetically. More exactly, the helical conformation of the Pro residue is more favored for the right-handed helix in the following segment, and the semiextended conformation for the left-handed helix. Furthermore, the latter case gave the lowest energy, being in good agreement with the experimental result that peptide **2** forming a left-handed helix adopts a semiextended conformation for the Pro residue [$\varphi_1 = -44(1)°$ and $\psi_1 = 131(1)°$]. Therefore, also in the theoretical aspect, the N-terminal L-Pro residue was found to favor a semiextended conformation, leading to a left-handed helix of the following achiral segment.

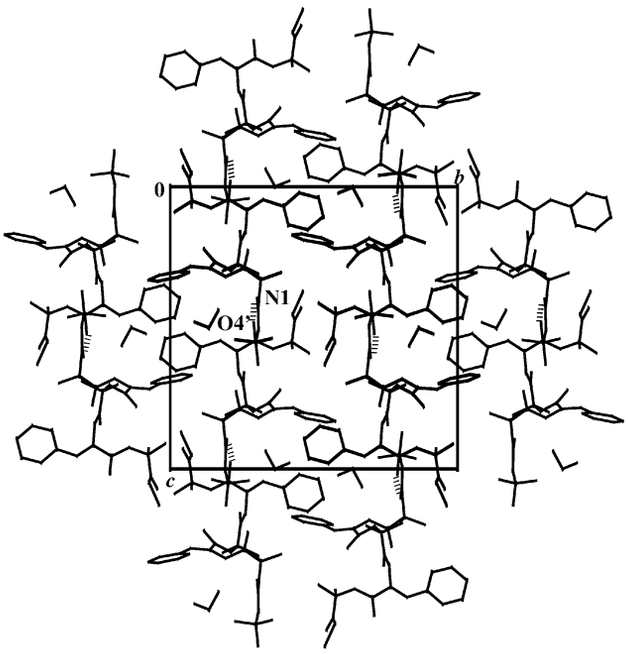


Fig. 4 Molecular packing of peptide **1**. View normal to *bc* planes. The dotted lines represent intermolecular hydrogen bonds between neighboring peptide pairs as shown in Table 3.

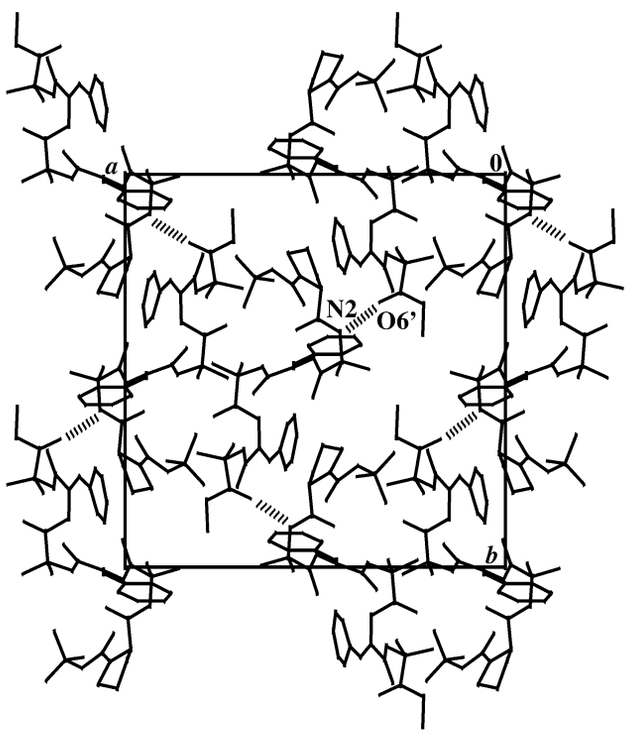


Fig. 5 Molecular packing of peptide **2**. View normal to *ab* planes. The dotted lines represent intermolecular hydrogen bonds between neighboring peptide pairs as shown in Table 3.

Molecular packing

The crystal structures of peptides **1** and **2** are shown in Figs. 4 and 5, respectively, and their hydrogen-bond parameters are listed in Table 3. (A stereoview of the packing of the crystal structures is available as supplementary data.†) The helical chains of peptide **1** are linked by intermolecular hydrogen bonds of N1–H [Aib(1)] with O4′ [Δ^{Z}Phe(4)] between symmetries (x, y, z) and ($x - 1/2$, $-y + 1/2$, $z + 1/2$). This leads to long columns of helical molecules running along the c axis in a head-to-tail fashion. The continuous helical columns are packed together along the b axis in an antiparallel fashion. A similar packing of molecules is seen in peptide **2**. There are intermolecular hydrogen bonds of N1–H [Aib(2)] with O6′ [Aib(6)] between symmetries (x, y, z) and ($-x$, $y + 1/2$, $-z + 3/2$). This head-to-tail hydrogen bonding leads to a continuous helical column running along the a axis. The helical columns are packed together along the b axis in an antiparallel fashion. Such head-to-tail hydrogen bonding found in peptides **1** and **2** has been commonly observed in helical conformations containing Aib or Δ^{Z}Phe residues.[10,30,33,39–43]

Conclusions

We here revealed the solid-state conformations of oligopeptides possessing an -(Aib-Δ^{Z}Phe)$_2$- segment to provide detailed conformational data for the segment. X-Ray crystallographic analysis was carried out for Boc-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe (**1**) and Boc-L-Pro-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe (**2**). Both peptides adopt a typical 3_{10}-helical conformation characterized by $\langle\varphi\rangle = 52.8°$, $\langle\psi\rangle = 29.3°$, and $\langle\psi\rangle = -173.8°$ for peptide **1** and $\langle\varphi\rangle = 54°$, $\langle\psi\rangle = 27°$, and $\langle\omega\rangle = -175°$ for peptide **2** in the average values of the four residues of -(Aib-Δ^{Z}Phe)$_2$- segment. The preference for a 3_{10}-helix in the -(Aib-Δ^{Z}Phe)$_2$- segment is consistent with the presence of Aib and Δ^{Z}Phe residues being strong inducers for the formation of a 3_{10}-helix. Therefore, the -(Aib-Δ^{Z}Phe)$_2$- segment is useful to rationally design achiral helical host peptides.

Second, the conformation of the N-terminal L-Pro residue in peptide **2** was presented here, which gives us the clear answer as to how the N-terminal L-residue induces a left-handed screw sense in the following achiral segment. As a result, a semi-extended conformation for the L-Pro residue (or type II β-bend for the -L-Pro-Aib- segment) led to a left-handed screw sense. This fact was fully supported by the conformational energy calculation in which the L-Pro residue leading to a left-handed 3_{10}-helical segment prefers a semiextended conformation rather than a right-handed helical conformation. These experimental and theoretical results were also consistent with our previous conclusion driven from conformational studies in solution.[1–3]

Acknowledgements

This work was partially supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan under Grant (No. 12650883) to Y. I.

References

1 Y. Inai, Y. Kurokawa and T. Hirabayashi, *Biopolymers*, 1999, **49**, 551.
2 Y. Inai, Y. Kurokawa, A. Ida and T. Hirabayashi, *Bull. Chem. Soc. Jpn.*, 1999, **72**, 55.
3 Y. Inai, S. Ashitaka and T. Hirabayashi, *Polym. J.*, 1999, **31**, 246.
4 Y. Inai, Y. Kurokawa and T. Hirabayashi, *Macromolecules*, 1999, **32**, 4575.
5 Y. Inai, K. Tagawa, A. Takasu, T. Hirabayashi, T. Oshikawa and M. Yamashita, *J. Am. Chem. Soc.*, 2000, **122**, 11731.
6 B. V. V. Prasad and P. Balaram, *CRC Crit. Rev. Biochem.*, 1984, **16**, 307.
7 E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, M. Crisma, G. M. Bonora and C. Toniolo, *J. Am. Chem. Soc.*, 1982, **104**, 2437.
8 R. Jain and V. S. Chauhan, *Biopolymers*, 1996, **40**, 105.
9 O. Pieroni, A. Fissi, C. Pratesi, P. A. Temussi and F. Ciardelli, *J. Am. Chem. Soc.*, 1991, **113**, 6338.
10 K. R. Rajashankar, S. Ramakumar and V. S. Chauhan, *J. Am. Chem. Soc.*, 1992, **114**, 9225.
11 C. Toniolo and E. Benedetti, *Trends Biochem. Sci.*, 1991, **16**, 350.
12 O. Pieroni, A. Fissi, R. M. Jain and V. S. Chauhan, *Biopolymers*, 1996, **38**, 97.
13 O. Pieroni, G. Montagnoli, A. Fissi, S. Merlino and F. Ciardelli, *J. Am. Chem. Soc.*, 1975, **97**, 6820.
14 Y. Inai, T. Ito, T. Hirabayashi and K. Yokota, *Biopolymers*, 1993, **33**, 1173.

15 A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori and M. Camalli, *J. Appl. Crystallogr.*, 1994, **27**, 435.
16 P. T. Beurskens, G. Admiraal, G. Beurskens, W. P. Bosman, R. de Gelder, R. Israel and J. M. M. Smits, The DIRDIF-94 program system, technical report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1994.
17 G. M. Sheldrick, SHELXL-93: Program for the Refinement of Crystal Structures, University of Goettingen, Germany, 1993.
18 Butch Software Studio FREE WHEEL for Windows: 0.60C for Molecular Modeling Software, Japan, 2000.
19 F. A. Momany, R. F. McGuire, A. W. Burgess and H. A. Scheraga, *J. Phys. Chem.*, 1975, **79**, 2361.
20 Y. Beppu, *Comput. Chem*, 1989, **13**, 101.
21 M. Sisido, *Peptide Chem. 1991*, 1992, **29**, 105.
22 Y. Inai, S. Kurashima, T. Hirabayashi and K. Yokota, *Biopolymers*, 2000, **53**, 484.
23 Y. Inai, T. Oshikawa, M. Yamashita, T. Hirabayashi and T. Hirako, *Biopolymers*, 2001, **58**, 9.
24 Y. Inai, S. Kurashima, Y. Okado, T. Hirabayashi and K. Yokota, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 1687.
25 Y. Paterson, S. M. Rumsey, E. Benedetti, G. Nemethy and H. A. Scheraga, *J. Am. Chem. Soc.*, 1981, **103**, 2947.
26 G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, 1968, **23**, 283.
27 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
28 J. J. P. Stewart, MOPAC97, Fujitsu Ltd, Tokyo, Japan, 1998.
29 C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone and C. Pedone, *Biopolymers*, 1983, **22**, 205.
30 C. Toniolo and E. Benedetti, *Macromolecules*, 1991, **24**, 4004, and references therein.
31 C. Schellman, in *Protein Folding*, ed. R. Jaenicke, Elsevier, Amsterdam, 1980, p. 53.
32 E. Benedetti, C. Pedone, C. Toniolo, G. Nemethy, M. S. Pottle and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1980, **16**, 156.
33 K. K. Bhandary and V. S. Chauhan, *Biopolymers*, 1993, **33**, 209.
34 S. N. Mitra, S. Dey, S. Karthikeyan and T. P. Singh, *Biopolymers*, 1997, **41**, 97.
35 T. Ashida and M. Kakudo, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 1129.
36 D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1354.
37 M. Crisma, G. Valle, F. Formaggio and C. Toniolo, *Z. Kristallogr.*, 1998, **213**, 599.
38 B. Pengo, F. Formaggio, M. Crisma, C. Toniolo, G. M. Bonora, Q. B. Broxterman, J. Kamphuis, M. Saviano, R. Iacovino, F. Rossi and E. Benedetti, *J. Chem. Soc., Perkin Trans. 2*, 1998, 165.
39 E. Benedetti, M. Saviano, R. Iacovino, C. Pedone, A. Santini, M. Crisma, F. Formaggio, C. Toniolo, Q. B. Broxterman and J. Kamphuis, *Biopolymers*, 1998, **46**, 433.
40 I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747.
41 I. L. Karle, J. L. Flippen-Anderson, K. Uma, H. Balaram and P. Balaram, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 765.
42 K. R. Rajashankar, S. Ramakumar, R. M. Jain and V. S. Chauhan, *Biopolymers*, 1997, **42**, 373.
43 M. R. Ciajolo, A. Tuzi, C. R. Pratesi, A. Fissi and O. Pieroni, *Int. J. Pept. Protein Res*, 1991, **38**, 539.