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Effects of N-Terminal L-Amino Acid Residues on Helical Screw Sense in Achiral Peptides

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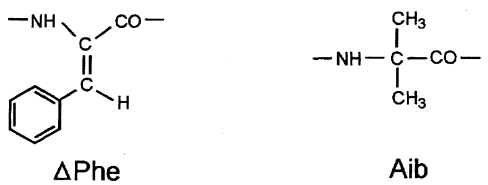
To understand the effects of N-terminal L-residues on dominating helical screw sense in achiral peptides, we adopted six kinds of peptides Boc–X–(Aib–ΔPhe)$_2$–Aib–OMe (Boc, *t*-butoxycarbonyl; OMe, methoxy), in which the X residue is an L-amino acid of alanine (Ala), leucine (Leu), valine (Val), phenylalanine (Phe), 1-naphthylalanine (Nap), or proline (Pro). The segment –(Aib–ΔPhe)$_2$– was used for a backbone composed of two "enantiomeric" (left-/right-handed) helices. Actually, this could be confirmed by ^{1}H NMR and CD spectroscopy on Boc–(Aib–ΔPhe)$_2$–Aib–OMe, which took left- and right-handed 3_{10}-helices with the same content. All peptides were also found to take 3_{10}-type helical conformations in $CDCl_3$ from solvent accessibility of NH resonances. Chloroform, acetonitrile, methanol, and tetrahydrofuran were used for solvents in CD measurement. All peptides in all solvents showed marked exciton couplets around 280 nm with a positive peak at longer wavelengths. Consequently, when an N-terminal L-residue, irrespective of types of L-residues, is introduced into a helical segment of achiral peptide, its main chain prefers the left-handed screw sense. The peptide with X = Ala showed the smallest amplitude of exciton couplets in each solvent, meaning that the Ala residue with the smallest side chain (methyl group) had the least effective chirality for taking a one-side helical screw sense preferentially, compared with the other residues used here.

Most of the L-amino acid residues are well recognized to prefer a right-handed screw sense in helical segments of proteins or peptides. On the other hand, an L-residue was rarely found to induce a left-handed screw sense for several peptides containing helicogenic achiral residues, (*Z*)-β-phenyl-α,β-dehydrophenylalanine (ΔPhe)[1—13] or α-aminoisobutyric acid (Aib) (Chart 1):[14,15] For example, a left-handed 3_{10}-helix in solution was seen in Ac–ΔPhe–Gly–ΔPhe-L-Ala–OMe (Ac, acetyl; OMe, methoxy)[13] or Boc–L-Pro–Aib–L-Ala–Aib–L-Ala–OMe (Boc, *t*-butoxycarbonyl).[16] Left-handed 3_{10}-helices have also been observed in the crystalline state for Aib peptides containing an L-residue in the C-terminal position.[16—19] Z–Aib–Aib–L-Ala–OMe (Z, benzyloxycarbonyl) took an incipient left-handed 3_{10}-helix (type III′ β-turn) in the solid state,[17] and Z–Aib–Aib–Aib–L-Val–OMe took a left-handed 3_{10}-helix in the solid state.[19] Also in proteins, Schellman noted that many right-handed helical segments ended with a residue in left-handed conformation.[20] Boc–L-Ala–ΔPhe–Gly–ΔPhe–L-Ala–OMe showed reversible screw sense inversion of 3_{10}-helix, depending on solvent and temperature conditions.[5,13] Also, Boc–L-Ala–ΔPhe–ΔPhe–NMA (NMA, N-methyl amide) took an incipient 3_{10}-helix of both left- and right-handed screw senses in the solid state.[12] Thus, the L-residue seems to show the character of left-handed screw sense when it will be relatively C-terminally introduced into achiral helical peptides.



ΔPhe Aib

Chart 1. ΔPhe and Aib residues.

On the other hand, little is known about the effects of an N-terminal L-residue on dominating the helical screw sense in achiral peptides. To discover these effects, we here adopted six kinds of peptides, Boc–X–(Aib–ΔPhe)$_2$–Aib–OMe **1**—**6**, in which an L-amino acid residue (X) is introduced into the N-terminal position in segment –(Aib–ΔPhe)$_2$– composed of achiral helicogenic residues, Aib and ΔPhe: X residue as an L-residue is alanine (Ala) for **1**, leucine (Leu) for **2**, valine (Val) for **3**, phenylalanine (Phe) for **4**, 1-naphthylalanine (Nap) for **5**, or proline (Pro) for **6** (Chart 2): We also intend to find how helical screw sense would be affected by steric factors of the X residue. Here the –(Aib–ΔPhe)$_2$– segment was used as "enantiomeric" (left-/right-handed) helical backbone. The conformations in solution were investigated by ^{1}H NMR and CD spectroscopy. Helical screw sense was identified by the sign of exciton couplets around 280 nm (assignable to ΔPhe residue), by the exciton chirality method.[21]

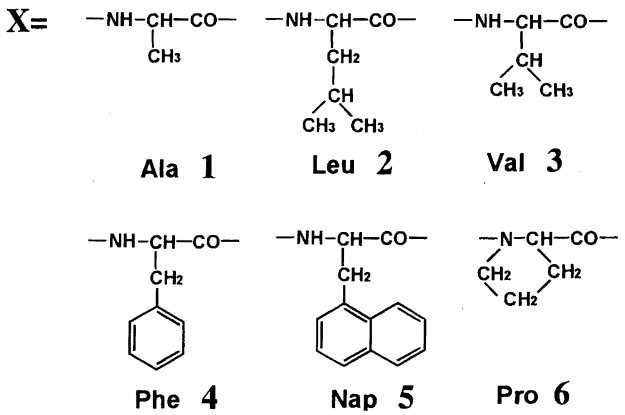


Chart 2.

Experimental

Peptide Synthesis. L-1-Naphthylalanine was prepared according to Refs. 22, 23, and 24, and the other amino acids were commercially available. Boc amino acid and amino acid methyl ester were prepared by the standard procedure. Boc–Aib–ΔPhe azlactone was prepared via Boc–Aib–DL-β-phenylserine–OH, based on Ref. 25. Boc–Aib–ΔPhe–Aib–OMe was prepared by ring-opening Boc–Aib–ΔPhe azlactone with the amino group of H–Aib–OMe. Likewise, Boc–(Aib–ΔPhe)$_2$–Aib–OMe (**7**) was prepared by ring-opening the azlactone with N-deprotected H–Aib–ΔPhe–Aib–OMe. The preparation and characterization of peptide **7** will be described in detail elsewhere. To obtain the final product, Boc–X–(Aib–ΔPhe)$_2$–Aib–OMe, Boc–X–OH was coupled with H–(Aib–ΔPhe)$_2$–Aib–OMe using dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole hydrate (HOBt). Peptides **1**—**6** were checked by ^{1}H NMR and IR spectroscopy, thin-layer chromatography (TLC), and gel permeation chromatography (GPC). TLC was done on precoated silica plates in the following solvent systems: (A) ethyl acetate, (B) methanol, (C) chloroform–methanol (9 : 1), and (D) 1-butanol–acetic acid–water (7 : 2 : 1). GPC was recorded on a Tosoh HLC-803-D equipped with G1000-, G2500-, and G3000-HLX columns in series, by using tetrahydrofuran (THF) eluent. Single spot in the TLC and single peak in the GPC were obtained for all peptides **1**—**6**.

Boc-L-Ala-(Aib-ΔPhe)$_2$-Aib-OMe (1). Boc–(Aib–ΔPhe)$_2$–Aib–OMe (0.80 g, 1.1 mmol) was dissolved in dichloromethane (5 mL)/trifluoroacetic acid (5 mL) at 0 °C, the solution stood for 24 h at room temperature, and concentrated in vacuo. After addition of 5% $NaHCO_3$ solution, the residue was extracted with ethyl acetate, the organic layer was washed with 5% $NaHCO_3$ and 10% NaCl solutions, and dried over $MgSO_4$. Evaporation of solvent gave white crystals of H–(Aib–ΔPhe)$_2$–Aib–OMe [Yield: 0.56 g (82%)].

To a solution of Boc–Ala–OH (155 mg, 0.82 mmol) and HOBt (130 mg, 0.82 mmol) in *N,N*-dimethylformamide (0.5 mL) was added DCC (170 mg, 0.82 mmol) at 0 °C, and the mixture was stirred for 0.5 h at 0 °C. After addition of H–(Aib–ΔPhe)$_2$–Aib–OMe (300 mg, 0.54 mmol), the mixture was stirred for 2 h at 0 °C, and for 48 h at room temperature, concentrated in vacuo, and the residue was redissolved in ethyl acetate. After dicyclohexylurea was removed by filtration, the solution was washed with 10% NaCl, 5% $KHSO_4$, 10% NaCl, 5% $NaHCO_3$, and 10% NaCl solutions, and dried over $MgSO_4$. The product was purified by eluting through a silica-gel column with ethyl acetate. Yield 280 mg (70%); mp 227—229 °C; R_f^A = 0.63; R_f^B = 0.96; R_f^C = 0.56; R_f^D = 0.99; retention time (t_R) = 22.6 min. 400 MHz ^{1}H NMR (in $CDCl_3$) δ = 8.57 (2H, s, 2×NHΔPhe), 7.96 (1H, s, NH Aib), 7.83 (1H, s, NH Aib), 7.5—7.2 (12H, m, 2×(phenyl H + C$^\beta$H) ΔPhe), 6.86 (1H, s, NH Aib), 5.20 (1H, d, Boc–NH), 3.84—3.72 (1H, m, C$^\alpha$H Ala), 3.70 (3H, s, $COOCH_3$), 1.60+1.60+1.58+1.57+1.42+1.40 (18H, s+s+s+s+s+s, 6×CH_3 Aib), 1.34 (3H, d, CH_3 Ala), and 1.16 (9H, s, 3×CH_3 Boc). IR (on NaCl) 3260, 1735, 1655, 1620, and 1525 cm^{-1}.

Peptides **2**—**6** were prepared in a similar manner to the above preparation of peptide **1**.

Boc-L-Leu-(Aib-ΔPhe)$_2$-Aib-OMe (2). Yield: 77%; mp 208—211 °C; R_f^A = 0.75; R_f^B = 0.95; R_f^C = 0.76; R_f^D = 0.99; t_R = 22.2 min. 400 MHz ^{1}H NMR (in $CDCl_3$) δ = 8.54 (2H, s, 2×NHΔPhe), 7.91 (1H, s, NH Aib), 7.79 (1H, s, NH Aib), 7.25—7.2 (12H, m, 2×(phenyl H+C$^\beta$H) ΔPhe), 6.58 (1H, s, NH Aib), 5.01 (1H, s, Boc–NH), 3.8—3.7 (1H, m, C$^\alpha$H Leu), 3.72 (3H, s, $COOCH_3$), 1.75—1.50 (15H, C$^\beta$H$_2$–C$^\gamma$H Leu and 4×CH_3 Aib), 1.72 (9H, s, 3×CH_3 Boc), 1.43+1.41 (6H, s+s, 2×CH_3 Aib), and 0.98+0.94 (6H, d+d, 2×CH_3 Leu). IR (on NaCl) 3230, 1725, 1645, 1610, and 1525 cm^{-1}.

Boc-L-Val-(Aib-ΔPhe)$_2$-Aib-OMe (3). Yield: 83%; mp 243—249 °C; R_f^A = 0.73; R_f^B = 0.94; R_f^C = 0.25; R_f^D = 0.99; t_R = 22.6 min. 400 MHz ^{1}H NMR (in $CDCl_3$) δ = 8.60 (1H, s, NH ΔPhe), 8.56 (1H, s, NH ΔPhe), 7.96 (1H, s, NH Aib), 7.80 (1H, s, NH Aib), 7.55—7.20 (12H, m, 2×(phenyl H + C$^\beta$H) ΔPhe), 6.63 (1H, s, NH Aib), 4.99 (1H, d, NH Val), 3.70 (3H, s, $COOCH_3$), 3.55—3.45 (1H, bs, C$^\alpha$H Val), 2.1—2.0 (1H, m, C$^\beta$H Val), 1.60+1.59+1.59+1.58+1.44+1.40 (18H, s+s+s+s+s+s, 6×CH_3 Aib), 1.18 (9H, s, 3×CH_3 Boc), and 1.01+0.99 (6H, d+d, 2×CH_3 Val). IR (on NaCl) 3270, 1730, 1655, 1620, and 1535 cm^{-1}.

Boc-L-Phe-(Aib-ΔPhe)$_2$-Aib-OMe (4). Yield: 82%; mp 256—259 °C; R_f^A = 0.73; R_f^B = 0.96; R_f^C = 0.76; R_f^D = 0.97; t_R = 22.4 min. 400 MHz ^{1}H NMR (in $CDCl_3$) δ = 8.56 (1H, s, NH ΔPhe), 8.40 (1H, s, NH ΔPhe), 7.91 (1H, s, NH Aib), 7.79 (1H, s, NH Aib), 7.60—7.15 (17H, m, 2×(phenyl H+C$^\beta$H) ΔPhe+phenyl H Phe), 6.49 (1H, s, NH Aib), 5.11 (1H, d, NH Phe), 4.05—3.95 (1H, m, C$^\alpha$H Phe), 3.69 (3H, s, $COOCH_3$), 3.1—2.9 (2H, m, C$^\beta$H$_2$ Phe), 1.62+1.61+1.59+1.57+1.33+1.26 (18H, s+s+s+s+s+s, 6×CH_3 Aib), and 1.17 (9H, s, 3×CH_3 Boc). IR (on NaCl) 3270, 1730, 1660, 1620, and 1530 cm^{-1}.

Boc-L-Nap-(Aib-ΔPhe)$_2$-Aib-OMe (5). Yield: 80%; mp 161—164 °C; R_f^A = 0.74; R_f^B = 0.90; R_f^C = 0.71; R_f^D = 0.95; t_R = 22.4 min. 400 MHz ^{1}H NMR (in $CDCl_3$) δ = 8.53 (1H, s, NH ΔPhe), 8.42 (1H, s, NH ΔPhe), 7.93 (1H, s, NH Aib), 7.78 (1H, s, NH Aib), 8.1—7.2 (19H, m, 2×(phenyl H+C$^\beta$H) ΔPhe+naphthyl H Nap), 6.19 (1H, s, NH Aib), 5.17 (1H, d, NH Nap), 4.2—4.1 (1H, m, C$^\alpha$H Nap), 3.69 (3H, s, $COOCH_3$), 3.59 − 3.54 + 3.42 − 3.37 (2H, m+m, C$^\beta$H$_2$ Nap), 1.64+1.63+1.59+1.57+1.23+1.11 (18H, s+s+s+s+s+s, 6×CH_3 Aib), and 1.17 (9H, s, 3×CH_3 Boc). IR (on NaCl) 3230, 1725, 1650, 1615, and 1525 cm^{-1}.

Boc-L-Pro-(Aib-ΔPhe)$_2$-Aib-OMe (6). Yield: 75%; mp 246—249 °C; R_f^A = 0.63; R_f^B = 0.90; R_f^C = 0.67; R_f^D = 0.93; t_R = 22.7 min. 400 MHz ^{1}H NMR (in $CDCl_3$) δ = 8.73 (1H, s, NH ΔPhe), 8.51 (1H, s, NH ΔPhe), 7.88 (1H, s, NH Aib), 7.79 (1H, s, NH Aib), 7.55—7.35 (12H, m, 2×(phenyl H+C$^\beta$H) ΔPhe), 6.68 (1H, s, NH Aib), 4.05—3.85 (1H, t, C$^\alpha$H Pro), 3.70 (3H, s, $COOCH_3$), 3.47—3.37 (2H, m, C$^\varepsilon$H$_2$ Pro), 2.25—1.8 (4H, m, C$^\beta$H$_2$+C$^\gamma$H$_2$ Pro), 1.66+1.60+1.58+1.56+1.43+1.38 (18H, s+s+s+s+s+s, 6×CH_3 Aib), and 1.22 (9H, s, 3×CH_3 Boc). IR (on NaCl) 3275, 1740, 1660, 1625, and 1535 cm^{-1}.

Spectroscopy. ^{1}H NMR spectra were recorded using a JEOL JNM-GX400 (400 MHz), and a Hitachi R-90 spectrometer (90 MHz). The measurements were done with a peptide concentration

of 10—20 mg ml^{-1} in $CDCl_3$, and a mixture of $CDCl_3/(CD_3)_2SO$. All of the chemical shifts were expressed as δ downfield from tetramethylsilane (TMS). A difference nuclear Overhauser effect (NOE) experiment was done on a Varian XL-200 spectrometer (200 MHz) using the standard Varian software library. The typical acquisition parameters were a 12.0-μs pulse width, a 5.0-s acquisition time, a 4.0-s delay time, and 200—500 accumulations. The difference NOE experiment was done in $CDCl_3$ at 27 °C for peptide **7**, and at 40 °C for peptide **2**. The IR spectra were recorded using a JASCO IR Report 100 spectrometer. CD and UV absorption spectra were simultaneously recorded using a JASCO J-600 in chloroform, acetonitrile, methanol, and THF. These solvents were purified by distillation before use. The ΔPhe concentration was determined using maximum absorbance (A_{max}) around 280 nm (assigned to a ΔPhe residue) and its molar extinction coefficient ($\varepsilon_{max} = 1.8\times10^4$).[26,27] For peptide **5**, A_{max} value for ΔPhe concentration was obtained after removing the contribution of ^1L_a band around 280 nm characteristic of 1-naphthyl group.

Results and Discussion

Confirmation of Helix. In this study, segment –(Aib–ΔPhe)$_2$– was used for a backbone composed of two "enantiomeric" (left-/right-handed) helices. The segment can be expected to be a helical structure, since Aib[14,15] and ΔPhe[1—13] residues are known to be strong inducers that create a 3_{10}-helix even in small peptides (or sometimes form an α-helix, depending on sequence, chain length, or solvent). Actually, this could be confirmed by ^{1}H NMR spectroscopy on Boc–(Aib–ΔPhe)$_2$–Aib–OMe (**7**). The difference NOE experiment in $CDCl_3$ showed marked signals between neighboring NH resonances (Table 1), indicating that peptide **7** takes a 3_{10}- or α-helix.[28—30] Figure 1 shows variation in NH chemical shifts for peptide **7** with concentration of $(CD_3)_2SO$. Two NH resonances, Aib(1) NH and ΔPhe(2) NH, were markedly shifted to a lower field with increasing concentration of $(CD_3)_2SO$, meaning the absence of hydrogen-bonds in Aib(1) and ΔPhe(2) NHs.[31] Namely, the other three NHs were relatively unaffected by the addition of strong hydrogen accepting $(CD_3)_2SO$, and thus should be shielded from solvent due to intramolecular hydrogen-bonding. Therefore, peptide **7** took a 3_{10}-type helical structure supported by an (i+3) → i hydrogen-bonding pattern. A helix-forming tendency in segment –(Aib–ΔPhe)$_2$– was also confirmed by a conformational energy calculation. The program PEPCON,[32,33] which was written by Sisido,[34] was modified to be applicable to ΔPhe-containing peptides,[26,35—37] and used. Figure 2 shows a main-chain energy contour map of Ac–(Aib–ΔPhe)$_2$–NMA, indicating left- and right-handed (3_{10}- or α-) helices as the most stable region.

A similar tendency in solvent dependence of NH chemical

Table 1. Difference NOEs of NH Resonance Observed for Peptide **7** in $CDCl_3$[a]

Irradiated	% NOE for NH resonance				
NH resonance	Aib(1)	ΔPhe(2)	Aib(3)	ΔPhe(4)	Aib(5)
Aib(1)	×	2.0			
ΔPhe(2)[b]	1.9	×	1.7	(1.7)	(×)
Aib(3)		1.3	×	3.8	
ΔPhe(4)			2.9	×	4.1
Aib(5)[b]	(0.7)	(×)	(1.3)	3.1	×

a) × shows irradiated NH resonance. b) The irradiation of ΔPhe-(2) resonance contained that of Aib(5) resonance due to partial overlap of both resonances.

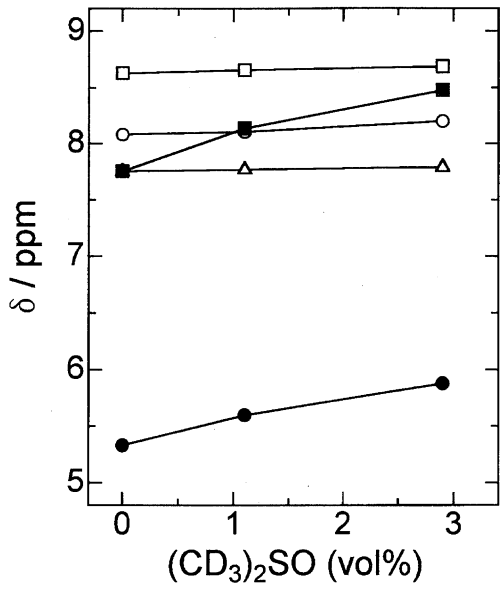


Fig. 1. Solvent dependence of NH chemical shifts in peptide **7** in $CDCl_3$–$(CD_3)_2SO$ mixtures of varying concentrations: (●) for Aib(1), (■) for ΔPhe(2), (○) for Aib(3), (□) for ΔPhe(4), and (△) for Aib(5).

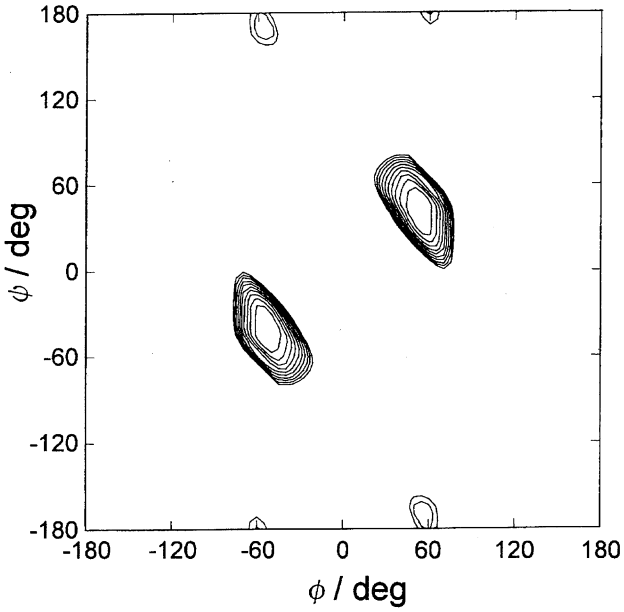


Fig. 2. Main-chain energy contour map of Ac–(Aib–ΔPhe)$_2$–NMA. The contours (10 lines) are drawn in 1.0 kcal [mol (per residues)]$^{-1}$ increments from the energy minimum points (−55°, −35°) and (55°, 35°). In each (ϕ,ψ), each χ^2 angle of ΔPhe side chain was taken as the value to give the minimal conformational energy, while its χ^1 was fixed to 0°.

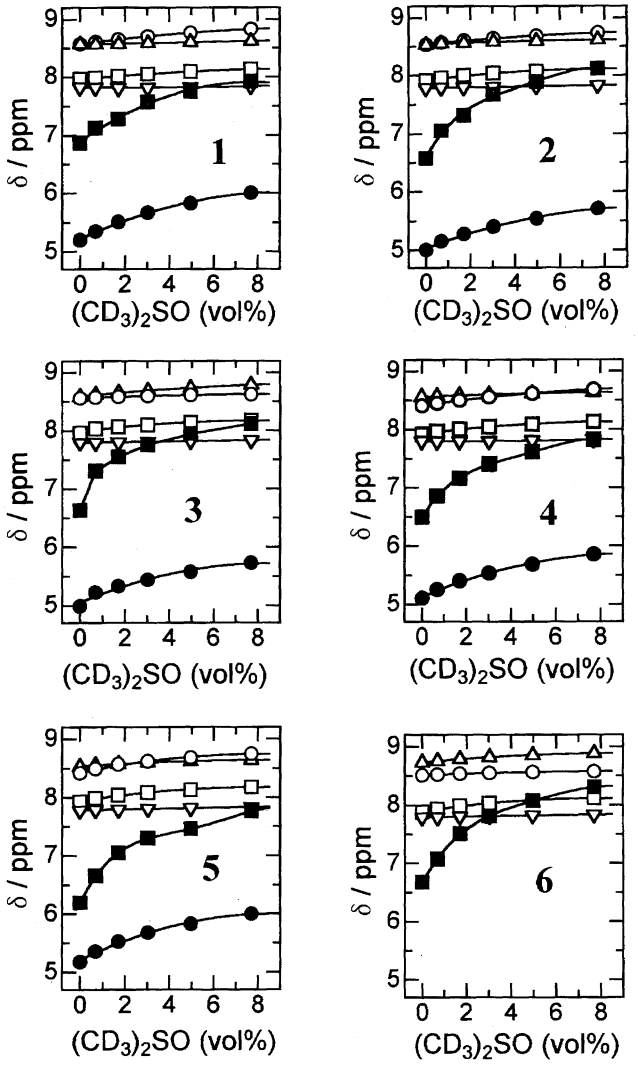


Fig. 3. Solvent dependence of NH chemical shifts in peptides **1**—**6** in $CDCl_3$–$(CD_3)_2SO$ mixtures of varying concentrations: (●) for X(1), (■) for Aib(2), (○) for ΔPhe(3), (□) for Aib(4), (△) for ΔPhe(5), and (▽) for Aib(6).

shifts was observed for peptides **1**—**6** having a common helical segment –(Aib–ΔPhe)$_2$–, as shown in Fig. 3. Namely, for peptides **1**—**5**, two NH resonances, X(1) and Aib(2) NHs, markedly changed with the concentration of $(CD_3)_2SO$. These shifts can be ascribed to the absence of hydrogen-bonds in X(1) and Aib(2) NHs, since CD spectra of peptides **1**—**6** in chloroform did not change (i.e., showed exciton couplets around 280 nm) with the addition of 0—8 vol% dimethyl sulfoxide, essentially. (The CD patterns in chloroform were retained even in 100% dimethyl sulfoxide, thus indicating the strong helix-forming tendency for peptides **1**—**6** due to the introduction of segment –(Aib–ΔPhe)$_2$–.) Peptide **6** showed a marked shift for only an Aib(2) NH resonance, since peptide **6** has no NH group due to the Pro residue as X residue. Thus, 3_{10}-type helical conformations in $CDCl_3$ were retained for peptides **1**—**6** in which the X residue is introduced into the N-terminal position of an achiral helical segment –(Aib–ΔPhe)$_2$–Aib–OMe. Also, peptide **2** (X = Leu) in $CDCl_3$ showed NOEs for each $NH_i \Longleftrightarrow NH_{i+1}$ pair characteristic of a 3_{10}- or α-helix,[28—30] similarly to peptide **7**.

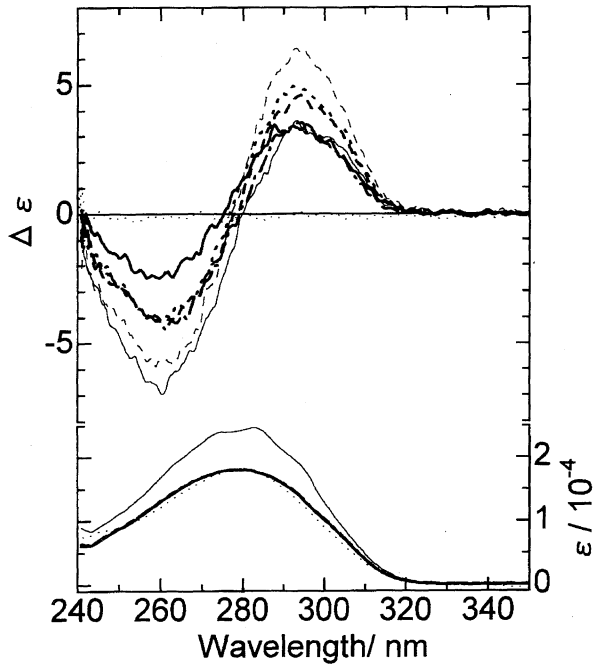


Fig. 4. CD (top) and UV absorption (bottom) spectra of peptides **1** (—), **2** (- - -), **3** (····), **4** (–·–), **5** (—), **6** (- - -), and **7** (···) in chloroform.

Identification of Helical Screw Sense. As expected, peptide **7** showed no CD signals due to no chiral residues being included, indicating peptide **7** took left- and right-handed 3_{10}-helices with the same content. On the other hand, marked CD signals were seen for peptides **1**—**6**, in which a chiral X residue is attached to the N-terminal position of the enantiomeric helical segment –(Aib–ΔPhe)$_2$–Aib–OMe. Figure 4 shows CD and UV absorption spectra for peptides **1**—**6** in chloroform. The $\Delta\varepsilon$ ($= \varepsilon_L - \varepsilon_R$) is expressed with respect to the molar concentration of the ΔPhe residue.

All absorption spectra except for peptide **5** showed intense maxima (λ_{max}) around 280 nm (band I) assignable to ΔPhe residue. The band I of peptide **5** is overlapped with the ^1L_a band of the 1-naphthyl group, but peptide **5** showed an absorption pattern similar to the other peptides by removing the contribution of the ^1L_a band (data not shown). Accordingly, the UV absorption pattern did not change essentially in all of the peptides **1**—**6**, but resembled that of Boc–L-Leu–ΔPhe–L-Leu–OMe[25] having a single ΔPhe residue. Thus, no strong ground-state interaction between the ΔPhe–ΔPhe pair exists in peptides **1**—**6**. Such interaction was also not observed in 3_{10}-helical peptides containing ΔPhe residues.[26,27,36]

The corresponding CD spectra of peptides **1**—**6** showed marked exciton couplets with a positive peak at longer wavelengths, as shown in Fig. 4. Peptides containing ΔPhe residues show intense absorption maxima around 220 nm and around 280 nm (band I). The former absorption band precludes a far-UV CD analysis usually used to investi-

gate conformations of peptides and proteins. On the other hand, the latter absorption band has been assigned to charge transfer from the highest occupied orbital localized on the styryl moiety to the vacant orbital of the carbonyl group in ΔPhe residue.[38,39] The transition moment was estimated from molecular orbital calculations to lie on the styryl-carbonyl line.[26] By applying the exciton chirality method[21] to this system, the sign of the split CD pattern corresponds to a left-handed helical arrangement of the transition moment, meaning left-handed helical main chain. This assignment has been also used for 3_{10}-helical peptides containing ΔPhe–X–ΔPhe unit(s):[1,2,5,13,27,36] i.e., the sign of exciton couplets at band I is a positive peak at longer wavelengths for a left-handed helix and a negative peak at longer wavelengths for a right-handed helix. Moreover, the sign of exciton couplets (a positive peak at longer wavelengths) should be ascribed to left-handed 3_{10}- or α-helices, based on theoretical CD calculation.[26,35] Namely, exciton couplets with a positive peak at longer wavelengths were obtained at band I for Boc–(Aib–ΔPhe)$_2$–Aib–OMe in five left-handed 3_{10}-type (4→1 hydrogen bonded) helices (1)—(5) and in three left-handed α-type (5→1 hydrogen bonded) helices (6)—(8) : (1) $\phi = 44^\circ$, $\psi = 33^\circ$; (2) $\phi = 54^\circ$, $\psi = 28^\circ$; (3) $\phi = 71^\circ$, $\psi = 18^\circ$; (4) $\phi = 53^\circ$, $\psi = 36^\circ$; (5) $\phi = 60^\circ$, $\psi = 30^\circ$; (6) $\phi = 53^\circ$, $\psi = 52^\circ$; (7) $\phi = 57^\circ$, $\psi = 47^\circ$; (8) $\phi = 63^\circ$, $\psi = 42^\circ$. These eight left-handed helices (ϕ, ψ) correspond to the right-handed ones ($-\phi$, $-\psi$) reported.[40—48] Therefore, the main chains of peptides **1**—**6** prefer the left-handed screw sense to the right-handed one. Obviously, irrespective of the types of L-residues (X), N-terminal L-residues in chloroform tend to induce the left-handed screw sense in two enantiomeric helices preferentially.

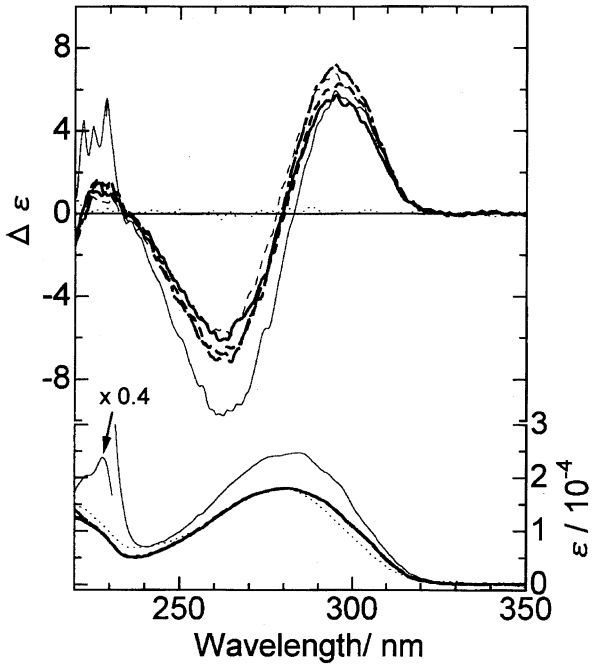


Fig. 5. CD (top) and UV absorption (bottom) spectra of peptides **1** (—), **2** (- - -), **3** (····), **4** (–·–), **5** (—), **6** (- - -), and **7** (···) in acetonitrile.

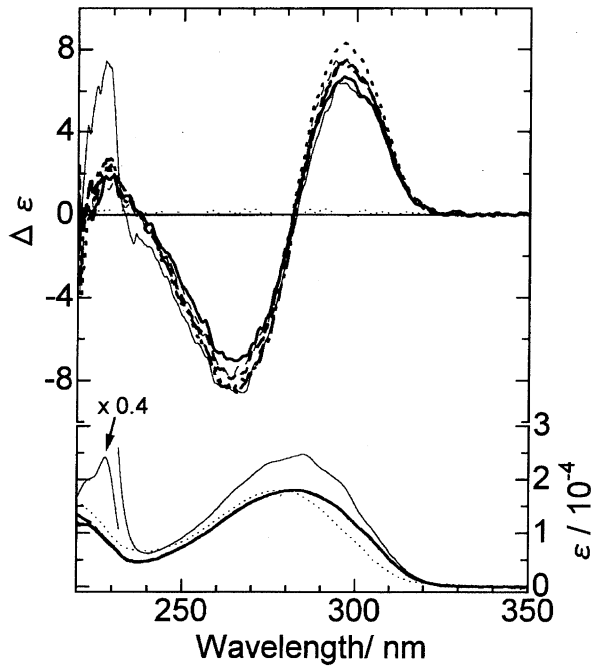


Fig. 6. CD (top) and UV absorption (bottom) spectra of peptides **1** (—), **2** (- - -), **3** (····), **4** (–·–), **5** (—), **6** (- - -), and **7** (···) in methanol.

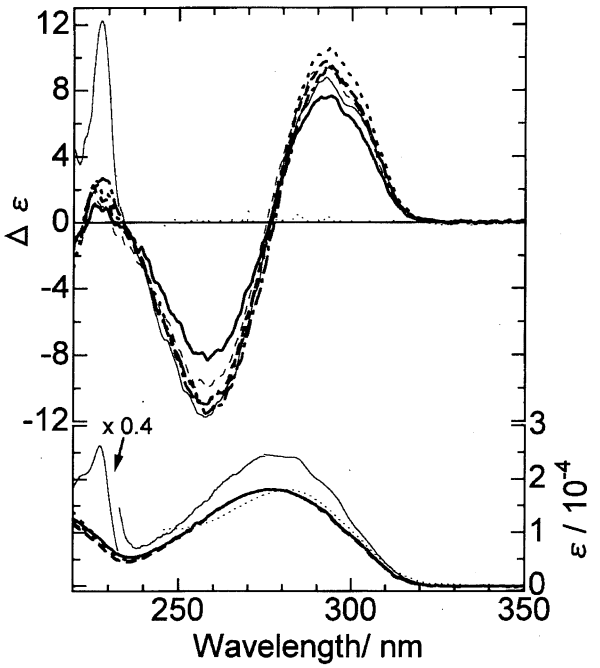


Fig. 7. CD (top) and UV absorption (bottom) spectra of peptides **1** (—), **2** (- - -), **3** (····), **4** (–·–), **5** (—), **6** (- - -), and **7** (···) in THF.

Figures 5, 6, and 7 show CD and UV absorption spectra of peptides **1**—**6** in acetonitrile, methanol, and THF, respectively. In these solvents, CD patterns at band I were similar to those in chloroform. It should also be concluded that N-terminal L-residues induce a left-handed screw sense preferentially. On the other hand, a variety of CD intensities was seen, depending on X residues and on solvents. Here the

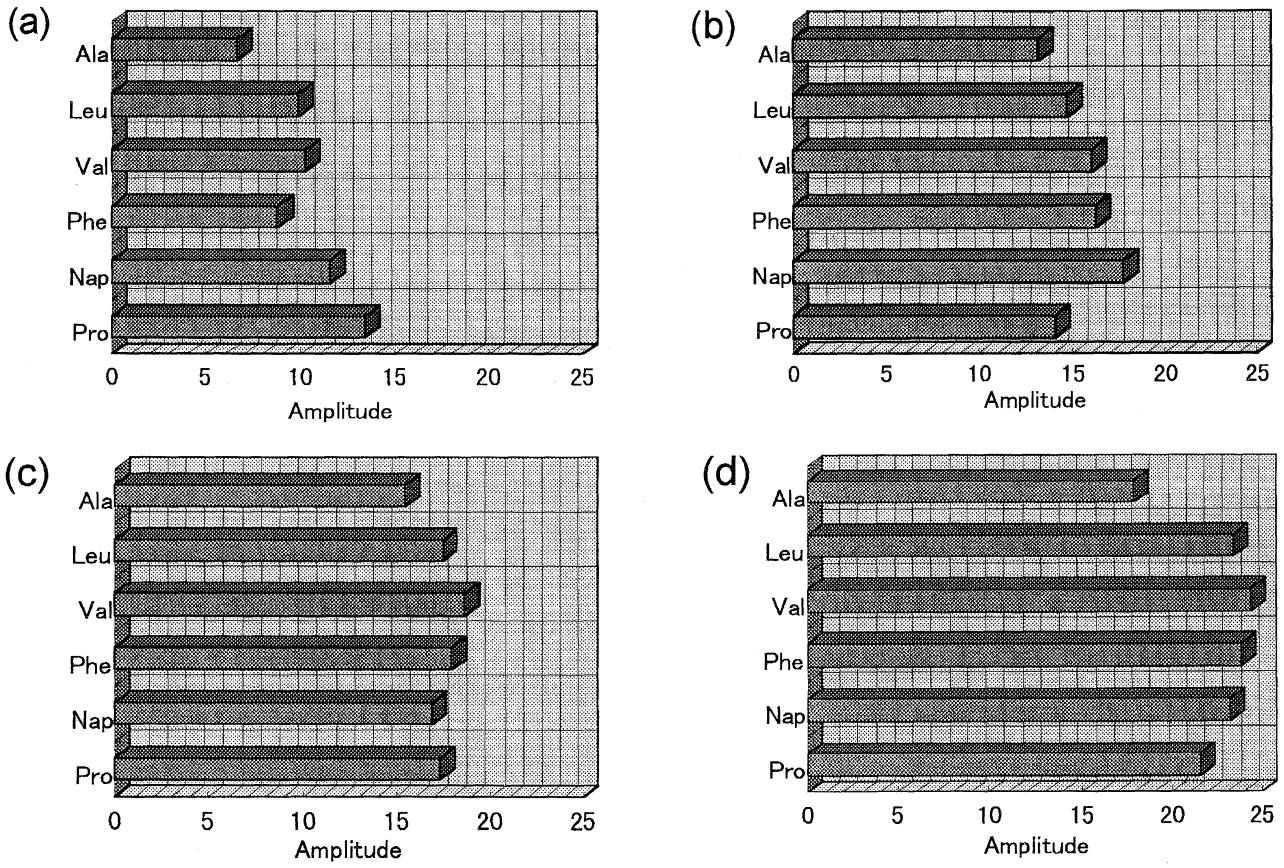


Fig. 8. Amplitude (A) of exciton couplets for Boc–X–(Aib–ΔPhe)$_2$–Aib–OMe (X = Ala, Leu, Val, Phe, Nap, and Pro) in (a) chloroform, (b) acetonitrile, (c) methanol, and (d) THF.

split amplitude (A)[26,35] is defined as the difference between the maximum and minimum $\Delta\varepsilon$ ($\Delta\varepsilon_{max}$ and $\Delta\varepsilon_{min}$) values in the couplets of band I. The A values for peptides **1**—**6** in each solvent are illustrated in Fig. 8. Interestingly, peptide **1** with X = Ala showed the smallest A values in all the solvents used here. The A value should depend on thermal fluctuation of helical structure and on the difference between contents of left- and right-handed helical screw senses in an equilibrium state. The former contribution to the A value in chloroform will be small, since all of peptides **1**—**6** take a 3_{10}-type helix of which stability should be mainly ascribed to a common helical segment –(Aib–ΔPhe)$_2$–Aib–OMe in chloroform. Thus, the A values here should depend on how much the left-handed helical screw sense dominates over the right-handed one for the peptide chain in an equilibrium state. Also in the other solvents, peptide **1** showed the smallest A values among all peptides. Consequently, the Ala residue showed the least effective chirality for taking a one-side helical screw sense preferentially, compared with the other residues used here. One reason for this should be ascribed to the side chain of the Ala residue being the smallest (methyl group), which gives the weakest interaction between methyl group and peptide backbone to induce a one-side helical screw sense preferentially.

Main-chain contour maps of X residue (X = Ala, Leu, Val, Phe, and Nap) were preliminarily calculated on Ac–X–(Aib–ΔPhe)$_2$–Aib–OMe. Here the segment –(Aib–ΔPhe)$_2$–Aib–OMe was fixed to the most stable left-handed helix, and side chain of X residue in each (ϕ, ψ) was taken as the value that gives the minimal conformational energy. These maps express the degree of freedom for conformation of the N-terminal X residue in left-handed helical segment. The map for X = Ala with the smallest side chain showed the widest contour area (i.e., the largest degree of freedom) among the five, thus indicated that the left-handed screw sense should be ascribed to steric interaction between the side chain of the X residue and the helical segment. Also, such steric interaction might work similarly for all L-residues to induce a left-handed screw sense preferentially, since all maps were essentially similar to each other in their patterns.

Except for peptide **1** (Ala), a marked order in the A values could not be seen through all solvents used here: e.g., peptide **5** (Nap) was in the fifth position in chloroform, but in the second position in THF. Also peptide **4** (Phe) was in the second position in chloroform, but in the fifth position in acetonitrile. In peptides **2**—**6**, the change of A order with solvents might be due to thermal fluctuation depending on solvents. To clarify this reason, further data on helical screw sense should be accumulated using helical peptides with varying their chain-lengths, sequences, environments (solvent or temperature), and so on.

However, we here could provide a systematic data of the left-handed screw sense induced by N-terminal L-residue in achiral peptides, although a number of screw sense data about

helical peptides with varying their chain-lengths, sequences, and environments should be accumulated to generalize the rule that N-terminal L-residue induces a left-handed screw sense preferentially. First, when an N-terminal L-residue, irrespective of types of L-residues, is introduced into a helical segment of achiral peptide, its main chain prefers the left-handed screw sense. Second, Ala residue, having the smallest side chain (methyl group), had the least effective chirality for taking a one-side helical screw sense preferentially, compared with the other residues used here.

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