Screw sense preference of non-polar L-amino acid residues second from the *N*-terminal position †

Yoshihito Inai,* Yuji Kurokawa and Nobumitsu Kojima

Department of Environmental Technology and Urban Planning, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan. E-mail: inai@mse.nitech.ac.jp

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To understand the positional effects of a single chiral residue on the helical screw sense of an achiral segment, we adopted five kinds of peptides Boc-Aib-X*-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe (Boc, *t*-butoxycarbonyl; Aib, α-aminoisobutyric acid; Δ^{Z}Phe, (*Z*)-dehydrophenylalanine; OMe, methoxy), wherein the X* residue is an L-residue of leucine (Leu), alanine (Ala), valine (Val), phenylalanine (Phe), or 1-naphthylalanine (Nap). Here the segment -(Aib-Δ^{Z}Phe)$_2$-Aib-OMe was employed as an achiral backbone for generating two "enantiomeric" (left-handed/right-handed) helices. All of the peptides are folded into a 3_{10}-type helical conformation in chloroform, as evidenced by FT-IR and ^{1}H NMR techniques. A CD analysis of these peptides indicates that they adopt both left-handed and right-handed helices, and that the prevailing screw sense as well as the screw sense bias depend on the type of solvent. Thus, the L-residue located at the position second from the *N*-terminus plays a unique role for energetically permitting both helices. These peptides also undergo a solvent-induced interconversion between both helices. The rank order of the penultimate L-residues for inducing a right-handed screw sense is Val > Leu ~ Ala > Phe > Nap, of which the reverse order represents the tendency to promote a left-handed screw sense. The prevailing screw sense induced by the penultimate L-residue is also discussed on the basis of conformational energy calculations. In conclusion, we here have been able to express experimentally a unique screw sense preference of these non-polar L-amino acids.

Introduction

The clarification of which factors govern the helical screw sense of biological macromolecules and synthetic polymers is a significant and common issue covering a wide range of chemical fields including biological chemistry, polymer chemistry, supramolecular chemistry, and chiral separation technologies. A crucial factor is the homochirality of the constituents of the helical polymer chains; *e.g.*, most naturally occurring helical peptides choose a right-handed screw sense due to their L-amino acid residues. Thus, the chemical structure of chiral constituents in a helical chain as well as their position in the helix should largely govern the whole helical screw sense. The most efficient approach for revealing the positional effects of various chiral constituents of a polymer on the helical screw sense is to utilize an appropriate model helix wherein a single chiral constituent can be covalently introduced into an arbitrary position of the achiral helical chain.

A large number of outstanding studies on the relationship between the primary structure of polymers and the resulting helical screw sense has been reported for synthetic helical polymers such as polymethacrylates,[1] polyisocyanates,[2] polyisocyanides,[3] polyacetylenes,[4] poly(aryleneethynylene)s,[5] polysilanes,[6] and so on. As for helical peptides possessing a single chiral center, such a systematic approach has been proposed using homooligopeptides of achiral helicogenic α-aminoisobutyric acid (Aib) residues[7–10] or achiral peptide nucleic acid backbones.[11,12] In contrast to most synthetic polymer systems, the peptide systems have the great advantage of being able to introduce covalently a specific chiral residue into an arbitrary position of the original achiral chain, because of the stepwise condensation applicable to peptide synthesis. Thus, a unique model compound can be provided using achiral peptide backbones to elucidate the positional effects of various amino acid residues on the helical screw sense. In the above Aib-peptides, N^{α}-blocked pentapeptide esters containing four Aib residues and one chiral L-valine (L-Val) or C^{α}-methyl-L-Val [L-(αMe)Val] residue in the *C*-terminal position of the sequence formed a left-handed helix in solution.[7] In the solid state, the pentapeptides adopted a right-handed helix for the L-Val residue, and both right-handed and left-handed helices for L-(αMe)Val residue.[9] On the other hand, a right-handed 3_{10}-helix in solution was found for a variety of N^{α}-blocked pentapeptide esters containing one chiral L-Val or L-(αMe)Val residue in the *N*-terminal or the internal (third from the *N*-terminus) position of the sequence, *i.e.*, Bz-Y-(Aib)$_4$-O*t*Bu or Bz-(Aib)$_2$-Y-(Aib)$_2$-O*t*Bu [Bz, benzoyl or *p*-substituted benzoyl; Y, L-Val or L-(αMe)Val residue; O*t*Bu, *t*-butoxy].[7]

Recently, we also have attempted to reveal dominant positional effects of a chiral amino acid residue on the helical screw sense of an achiral peptide segment, using another achiral sequence.[13–17] The sequence consisting of achiral helicogenic Aib and (*Z*)-dehydrophenylalanine (Δ^{Z}Phe) residues, -(Aib-Δ^{Z}Phe)$_n$- (n = 2–4), has been employed as a common scaffold for the achiral helix.[13–19] As a result, an L-residue incorporated into the *N*-terminal position induces a predominantly left-handed screw sense for the remaining achiral segment,[13–16] in contrast to the common preference for a right-handed screw sense in an L-residue. The left-handed screw sense induced by an *N*-terminal L-residue was observed regardless of the type of L-residue,[13,14] type of solvent,[13,14] and chain length of the

† Electronic supplementary information (ESI) available: main-chain energy contour map of X* for Ac-Aib-X*-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe (X* = L-Ala, L-Val, L-Phe, and L-Nap) in the lowest-energy left-handed helix (a) or right-handed helix (b) shown in Table 3. The contours are drawn in 0.5 kcal mol^{-1} increments from the minimum points (55°, 65°) for (a) and (−65°, −50°) for (b) to 5 kcal mol^{-1}. For X* = L-Leu, see ref. 17. See http://www.rsc.org/suppdata/p2/b2/b206664e/

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achiral segment.[16] Detailed conformational studies[13,15] on the above peptides demonstrated that the *N*-terminal L-residue adopts a semi-extended conformation, in turn leading to a left-handed screw sense for the remaining achiral segment.

The real tendency to generate a given helical screw sense cannot be extracted from a study of a residue at the *N*-terminus of the peptide chain, but rather of a residue in an internal position. Accordingly, our attention was focused on another positional effect, namely, a heptapeptide possessing an L-leucine (L-Leu) residue at the position second from *N*-terminus, Boc-Aib-L-Leu-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe, the helical screw sense of which exhibits a unique solvent dependence.[17] This heptapeptide shows a preference for a right-handed screw sense in chloroform, but adopts predominantly a left-handed helix in methanol or in tetrahydrofuran (THF). Our results differ from the other groups' results obtained for Aib-oligopeptides[7] essentially in the two points. First, the *N*-terminal L-Leu residue in peptide Boc-L-Leu-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe, and another L-residue substituted for the L-Leu induce a left-handed screw sense, whereas an *N*-terminal L-Val or L-(αMe)Val residue (Y) in Bz-Y-(Aib)$_4$-O*t*Bu induces a right-handed screw sense.[7] Second, the shift of the L-Leu residue from the *N*-terminal to second position in our sequence affects the prevailing screw sense dramatically, whereas a right-handed screw sense is observed for both the *N*-terminal and internal L-residues in the pentapeptides containing four Aib residues.[7] The discrepancy might be ascribed to the difference in the chemical structure of the two achiral helical segments: *i.e.*, -(Aib-Δ^{Z}Phe)$_2$-Aib- for our sequence, and -(Aib)$_4$-.[7–10] Or, what might be relevant is the different tendency of L-Leu *versus* L-Val or L-(αMe)Val [L-Leu > L-Val or L-(αMe)Val] to adopt the semi-extended conformation at the *N*-terminus. This also suggests that accumulation of other related data should be important for a comprehensive understanding of the positional effects of a single chiral residue.

As for our previous data, it remains open whether or not a unique solvent-induced helix-to-helix inversion can be observed for another L-amino acid residue second from the *N*-terminus. To clarify this point, we here have adopted the following five kinds of peptide **1**–**5** possessing a non-polar L-amino acid residue (X*) at the penultimate position of the *N*-terminus: Boc-Aib-X*-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe.

The X* residue is Leu for **1**,[17] alanine (Ala) for **2**, Val for **3**, phenylalanine (Phe) for **4**, and 1-naphthylalanine (Nap) for **5**. As mentioned above, peptide **1** with X* = L-Leu residue does not induce exclusively a one-handed screw sense. Likewise, a different L-residue at the penultimate position might not lead to the predominant formation of one-handed helix, thereby offering a different bias towards either helix. As a result, the overall comparison of the prevailing screw senses for peptides **1**–**5** will also allow us to obtain a unique conformational parameter of each L-residue, *i.e.*, a screw sense preference wherein each L-residue induces an excess of a one-handed helix with a different bias for the remaining achiral segment. For peptides **2**–**5** prepared in the present work, their solution conformations were investigated by ^{1}H NMR and CD spectroscopy. The helical screw senses were identified by CD spectroscopy, which provides the sign of the exciton couplets around 280 nm for the Δ^{Z}Phe residue,[20–22] according to the exciton chirality method.[23] These screw sense data were compared with the previous data for peptide **1**,[17] and the rank order for inducing a one-handed screw sense is presented for the five kinds of L-residue as a unique conformational parameter.

Experimental

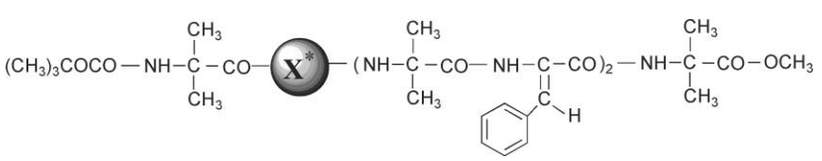
Measurements

^{1}H NMR spectra were recorded using a Bruker DRX-600 (600 MHz) or a DPX-200 (200 MHz) spectrometer for samples of peptide concentration of 10–11 mM in $CDCl_3$ or $CDCl_3$–$(CD_3)_2SO$ at 299 K. All chemical shifts in parts per million (ppm) were determined using tetramethylsilane as an internal standard, and the assignment of NH and C$^{\alpha}$H resonances was based on correlated spectroscopy (COSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY). COSY spectra were acquired and processed using a standard software library in XWINNMR software (version 2.5). ROESY spectra were measured on the Bruker DRX-600 (600 MHz) spectrometer using a Bruker standard pulse program (roesytp)[24] with a mixing time of 400 ms, 8 transients per t_1, 2K data points in the t_2 domain, and 256 points in the t_1 domain. The data processing and analysis were also performed with the XWIN-NMR software. FT-IR spectra were recorded for samples in chloroform using a JASCO FT/IR-430 spectrometer. A chloroform solution of peptide (1.5 mM) was prepared and transferred to an NaCl cell with 0.1 mm optical path length, and 100% chloroform was used as a blank. CD and UV spectra were recorded for samples in chloroform, acetonitrile, methanol, and THF on JASCO J-500 and JASCO V-550 spectrometers, respectively. These solvents were purified by distillation before use. The Δ^{Z}Phe concentration was determined using a maximum absorbance around 280 nm (assignable to a Δ^{Z}Phe residue) and its molar extinction coefficient (ε_{max} = 1.8 × 10^4 dm^3 mol^{-1} cm^{-1}). MALDI-TOF mass spectra of the final peptides were acquired on PerSeptive Biosystems Voyager RP in reflectron mode, using anthracene-1,8,9-triol (dithranol) matrix and NaI salt for the sample preparation. Thin-layer chromatography (TLC) was carried out on precoated silica plates in the following solvent systems: (A) ethyl acetate, (B) methanol, (C) chloroform-methanol (9:1), and (D) *n*-butanol-acetic acid-water (7:2:1). A single spot in the TLC was obtained for each of the peptides **2**–**5**.

Peptide synthesis

Peptides **2**–**5** were prepared in the similar manner to that described for peptide **1**:[17] Boc-Aib-OH was coupled with *N*-deprotected H-X*-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe using dicyclohexylcarbodiimide-1-hydroxybenzotriazole. The *N*-deprotected hexapeptide was obtained from Boc-X*-(Aib-Δ^{Z}Phe)$_2$-Aib-OMe prepared according to refs. 13 and 14. Characterization data for peptides **2**–**5** are as follows.

2 (X* = Ala): mp 148–151 °C. R_f^A = 0.45; R_f^B = 0.74; R_f^C = 0.50; R_f^D = 0.81. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.70 [1H, s, NH Δ^{Z}Phe(6)], 8.66 [1H, s, NH Δ^{Z}Phe(4)], 7.95 [1H, s, NH Aib(5)], 7.89 [1H, s, NH Aib(3)], 7.87 [1H, s, NH Aib(7)], 7.53–7.19 [12H, m, 2 × (C$^{\beta}$H + phenyl) Δ^{Z}Phe], 6.88 [1H, br s, NH Ala(2)], 5.27 [1H, s, NH Aib(1)], 3.90 [1H, m, C$^{\alpha}$H Ala(2)], 3.70 (3H, s, $COOCH_3$), 1.65 + 1.64 + 1.60 + 1.58 + 1.58 + 1.42 + 1.20 + 1.08 (24H, 8 × s, 8 × CH_3 Aib), 1.47 (9H, s, 3 × CH_3 Boc), 1.40 [3H, d, J = 7.3 Hz, C$^{\beta}$H$_3$ Ala(2)]. FT-IR (cm^{-1}, in KBr): 3286, 1733, 1659, 1627, 1534. MS (MALDI-TOF) (*m*/*z*), [M + Na]$^+$ (calcd. = 856.96): found 856.94. In the ROESY spectrum, the relative intensity (%) of N_iH–$N_{i+1}H$ (i – i + *1*) cross-peaks on setting the diagonal volume of the Δ^{Z}Phe(4) NH to 100% was as follows: 3.3 (3–4), 3.2 (4–5), 2.1 (5–6), 5.9 (6–7); in other cross peaks, 2.3 for C$^{\alpha}{}_2$H–N$_3$H.



3 (X* = Val): mp 138–141 °C. R_f^A = 0.62; R_f^B = 0.80; R_f^C = 0.54; R_f^D = 0.82. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.69 [1H, s, NH Δ^ZPhe(6)], 8.61 [1H, s, NH Δ^ZPhe(4)], 8.00 [1H, s, NH Aib(5)], 7.89 [1H, s, NH Aib(3)], 7.85 [1H, s, NH Aib(7)], 7.53–7.19 [12H, m, 2 × (C^βH + phenyl) Δ^ZPhe], 6.63 [1H, d, J = 3.58 Hz, NH Val(2)], 5.09 [1H, s, NH Aib(3)], 3.81 [1H, s, C^αH Val(2)], 3.71 (3H, s, $COOCH_3$), 2.25 [1H, s, C^βH Val(2)], 1.65 + 1.61 + 1.57 + 1.56 + 1.47 + 1.41 + 1.24 + 1.09 (24H, 8 × s, 8 × CH_3 Aib), 1.47 (9H, s, 3 × CH_3 Boc), 1.01 + 0.97 [6H, d + d, 2 × CH_3 Val(2)]. FT-IR (cm^{-1}, in KBr): 3289, 1731, 1661, 1628, 1534. MS (MALDI-TOF) (*m*/*z*), $[M + Na]^+$ (calcd. = 885.01): found 884.93. In the ROESY spectrum, the relative intensity (%) of N_iH–$N_{i+1}H$ (i – i + 1) cross-peaks on setting the diagonal volume of the Δ^ZPhe(4) NH to 100% was as follows: 1.4 (1–2), 0.6 (2–3), 2.3 (3–4), 3.2 (4–5), 3.6 (5–6), 4.0 (6–7); in other cross-peaks, 2.9 for N_2H–$C^\alpha{}_2H$.

4 (X* = Phe): mp 132–136 °C. R_f^A = 0.68; R_f^B = 0.82; R_f^C = 0.55; R_f^D = 0.89. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.69 [1H, s, NH Δ^ZPhe(6)], 8.54 [1H, s, NH Δ^ZPhe(4)], 7.94 [1H, s, NH Aib(5)], 7.84 [1H, s, NH Aib(7)], 7.78 [1H, s, NH Aib(3)], 7.56–7.11 [17H, m, 2 × (C^βH + phenyl) Δ^ZPhe + phenyl Phe], 6.65 [1H, d, J = 4.9 Hz, NH Phe(2)], 4.87 [1H, s, NH Aib(1)], 4.15 [1H, m, C^αH Phe(2)], 3.71 (3H, s, $COOCH_3$), 3.3–3.1 [2H, m, $C^\beta H_2$ Phe(2)], 1.67 + 1.62 + 1.61 + 1.58 + 1.44 + 1.40 + 1.10 + 1.06 (24H, 8 × s, 8 × CH_3 Aib), 1.35 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3289, 1725, 1660, 1628, 1534. MS (MALDI-TOF) (*m*/*z*), $[M + Na]^+$ (calcd. = 933.06): found 933.17. In the ROESY spectrum, the relative intensity (%) of N_iH–$N_{i+1}H$ (i – i + 1) cross-peaks on setting the diagonal volume of the Δ^ZPhe(4) NH to 100% was as follows: 2.2 (1–2), 1.7 (2–3), 2.0 (3–4), 2.8 (4–5), 3.2 (5–6), 3.8 (6–7).

5 (X* = Nap): mp 144–146 °C. R_f^A = 0.70; R_f^B = 0.88; R_f^C = 0.53; R_f^D = 0.90. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.69 [1H, s, NH Δ^ZPhe(6)], 8.60 [1H, s, NH Δ^ZPhe(4)], 7.99 [1H, s, NH Aib(5)], 7.85 [2H, s, NH Aib(3) + NH Aib(7)], 8.05–7.20 [19H, m, 2 × (C^βH + phenyl) Δ^ZPhe + naphthyl Nap(2)], 6.67 [1H, d, J = 5.3 Hz, NH Nap(2)], 4.72 [1H, s, NH Aib(1)], 4.29 [1H, m, C^αH Nap(2)], 3.88 + 3.42 [2H, d + q, $C^\beta H_2$ Nap(2)], 3.71 (3H, s, $COOCH_3$), 1.69 + 1.65 + 1.61 + 1.59 + 1.43 + 1.41 + 1.02 + 1.00 (24H, 8 × s, 8 × CH_3 Aib), 1.32 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3283, 1733, 1661, 1627, 1533. MS (MALDI-TOF) (*m*/*z*), $[M + Na]^+$ (calcd. = 983.1): found 982.83. In the ROESY spectrum, the relative intensity (%) of N_iH–$N_{i+1}H$ (i – i + 1) cross-peaks on setting the diagonal volume of the Δ^ZPhe(4) NH to 100% was as follows: 3.4 (1–2), 2.8 (2–3), 4.1 (3–4), 2.5 (4–5), 2.3 (5–6), 2.5 (6–7); in other cross peaks, 2.6 for N_2H–$C^\alpha{}_2H$.

Conformational energy calculation

An empirical conformational energy calculation was carried out using structural and energy parameters that are based on the ECEPP system.[25] The program PEPCON[26,27] for conformational energy calculation and graphics of a given peptide was modified to be applicable to β-aryldehyroalanine-containing peptides.[28–30]

On the basis of the crystallographic structures of analogous peptides containing an -(Aib-Δ^ZPhe)$_2$-Aib- segment,[15] all amide groups were fixed in the *trans* conformation (ω = 180°) and each Δ^ZPhe side chain was fixed in the *Z*-configuration (χ^1 = 0°). Energy minimization was carried out for Ac-Aib-X*-(Aib-Δ^ZPhe)$_2$-Aib-OMe (Ac, acetyl) to predict the stable conformations of the *N*-terminal moiety, Ac-Aib-X*-, attached to a left-handed or right-handed 3_{10}-helical segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe: all the main-chain and side-chain torsion angles of the Ac-Aib-X*- segment (*e.g.*, for X* = Nap, ϕ_{Aib}, ψ_{Aib}, $\chi^{1,1}{}_{Aib}$, $\chi^{1,1'}{}_{Aib}$, ϕ_{Nap}, ψ_{Nap}, $\chi^1{}_{Nap}$, and $\chi^2{}_{Nap}$) were varied with the Simplex algorithm for the optimization, whereas the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was fixed to a standard left-handed or right-handed 3_{10}-helix: (ϕ, ψ) = (60°, 30°) or (–60°, −30°).[31,32] Here all combinations of energy minima of Aib and X* residues were used as starting conformations of the -Aib-X*-: *i.e.*, 28 × 81 for **1**,[17] 28 × 9 for **2**, 28 × 30 for **3**, 28 × 81 for **4**, and 28 × 45 for **5**. The conformations of the -Aib-X*- segment were expressed by the conformational letter code (CLC) that divides 16 regions in conformational space.[33]

Results and discussion

Confirmation of helical conformation in peptides 2–5

The achiral segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe in peptides **2**–**5** can be expected to generate two "enantiomeric" (left-handed and right-handed) helices, based on the fact that the analogous peptide **1** was found to take a 3_{10}-helical conformation in solution.[17] In addition, oligopeptides containing an -(Aib-Δ^ZPhe)$_m$-Aib-OMe (m = 2 or 4) segment tend to adopt a 3_{10}-helical conformation in solution and in the solid states.[13–19] In fact, a helical conformation for peptides **2**–**5** is evidenced by ^{1}H NMR and FT-IR spectroscopy in solution. ROESY spectra of peptides **2**–**5** in $CDCl_3$ gave marked cross-peaks of N_iH–$N_{i+1}H$ resonances in the segment Aib(3) to Aib(7), thus indicating the presence of a 3_{10}-helix or an α-helix.[34,35] Fig. 1 shows the

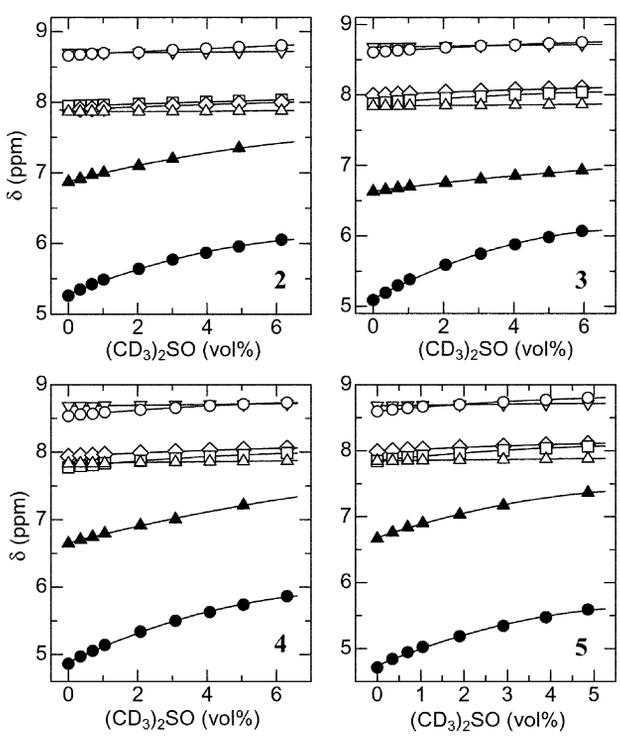


Fig. 1 Solvent dependence on NH chemical shifts in peptides **2**–**5** in $CDCl_3$–$(CD_3)_2SO$ mixtures of varying concentrations: Aib(1) (●), X*(2) (▲), Aib(3) (□), Δ^ZPhe(4) (○), Aib(5) (open diamond), Δ^ZPhe(6) (▽), and Aib(7) (△).

variation in NH chemical shifts of peptides **2**–**5** with concentration of $(CD_3)_2SO$[36] in $CDCl_3$.

The five NH resonances of Aib(3) to Aib(7) residues in each of peptides **2**–**5** are shielded from the solvent due to intramolecular hydrogen bonding. These hydrogen-bonding patterns correspond to a 3_{10}-helix[37] supported by consecutive (i+3)⟶i hydrogen bonds starting from NH Aib(3)⟶CO Boc. The helical conformation is also supported by the positions of the amide I absorption bands of their FT-IR spectra in solution, as shown in Table 1. Unlike for helical oligopeptides consisting only of saturated amino acids,[38] two characteristic peaks in the amide I region were observed: *i.e.*, first peak at *ca.* 1664–1662 cm^{-1} and second peak at *ca.* 1627–1625 cm^{-1}, which are assigned to saturated amino acid and Δ^ZPhe residues in helical segments, respectively.[19,39] A shift to lower wavenum-

Table 1 Peak positions of amide I and II bands of peptides **1**–**5** in chloroform

Peptide/X*	Amide I (ν/cm^{-1})[a]	Amide II (ν/cm^{-1})[a]
1/Leu[17]	1662 (s) and 1627 (m)	1533 (s)
2/Ala	1664 (s) and 1625 (m)	1535 (s)
3/Val	1663 (s) and 1627 (m)	1535 (s)
4/Phe	1663 (s) and 1627 (m)	1534 (s)
5/Nap	1664 (s) and 1627 (m)	1534 (s)

[a] s = strong, m = medium.

ber in the second peak is ascribed to the contribution of partial resonance between the carbonyl and styryl groups in the Δ^{Z}Phe residue.[39] Consequently, peptides **2**–**5** were found to adopt a 3_{10}-helical conformation in solution, similarly to peptide **1**. The helicity of the segment -(Aib-Δ^{Z}Phe)$_m$-Aib-OMe is essentially retained by different types of L-residue located at the penultimate position.

Identification of helical screw sense

Fig. 2 shows CD and UV absorption spectra of peptides **1**–**5** in

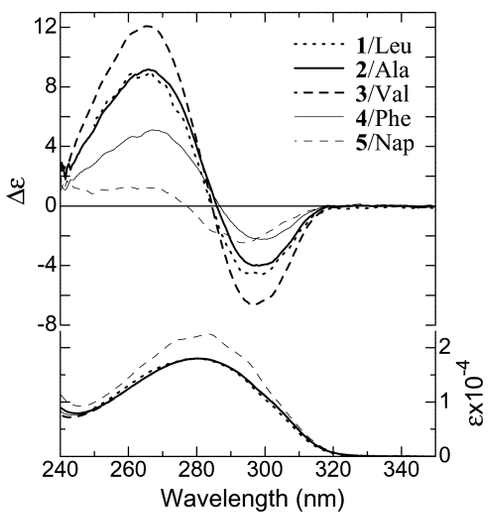


Fig. 2 CD (top) and UV (bottom) absorption spectra of peptides **1**–**5** in chloroform; ref. 17 for peptide **1**.

chloroform. The UV spectra of peptides **1**–**4** exhibit intense maxima (λ_{max}) around 280 nm (band I) assignable to the Δ^{Z}Phe residue. Band I of peptide **5** is fully overlapped with the $^{1}L_{a}$ band of the naphthyl group, but the absorption pattern obtained after removal of the contribution of the $^{1}L_{a}$ band on the spectral chart is quite similar to those of peptides **1**–**4**. Thus, the absorption spectra of all of the peptides essentially do not change, but resemble that of peptide Boc-Leu-Δ^{Z}Phe-Leu-OMe containing only a single Δ^{Z}Phe residue.[40] This tendency was also observed in the other solvents, as shown in Figs. 3–5. Therefore, no strong ground state interactions between the Δ^{Z}Phe–Δ^{Z}Phe pairs are present in peptides **1**–**5** in solution.

The corresponding CD spectra of peptides **1**–**5** in chloroform exhibit marked exciton couplets centered at around 280 nm, as shown in Fig. 2. The $\Delta\varepsilon$ ($= \varepsilon_L - \varepsilon_R$) in the ordinate is expressed with respect to the molar concentration of Δ^{Z}Phe residue. The achiral pentapeptide segment -(Aib-Δ^{Z}Phe)$_2$-Aib- cannot show any CD signals due to the lack of any chiral residues included, thereby taking both left-handed and right-handed helices with the same content in an equilibrium state. Thus, the CD signals observed for peptides **1**–**5** originate from chiral induction of the achiral segment through the L-residue covalently incorporated into the penultimate position.

In general, Δ^{Z}Phe-containing peptides offer two characteristic absorption bands around 220 and 280 nm. The former band precludes conventional far-UV CD analysis for obtaining secondary structures of peptides or proteins. On the other hand, the latter band has been assigned to charge transfer between the styryl and carbonyl groups;[20,21] the transition

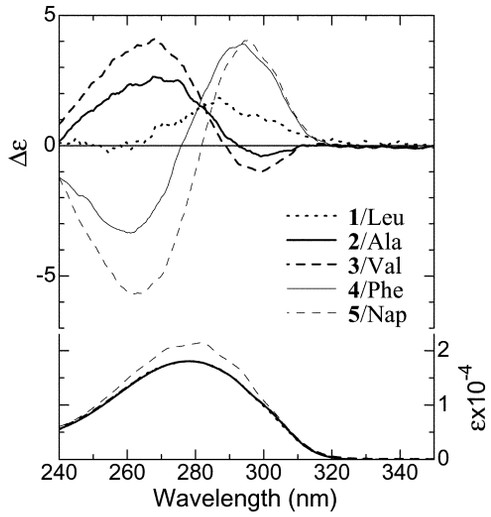


Fig. 3 CD (top) and UV (bottom) absorption spectra of peptides **1**–**5** in acetonitrile; ref. 17 for peptide **1**.

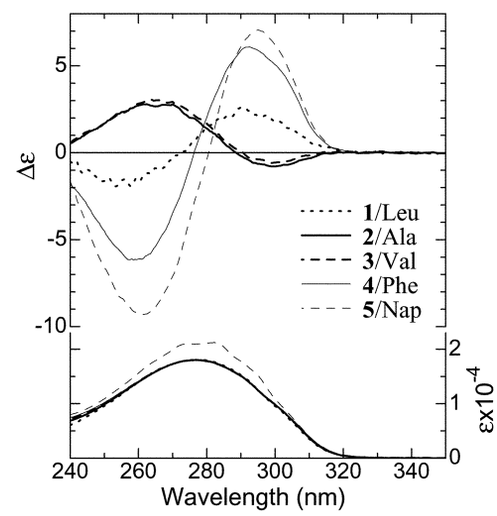


Fig. 4 CD (top) and UV (bottom) absorption spectra of peptides **1**–**5** in THF; ref. 17 for peptide **1**.

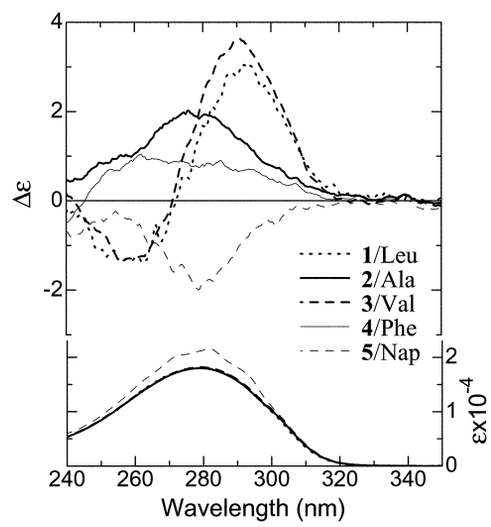


Fig. 5 CD (top) and UV (bottom) absorption spectra of peptides **1**–**5** in methanol; ref. 17 for peptide **1**.

moment was estimated from MO calculation to lie on the styryl and carbonyl line.[22] On the basis of the exciton chirality method,[23] the sign of the split CD of peptides **1**–**5** in chloroform, with a negative peak at longer wavelength, indicates a left-handed helical arrangement of the transition moment at 280 nm, thereby corresponding to a right-handed screw sense for a 3_{10}-helical or an α-helical backbone containing a -Δ^ZPhe-X-Δ^ZPhe- unit. This assignment has also been applied to 3_{10}-helical peptides containing -Δ^ZPhe-X-Δ^ZPhe- unit(s).[17,20] Moreover, the assignment of a split CD sign to the helical screw sense was evidenced by theoretical CD calculations on 3_{10}-helical or α-helical peptides containing -(Aib-Δ^ZPhe)$_n$- unit(s).[13,22]

Therefore, peptides **1**–**5** in chloroform prefer a right-handed helix, which might arise from the screw sense preference commonly expected for an L-residue. However, there are various split CD amplitudes observed for peptides **1**–**5**; in particular, peptides **4** and **5** give much weaker amplitudes than peptides **1**–**3**. As described in the preceding section, the NMR and FT-IR results have revealed that all of the peptides adopt a 3_{10}-helical backbone in solution. Thus, the reason for the different split-CD amplitudes should be ascribed not to helical structural stability, but to a different bias towards a right-handed screw sense. Accordingly, peptides **4** and **5** possessing an aromatic L-residue should adopt the left-handed helix more clearly, in comparison to peptides **1**–**3** having an alkyl L-residue.

This interesting finding becomes more remarkable in acetonitrile (Fig. 3) and in THF (Fig. 4). As mentioned before, the presence of the ^1L_a band of the naphthyl group in peptide **5** might affect its CD spectra around 280 nm (based on band I of Δ^ZPhe). However, this contribution should be negligible, because the ε_{max} of ^1L_a band ($\varepsilon_{280} \sim 6.3 \times 10^3$ dm^{-3} mol^{-1} cm^{-1}) is considerably smaller than that of band I of two Δ^ZPhe residues ($2\varepsilon_{280} \sim 3.6 \times 10^4$ dm^{-3} mol^{-1} cm^{-1}). In fact, the vibronic CD pattern based on the ^1L_a band was hardly observed in the split-CD patterns of peptide **5**.

In acetonitrile, peptides **2** and **3** show an exciton splitting with a negative peak at longer wavelength, but peptides **4** and **5** give a split CD with the opposite sign. Moreover in THF, peptides **2** and **3** show a split CD with a negative peak at longer wavelength, whereas peptides **1**, **4** and **5** give the opposite-sign CD pattern. These results clearly prove that the preferred screw sense is strongly governed by types of solvent and of the L-residue located at the penultimate position. That is to say, X* = Ala and Val induce a right-handed screw sense for the remaining achiral segment in acetonitrile and in THF, whereas X* = Phe and Nap prefer a left-handed screw sense in these solvents. X* = Leu shows a non-split CD pattern in acetonitrile, implying that there is no strong bias towards a one-handed helix, but induces a left-handed helix in THF. In methanol (Fig. 5), X* = Val and Leu induce a small excess of a left-handed helix, but X* = Ala, Phe, and Nap offer no strong bias towards a one-handed helix. The detailed UV and CD data are summarized in Table 2.

The relationship between the penultimate L-residue and the observed screw sense bias is demonstrated in Fig. 6, which illustrates the split-CD amplitudes (*A*) observed for peptides **1**–**5** in the four solvents. The *A* value is defined by $\Delta\varepsilon_2 - \Delta\varepsilon_1$, wherein $\Delta\varepsilon_1$ is the positive or negative maximum value for the first Cotton effect, and $\Delta\varepsilon_2$ is that for the second Cotton effect. Negative and positive signs of *A* values correspond to left-handed and right-handed screw senses, respectively. Thus, the *A* value expresses the degree of bias towards a one-handed helix, *i.e.*, to what extent each amino acid residue can induce a one-handed helix. On the whole, penultimate Ala, Leu, and Val residues have a marked tendency to induce a right-handed helix, which corresponds with the screw sense preference expected for these L-amino acid residues. Except for the CD data in methanol, the Val residue has the strongest effect for

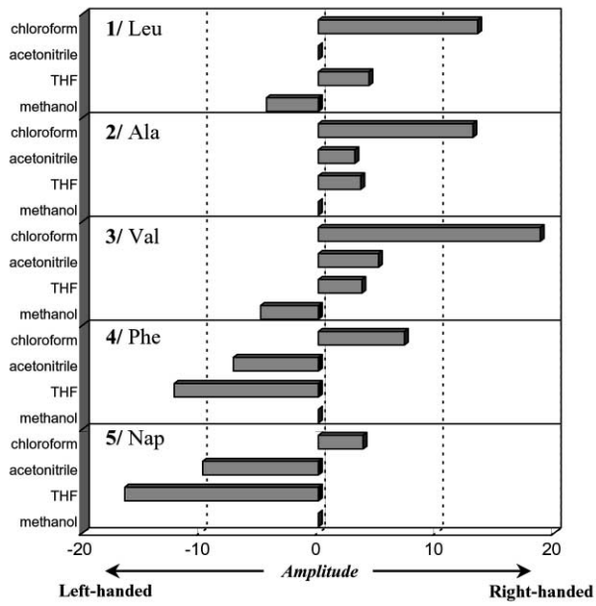


Fig. 6 Split CD amplitude (*A*) of peptides **1**–**5** with the five kinds of L-residue (X* = Leu,[17] Ala, Val, Phe, and Nap) in each solvent; negative and positive *A* values represent left-handed and right-handed helices, respectively.

inducing a right-handed helix, whereas the Ala and Leu residues are similar to each other in their preference for a right-handed helix. In contrast with these amino acids, Phe and Nap tend to prefer a left-handed helix, in particular more prominently in acetonitrile and THF. On the whole, Nap residue shows a stronger bias towards a left-handed helix than Phe. It should be noted that the screw sense preference observed for these analogous peptides is governed only by the subtle difference in the chemical structure of the penultimate L-residues (more specifically, the β-substituent of the L-residues). In summary, the rank order of the penultimate L-residues for inducing a right-handed screw sense is Val > Leu ~ Ala > Phe > Nap, of which the reverse order also represents the tendency to promote a left-handed screw sense.

Recently, we reported the effects of an *N*-terminal non-polar L-residue on the predominance of a helical screw sense of the remaining achiral segment, using six kinds of peptide: Boc-Y*-(Aib-Δ^ZPhe)$_2$-Aib-OMe,[14] wherein the Y* residue is a non-polar L-residue of Ala, Leu, Val, Phe, Nap or proline. NMR and CD spectroscopy revealed that all of the peptides in solution adopt a left-handed 3_{10}-helical conformation that shows exciton couplets centered at around 280 nm, with a positive peak at longer wavelength. This clearly demonstrates that the *N*-terminal L-residues employed induce the same (left-handed) screw sense for the remaining segment. Herein the split CD amplitudes (*A*), which mean a preference for a left-handed helix, are compared for these peptides in solution (chloroform, acetonitrile, methanol and THF). However, a clear rank order of the *A* values could not be observed through all of the solvents, although Y* = Ala gave somewhat smaller *A* values than the others. Consequently, we now can explain the dramatic difference between the *N*-terminal and penultimate positions in the positional effects of a single chiral center on helical screw sense. The non-polar L-residues at the *N*-terminal position work as left-handed helix inducers, but these L-residues prefer right-handed helices when shifted to an inner position. Therefore, an L-residue located at the position second from the *N*-terminus is less sensitive for determining a one-handed helix, thus playing a role for energetically permitting both helices. Through this interesting phenomenon arising from the penultimate position, we have been able to express experimentally a unique screw sense preference for these non-polar L-amino acids.

Table 2 Spectroscopic properties of peptides **1**–**5** in solution [a]

Peptide/X*	Solvent	UV data (λ_{max}/nm)	CD data		
			First Cotton effect [b] $\Delta\varepsilon_1/(\lambda_{max}/nm)$	Second Cotton effect [c] $\Delta\varepsilon_2/(\lambda_{max}/nm)$	A [d]
1/Leu	Chloroform	280	−4.5/297	+9.0/264	+13.5
2/Ala	Chloroform	280	−4.0/297	+9.1/266	+13.1
3/Val	Chloroform	281	−6.7/297	+12.1/266	+18.8
4/Phe	Chloroform	281	−2.2/300	+5.1/268	+7.3
5/Nap	Chloroform	283	−2.5/294	+1.3/268	+3.8
1/Leu	Acetonitrile	278	+1.8/286		—[e]
2/Ala	Acetonitrile	278	−0.4/299	+2.7/262	+3.1
3/Val	Acetonitrile	278	−1.0/299	+4.1/268	+5.1
4/Phe	Acetonitrile	278	+3.9/293	−3.3/261	−7.2
5/Nap	Acetonitrile	282	+4.1/295	−5.7/263	−9.8
1/Leu	Methanol	280	+3.0/292	−1.4/259	−4.4
2/Ala	Methanol	279	+2.0/278		—[e]
3/Val	Methanol	279	+3.6/291	−1.3/259	−4.9
4/Phe	Methanol	279	+0.9/276		—[e]
5/Nap	Methanol	282	−1.9/279		—[e]
1/Leu	THF	278	+2.5/292	−1.8/256	−4.3
2/Ala	THF	277	−0.8/299	+2.8/265	+3.6
3/Val	THF	277	−0.6/299	+3.1/266	+3.7
4/Phe	THF	277	+6.1/292	−6.1/259	−12.2
5/Nap	THF	282	+7.1/295	−9.3/261	−16.4

[a] Ref. 17 for **1**/Leu. [b] $\Delta\varepsilon_1$ is the positive or negative maximum value for the first Cotton effect. [c] $\Delta\varepsilon_2$ is the positive or negative maximum value for the second Cotton effect. [d] $A = \Delta\varepsilon_2 - \Delta\varepsilon_1$. Negative and positive signs of A values correspond to left-handed and right-handed screw senses, respectively. [e] Could not be estimated due to non-split CD pattern.

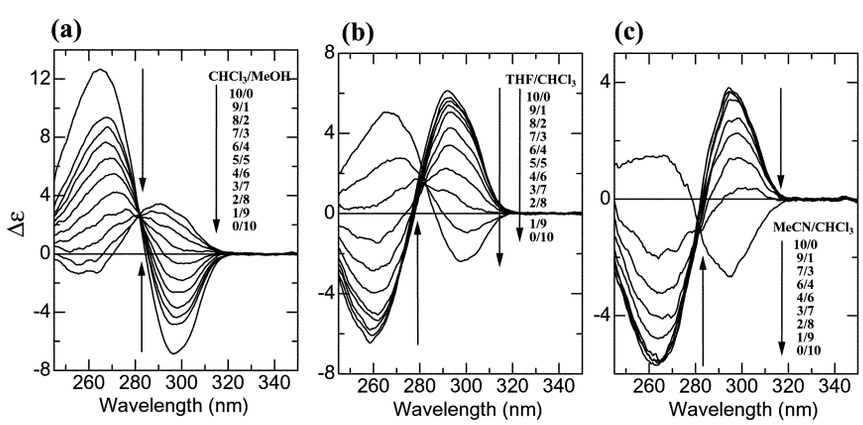


Fig. 7 CD (top) and UV (bottom) absorption spectra of (a) peptide **3** (X* = Val) in chloroform-methanol mixtures of varying composition (vol%); (b) peptide **4** (X* = Phe) in THF-chloroform mixtures; (c) peptide **5** (X* = Nap) in acetonitrile-chloroform mixtures.

Solvent-induced helix-to-helix interconversion

Fig. 7(a) shows the solvent dependence in the CD spectra of peptide **3** (X* = Val) in chloroform-methanol mixtures of varying compositions (vol%). As shown in the preceding section, peptide **3** adopts predominantly a right-handed helix in pure chloroform, whereas it favors a left-handed helix in pure methanol. The split CD amplitude in 100% chloroform, with a negative peak at longer wavelengths, decreases with increased methanol content (0–70 vol%). Then, an opposite split pattern (with a positive peak at longer wavelengths) begins to appear at 90 vol% methanol, and the amplitude increases further at 100% methanol. Thus, the right-handed helix predominant in chloroform gradually shifts to the left-handed helix that is preferred in methanol, with increased methanol content. Conversely, the left-handed helix in pure methanol changes to a right-handed helix with increased chloroform content. Obviously, peptide **3** exhibits a unique helix-to-helix interconversion induced by chloroform-methanol compositions. A similar helix-to-helix interconversion is observed for peptide **4** (X* = Phe) in THF-chloroform mixtures [Fig. 7(b)], and for peptide **5** (X* = Nap) in acetonitrile-chloroform mixtures [Fig. 7(c)]. Such reversible screw sense inversion has only rarely been found in oligopeptides: *e.g.*, Boc-L-Ala-Δ^ZPhe-Gly-Δ^ZPhe-L-Ala-OMe [41,42] and Boc-L-Val-Δ^ZPhe-Gly-Δ^ZPhe-L-Val-OMe [43] showed reversible screw sense inversion of the 3_{10}-helix, depending on solvent or temperature conditions.

We have reported that peptide **1** (X* = Leu) undergoes a helix-to-helix interconversion in chloroform-methanol mixtures of varying composition.[17] Thus, this unique solvent-induced interconversion is commonly observed for peptides possessing a non-polar L-residue at the penultimate position of the *N*-terminus. These peptides should show no marked energy difference between left-handed and right-handed helices. In other words, the incorporation of an L-residue into the penultimate position of achiral segment should not cause a strong screw sense bias.

Conformational preference of penultimate L-residues

The preceding results demonstrate that peptides **1**–**5**, possessing one non-polar L-residue at the penultimate position, can adopt

Table 3 Energy-minimized conformations for L-residues (X*) in Ac-Aib-X*-(Aib-Δ^ZPhe)$_2$-Aib-OMe in a standard 3_{10}-helix[a]

Peptide/X*	CLC[b]	Aib		X*		Helical screw sense[c]	ΔE_{res}/kcal mol^{-1}[d]
		φ	ψ	φ	ψ		
1/Leu	AA	−58	−34	−67	−51	RH	0.00
	A*A*	57	34	58	63	LH	0.18
	A*C	53	46	−64	109	LH	0.28
2/Ala	AA	−57	−35	−68	−50	RH	0.00
	A*A*	56	35	57	62	LH	0.13
	A*C	53	47	−64	110	LH	0.24
3/Val	AA	−58	−34	−71	−48	RH	0.00
	A*A*	56	33	55	67	LH	0.22
	A*C	53	46	−68	111	LH	0.24
4/Phe	AA	−55	−41	−67	−45	RH	0.00
	A*A*	53	39	51	66	LH	0.30
	A*C	53	46	−61	110	LH	0.35
5/Nap	AA	−56	−41	−69	−45	RH	0.00
	A*A*	53	40	50	67	LH	0.34
	A*C	53	45	−63	113	LH	0.34

[a] Energy minimization was carried out with the main-chain and side-chain torsion angles of the segment -Aib-X*- in Ac-Aib-X*-(Aib-Δ^ZPhe)$_2$-Aib-OMe, while the other segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was fixed to a standard left-handed or right-handed 3_{10}-helix: (60°, 30°) or (−60°,−30°),[31,32] respectively. [b] Conformational letter code[33] of the segment -Aib-X*-. [c] LH and RH represent left- and right-handed helices for the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe, respectively. [d] $\Delta E_{res} = (E - E_0)/7$. E_0 is the lowest energy.

both helical screw senses with different bias. In addition, the rank order of the L-residues for inducing a right-handed screw sense is Val > Leu ~ Ala > Phe > Nap. The most likely question raised at this point is why these penultimate L-residues do not choose only a one-handed helix, or what is relevant to the rank order.

A rational explanation for the rank order could be ascribed to the conventional helix-forming tendencies of naturally occurring amino acids. These parameters have been estimated from the 'host-guest' experiments, in which copolymers are composed of the target amino acid (the 'guest') and a host residue.[44,45] According to the helix-flexible chain transition theory, the helix propagation parameter decreases in the order Phe > Leu > Ala > Val.[44] Herein the helix-forming tendency should correspond more closely to a preference for a right-handed helix. Another system, in which three non-polar amino acids are substituted for an Ala-based 17-residue peptide, revealed that the right-handed helix content decreases in the order Leu ~ Ala > Phe > Val for the substitution.[45] In both cases, Val is shown to be the least effective residue for stabilizing a right-handed helix. Interestingly, this tendency does not meet our rank order that a Val residue induces a right-handed helix the most effectively. The tendency found here might imply that γ-substituted L-residues (Leu, Phe, and Nap) have a lower propensity to give rise to right-handed helices than the β-substituted L-residues (Val) that have already been published for the Aib-based peptides.[7,10]

For more information, Table 3 shows energy-minimized conformations of the segment -Aib-X*- in Ac-Aib-X*-(Aib-Δ^ZPhe)$_2$-Aib-OMe, in which the achiral segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe is fixed to a standard left-handed or right-handed 3_{10}-helix, and lists the lowest-energy to third lowest energy conformations in ones giving dissimilar CLC.[33]

ΔE_{res} is the energy difference per residue from the lowest energy. From Table 3, two characteristic tendencies are commonly obtained for peptides **1**–**5**. First, all of the peptides show a right-handed helix as the lowest-energy conformation, whereas a left-handed helix is found in second lowest energy and third lowest energy conformations. Herein, ΔE_{res} values of second lowest energy conformation are relatively small (0.13–0.34 kcal mol^{-1}). This small energy difference might imply that these peptides can adopt both helical screw senses, and thus that the prevailing screw sense should be largely affected by a change of environment around a peptide molecule (*e.g.*, types of solvent).

Second, the *N*-terminal Ac-Aib-X*- of peptides **1**–**5** shows a similar conformational preference. When the achiral segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe adopts a right-handed helix, the Ac-Aib-X*- takes the conformational letter code AA, corresponding to a right-handed helix. Thus this incipient right-handed helix promotes a right-handed helix for the following segment. On the other hand, a left-handed helix is induced by two types of conformation of the Ac-Aib-X*-, *i.e.*, A*A* and A*C. The former corresponds to a left-handed helix, and the latter to an irregular conformation deviating from both helices. For further information, the main-chain energy contour map (ϕ, ψ) of the X* residue of peptides **1**–**5** has been computed. (The contour maps are available as supplementary data†). In this calculation, the fragment other than the X* residue is fixed to the right-handed helix (the lowest energy) or the left-handed helix (second lowest energy) shown in Table 3. In each (ϕ, ψ) point, the side chain of X* residue (χ^1 for Ala and Val; χ^1 and χ^2 for Leu, Phe, and Nap) is taken as the conformer that gives the minimal conformational energy obtained by changing χ^1 and χ^2 in steps of 5° from 0° to 360°. When the right-handed 3_{10}-helix is being induced for the achiral -(Aib-Δ^ZPhe)$_2$-Aib-OMe, the conformational space (ϕ, ψ) of the X* residue is severely restricted to only a right-handed helical region characterized by A (−70°, −40°) shown in Table 3. On the other hand, a left-handed helix of the achiral segment is induced by the two stable regions of the X* residue characterized by A* (50°, 60°) and C (−60°, 110°) shown in Table 3. The latter region (C) is energetically somewhat higher than the former (A*), but is wider (entropically favorable). The preferred conformations of the X* residue (A for right-handed helix, and A* and C for left-handed helix) are essentially common to the five non-polar L-residues. On the other hand, interestingly, the Phe and Nap residues tend to show a somewhat larger conformational freedom for induction of a left-handed helix than the Ala, Leu, and Val residues: *i.e.*, an additional region appears around E (−150°, 140°) for the Phe and Nap residues. This theoretical prediction might imply that the two aromatic residues have a higher propensity to lead to the induction of a left-handed helix, compared with the three alkyl residues.

Conclusions

To understand the positional effects of a single L-residue on predominant helical screw sense, we investigated the prevailing screw sense for peptides **1**–**5** possessing a non-polar L-residue at the penultimate position of the *N*-terminus. All of the peptides are folded into a 3_{10}-type helical conformation in solution, as

discerned from FT-IR and ^{1}H-NMR techniques. CD studies of these peptides strongly indicate that they adopt both left-handed and right-handed helices, and that the prevailing screw sense, as well as the screw sense bias, depends on the type of solvent. Thus, the L-residue located at the penultimate position plays a unique role for energetically permitting both helices. These peptides also undergo a solvent-induced helix-to-helix interconversion. The rank order of the penultimate L-residues for inducing a right-handed screw sense is Val > Leu ~ Ala > Phe > Nap, of which the reverse order represents the tendency to promote a left-handed screw sense. In conclusion, we have been able experimentally to express a unique screw sense preference of these non-polar L-amino acids. The present findings also provide important basic information about the helical propensity of non-polar amino acids and protein folding mechanisms.

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