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Noncovalent Domino Effect on Helical Screw Sense of Chiral Peptides Possessing C-Terminal Chiral Residue

Yoshihito Inai,* Yuya Ishida, Kenichi Tagawa, Akinori Takasu, and Tadamichi Hirabayashi

Contribution from the Department of Environmental Technology and Urban Planning, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan

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Abstract: Recently, a novel chiral intermolecular interaction was found in an N-deprotected achiral nonapeptide that undergoes the predominance of one-handed screw sense through the addition of chiral small carboxylic acid (Inai, Y.; Tagawa, K.; Takasu, A.; Hirabayashi, T.; Oshikawa, T.; Yamashita, M. *J. Am. Chem. Soc.* **2000**, *122*, 11731). We here clarify to what extent such noncovalent chiral domino effect affects the helical screw sense of an N-deprotected *chiral* peptide. Two chiral peptides consisting of C-terminal L-Leu (**1**) or L-Leu_2 (**2**) and the preceding achiral helical octapeptide segment were employed. NMR and IR spectroscopy, and energy calculation indicated that both peptides adopt a helical conformation in chloroform. Peptide **1** showed a small excess of a left-handed screw sense for the achiral helical octapeptide, but peptide **2** strongly preferred a right-handed screw sense. The addition of chiral Boc amino acid to a chloroform solution of peptide **1**, depending on its chirality, underwent a unique helix-to-helix transition or led to remarkable stabilization of the original left-handed screw sense. Peptide **2** retained the original right-handed screw sense on addition of chiral Boc-amino acid, but its helical stability changed to some extent depending on its added chirality. Therefore, the importance of noncovalent domino effect for controlling the helical screw sense or helical stability of a chiral peptide has been demonstrated here for the first time. In addition, we here have presented a unique system that both N-terminal noncovalent and C-terminal covalent domino effects operate simultaneously on the helical screw sense of a single achiral segment and have compared both powers for inducing the screw sense bias.

Introduction

To control the helical screw sense of biological macromolecules[1] or synthetic polymers[2] through external stimuli such as pH, light, temperature, solvent, and chiral molecules is of academic as well as of practical importance in a wide range of chemical fields such as biochemistry, polymer chemistry, supramolecular chemistry, analytical chemistry, and chiral separation and chiral pharmaceutical technologies. In most of previous studies on the control of a helical screw sense, such external stimuli act on a whole polymer molecule, including the main and/or side chains. On the other hand, little is known about the possibility that the site-specific action of external stimulus upon a terminal moiety of a helical polymer can control the whole helical screw sense. Recently, we found that the predominance of one-handed screw sense is induced for an N-deprotected achiral nonapeptide by the addition of a chiral small carboxylic acid, of which chiral stimulus acts on the N-terminal amino group to generate the helical screw sense bias.[3] This noncovalent domino effect will provide new insight into the nature of chiral interactions between a helical segment and a chiral molecule in peptide and protein science. However, one might ask at this point to what extent such noncovalent domino effect, in fact, affects the helical screw sense or helical stability of a *chiral* peptide.

To demonstrate the importance of the novel chiral interaction in biopolymers, we here adopted the following N-deprotected nonapeptide **1** and decapeptide **2** containing achiral helicogenic residues[4] of α-aminoisobutyric acid (Aib) and α,β-

* To whom correspondence should be addressed: inai@mse.nitech.ac.jp.

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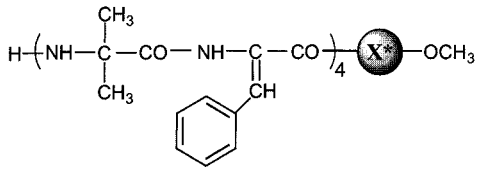
didehydrophenylalanine (Δ^ZPhe).



H-(Aib-Δ^ZPhe)$_4$-X*-OMe X* = L-Leu (**1**) and L-Leu$_2$ (**2**)

(OMe=methoxy)

These peptides possess a C-terminal chiral residue (L-Leu) or segment (L-Leu$_2$) that will induce the excess of one-handed screw sense for the preceding achiral helical segment -(Aib-Δ^ZPhe)$_4$- through the C-terminal covalent domino effect. Here, addition of chiral carboxylic acid will give rise to the N-terminal noncovalent domino effect to lead to the terminal control of the original helical screw sense. This is also a very unique system for understanding novel factors governing the helical screw sense of polymer molecules, i.e., the covalent chiral effect from one-side terminal of a helical segment, and noncovalent chiral effect generating from the other terminal operate simultaneously on the helical screw sense of an achiral segment, as if it were a *tug-of-war* game on the achiral helical chain.

Experimental Section

Materials. All amino acids and coupling reagents were purchased from Tokyo Kasei Co. (Tokyo, Japan) or Kokusan Chemical Works Ltd. (Tokyo, Japan). Boc-amino acid (Boc = *t*-butoxycarbonyl) was prepared by a standard procedure with $(Boc)_2O$, or was purchased from Kokusan Chemical Works Ltd. Size exclusion column (TOYOPEARL HW-40) for purification of products was commercially available from TOSOH Co. (Tokyo, Japan). Chloroform dried over $CaSO_4$ was distilled onto $CaSO_4$ before use. *N*,*N*-Dimethylformamide (DMF) was purified by distillation with ninhydrin under a reduced pressure. Thin-layer chromatography (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): single spot in the TLC was obtained for each of the final products and their intermediates, as shown below.

Boc-(Aib-Δ^ZPhe)$_3$-L-Leu-OMe. Boc-(Aib-Δ^ZPhe)$_2$-L-Leu-OMe[5] (2.6 g, 3.3 mmol) was dissolved in trifluoroacetic acid (5 mL)/dichloromethane (15 mL) at 0 °C, and then the solution was allowed to stand for 3 h at 0 °C, and concentrated in vacuo. After addition of 5% $NaHCO_3$ solution, the residue was extracted with chloroform, and the organic solution was dried over $MgSO_4$. After removing the solvent, the residue was dissolved in DMF (10 mL), and to the solution was added Boc-Aib-Δ^ZPhe azlactone[5] (1.1 g, 3.3 mmol) at 0 °C. The mixture was stirred for 2 h at 0 °C, then for 24 h at room temperature, concentrated in vacuo, and the residue was dissolved in chloroform. 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 recrystallization from chloroform/*n*-hexane to give the heptapeptide in 1.5 g yield (50%). R_f^A 0.70; R_f^B 0.87; R_f^C 0.53; R_f^D 0.82. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.95 (1H, s, NH Δ^ZPhe(4)), 8.74 (1H, s, NH Δ^ZPhe(6)), 8.12 (1H, s, NH Aib(3)), 8.02 (1H, s, NH Aib(5)), 7.97 (1H, d, J = 7.9 Hz, NH Leu(7)), 7.74 (1H, s, NH Δ^Z-Phe(2)), 7.63−7.18 (18H, m, 3 × (C$^\beta$H + phenyl) Δ^ZPhe), 5.14 (1H, s, NH Aib(1)), 4.68 (1H, m, C$^\alpha$H Leu(7)), 3.64 (3H, s, $COOCH_3$), 1.99−1.6 (3H, m, C$^\beta$H$_2$ + C$^\gamma$H Leu(7)), 1.64 + 1.62 + 1.50 + 1.48 + 1.30 + 1.28 (18H, s + s + s + s + bs + bs, 6 × CH_3 Aib), 1.44 (9H, s, 3 × CH_3 Boc), 0.90 (6H, bs, 2 × CH_3 Leu(7)). FT-IR (cm^{-1}, in KBr): 3280, 1741, 1686, 1661, 1626, 1536.

Boc-(Aib-Δ^ZPhe)$_4$-L-Leu-OMe (3). The nonapeptide was prepared by ring-opening reaction of Boc-Aib-Δ^ZPhe azlactone[5] with H-(Aib-Δ^ZPhe)$_3$-L-Leu-OMe prepared from Boc-(Aib-Δ^ZPhe)$_3$-L-Leu-OMe, in a manner similar to Boc-(Aib-Δ^ZPhe)$_3$-L-Leu-OMe. Yield 42%. R_f^A 0.66; R_f^B 0.87; R_f^C 0.49; R_f^D 0.81. Anal. Calcd. for $C_{64}H_{79}N_9O_{12}$ H_2O: C 64.90, H 6.89, N 10.64. Found: C 65.09, H 6.80, N 10.48. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 9.02 (1H, s, NH Δ^ZPhe(4)), 8.99 (1H, s, NH Δ^ZPhe(6)), 8.70 (1H, bs, NH Δ^ZPhe(8)), 8.10 (2H, s, 2 × NH Aib(3) + Aib(7)), 8.05 (1H, s, NH Aib(5)), 7.95 (1H, d, J = 7.8 Hz, NH Leu(9)), 7.73 (1H, s, NH Δ^ZPhe(2)), 7.56−7.10 (24H, m, 4 × (C$^\beta$H + phenyl) Δ^ZPhe), 5.17 (1H, s, NH Aib(1)), 4.60 (1H, m, C$^\alpha$H Leu(9)), 3.56 (3H, s, $COOCH_3$), 1.93−1.6 (3H, m, C$^\beta$H$_2$ + C$^\gamma$H Leu(9)), 1.58 + 1.56 + 1.49 + 1.29 + 1.21 (24H, s + s + bs + bs + bs, 8 × CH_3 Aib), 1.36 (9H, s, 3 × CH_3 Boc), 0.83 (6H, bs, 2 × CH_3 Leu(9)). FT-IR (cm^{-1}, in KBr): 3277, 1737, 1660, 1625, 1536.

H-(Aib-Δ^ZPhe)$_4$-L-Leu-OMe (1). Nonapeptide **3** (60 mg, 52 μmol) was dissolved in trifluoroacetic acid (0.6 mL)/dichloromethane (0.6 mL) at 0 °C, and then the solution was allowed to stand at 0 °C for 5 h, and concentrated in vacuo. After addition of 5% $NaHCO_3$ solution, the residue was extracted with chloroform, and the organic solution was dried over $MgSO_4$. The product was purified by precipitation from chloroform/*n*-hexane to give **1** in 45 mg yield (82%). R_f^A 0−0.21; R_f^B 0.51−0.81; R_f^C 0.42; R_f^D 0.61. Anal. Calcd. for $C_{59}H_{71}N_9O_{10}$ H_2O: C 65.36, H 6.79, N 11.63. Found: C 65.51, H 6.54, N 11.51. 600 MHz ^{1}H NMR (δ, in $CDCl_3$ containing 8.9 vol % $(CD_3)_2SO$): 9.40 (1H, s, NH Δ^ZPhe(8)), 9.13 (1H, s, NH Δ^ZPhe(6)), 8.80 (1H, s, NH Δ^ZPhe-(4)), 8.24 (1H, s, NH Aib(7)), 8.21 (1H, s, NH Aib(5)), 8.06 (1H, s, NH Aib(3)), 8.04 (1H, d, J = 7.7 Hz, NH Leu(9)), 7.58−7.21 + 6.75 (24H, m + s, 4 × (C$^\beta$H + phenyl) Δ^ZPhe), 4.63 (1H, m, C$^\alpha$H Leu(9)), 3.64 (3H, s, $COOCH_3$), 1.96 + 1.85 (2H, m + bs, C$^\beta$H$_2$ Leu(9)), 1.68−1.6 (1H, m, C$^\gamma$H Leu(9)), 1.65 + 1.63 + 1.53 + 1.31 + 1.21 (24H, s + s + bs + bs + s, 8 × CH_3 Aib), 0.90 (6H, bs, 2 × CH_3 Leu(9)). FT-IR (cm^{-1}, in chloroform containing 9 vol %-$(CH_3)_2SO$; [**1**] = 1.0 mM): 1730, 1659, 1627, 1537; (cm^{-1}, in KBr): 3269, 1738, 1658, 1622, 1538. In the NOESY 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: 1.2 (*3*-*4*), 1.3 (*4*-*5*), 1.2 (*5*-*6*), 1.0 (*6*-*7*), 1.2 (*7*-*8*), and 1.4 (*8*-*9*).

Boc-Aib-Δ^ZPhe-OMe. The dipeptide was prepared by ring-opening reaction of Boc-Aib-Δ^ZPhe azlactone[5] with methanol.[6] Yield 98%. R_f^A 0.78; R_f^B 0.83; R_f^C 0.73; R_f^D 0.73. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.19 (1H, s, NH Δ^ZPhe), 7.55−7.29 (6H, m, C$^\beta$H + phenyl Δ^ZPhe), 4.94 (1H, s, NH Aib), 3.83 (3H, s, $COOCH_3$), 1.56 (6H, s, 2 × CH_3 Aib), 1.45 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3401, 3378, 1714, 1699, 1685, 1641, 1504.

Boc-(Aib-Δ^ZPhe)$_m$-OMe (m = 2−4). Peptides (m = 2−4) were prepared by ring-opening reaction of Boc-Aib-Δ^ZPhe azlactone[5] with H-(Aib-Δ^ZPhe)$_n$-OMe (n = 1−3) prepared from Boc-(Aib-Δ^ZPhe)$_n$-OMe, in a manner similar to the preparation of Boc-(Aib-Δ^ZPhe)$_3$-L-Leu-OMe.

Boc-(Aib-Δ^ZPhe)$_2$-OMe. Yield 78%. R_f^A 0.73; R_f^B 0.81; R_f^C 0.58; R_f^D 0.73. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.71 (1H, s, NH Δ^ZPhe-(4)), 7.75 (1H, s, NH Aib(3)), 7.52 (1H, s, NH Δ^ZPhe(2)), 7.71−7.19 (12H, m, 2 × (C$^\beta$H + phenyl) Δ^ZPhe), 4.87 (1H, s, NH Aib(1)), 3.82 (1H, s, $COOCH_3$), 1.67 + 1.46 (12H, s + s, 4 × CH_3 Aib), 1.44 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3313, 3286, 1718, 1670, 1636, 1528, 1503.

Boc-(Aib-Δ^ZPhe)$_3$-OMe. Yield 69%. R_f^A 0.65; R_f^B 0.85; R_f^C 0.53; R_f^D 0.69. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 8.77 (2H, s, 2 × NH Δ^ZPhe(4) + Δ^ZPhe(6)), 8.03 (1H, s, NH Aib(3)), 7.76 (1H, s, NH Aib-(5)), 7.63 (1H, s, NH Δ^ZPhe(2)), 7.74−7.18 (18H, m, 3 × (C$^\beta$H +

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phenyl) Δ^ZPhe), 4.96 (1H, s, NH Aib(1)), 3.75 (1H, s, $COOCH_3$), 1.69 + 1.55 + 1.24 (18H, s + s + s, 6 × CH_3 Aib), 1.43 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3296, 1715, 1686 (sh), 1666, 1632, 1531.

Boc-(Aib-Δ^ZPhe)$_4$-OMe (4). Yield 76%. R_f^A 0.65; R_f^B 0.85; R_f^C 0.53; R_f^D 0.75. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 9.06 (1H, s, NH Δ^ZPhe-(4)), 8.88 (1H, s, NH Δ^ZPhe(6)), 8.77 (1H, s, NH Δ^ZPhe(8)), 8.08 (1H, s, NH Aib(3)), 8.06 (1H, s, NH Aib(5)), 7.90 (1H, s, NH Aib(7)), 7.65 (1H, s, NH Δ^ZPhe(2)), 7.75−7.17 (24H, m, 4 × (C^βH + phenyl) Δ^Z-Phe), 5.05 (1H, s, NH Aib(1)), 3.69 (3H, s, $COOCH_3$), 1.70 + 1.60 + 1.30 + 1.25 (24H, s + s + bs + bs, 8 × CH_3 Aib), 1.43 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3295, 1729, 1688, 1660, 1628, 1532.

Boc-(Aib-Δ^ZPhe)$_4$-OH (5). To a solution of peptide **4** (650 mg, 0.62 mmol) in methanol (75 mL) and dioxane (75 mL) was added 1 M NaOH solution (3.1 mL, 3.1 mmol) at 0 °C. Then the reaction mixture was stirred at room temperature for 2 days until TLC (ethyl acetate) indicated that the saponification process was complete. After concentration in vacuo, the mixture was inserted into a $KHSO_4$ solution (pH = 2−3) to obtain a white precipitate. The precipitate was washed with distilled water until the washed water became neutral, and was dried in vacuo to give **5** in 480 mg yield (75%). R_f^A 0−0.24; R_f^B 0.90; R_f^C 0.37−0.54; R_f^D 0.80. 600 MHz ^{1}H NMR (δ, in $CDCl_3$ containing 20 vol %-$(CD_3)_2SO$): 9.69 (1H, s, NH Δ^ZPhe(2)), 9.21 (1H, s, NH Δ^Z-Phe(4)), 9.09 (1H, s, NH Δ^ZPhe(6)), 8.83 (1H, s, NH Δ^ZPhe(8)), 8.49 (1H, s, NH Aib(3)), 8.30 (1H, s, NH Aib(7)), 8.23 (1H, s, NH Aib(5)), 7.70−7.17 (24H, m, 4 × (C^βH + phenyl) Δ^ZPhe), 6.68 (1H, s, NH Aib(1)), 1.66 + 1.57 + 1.34 + 1.25 (24H, s + s + bs + bs, 8 × CH_3 Aib), 1.45 (9H, s, 3 × CH_3 Boc). FT-IR (cm^{-1}, in KBr): 3273, 1721, 1663, 1627, 1534.

Boc-(Aib-Δ^ZPhe)$_4$-L-Leu$_2$-OMe (6). Peptide **5** (400 mg, 0.39 mmol) and HCl·H-L-Leu_2-OMe (125 mg, 0.42 mmol) were dissolved in DMF (5 mL), cooled to 0 °C. To the solution were added 1-hydroxybenzotriazole monohydrate (65 mg, 0.42 mmol), dicyclohexylcarbodiimde (88 mg, 0.42 mmol), and *N*-methylmorphorine (47 μL, 0.42 mmol). The reaction mixture was stirred at 0 °C for 3 h, and at room temperature for 4 days. The mixture was concentrated in vacuo, and the residue was redissolved in chloroform. 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 then dried over $MgSO_4$. The product was purified using a silica gel column eluted with ethyl acetate, and then a size-exclusion column (TOYOPEARL HW-40) eluted with DMF. The resulting residue was subjected to precipitation from chloroform/diethyl ether to give **6** in 230 mg yield (47%). R_f^A 0.52; R_f^B 0.85; R_f^C 0.44; R_f^D 0.80. Anal. Calcd. for $C_{70}H_{90}N_{10}O_{13}$ H_2O: C 64.80, H 7.15, N 10.80. Found: C 65.01, H 7.07, N 10.78. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 9.15 (1H, s, NH Δ^ZPhe(6)), 9.06 (1H, s, NH Δ^ZPhe(8)), 9.05 (1H, s, NH Δ^ZPhe(4)), 8.34 (1H, s, NH Aib(7)), 8.19 (1H, s, NH Aib(3)), 8.14 (1H, s, NH Aib(5)), 7.94 (1H, bs, NH Leu(9)), 7.85 (1H, bs, NH Δ^ZPhe(2)), 7.63 (1H, bs, NH Leu(10)), 7.60−7.21 (24H, m, 4 × (C^βH + phenyl) Δ^Z-Phe), 5.30 (1H, s, NH Aib(1)), 4.56 (2H, m, 2 × C^αH Leu(9) + Leu-(10)), 3.67 (3H, s, $COOCH_3$), 2.0−1.7 (6H, m, 2 × ($C^\beta H_2$ + C^γH) Leu(9) + Leu(10)), 1.71 + 1.64 + 1.60 + 1.46 + 1.38 + 1.26 + 1.19 (24H, s + s + s + bs + bs + s + bs, 8 × CH_3 Aib), 1.43 (9H, s, 3 × CH_3 Boc), 0.92 + 0.84 (12H, m, 4 × CH_3 Leu(9) + Leu(10)). FT-IR (cm^{-1}, in KBr): 3277, 1740, 1659, 1624, 1534.

H-(Aib-Δ^ZPhe)$_4$-L-Leu$_2$-OMe (2). Boc group of decapeptide **6** was removed in a manner similar to that of peptide **1**. Yield 83%. R_f^A 0−0.12; R_f^B 0.59−0.80; R_f^C 0.37; R_f^D 0.53. Anal. Calcd. for $C_{65}H_{82}N_{10}O_{11}$ H_2O: C 65.20, H 7.07, N 11.70. Found: C 65.09, H 7.01, N 11.56. 600 MHz ^{1}H NMR (δ, in $CDCl_3$): 9.36 (1H, s, NH Δ^ZPhe(4)), 9.12 (1H, s, NH Δ^ZPhe(6)), 9.09 (1H, s, NH Δ^ZPhe(8)), 8.40 (1H, s, NH Aib(7)), 8.17 (1H, s, NH Aib(5)), 8.06 (1H, s, NH Leu(9)), 7.68 (1H, s, NH Leu(10)), 7.47 (1H, s, NH Aib(3)), 7.57−7.20 + 6.93 (24H, m + s, 4 × (C^βH + phenyl) Δ^ZPhe), 4.51 (1H, m, C^αH Leu(10)), 4.42 (1H, m, C^αH Leu(9)), 3.66 (3H, s, $COOCH_3$), 2.0−1.6 (6H, m, 2 × ($C^\beta H_2$ + C^γH) Leu(9) + Leu(10)), 1.7 + 1.61 + 1.57 + 1.39 + 1.32 + 1.22 (24H, s + s + s + s + s + s, 8 × CH_3 Aib), 0.96−0.76 (12H, m, 4 × CH_3 Leu(9) + Leu(10)). FT-IR (cm^{-1}, in chloroform; [**2**] = 1.0 mM): 3285 (br), 1732, 1659, 1626, 1537; (cm^{-1}, in KBr): 3275, 1741, 1659, 1625, 1536. In the NOESY 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(6) NH to 100% was as follows: 6.9 (*3-4*), 6.7 (*4-5*), 11.8 (*5-6*), 10.6 (*6-7*), 12.2 (*7-8*), 6.9 (*8-9*), and 12.2 (*9-10*); in other cross-peaks, 10.8 for $N_9H-C^\alpha{}_9H$, 8.7 for $N_{10}H-C^\alpha{}_{10}H$, and 6.0 for $C^\alpha{}_9H-N_{10}H$.

Spectroscopic Measurements. ^{1}H NMR spectra were recorded on Bruker DRX-600 (600 MHz) or DPX-200 (200 MHz) spectrometers at 299 K for peptide **1** (5 mM) in 8.9 vol %-$(CD_3)_2SO/CDCl_3$ and for peptide **2** (9 mM) in $CDCl_3$. All chemical shifts in parts per million (ppm) were determined using tetramethylsilane as an internal standard. NOESY spectra were measured on Bruker DRX-600 (600 MHz) using a Bruker standard pulse program (noesytp)[7] with a mixing time of 700 ms, 64 transients per t_1, 2 K data points in the t_2 domain, and 256 points in the t_1 domain. The data processing and analysis were performed with the XWINNMR software (ver 2.5). FT-IR spectra were recorded on a JASCO FT/IR-430 spectrometer in KBr, in 9 vol %-$(CH_3)_2SO$/chloroform for peptide **1**, and in chloroform for peptide **2**. CD and UV spectra were recorded at ambient temperature (17−20 °C) for a chloroform solution of peptide (0.14 mM) containing various amounts of carboxylic acid (0−200 mM) on JASCO J-500 and JASCO V-550 spectrometers, respectively.

Conformational Energy Calculation. Energy-minimized conformations of peptides **1** and **2** were obtained using the semiempirical molecular orbital (MO) calculation (AM1 method)[8] in MOPAC97.[8] The minimization with a MOPAC97 keyword of MMOK was carried out for the variables of all bond lengths, bond angles, and torsion angles. An initial conformation of L-Leu and L-Leu_2 moieties for the AM1 calculation was obtained using the modified PEPCON[9] for conformational energy calculation on Δ^ZPhe-containing peptides, and that of -(Aib-Δ^ZPhe)$_4$- was set to a standard left-handed 3_{10}-helix for **1** (ϕ = 60.0°, ψ = 30.0°, and ω = 180.0°) and to a standard right-handed one for **2** (ϕ = −60.0°, ψ = −30.0°, and ω = 180.0°)[10] on the basis of the experimental data.

Results and Discussion

Conformation of Peptides 1 and 2. The achiral segment -(Aib-Δ^ZPhe)$_4$- in both peptides can be expected to generate two "enantiomeric" (left- and right-handed) helices, since oligopeptides bearing -(Aib-Δ^ZPhe)$_m$- (m = 2 or 4) were found to form a 3_{10}-helical structure in solution and in the solid states.[3,5,11] Actually, a helical conformation of peptides **1** and **2** was evidenced by ^{1}H NMR and FT-IR spectroscopy in solution. NOESY spectra of both peptides gave marked cross-peaks of

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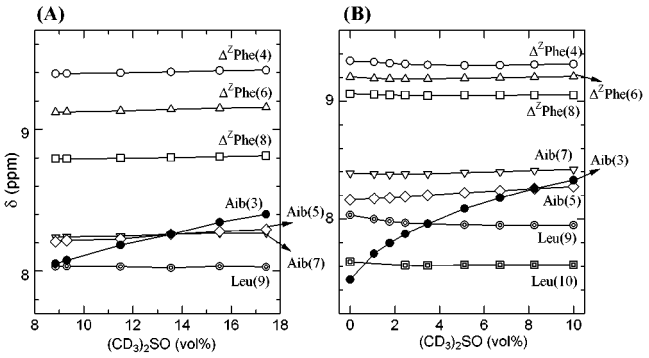

Figure 1. Solvent dependence on NH chemical shifts of **(A)** peptides **1** and **(B) 2** in $CDCl_3/(CD_3)_2SO$ mixtures. The Δ^ZPhe(2) NH resonance could not be observed due to its broadening. The titration experiment for peptide **1** started at 8.9 vol % of $(CD_3)_2SO$, because of its less solubility in pure $CDCl_3$.

$N_iH-N_{i+1}H$ resonances in the segment of Aib(3) to Δ^ZPhe(8), thus indicating the presence of 3_{10}- or α-helix.[12] Figure 1 shows the variation in NH chemical shifts of peptides **1** and **2** with concentration of $(CD_3)_2SO$[13] in $CDCl_3$.

Six NH resonances of Δ^ZPhe(4) to Leu(9) residues for peptide **1** and seven NHs of Δ^ZPhe (4) to Leu(10) residues for **2** are shielded from solvent due to intramolecular hydrogen bonding, of which the pattern corresponds to a 3_{10}-helix[14] supported by consecutive $(i + 3) \rightarrow i$ hydrogen bonds starting from NH Δ^Z-Phe(4) → CO Aib(1). The helical conformation was also supported by the positions of amide I absorption bands of their FT-IR spectra in solution: 1659 and 1627 cm^{-1} for **1**, and 1659 and 1625 cm^{-1} for **2**, which can be assigned to saturated amino acid and Δ^ZPhe residues,[15] respectively. Furthermore, as shown in Figure 2,[16] energy minimization of both peptides gave a 3_{10}-helical conformation for the achiral heptapeptide segment Δ^Z-Phe(2)−Δ^ZPhe(8): average values for Δ^ZPhe(2)−Δ^ZPhe(8) residues are $\langle\phi\rangle = 38.3°$, $\langle\psi\rangle = 41.3°$, and $\langle\omega\rangle = 178.9°$ for peptide **1**, and $\langle\phi\rangle = -39.1°$, $\langle\psi\rangle = -41.2°$, and $\langle\omega\rangle = 179.7°$ for peptide **2**. In both energy-minimized peptides, six NHs of Δ^ZPhe(4)-Leu(9) residues participated in consecutive $(i + 3) \rightarrow i$ hydrogen bonds starting from NH Δ^ZPhe(4) → CO Aib(1), appropriate for a 3_{10}-helix.

Covalent Domino Effect on Helical Screw Sense. The preferred screw sense of peptides **1** and **2** prior to addition of chiral carboxylic acid was investigated. Peptide **1** in chloroform showed an exciton splitting centered at around 280 nm assignable to Δ^ZPhe residue (Figure 3).

On the basis of the exciton chirality method[17] and theoretical CD calculation,[3,11a,18] the split CD sign corresponds to a left-handed screw sense for a 3_{10}- or α-helix. Thus, the C-terminal L-Leu residue induces a left-handed screw sense for the achiral helical segment -(Aib-Δ^ZPhe)$_4$- through the covalent domino effect. The left-handedness induced by a C-terminal L-residue has also been observed in Aib peptides containing one L-residue in the C-terminal position,[19] or in an analogous peptide Boc-(Aib-Δ^ZPhe)$_2$-L-Leu-OMe in solution.[5] Schellman also noted that many right-handed helical segments in proteins ended with a residue in a left-handed conformation.[20] According to the rational mechanism proposed in ref 19a and 19b, the left-handed screw sense induced by a C-terminal L-residue ester should be interpreted on the basis of an unfavorable $O_{(i-2)}\cdots O_i$ interaction taking place between the carbonyl oxygen atom of (*i-2*)th residue from the C-terminus and either oxygen atom of the ester group of the C-terminal *i*th residue if the sign of the ϕ_i angle of the *i*th residue is the same as that of the preceding 3_{10}-helical segment. This unfavorable interaction can be removed by the ϕ_i angle with the opposite sign of the ϕ_{i-2} angle. Thus, because an L-amino acid strongly tends to adopt negative ϕ_i values, a left-handed screw sense (positive ϕ_{i-2}) should be induced for the preceding achiral segment. A similar mechanism should be applied to the left-handed screw sense of peptide **1** because the energy-minimized conformation (Figure 2A) gives $\phi_9 = -124°$ for the C-terminal L-Leu(9) residue and $\phi_7 = +43°$ for the preceding Aib(7) residue, leading to the disappearance of an unfavorable $O_7\cdots O_9$ interaction. This negatively large ϕ_9 value should be supported by a large J_{NH-CH} value of L-Leu(9) (7.7 Hz), which corresponds to $\phi_9 = -88°$.[21]

On the other hand, peptide **2** showed a strong exciton splitting assignable to a right-handed screw sense, thus indicating that the chiral dipeptide segment induces predominantly a right-handed screw sense for the preceding achiral segment. Interestingly, the covalent chiral domino effect induced by the C-terminal L-Leu$_2$ segment is dramatically different from that by the C-terminal L-Leu residue in the determination of the whole screw sense. This finding might also clarify a role of C-terminal chiral L−L doublet in the choice of a right-handed screw sense for the preceding segment, or more generally in one of protein folding mechanisms. In the energy-minimized conformation (Figure 2B), the L-Leu$_2$ segment adopts a non-helical conformation characterized by $\phi_9 = -89°$, $\psi_9 = 50°$, $\phi_{10} = -119°$, and $\psi_{10} = -49°$. Here, the proton pair of $C^\alpha H$ Leu(9)−NH Leu(10) is close to each other (2.8 Å), which is supported by the observation of a marked NOE signal for the corresponding proton pair (6.0%). In addition, the negatively large ϕ_9 value should be supported by a large J_{NH-CH} value of L-Leu(9) (7.9 Hz in 200 MHz ^{1}H NMR spectrum in $CDCl_3$), which corresponds to $\phi_9 = -90°$.[21] Further systematic investigations on covalent chiral domino effects on helical screw

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(15) FT-IR absorption data of Δ^ZPhe-containing peptides taking a 3_{10}-helical conformation in solution were presented.[15a] Unlike helical oligopeptides consisting of only saturated amino acids,[15b] two characteristic peaks in an amide I band region were observed: first peak at ca. 1665−1655 cm^{-1} and second peak at ca. 1628−1625 cm^{-1}, of which are assigned to saturated amino acid and Δ^ZPhe residues in helical segments, respectively. A shift to lower wavenumbers in the second peak should be ascribed to partial contribution of resonance between carbonyl and styryl groups in a Δ^ZPhe residue. See: (a) Inai, Y.; Sakakura, Y.; Hirabayashi, T. *Polym. J.* **1998**, *30*, 828. (b) Kennedy, D. F.; Crisma, M.; Toniolo, C.; Chapman, D. *Biochemistry* **1991**, *30*, 6541. Furthermore, two dehydropeptides adopting a typical 3_{10}-helix in the crystalline state[11b] showed similar absorption bands in the solid state: i.e., for Boc-(Aib-Δ^ZPhe)$_2$-Aib-OMe, 1662 and 1628 cm^{-1}; for Boc-L-Pro-(Aib-Δ^ZPhe)$_2$-Aib-OMe, 1660 and 1629 cm^{-1}.
(16) The molecular graphics were illustrated using the molecular modeling software: Butch Software Studio *FREE WHEEL for Windows: 0.60E* for Molecular Modeling Software, Japan, 2001.
(17) (a) Harada, N.; Chen, S. L.; Nakanishi, K. *J. Am. Chem. Soc.* **1975**, *97*, 5345. For CD analysis of Δ^ZPhe-containing peptides, see: (b) Pieroni, O.; Fissi, A.; Jain, R. M.; Chauhan, V. S. *Biopolymers* **1996**, *38*, 87.
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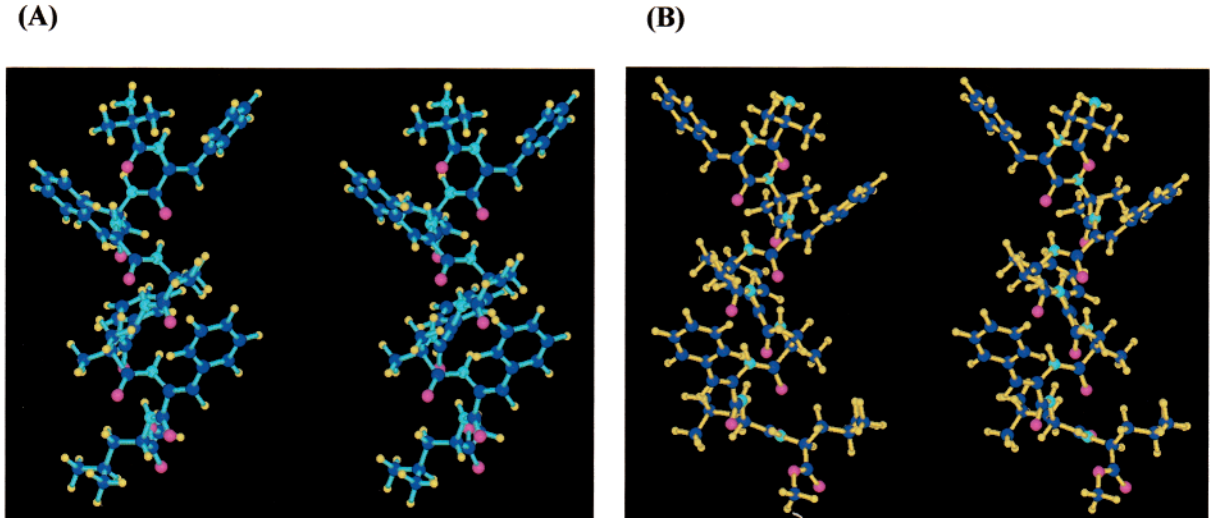


Figure 2. Stereoviews of energy-minimized conformations of **(A)** peptides **1** and **(B) 2** by the semiempirical MO calculation (AM1 method).[8] Peptide **1** gives a left-handed 3_{10}-helical conformation for the achiral heptapeptide segment Δ^ZPhe(2)-Δ^ZPhe(8), and peptide **2** gives a right-handed 3_{10}-helical conformation for the achiral segment.

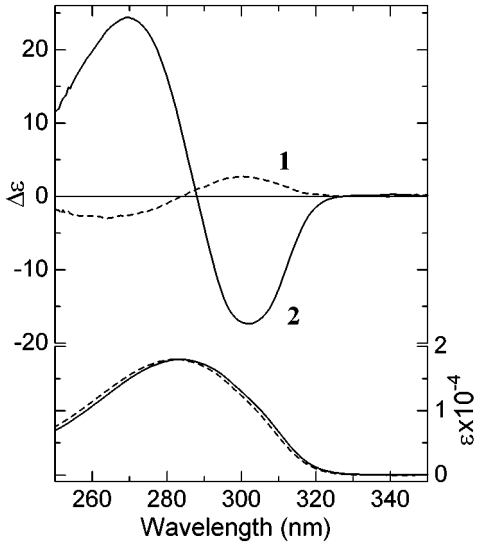


Figure 3. CD (top) and UV absorption (bottom) spectra of peptides **1** (dashed line) and **2** (solid line) in chloroform.

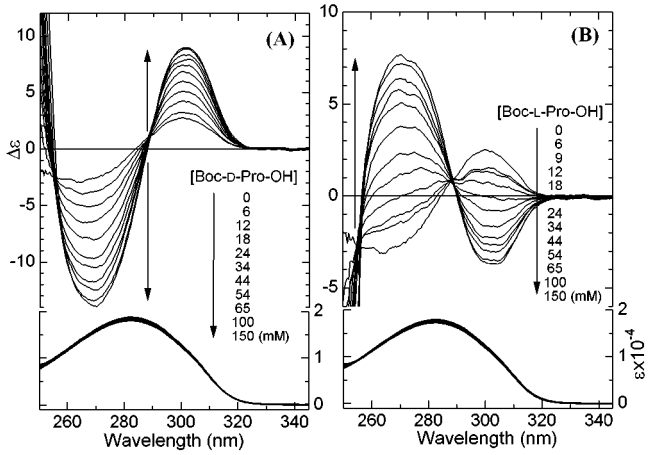


Figure 4. CD (top) and UV absorption (bottom) spectra of peptide **1** in chloroform containing **(A)** Boc-D-Pro-OH or **(B)** Boc-L-Pro-OH: [**1**] = 0.14 mM and [Boc-Pro-OH] = 0−150 mM. $\Delta\epsilon$ and ϵ are expressed with respect to the molar concentration of Δ^ZPhe residues.

sense are in progress. However, we can conclude that the single C-terminal L-Leu residue gives a small excess of a left-handed screw sense for the preceding achiral segment, and that the C-terminal L-Leu doublet induces strongly a right-handed screw sense.

Noncovalent Domino Effect in Peptide 1. As described above, peptide **1** alone prefers a left-handed screw sense in chloroform through the C-terminal covalent domino effect. Here, the addition of chiral carboxylic acid (Boc-Pro-OH) leads to a dramatic change in the original CD spectrum, as shown in Figure 4.

On the addition of Boc-D-Pro-OH (Figure 4A), the split CD sign corresponding to a left-handed screw sense was retained, but the split amplitude markedly increased: ca. 5-fold at [Boc-D-Pro-OH] = 150 mM. Obviously, the original left-handed helix induced by the C-terminal covalent domino effect can be markedly stabilized by the chiral stimulus of Boc-D-Pro-OH. Conversely, the addition of Boc-L-Pro-OH gives rise to a remarkable helix-to-helix transition from left- to right-handed screw sense, as shown in Figure 4B. The amplitude of split CD signals with a positive peak at longer wavelengths decreased with an increase in [Boc-L-Pro-OH] of 0−12 mM. Then an opposite split CD pattern with a negative peak at longer wavelengths began to appear at [Boc-L-Pro-OH] = 18 mM, and the split amplitude increased with further addition of Boc-L-Pro-OH (24−150 mM). Therefore, the chiral stimulus of Boc-L-Pro-OH gives rise to destabilization of the original left-handed helix, and subsequently leads to a right-handed helix. The directions of the helical screw senses induced by Boc-D-Pro-OH and Boc-L-Pro-OH agreed with those observed for achiral peptide H-(Aib-Δ^ZPhe)$_4$-Aib-OMe.[3] Similar tendency was observed for addition of other chiral Boc-amino acids (Ala, Leu, Val, and Phe), as shown in Figure 5 and Table 1. Namely, Boc-L-amino acid tends to destabilize the original left-handed helix or lead to a helix-to-helix transition, whereas the corresponding Boc-D-amino acid stabilizes the left-handed helix.

N-Boc-protected peptide **1**, Boc-(Aib-Δ^ZPhe)$_4$-L-Leu-OMe, also showed a split CD spectrum similar to that of peptide **1** preferring a left-handed screw sense. However, the CD pattern and intensity were unaffected by the addition of a large excess (480-fold) of chiral Boc-Pro-OH. Obviously, the N-terminal

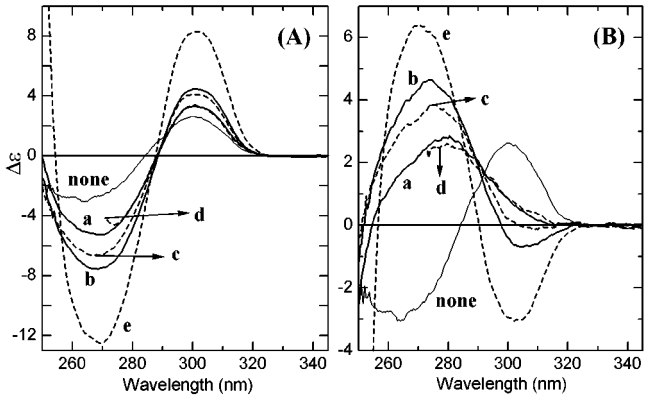


Figure 5. CD spectra of peptide **1** in chloroform containing **(A)** Boc-D-amino acid (**(a)** Ala, **(b)** Leu, **(c)** Val, **(d)** Phe, and **(e)** Pro) or **(B)** the corresponding Boc-L-amino acid: [**1**] = 0.14 mM; [Boc-amino acid] = 65 mM. The CD spectra of the mixtures containing Boc-L (or D)-Phe-OH are not shown below 272 nm due to overlap of the peptide and Boc-L (or D)-Phe-OH signals.

Table 1. Signs of Splitting Cotton Effects and $\Delta\epsilon$ Values for Induced CD of Peptide **1** with Chiral Carboxylic Acid[a]

	first Cotton effect		second Cotton effect	
acid	sign	$\Delta\epsilon$ (λ/nm)	sign	$\Delta\epsilon$ (λ/nm)
none	+	2.5/300	−	2.8/264
Boc-D-Pro-OH	+	8.3/301	−	12.4/270
Boc-D-Ala-OH	+	3.4/301	−	5.3/269
Boc-D-Val-OH	+	4.1/301	−	6.6/268
Boc-D-Leu-OH	+	4.5/301	−	7.5/268
Boc-D-Phe-OH	+	3.5/301	−	[b]
Boc-L-Pro-OH	−	3.0/302	+	6.4/270
Boc-L-Ala-OH		[c]	+	2.8/279
Boc-L-Val-OH	−	0.1/309	+	3.9/275
Boc-L-Leu-OH	−	0.7/304	+	4.7/273
Boc-L-Phe-OH		[c]	+	2.6/279

[a] [**1**] = 0.14 mM and [Boc-amino acid] = 65 mM in chloroform. [b] Overlapped with CD signal of Boc-D-Phe-OH. [c] Not observed.

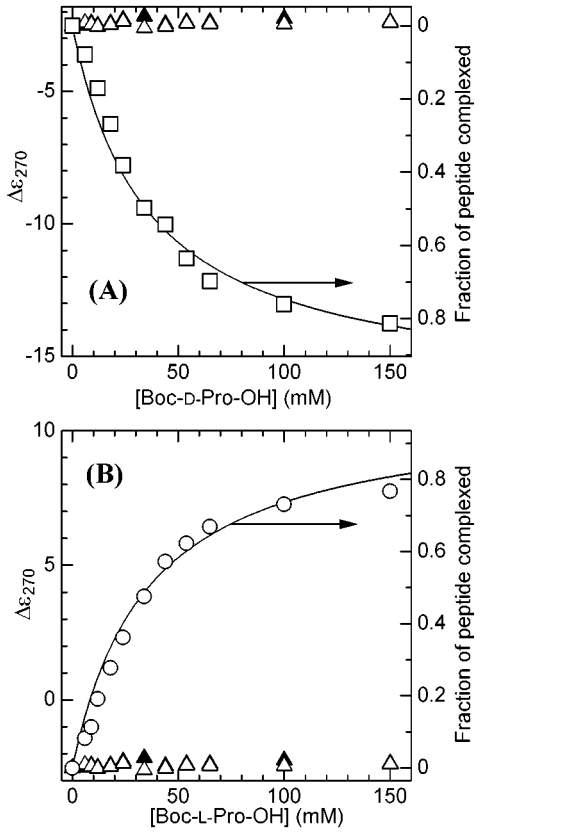


Figure 6. Titration curves of the values of induced CD signals at 270 nm ($\Delta\epsilon_{270}$) in the complexation of peptide **1** (0.14 mM) with **(A)** Boc-D-Pro-OH (open square), or **(B)** Boc-L-Pro-OH (open circle) in chloroform. Titration curves of $\Delta\epsilon_{270}$ in the mixture of peptide **1** with Boc-Gly-OH (closed triangle) and with Boc-DL-Pro-OH (D/L = 50/50; open triangle) are plotted. The fraction of peptide **1** complexed (solid line) is also superimposed; the fraction was calculated using the binding constant ($K = 28\ M^{-1}$)[3] between Boc-L-Pro-OH and H-Aib-OMe for the model compound of an N-terminal moiety of peptide **1**, according to ref 3.

amino group for interacting with a chiral carboxylic acid is required for controlling the original helical screw sense of chiral peptide **1**. Figure 6 shows the CD titration curves upon the complexation of peptide **1** with Boc-D-Pro-OH (Figure 6A) or Boc-L-Pro-OH (Figure 6B) in chloroform.

The CD intensity increased remarkably with an increase in the concentration of chiral Boc-Pro-OH and reached a saturation value over 100 mM essentially. Also, the CD spectrum of peptide **1** was almost unaffected by adding a large excess of achiral Boc-Gly-OH (ca. 40−1100-fold) instead of chiral Boc-amino acid (Figure 6), thus indicating that achiral acid−base interaction does not influence the helical screw sense or helical stability of chiral peptide **1** essentially. Racemic Boc-DL-Pro-OH (D/L = 50/50) also gave no marked changes in the original CD spectrum (Figure 6), implying that the binding affinity of peptide **1** to Boc-D-Pro-OH is almost the same as that to Boc-L-Pro-OH. Moreover, the CD amplitudes ($\Delta\epsilon_{270}$) induced by chiral Boc-Pro-OH could be essentially attributed to the fraction of peptide **1** complexed (Figure 6); the fraction was calculated using the binding constant ($K = 28\ M^{-1}$)[3] between Boc-L-Pro-OH and H-Aib-OMe for the model compound of an N-terminal moiety of peptide **1**, according to ref 3. Therefore, the chiral acid−base interaction at the N-terminal position, i.e., noncovalent domino effect, controls the original left-handed screw sense of chiral peptide **1**, as shown in Chart 1A. In peptide **1**, the N-terminal noncovalent effects give a tug at the original screw sense arising from the C-terminal covalent effect toward their preferred ones.

Noncovalent Domino Effect in Peptide 2. Figure 7 shows the effect of chiral carboxylic acid on CD spectra of peptide **2** in chloroform.

In contrast to peptide **1**, peptide **2** retains the original right-handed screw sense on the addition of Boc-D-Pro-OH or Boc-L-Pro-OH. This should be ascribed to a strong propensity of peptide **2** to adopt a right-handed helical conformation. Thus, noncovalent chiral interaction seems not to be strong enough to induce a dramatic helix-to-helix transition in a chiral peptide adopting a stable one-handed helix. However, the split amplitude of the original CD spectrum increased markedly with an increase in the Boc-L-Pro-OH concentration, whereas the addition of Boc-D-Pro-OH did not change the amplitude essentially. This strongly implies that the original right-handed helix is more stabilized by the chiral stimulus of Boc-L-Pro-OH. Similar tendency was observed for addition of other chiral Boc-amino acids (Ala, Leu, Val, and Phe), as shown in Figure 8 and Table 2. Namely, Boc-L-amino acids tend to more stabilize the original right-handed helix, compared with the corresponding Boc-D-amino acids.

To eliminate effect of achiral acid−base interaction on CD spectra, the split CD amplitude was plotted against concentra-

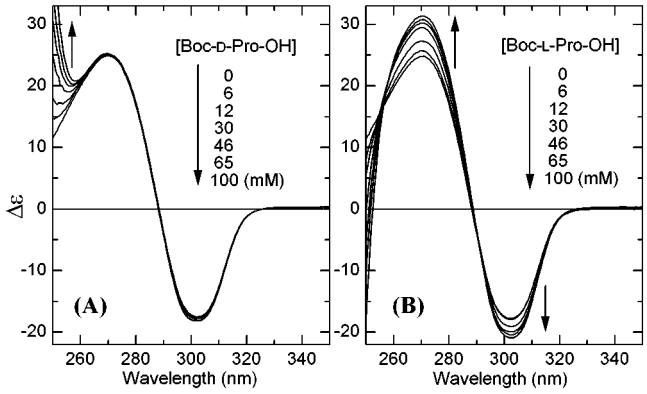


Figure 7. CD spectra of peptide **2** in chloroform containing **(A)** Boc-D-Pro-OH and **(B)** Boc-L-Pro-OH: [**2**] = 0.14 mM and [Boc-Pro-OH] = 0−100 mM.

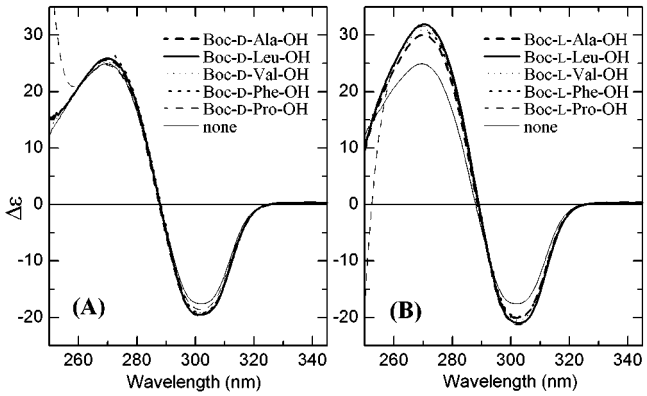
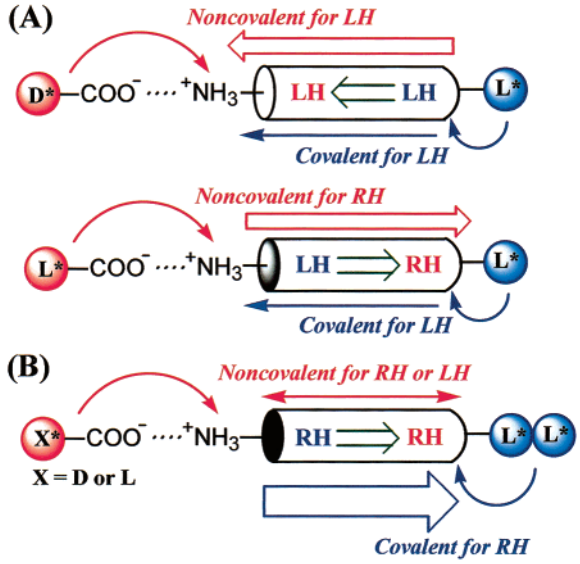


Figure 8. CD spectra of peptide **2** in chloroform containing **(A)** Boc-D-amino acid (Ala, Leu, Val, Phe, and Pro) or **(B)** the corresponding Boc-L-amino acid: [**1**] = 0.14 mM; [Boc-amino acid] = 65 mM. The CD spectra of the mixtures containing Boc-L (or D)-Phe-OH are not shown below 272 nm due to overlap of the peptide and Boc-L (or D)-Phe-OH signals.

Chart 1. Preferred Helical Screw Sense of Chiral Peptides **1 (A)** and **2 (B)** through Both Noncovalent and Covalent Domino Effects; the Directions of Arrows Represent the Induction of Left- (LH) or Right-handed (RH) Screw Sense.



tions of Boc-D-Pro-OH, Boc-L-Pro-OH, and achiral Boc-Gly-OH, as shown in Figure 9A.

Compared with the titration curve of Boc-Gly-OH, Boc-D-Pro-OH destabilizes somewhat the original right-handed helix,

Table 2. Signs of Splitting Cotton Effects and Δε Values for Induced CD of Peptide **2** with Chiral Carboxylic Acid[a]

	first Cotton effect		second Cotton effect	
acid	sign	Δε (λ/nm)	sign	Δε (λ/nm)
none	−	17.7/302	+	24.8/270
Boc-D-Pro-OH	−	18.5/302	+	24.6/270
Boc-D-Ala-OH	−	19.5/301	+	25.6/270
Boc-D-Val-OH	−	19.1/301	+	25.1/270
Boc-D-Leu-OH	−	19.4/301	+	25.9/270
Boc-D-Phe-OH	−	19.1/302	+	[b]
Boc-L-Pro-OH	−	21.3/302	+	31.6/270
Boc-L-Ala-OH	−	20.0/302	+	30.1/270
Boc-L-Val-OH	−	20.5/302	+	31.0/270
Boc-L-Leu-OH	−	21.0/303	+	31.9/271
Boc-L-Phe-OH	−	20.0/302	+	[b]

[a] [**2**] = 0.14 mM and [Boc-amino acid] = 100 mM in chloroform. [b] Overlapped with CD signal of Boc-L (or D)-Phe-OH.

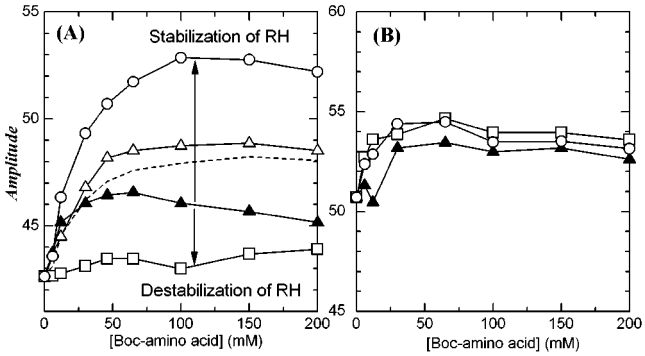


Figure 9. **(A)** Titration curves of the amplitudes of split CD signals in the complexation of peptide **2** (0.14 mM) with Boc-D-Pro-OH (open square), Boc-L-Pro-OH (open circle), Boc-DL-Pro-OH (D/L = 50/50; open triangle), or Boc-Gly-OH (closed triangle) in chloroform. The dashed line represents the arithmetic mean of the two amplitudes induced by Boc-D-Pro-OH (open square) and Boc-L-Pro-OH (open circle). **(B)** Titration curves of the amplitudes of split CD signals in the complexation of N-Boc-protected peptide **2** (0.14 mM) with Boc-D-Pro-OH (open square), Boc-L-Pro-OH (open circle), or Boc-Gly-OH (closed triangle) in chloroform.

whereas Boc-L-Pro-OH stabilizes it markedly. The directions of the helical screw senses induced by Boc-L-Pro-OH and Boc-D-Pro-OH agreed with those observed for achiral peptide H-(Aib-Δ^ZPhe)$_4$-Aib-OMe.[3] In Figure 9A, the CD amplitude induced by Boc-DL-Pro-OH (D/L = 50/50) was slightly larger over the concentration range of Boc-Pro-OH (0−200 mM) than the arithmetic mean (dashed line) of the two amplitudes induced by Boc-D-Pro-OH and Boc-L-Pro-OH. Because Boc-L-Pro-OH induces a right-handed screw sense in peptide **1** and achiral peptide H-(Aib-Δ^ZPhe)$_4$-Aib-OMe,[3] peptide **2** exhibiting a strong bias in favor of a right-handed screw sense might slightly prefer the binding to Boc-L-Pro-OH rather than to Boc-D-Pro-OH. N-Boc-protected peptide **2**, Boc-(Aib-Δ^ZPhe)$_4$-L-Leu$_2$-OMe, also showed a split CD spectrum corresponding to a right-handed screw sense. Although addition of Boc-L-Pro-OH, Boc-D-Pro-OH, or Boc-Gly-OH slightly increased its original CD amplitude, the three titration curves (Figure 9B) did not differ from one another essentially. Obviously, the N-terminal amino group is required for controlling the stabilization of the original right-handed helix. Therefore, noncovalent chiral domino effect in peptide **2** can contribute even to the helical stability of a chiral peptide prevailing one-handed helix strongly, as shown in Chart 1B.

Conclusions

In the present work, we have demonstrated, for the first time, the importance of noncovalent chiral domino effect operating on the N-terminal position of chiral peptides. This effect is capable of controlling the original helical screw sense or helical stability of a chiral peptide, thus providing novel insights into the nature of chiral intermolecular interactions of a helical peptide with a chiral molecule in peptide and protein science. In particular, the effect is sensitive to a chiral peptide exhibiting a small excess of one-handed helix; i.e., peptide **1** possessing only one chiral residue undergoes a dramatic helix-to-helix transition or remarkable stabilization of one-handed helix upon complexation with chiral carboxylic acid, depending on its chirality. Second, we here have presented a unique system that both noncovalent and covalent domino effects operate simultaneously on the helical screw sense of a single achiral segment and have compared both powers for inducing the screw sense bias. As a result of the tug-of-war game on the achiral helical chain, the chiral domino effect governing the whole screw sense is the N-terminal noncovalent type for peptide **1**, and the C-terminal covalent type for peptide **2**, as shown in Chart 1.

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