Conformational Preference in β-Aryldehydroalanine. Synthesis and Conformational Study of Tripeptides Containing β-Aryldehydroalanine Residues

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(Received December 18, 1995)

In order to investigate the effect of bulky β-substituents on the conformational preference in α, β-dehydroamino acid residues, three kinds of tripeptides containing (*Z*)-β-phenyldehydroalanine, (*Z*)-β-(1-naphthyl)dehydroalanine, or (*Z*)-β-(1-pyrenyl)dehydroalanine residue were synthesized: Boc–Ala–Δ^ZAA–Val–OMe (Boc, *t*-butoxycarbonyl; Ala, L-alanine; Δ^ZAA, (*Z*)-β-aryldehydroalanine; Val, L-valine; OMe, methoxy). Their conformations in solution were investigated using ^{1}H NMR spectroscopy. The solvent accessibility of the NH resonances and the nuclear Overhauser effect (NOE) indicated that the three peptides in $CDCl_3$ form a type II β-turn conformation supported by hydrogen bonding between CO (Boc) and NH (Val). From a conformational energy calculation, the three kinds of Δ^ZAA residues were also shown to favor a type II β-turn conformation. In each β-turn, the orientation of the aryl plane was found to be non planar relative to the C^α=C^β–C^γ plane, suggesting that a steric interaction between the β-aryl group and the peptide backbone leads to the internal rotation angles preferred for the β-turn backbone.

α,β-Dehydroamino acid (unsaturated) residues are naturally present in many peptides having biological activity as well as in some proteins.[1—5] When these residues are introduced into a peptide composed of saturated residues, the biological activity and conformation change remarkably. This is mainly based on the presence of a C^α=C^β double bond that gives not only a specific chemical property, but also an inherent conformational preference. Thus, dehydroresidues attract much interest as an important element for effectively designing specific secondary structures in peptide chemistry.

Their conformational preference is mainly ascribed to the structural feature of the double bond, e.g., the planarity around C^α=C^β and the trigonal geometry on C^α. In addition, the size of the β-substituent in dehydroresidues (X in Chart 1) affects their conformational preference. When a dehydroresidue is introduced into a small saturated peptide (mostly, di- or tripeptides), its conformation depends on the β-substituent as follows. (*Z*)-β-Phenyl-α,β-dehydroalanine (Δ^ZPhe) residue having a β-phenyl group favors a β-turn conformation.[6—9] (*Z*)-α,β-Dehydroleucine (Δ^ZLeu) residue having a β-isopropyl group also induces a β-turn conformation.[10,11] On the other hand, dehydroalanine (Δ Ala) without a β-substituent adopts an extended conformation,[12—14] and induces an inverse γ-turn in the preceding residue.[15,16] Peptides containing (*Z*)- or (*E*)-2-amino-2-butenoic acids (Δ^ZAbu or Δ^EAbu) with a β-methyl group tend to take an extended conformation.[17] On the other hand, little is known about the conformational preference in dehydroresidues having a larger β-substituent than a phenyl group. Such a β-substituent effect on the conformational preference in dehydroresidues is important for rationally designing a wide variety of secondary structures and bioactive peptides containing β-functional groups.

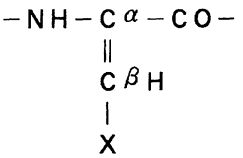
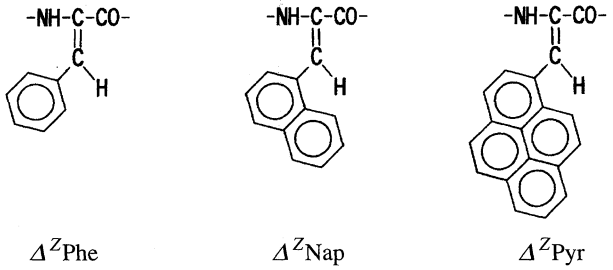


Chart 1. α,β-Dehydroresidue.

For this purpose, we adopt here three kinds of tripeptides **1**—**3** containing Δ^ZPhe, (*Z*)-β-(1-naphthyl)dehydroalanine (Δ^ZNap), and (*Z*)-β-(1-pyrenyl)dehydroalanine (Δ^ZPyr)



Δ^ZPhe Δ^ZNap Δ^ZPyr

Boc–Ala–Δ^ZPhe–Val–OMe **1**
Boc–Ala–Δ^ZNap–Val–OMe **2**
Boc–Ala–Δ^ZPyr–Val–OMe **3**
(Boc, *t*-butoxycarbonyl; Ala, L-alanine; Val, L-valine; OMe, methoxy)

Chart 2.

residues, respectively (Chart 2). Tripeptides containing a Δ^ZPhe residue at the second position, an analog of peptide **1**, have been found to take a β-turn conformation in both the solid state and in solution.[6—9] We intend to reveal whether tripeptides **2** and **3** having more bulky β-substituents, 1-naphthyl and 1-pyrenyl groups, retain such a β-turn structure. In addition, the naphthyl and pyrenyl groups would expand the π-conjugation system, compared with the phenyl group. According to theoretical studies,[18,19] dehydroresidues have large torsional barriers about the N–C^α and C^α–CO bonds due to π-conjugation between C^α=C^β and the amide group. These barriers remarkably affect the conformational preference. Thus, we also note the effect of the π-conjugation size on the conformational preference in the (*Z*)-β-aryldehydroalanine (Δ^ZAA) residue. Peptides **1**—**3** have been synthesized, and their conformations have been investigated by ^{1}H NMR studies, such as NH accessibility and nuclear Overhauser effect (NOE), and conformational energy calculation.

Experimental

Spectroscopy. ^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer (200 MHz). The pulse sequence was 6.0-μs pulse width, 5.0-s acquisition time, and 8.0-s delay time. A Hitachi H-90 spectrometer was also used for 90-MHz ^{1}H NMR. Most of the spectra were determined at 23 °C for $CDCl_3$ solutions of 20 mM peptide concentrations (1 M=1 mol dm^{-3}). All of the chemical shifts were expressed as δ downfield from internal tetramethylsilane (TMS). The temperature dependence was observed in the range of 23—38 °C. A difference NOE experiment was carried out on an XL-200 spectrometer using the standard Varian software library. Each NH resonance on the main chain of the peptides was irradiated. 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.

Conformational Energy Calculation. An empirical conformational energy calculation was carried out using structural and energy parameters based on the ECEPP system.[20] The parameters for the Δ^ZPhe residue were determined in previous studies.[21,22] On the other hand, no available parameters for the Δ^ZNap and Δ^ZPyr residues have been reported. To obtain qualitative information concerning the conformational preference in the Δ^ZNap and Δ^ZPyr residues, a tentative adaptation was used. The atomic coordinates and partial charges of the β-aryl group in 1-naphthylalanine[23] and 1-pyrenylalanine[24] were used for those of the β-aryl group in the Δ^ZNap and Δ^ZPyr residues, respectively, while those of the Δ^ZPhe moiety, except for the β-phenyl group, were used for the Δ^ZNap and Δ^ZPyr residues. The twofold rotational barriers were taken to have the same values as the Δ^ZPhe residue: 10 kcal mol^{-1} about the N–C^α bond, 8 kcal mol^{-1} about the C^α–CO, and 6.4 kcal mol^{-1} about the C^β–C^γ.[13,15,25] The program PEPCON, written by M. Sisido[26] for a conformational-energy calculation[20] and graphics[27] of a given peptide, was modified to be applicable to β-aryldehydroalanine-containing peptides.

The conformational energy of peptides **1**—**3** was calculated for Ac–Ala–Δ^ZAA–NMA (Ac, acetyl; NMA, *N*-methylamide). Here, all combinations of the energy minima in Ala and Δ^Z AA residues were used as the starting conformations. Minimization was carried out for five variables (ϕ_{Ala}, ψ_{Ala}, ϕ_{Δ^ZAA}, ψ_{Δ^ZAA}, and χ_{2,Δ^ZAA}) using the Simplex algorithm (Chart 3). Based on crystallographic data

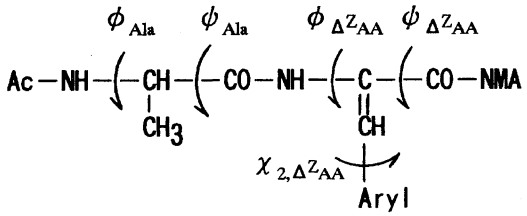


Chart 3.

of the Δ^ZPhe–containing peptides,[8,9] each amide group was fixed to the *trans* conformation (ω=180°), and each Δ^ZAA side chain (χ_1) was fixed to 0°. The minimized conformations were also expressed by the conformational letter code[28] that divides 16 regions in conformational space. A similar energy minimization was also carried out on Ac–Ala–Δ^ZAA–Val–OMe with ten variables (ϕ_{Ala}, ψ_{Ala}, ϕ_{Δ^ZAA}, ψ_{Δ^ZAA}, χ_{2,Δ^ZAA}, ϕ_{Val}, ψ_{Val}, $\chi_{1,Val}$, $\chi_{2,Val}$, and $\chi_{3,Val}$) using all combinations of the energy minima in the Ala, Δ^ZAA, and Val residues as the starting conformations.

Synthesis. Peptides **1**—**3** were synthesized in a similar manner to a previously reported procedure.[6,21,22] DL-β-(1-naphtyl)serine (napSer) and DL-β-(1-pyrenyl)serine (pyrSer) were synthesized according to the preparation of DL-β-phenylserine.[29] Boc–Ala–OH, H–napSer–OMe, H–pyrSer–OMe, and TosOH·H–Val–OMe (TosOH, *p*-toluenesulfonic acid) were prepared by the standard procedure. Amino acid couplings were performed by a mixed-anhydride method[30,31] or a dicyclohexylcarbodiimide (DCC)-hydroxybenzotriazole hydrate (HOBt) method.[32,33] The Δ^ZAA residue was introduced according to Ref. 34. All of the final peptides **1**—**3** were checked by ^{1}H NMR and IR spectroscopy, thin-layer chromatography (TLC), and gel permeation chromatography (GPC), as well as elemental analysis. TLC was carried out on precoated silica plates in the following solvent systems: (A) ethyl acetate, (B) chloroform–methanol (9 : 1), and (C) 1-butanol–acetic acid–water (4 : 1 : 1). GPC was recorded on a Tosoh HLC-803D equipped with G1000-, G2500-, G3000-HLX columns in series, using tetrahydrofuran (THF) as an eluent. A single spot in the TLC and a single peak in the GPC were obtained for each of peptides **1**—**3**.

Boc–Ala–Δ^ZPhe–Val–OMe 1. Peptide **1** was synthesized according to Refs. 6, 21, and 22. Mp 154 °C; R_f^A, 0.78; R_f^B, 0.57; R_f^C, 1.0; GPC retention time=25 min. Found: C, 61.63; H, 7.61; N, 9.18%. Calcd for $C_{23}H_{33}N_3O_6$: C, 61.73; H, 7.43; N, 9.39%. IR (NaCl) 3250 (NH), 1740 (C=O), 1700 (C=O), 1650 (C=O), 1620 (C=O), and 1520 cm^{-1} (NH). ^{1}H NMR (200 MHz) δ=0.97 (dd, 6H, 2×CH_3 Val), 1.42 (s, 9H, 3×CH_3 Boc), 1.43 (d, 3H, CH_3 Ala), 2.22 (m, 1H, C^βH Val), 3.74 (s, 3H, OCH_3), 4.24 (m, 1H, C^αH Ala), 4.61 (dd, 1H, C^αH Val), 5.08 (d, 1H, NH Ala), 6.91 (d, 1H, NH Val), 7.2—7.5 (m, 6H, phenyl H+H$^\beta$ Δ^ZPhe), and 7.83 (s, 1H, NH Δ^ZPhe).

H–napSer–OMe. 1-Naphthalenecarbaldehyde (35 g, 0.23 mol) and Gly (9 g, 0.12 mol) were suspended in 1,4-dioxane. To the mixture was added a 6 M NaOH solution (30 mL, 0.18 mol) at 5 °C with vigorous stirring. After the mixture became homogeneous, it was first stirred at 5 °C for 30 min, and then at 15 °C for 1 h to precipitate a white solid. After the mixture was left standing at room temperature for 48 h, the resulting solid mass was treated with a 6 M HCl solution (20 mL) under cooling at 0 °C, and broken up with a stirring rod. To the mixture was added water (50 mL); it was then stirred at 0 °C for 24 h, and neutralized with a 3 M NaOH solution under cooling at 0 °C. The resulting solid was collected, washed successively with water, methanol, and diethyl ether, and dried in vacuo. The yield for the crude product was 20 g (77%).

The crude product (17 g, 0.07 mol) was dissolved in a 6 M HCl methanol solution (100 mL), and then refluxed at 65—70 °C for 72 h. The solvent was evaporated, and the residue was suspended in a 5% $NaHCO_3$ solution, and extracted with ethyl acetate. The extract was washed with 5% $NaHCO_3$, and 10% NaCl solutions, and then dried over $MgSO_4$. Evaporation of the solvent gave white crystals: Yield 10 g (60%); mp 130—134; R_f^A, 0.34; R_f^B, 0.50 R_f^C, 0.66. IR (NaCl) 3100 (NH), and 1740 cm^{-1} (C=O). ^1H NMR (90 MHz) δ=1.8—2.4 (br.s, 2H, NH_2), 3.76 (s, 3H, OCH_3), 3.95 (m, 1H, $C^{\alpha}H$), 5.83 (d, 1H, $C^{\beta}H$), and 7.3—8.2 (m, 7H, naphthyl H).

Boc–Ala–napSer–OMe. Boc–Ala–OH (0.96 g, 5.1 mmol) and H–napSer–OMe (1.2 g, 4.8 mmol) were dissolved in *N,N*-dimethylformamide (10 mL) containing HOBt (0.81 g, 5.3 mmol). To the mixture was added DCC (1.1 g, 5.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h, and then at room temperature for 24 h. The solvent was evaporated, and to the residue was added ethyl acetate. The insoluble part was filtered off, and the filtrate was washed with 10% $KHSO_4$, 10% NaCl, 5% $NaHCO_3$, and 10% NaCl solutions, and dried over $MgSO_4$. After evaporation of the solvent, the residue was recrystallized from ethyl acetate/hexane: Yield 1.7 g (86%); mp 80—83 °C; R_f^A, 0.75; R_f^B, 0.54; R_f^C, 0.96. IR (NaCl) 3350 (NH), 1740 (C=O), 1670 (C=O), and 1510 cm^{-1} (NH). ^1H NMR (90 MHz) δ=1.15 (dd, 3H, CH_3 Ala), 1.40 (s, 9H, 3×CH_3 Boc), 3.88 (d, 3H, OCH_3), 4.09 (m, 1H, $C^{\alpha}H$ Ala), 4.82 (d, 1H, NH Ala), 5.02 (m, 1H, $C^{\alpha}H$ napSer), 6.05 (s, 1H, $C^{\beta}H$ napSer), 6.95 (dd, 1H, NH napSer), and 7.3—8.1 (m, 7H, naphtyl H).

Boc–Ala–napSer–OH. Boc–Ala–napSer–OMe (1.7 g, 4.1 mmol) was dissolved in methanol (5 mL)/1,4-dioxane (5 mL). To the solution was added a 1 M NaOH solution (5.3 mL, 5.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, neutralized with a 5% $KHSO_4$ solution, and concentrated in vacuo. The remaining solution was diluted with a 5% $NaHCO_3$ solution, and then washed with diethyl ether to remove any remaining ester. The aqueous solution was acidified with a 5% $KHSO_4$ solution, and extracted with ethyl acetate. The extract was washed with a 10% NaCl solution, and dried over $MgSO_4$: Yield 1.6 g (95%); mp 112—115 °C; R_f^A, 0—0.07; R_f^B, 0.03—0.12; R_f^C, 0.94. IR (NaCl)3350 (NH), 1720 (C=O), 1670 (C=O), and 1510 cm^{-1} (NH). ^1H NMR (90 MHz) δ=1—1.3 (m, 3H, CH_3 Ala), 1.32+1.38 (s+s, 9H, 3×CH_3 Boc), 4.15 (m, 1H, $C^{\alpha}H$ Ala), 5.05 (d, 1H, NH Ala), 6.24 (s, 1H, $C^{\beta}H$ napSer), and 7.2—8.2 (m, 8H, NH napSer+naphthyl H).

Boc–Ala–Δ^ZNap Azlactone. Boc–Ala–napSer–OH (0.78 g, 2.0 mmol) was dissolved at 0 °C in acetic anhydride (3.0 mL) containing sodium acetate (0.21 g, 2.5 mmol). The mixture was stirred at room temperature for 24 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate. The solution was washed with 5% $NaHCO_3$, and 10% NaCl solutions, and then dried over $MgSO_4$. Evaporation of the solvent gave a yellow solid: Yield 0.64 g (90%); mp 148—151 °C; R_f^A, 0.88; R_f^B, 0.85; R_f^C, 0.96. IR (NaCl) 3330 (NH), 1790 (C=O), 1700 (C=O), 1650 (C=O), and 1500 cm^{-1} (NH). ^1H NMR (90 MHz) δ=1.45 (s, 9H, 3×CH_3 Boc), 1.55 (d, 3H, CH_3 Ala), 4.80 (m, 1H, $C^{\alpha}H$ Ala), 5.16 (d, 1H, NH Ala), and 7.4—8.9 (m, 8H, naphthyl H+H^{β} Δ^ZNap).

Boc–Ala–Δ^ZNap–Val–OMe 2. To a solution of Boc–Ala–Δ^ZNap azlactone (0.31 g, 0.86 mmol) in THF (10 mL) was added TosOH·H–Val–OMe (0.26 g, 0.86 mmol) and *N*-methylmorpholine (85 μL, 0.90 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h, and at room temperature for 48 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate. The solution was washed with 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 0.15 g (34 %); mp 159—162 °C; R_f^A, 0.81; R_f^B, 0.57; R_f^C, 0.99; GPC retention time=25 min. Found: C, 65.26; H, 7.32; N, 8.24%. Calcd for $C_{27}H_{35}N_3O_6$: C, 65.17; H, 7.09; N, 8.45%. IR (NaCl) 3300 (NH), 1740 (C=O), 1680 (C=O), 1650 (C=O), 1610 (C=O), and 1520 cm^{-1} (NH). ^1H NMR (200 MHz) δ=1.01 (dd, 6H, 2×CH_3 Val), 1.21 (d, 3H, CH_3 Ala), 1.33 (s, 9H, 3×CH_3 Boc), 2.26 (m, 1H, $C^{\beta}H$ Val), 3.76 (s, 3H, OCH_3), 4.03 (m, 1H, $C^{\alpha}H$ Ala), 4.68 (dd, 1H, $C^{\alpha}H$ Val), 4.78 (d, 1H, NH Ala), 7.02 (d, 1H, NH Val), 7.41 (s, 1H, NH Δ^ZNap), and 7.4—8.0 (m, 8H, naphthyl H+H^{β} Δ^ZNap).

Boc–Ala–Δ^ZPyr–Val–OMe 3. Peptide **3** was synthesized in a similar manner to peptide **2**.

H–pyrSer–OMe. 1-Pyrenecarbaldehyde (4.0 g, 17 mmol) and Gly (0.69 g, 9.2 mmol) were suspended in 1,4-dioxane. To the mixture was added a 1 M NaOH solution (15 mL, 14 mmol) at 5 °C with vigorous stirring. The mixture was stirred at room temperature for 140 h. A subsequent procedure was carried out in a similar manner to H–napSer–OMe. H–pyrSer–OMe was obtained as yellow crystals: Yield 0.42 g (15%); mp 117—124 °C; R_f^A, 0.12; R_f^B, 0.32, R_f^C, 0.67. IR (NaCl) 3350 (NH), and 1730 cm^{-1} (C=O). ^1H NMR (90 MHz) δ=2.2 (br.s, 2H, NH_2), 3.55+3.70 (s+s, 3H, OCH_3), 4.10 (m, 1H, $C^{\alpha}H$), 6.04 (t, 1H, $C^{\alpha}H$), and 7.8—8.4 (m, 9H, pyrenyl H).

Boc–Ala–pyrSer–OMe. Yield 80%; mp 98—101 °C; R_f^A, 0.76; R_f^B, 0.40; R_f^C, 0.96. IR (NaCl) 3350 (NH), 1730 (C=O), 1660 (C=O), and 1500 cm^{-1} (NH). ^1H NMR (90 MHz) δ=1—1.5 (m, 12H, 3×CH_3 Boc+CH_3 Ala), 3.40+3.76 (s+s, 3H, OCH_3), 4.1 (m, 1H, $C^{\alpha}H$), 3.7—4.4 (m, 2H, NH Ala+$C^{\alpha}H$ pyrSer), 6.4 (br.s, 1H, $C^{\beta}H$ pyrSer), 7.2 (br.s, 1H, NH pyrSer), and 7.8—8.6 (m, 9H, pyrenyl H).

Boc–Ala–pyrSer–OH. Yield 80%; mp 124—130 °C; R_f^A, 0—0.07; R_f^B, 0.05—0.13; R_f^C, 0.89. IR (NaCl) 3400 (NH), 1710 (C=O), 1660 (C=O), and 1510 cm^{-1} (NH). ^1H NMR (90 MHz) δ=0.8—1.5 (m, 12H, 3×CH_3 Boc+CH_3 Ala), 4.2 (m, 1H, $C^{\alpha}H$ Ala), 5.2 (d, 1H, NH Ala), 5.7 (br.s, 1H, $C^{\alpha}H$ pyrSer), 6.2—6.5 (m, 1H, $C^{\beta}H$ pyrSer), 7.4 (m, 1H, NH pyrSer), and 7.8—8.6 (m, 9H, pyrenyl H).

Boc–Ala–Δ^ZPyr Azlactone. Yield 64%; mp 209—218 °C; R_f^A, 0.88; R_f^B, 0.86; R_f^C, 1.0. IR 3370 (NH), 1780 (C=O), 1680 (C=O) 1640 (C=O), and 1500 cm^{-1} (NH). ^1H NMR (90 MHz) δ=1.5 (s, 9H, 3×CH_3 Boc), 1.6 (d, 3H, CH_3 Ala), 4.85 (m, 1H, $C^{\alpha}H$ Ala), 5.18 (d, 1H, NH Ala), and 7.9—9.4 (m, 10H, pyrenyl H+H^{β} Δ^ZPyr).

Boc–Ala–Δ^ZPyr–Val–OMe 3. Yield 40%; mp 195—199 °C; R_f^A, 0.82; R_f^B, 0.57; R_f^C, 0.98; GPC retention time=25min. Found: C, 67.49; H, 6.52; N, 6.86%. Calcd for $C_{33}H_{37}N_3O_6H_2O$: C, 67.22; H, 6.67; N, 7.12%. IR (NaCl) 3250 (NH), 1730 (C=O), 1680 (C=O), 1620 (C=O), and 1510 cm^{-1} (NH). ^1H NMR (200 MHz) δ=1.05 (dd, 6H, 2×CH_3 Val), 1.25 (s, 9H, 3×CH_3 Boc), 1.26 (d, 3H, CH_3 Ala), 2.29 (m, 1H, $C^{\beta}H$ Val), 3.79 (s, 3H, OCH_3), 4.05 (m, 1H, $C^{\alpha}H$ Ala), 4.73 (dd, 1H, $C^{\alpha}H$ Val), 4.76 (d, 1H, NH Ala), 7.07 (d, 1H, NH Val), 7.52 (s, 1H, NH Δ^ZPyr), and 7.95—8.25 (m, 10H, pyrenyl H+H^{β} Δ^ZPyr).

Results and Discussion

Hydrogen-Bonding Mode. The presence of hydrogen-bonded NH resonances has usually been confirmed by measuring the degree of solvent exposure of each NH group in a given peptide. In the present study, two criteria were

used: (i) the temperature dependence of the NH chemical shifts,[35] and (ii) the solvent dependence of the NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures.[36]

The NH resonances in peptides **1**—**3** could be assigned straightforwardly. Δ^ZAA NH appeared as a singlet at a low field (7.4—7.8 ppm). On the other hand, Ala NH appeared as a doublet at a relatively high field (4.7—5.0 ppm), characteristic of urethane NH.[37—40] The remaining doublet NH resonance was assigned to Val NH. Table 1 summarizes the NH chemical shifts and their temperature coefficients ($d\delta/dT$). In each peptide, Val NH gave the smallest temperature coefficient, which belongs to that for solvent-shielded protons (<3 ppb K^{-1}).[41] This indicates that Val NH has a solvent-shielded nature due to intramolecular hydrogen bonding. The same tendency was also observed in experiment (ii). As shown in Fig. 1, Ala NH and Δ^ZAA NH resonances in $CDCl_3$ for each peptide were markedly shifted to a lower field upon

Table 1. ^{1}H NMR Parameters for NH Groups in Peptides **1**—**3** in $CDCl_3$ at 23 °C

Paramter	Residue	1	2	3
δ	Ala	5.08	4.78	4.76
(ppm)	Δ^ZAA	7.83	7.41	7.52
	Val	6.91	7.02	7.07
$J_{NHC^\alpha H}$	Ala	6.6	6.0	6.3
(Hz)	Val	8.1	8.3	8.3
$d\delta/dT$	Ala	3.3	2.9	a)
($-$ppb K^{-1})	Δ^ZAA	4.7	3.2	2.1
	Val	2.4	2.1	1.4

a) The value could not be determined due to overlap of NH and Val $C^\alpha H$ resonances.

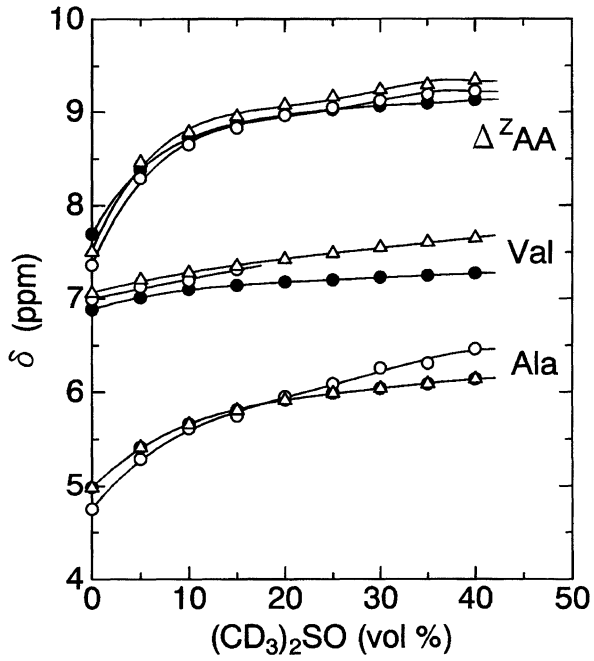


Fig. 1. Solvent dependence of NH chemical shifts for peptides **1** (●), **2** (○), and **3** (△) in $CDCl_3$–$(CD_3)_2SO$ mixtures.

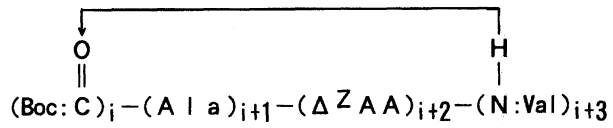


Chart 4.

adding a strong hydrogen-bond accepting $(CD_3)_2SO$. On the other hand, the Val NH resonance was less affected by adding $(CD_3)_2SO$. All of the NH chemical shifts changed monotonically up to a $(CD_3)_2SO$ concentration of 40% (v/v), suggesting that no major conformational change occurred in the present experiment.[36] Consequently, both experiments (i) and (ii) indicate that all the peptides **1**—**3** form a specific conformation supported by an intramolecular hydrogen bond with the Val NH group.

Linear small peptides containing a Δ^ZPhe residue at the (i+2)th position have been shown to form a β-turn conformation with an intramolecular hydrogen bond between the C=O (i) and NH (i+3) groups.[6—9] Similarly, peptides **1**—**3** containing a Δ^ZAA residue at the second position showed intramolecular hydrogen bonding with NH of the Val residue at the third position. Accordingly, peptides **1**—**3** should form a β-turn conformation supported by a hydrogen bond between C=O (Boc) and NH (Val): Chart 4.

NOE Studies. To obtain further information concerning the main-chain conformations, difference NOE experiments were carried out. NOEs are usually observed for proton pairs that are <3 Å apart, and can generally be used to probe specific conformations in peptides.[42] Figures 2 and 3 show the ^{1}H NMR spectra of peptides **1** and **2**, as well as the corresponding difference NOE spectra obtained by the irradiation of each NH resonance, respectively. All of the NOEs observed were positive, suggesting that the condition $\tau_C < 1$ is satisfied.[43,44] The NOE data are summarized in Table 2. For all of the peptides, a remarkable NOE (ca. 8—11%) was observed for Ala $C^\alpha H$ upon the irradiation of the Δ^ZAA NH resonance. Such NOEs indicate $\psi_{Ala} \approx 120° \pm 30°$,[45,46] which corresponds to standard values for the (i+1)th residue in a type II β-turn conformation. A similar $C^\alpha_{i+1}H$–$N_{i+2}H$ NOE was observed in their analogous peptides, Boc–Phe–Δ^ZPhe–X–OMe (X=Val, Ala, and Leu) **4**,[6] that favor a type II β-turn conformation in $CDCl_3$ essentially. Thus, peptides **1**—**3** should take a type II β-turn conformation in $CDCl_3$.

On the other hand, peptides **1**—**3** gave small NOEs, suggesting other conformations. First, the irradiation of the Ala NH and Δ^ZPhe NH resonances led to small NOEs on the Δ^ZPhe NH and Ala NH resonances, respectively. Such an $N_{i+1}H$–$N_{i+2}H$ NOE was also observed in peptides **4**.[6] In a peptide that takes the standard type II β-turn, the observation of such an NOE is not expected due to the longer interproton distance of $N_{i+1}H$–$N_{i+2}H$ (4.5 Å),[46] thus suggesting that peptides **1**—**3** in $CDCl_3$ contain a conformation with a shorter $N_{i+1}H$–$N_{i+2}H$ distance: e.g., a type III β-turn where an $N_{i+1}H$–$N_{i+2}H$ distance less than 3.0 Å[45] is considered, since peptide **4** (X=Val) takes a type III β-turn

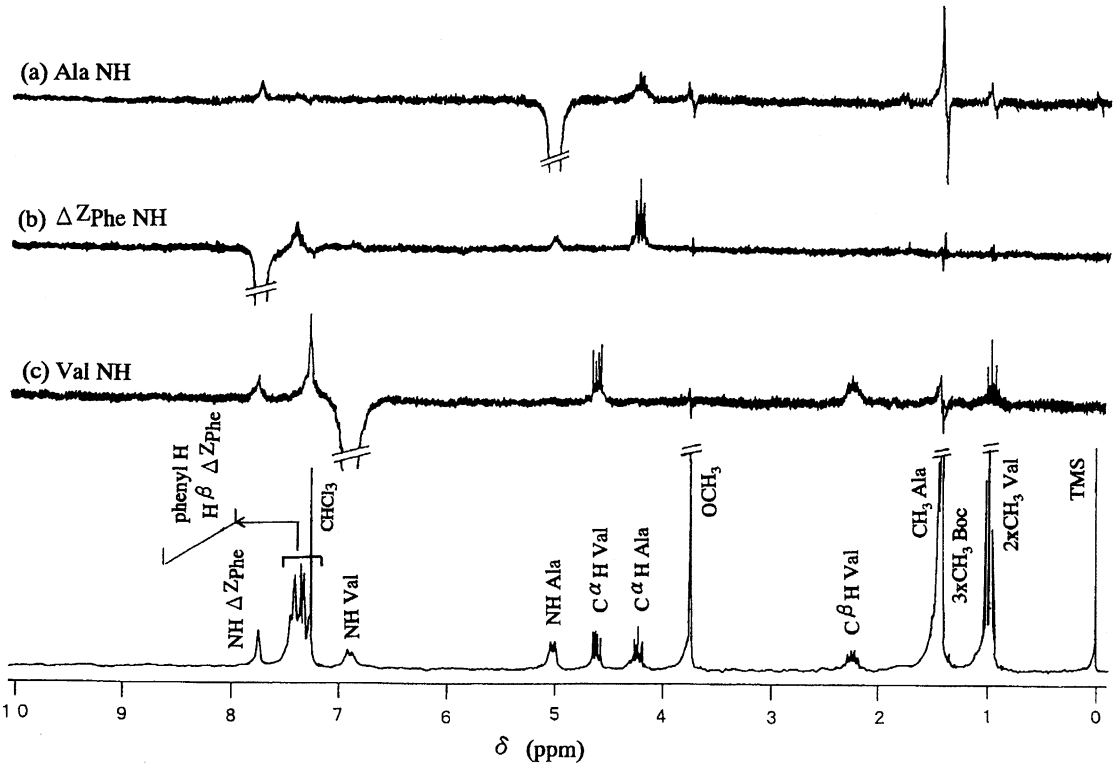


Fig. 2. ^{1}H NMR spectrum (bottom) of peptide **1** in $CDCl_3$ and the difference NOE spectra obtained by irradiation of (a) Ala NH, (b) Δ^ZPhe NH, and (c) Val NH resonances.

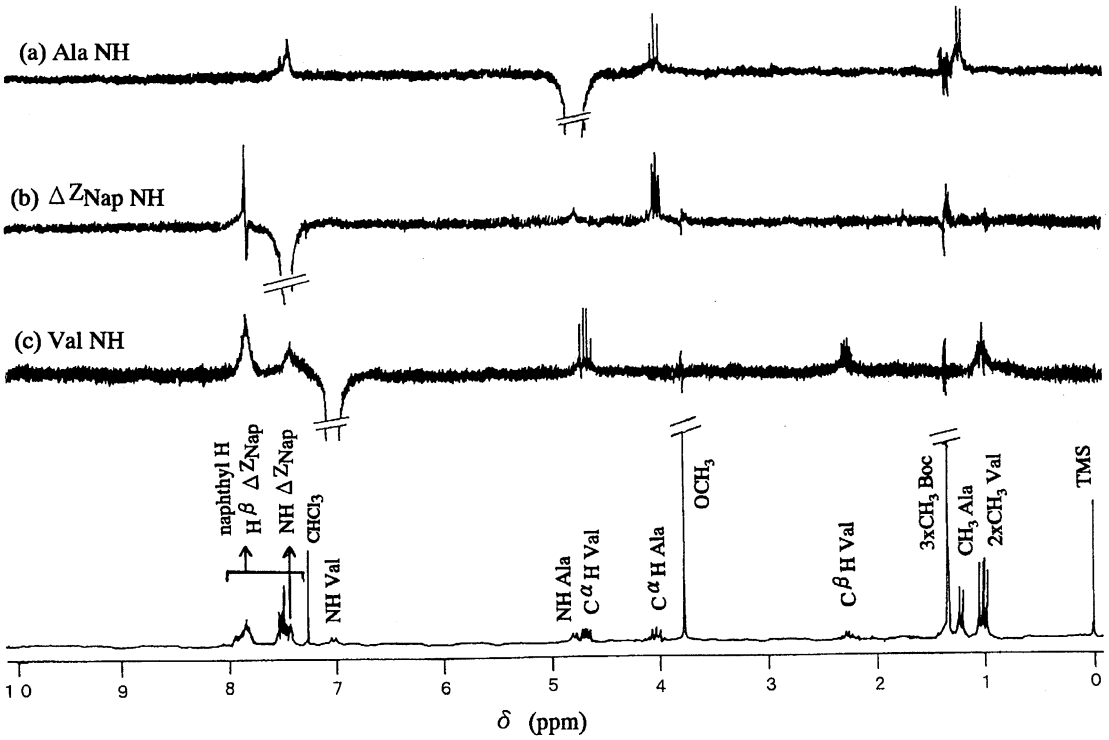


Fig. 3. ^{1}H NMR spectrum (bottom) of peptide **2** in $CDCl_3$ and the difference NOE spectra obtained by irradiation of (a) Ala NH, (b) Δ^ZNap NH, and (c) Val NH resonances.

structure in the solid state.[8] Secondly, the irradiation of Val NH led to NOEs on a aromatic H and C^βH resonances in the Δ^ZAA residue, suggesting the presence of partially extended conformations.[6] Therefore, strictly speaking, peptides **1**—**3** in $CDCl_3$ take a type II β-turn as a major conformation, and other conformations as minor ones, while all conformations are averaged by a rapid equilibrium among themselves.

Conformational Energy Calculation. Based on the

above ^{1}H NMR results, the three kinds of Δ^ZAA residues were found to have essentially the same conformational feature, in which a tripeptide containing a Δ^ZAA residue at the second position favors a type II β-turn as a major conformation. Here, the experimental results were discussed on the basis of the conformational energy on Ac–Ala–Δ^ZAA–NMA. Table 3 shows the energy-minimized conformations giving the lowest-energy to fifth-energy ones. ΔE_{res} is the energy difference per residue from the lowest energy. The three kinds of Δ^ZAA residues showed a tendency to favor a type II or type II′β-turn conformation (B or B*). In particular, the lowest-energy conformation in all cases was found to be a type II β-turn supported by a hydrogen bond between C=O (Ac) and NH (NMA), as shown in Figs. 4 and 5. (These type II β-turns are slightly different from the standard one:[47] $\phi_{Ala}=-60°$, $\psi_{Ala}=120°$, $\phi_{\Delta^ZAA}=80°$, and $\psi_{\Delta^ZAA}=0°$.) A similar energy calculation was carried out on Ac–Ala–Δ^ZAA–Val–OMe, corresponding to the experimental sequence. Similarly to Ac–Ala–Δ^ZAA–NMA, the three kinds of peptides showed type II β-turns as the lowest-energy conformation: for Δ^ZAA=Δ^ZPhe, $\phi_{Ala}=-71°$, $\psi_{Ala}=97°$, $\phi_{\Delta^ZPhe}=124°$, $\psi_{\Delta^ZPhe}=-9°$, $\chi_{2,\Delta^ZPhe}=138°$, $\phi_{Val}=-81°$, and $\psi_{Val}=125°$; for Δ^ZNap, $\phi_{Ala}=-70°$, $\psi_{Ala}=97°$, $\phi_{\Delta^ZNap}=125°$, $\psi_{\Delta^ZNap}=-9°$, $\chi_{2,\Delta^ZNap}=314°$, $\phi_{Val}=-82°$, and $\psi_{Val}=129°$; for Δ^ZPyr, $\phi_{Ala}=-70°$, $\psi_{Ala}=97°$, $\phi_{\Delta^ZPyr}=125°$, $\psi_{\Delta^ZPyr}=-9°$, $\chi_{2,\Delta^ZPyr}=314°$, $\phi_{Val}=-81°$, and $\psi_{Val}=127°$. This prediction agrees well with the result of an NMR study. Experimentally as well as theoretically, the three kinds of tripeptides favor a type II β-turn conformation. Thus, dehydroresidues having such β-substituent sizes as 1-naphthyl and 1-pyrenyl groups should retain the type II β-turn conformation preferred for

Table 2. NOEs Observed for Peptides **1**—**3** in $CDCl_3$

Resonance irradiated	Resonance observed	%NOE **1**	**2**	**3**
Ala NH	Ala C$^\alpha$H	2.7	1.7	1.8[a]
	Ala C$^\beta$H	1.3	1.2	1.1[a]
	Δ^ZAA NH	1.5	1.5	1.4[a]
Δ^ZAA NH	Ala NH	1.7	1.7	2.0
	Ala C$^\alpha$H	11.4	8.7	11.3
	Aromatic H and C$^\beta$H in Δ^ZAA	4.9	3.1	7.6
	Val NH	0.9	1.9	1.3
Val NH	Δ^ZAA NH	1.4	2.8	1.6
	Aromatic H and C$^\beta$H in Δ^ZAA	6.7	6.9	5.1
	Val C$^\alpha$H	4.0	4.7	3.1
	Val C$^\beta$H	2.8	2.8	2.2
	Val C$^\gamma$H	0.4	0.7	0.5

a) The irradiation of Ala NH resonance slightly contained that of Val C$^\alpha$H resonance due to partial overlap of both resonances.

Table 3. Energy- Minimized Conformations for Ac–Ala–Δ^ZAA–NMA

Δ^ZAA	C.L.C.[a]	Ala ϕ	Ala ψ	Δ^ZAA ϕ	Δ^ZAA ψ	Δ^ZAA χ_2[c]	ΔE_{res}[b] kcal mol^{-1}
Δ^ZPhe	CB*	−69	99	126	−11	137	—(9.32)
	CB	−85	126	−126	12	45	0.63
	AB	−71	−41	−125	13	43	0.74
	AB*	−83	−49	125	−13	135	0.84
	EB*	−154	155	126	−13	136	0.87
Δ^ZNap	CB*	−70	98	126	−11	314	—(9.06)
	CB	−84	77	−126	13	46	0.30
	HB	−80	−92	−125	12	248	0.53
	CB	−83	125	−127	13	49	0.64
	AB	−71	−41	−126	14	47	0.72
Δ^ZPyr	CB*	−70	98	126	−11	313	—(8.92)
	CB	−80	97	−125	13	247	0.18
	AB*	−74	−45	126	−14	112	0.46
	CB	−83	126	−126	12	49	0.55
	CB*	−70	99	127	−12	116	0.69

a) Conformational letter code. b) $\Delta E_{res}=(E-E_0)/2$: E_0 is the lowest energy, which is shown in the parentheses. c) When $\chi_2=0$, aromatic C2 carbon is defined as the eclipsed position relative to C$^\alpha$, as shown in Chart 1.

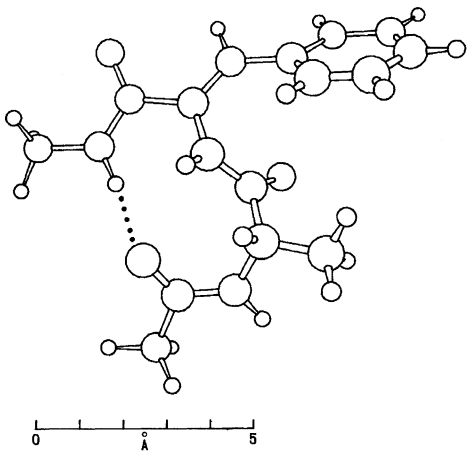


Fig. 4. The lowest-energy conformation of peptide **1**. The dot line (2.1 Å) shows a hydrogen bond between CO (Ac) and NH (NMA).

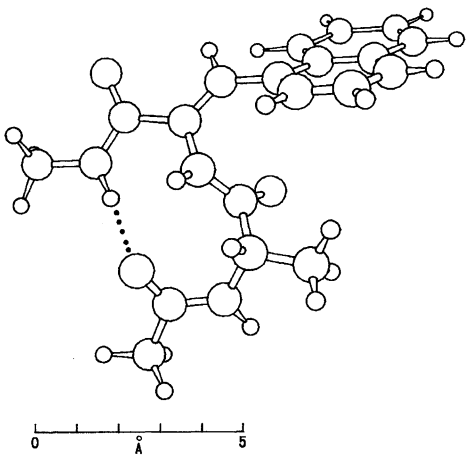


Fig. 5. The lowest-energy conformation of peptide **2**. The dot line (2.1 Å) shows a hydrogen bond between CO (Ac) and NH (NMA).

the Δ^ZPhe residue. In these β-turns, aryl group was found to be non planar ($\chi_2 \neq 0°$ or 180°) relative to the $C^\alpha{=}C^\beta{-}C^\gamma$ plane, irrespective of the aryl size. This suggests that a steric interaction between the β-aryl group and the peptide backbone leads to the internal rotation angles for the β-turn backbone.[7,11]

In the above calculation, the torsional barriers about the N–C^α, C^α–CO, and C^β–C^γ bonds in the Δ^ZNap and Δ^ZPyr residues were taken to be equal to those in the Δ^ZPhe residue. Accordingly, the effect of the π-conjugation size on the torsional barriers was not explicitly involved. However, the similarly in the conformations of peptides **1**—**3** should indicate that the effect of the π-conjugation sizes is small in the present system. The reason for this may be that the Δ^ZAA residue is non-planar. Figure 6 shows the dependence of the χ_2 angle in Δ^ZAA residues on the conformational energy of peptides **1**—**3** with the lowest-energy backbone (type II β-turn). In peptide **1**, the planar orientation of the phenyl group (χ_2=0° or 180°) showed an extremely high energy, compared with the most stable one (χ_2=−40° or 140°), thus being regarded as being energetically prohibited. This tendency is more prominent in peptides **2** and **3**, since steric interactions between the β-aryl group and the peptide backbone are enhanced due to the more bulky aryl groups. The non-planarity of the Δ^ZAA residues can also be supported experimentally. In the crystal structures of several Δ^ZPhe-containing peptides, the χ_2 values of the Δ^ZPhe residues were 20°—60° [8,48] or 140°—155°.[49,50] A NOE study of the 1-naphthylethylene derivatives with one methyl group on the ethylene indicated that they are essentially non-planar.[51] Thus, this prohibited coplanarity between the β-aryl plane and $C^\alpha{=}C^\beta$ should weaken the π-conjugation effect in the β-aryl group.

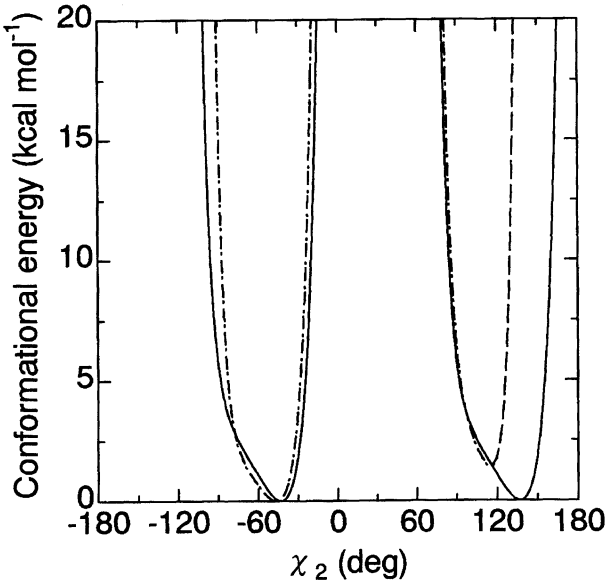


Fig. 6. Dependence of aryl orientation (χ_2 angle) on relative conformational energy of peptides **1** (—), **2** (- - -), and **3** (····) with the lowest-energy backbone (type II β-turn). Minimum energy for each case was set to 0 kcal mol^{-1}. When χ_2=0, aromatic C2 carbon was defined as the eclipsed position relative to C^α, as shown in Chart 1.

The Δ^ZPhe residue also has a specific conformational space for its side chain:[21,22] Its side-chain freedom would be smaller than those for naturally occurring amino acids, such as Phe, Asp, Glu, and Lys, because the variable side-chain dihedral angle is regarded as being single due to prohibited rotation about the $C^\alpha{=}C^\beta$ double bond. Thus, the Δ^ZPhe residue has been expected to be unique residues for spatially- and regularly-arranging a β-phenyl group on its peptide backbone. The present findings should mean that another bulky β-substituent can be arranged regularly on a peptide backbone having a conformational feature similar to the Δ^ZPhe residue. Thus, β-substituted α,β-dehydroalanine-containing peptides will be useful to provide a rigid molecular frame for regularly arranging β-functional groups, and to design functional bioactive peptides.

We thank Dr. A. Yoshino of the Department of Applied Chemistry, Nagoya Institute of Technology, for his valuable technical assistance in the NMR measurement.

References

1) K. Noda, Y. Shimohigashi, and N. Izumiya, "The Peptides," ed by E. Gross and J. Meienhofer, Academic Press, N. Y. (1983), Vol. 5, pp. 286—339.
2) E. Gross and J. L. Morell, *J. Am. Chem. Soc.*, **89**, 2791 (1967).
3) H. Allgaier, G. Jung, R. G. Werner, U. Schneider, and H. Zaehner, *Eur. J. Biochem.*, **160**, 9 (1986).
4) I. L. Givot, T. A. Smit, and R. H. Abeles, *J. Biol. Chem.*, **244**, 6341 (1969).
5) R. B. Wickner, *J. Biol. Chem.*, **244**, 6550 (1969).
6) V. S. Chauhan, A. K. Sharwa, K. Uma, P. K. C. Paul, and P. Balaram, *Int. J. Peptide Protein Res.*, **29**, 126 (1987).
7) P. Kaur, K. Uma, P. Balaram, and V. S. Chauhan, *Int. J. Peptide Protein Res.*, **33**, 103 (1989).
8) T. P. Singh, M. Haridas, V. S. Chauhan, and A. Kumar, *Biopolymers*, **26**, 819 (1987).
9) K. Uma and P. Balaram, *Indian J. Chem., Sect. B*, **1989**, 705, and references therein.
10) T. P. Singh, P. K. Narula, V. S. Chauhan, A. K. Sharma, and W. Hinrichs, *Int. J. Peptide Protein Res.*, **33**, 167 (1989).
11) K. Uma, P. Balaram, P. Kaur, A. Kumar, and V. S. Chauhan, *Int. J. Biol. Macromol.*, **11**, 169 (1989).
12) C. Pascard, A. Ducruix, J. Lunel, and T. Prangé, *J. Am. Chem. Soc.*, **99**, 6418 (1977).
13) D. Ajò, G. Granozzi, E. Tondello, A. D. Prà, and G. Zanotti, *J. Chem. Soc., Perkin Trans. 2*, **1979**, 927.
14) B. Anderson, D. C. Hodgkin, and M. A. Viswamitra, *Nature*, **225**, 233 (1970).
15) A. Gupta and V. S. Chauhan, *Biopolymers*, **30**, 395 (1990).
16) D. E. Palmer, C. Pattaroni, K. Nunami, R. K. Chadha, M. Goodman, T. Wakamiya, K. Fukase, S. Horimoto, M. Kitazawa, H. Fujita, A. Kubo, and T. Shiba, *J. Am. Chem. Soc.*, **114**, 5634 (1992).
17) A. Gupta and V. S. Chauhan, *Int. J. Peptide Protein Res.*, **41**, 421 (1993).
18) D. Ajò, G. Granozzi, and E. Tondello, *J. Mol. Struct.*, **41**, 131 (1977).
19) D. Ajò, G. Granozzi, E. Tondello, and A. D. Prà, *Biopoly-*

mers, **19**, 469 (1980).
20) F. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).
21) Y. Inai, T. Ito, T. Hirabayashi, and K. Yokota, *Biopolymers*, **33**, 1173 (1993).
22) Y. Inai, T. Ito, T. Hirabayashi, and K. Yokota, *Polym. J.*, **27**, 846 (1995).
23) M. Sisido, Y. Inai, and Y. Imanishi, *Macromolecules*, **23**, 1665 (1990).
24) M. Sisido, R. Tanaka, Y. Inai, and Y. Imanishi, *J. Am. Chem. Soc.*, **111**, 6790 (1989).
25) D. Ajò, M. Casarin, and G. Granozzi, *J. Mol. Struct.*, **86**, 420 (1982).
26) M. Sisido, private communications.
27) Y. Beppu, *Comput. Chem.*, **13**, 101 (1989).
28) S. S. Zimmerman, M. S. Pottle, G. Nemethy, and H. A. Scheraga, *Macromolecules*, **10**, 1 (1977).
29) G. Carrara and G. Weitnauer, *Gazz. Chim. Ital.*, **79**, 856 (1949).
30) J. R. Vaughan and R. L. Osato, Jr., *J. Am. Chem. Soc.*, **74**, 676 (1952).
31) G. W. Anderson, F. M. Callahan, and J. E. Zimmerman, *J. Am. Chem. Soc.*, **88**, 1338 (1966).
32) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
33) W. König and R. Geiger, *Chem. Ber.*, **103**, 2024 (1970).
34) D. G. Doherty, J. E. Tietzman, and M. Bergmann, *J. Biol. Chem.*, **147**, 617 (1943).
35) K. D. Kopple, A. Go, and P. R. Pilipauskas, *J. Am. Chem. Soc.*, **97**, 6830 (1975).
36) T. P. Pitner and D. W. Urry, *J. Am. Chem. Soc.*, **94**, 1399 (1972).
37) M. Iqbal and P. Balaram, *Biochemistry*, **20**, 4866 (1981).
38) R. Nagaraj, N. Shamala, and P. Balaram, *J. Am. Chem. Soc.*, **101**, 16 (1979).
39) R. Nagaraj and P. Balaram, *Biochemistry*, **20**, 2828 (1981).
40) M. Iqbal and P. Balaram, *J. Am. Chem. Soc.*, **103**, 5548 (1981).
41) V. J. Hruby, "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," ed by B. Weinstein, Dekker, N. Y. (1974), Vol. 3, pp. 1—188.
42) B. N. N. Rao, A. Kumar, H. Balaram, A. Ravi, and P. Balaram, *J. Am. Chem. Soc.*, **105**, 7423 (1983).
43) P. Balaram, A. A. Bothner-By, and J. Dadok, *J. Am. Chem. Soc.*, **94**, 4015 (1972).
44) J. D. Glickson, S. L. Gordon, T. P. Pinter, D. G. Agresti, and R. Walter, *Biochemistry*, **15**, 5721 (1976).
45) M. D. Shenderovich, G. V. Nikiforovich, and G. I. Chipens, *J. Magn. Reson.*, **59**, 1 (1984).
46) M. Billeter, W. Braun, and K. Wüthrich, *J. Mol. Biol.*, **155**, 321 (1982).
47) G. D. Rose, L. M. Gierash, and J. A. Smith, *Adv. Protein. Chem.*, **37**, 1 (1985).
48) O. Pieroni, G. Montagnoli, A. Fissi, S. Merlino, and F. Ciardelli, *J. Am. Chem. Soc.*, **97**, 6820 (1975).
49) A. Aubry, F. Allier, G. Boussard, and M. Marraud, *Biopolymers*, **24**, 639 (1985).
50) B. Padmanabhan and T. P. Singh, *Biopolymers*, **33**, 613 (1993).
51) J. E. Anderson, D. J. D. Barkel, and J. E. Parkin, *J. Chem. Soc., Perkin Trans. 2*, **1987**, 955.