First Observation of Left-Handed Helical Conformation in a Dehydro Peptide Containing Two L-Val Residues. Crystal and Solution Structure of Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe[†,⊥]

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Abstract: The solution and solid structure of Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe, containing three consecutive ΔPhe residues, have been determined by X-ray diffraction, nuclear magnetic resonance, and circular dichroism methods. The crystals grown from aqueous methanol are orthorhombic, space group $P2_12_12_1$, $a = 11.624(2)$, $b = 17.248(2)$, $c = 21.532$ Å, $V = 4216$ (1) Å^3, $Z = 4$. In the solid state, the peptide exhibits a left-handed 3_{10}-helical conformation, in spite of the presence of two L-Val residues. NMR and CD studies in different solvents also support the crystal structure data, suggesting that the solid state structure is maintained in solution as well. This is the first report of a dehydropeptide containing three consecutive ΔPhe residues and exhibiting left-handed 3_{10}-helical conformation, which demonstrates the remarkable conformational consequences produced by consecutive occurrence of ΔPhe residues in a peptide.

Introduction

Peptide and protein mimicry aims to transfer some of the complex structural and functional properties of this class of bioactive molecules to simplified, synthetically accessible compounds. To this end, substitution with α,β-dehydroamino acid (Δa.a) comes in use for producing well defined structural motifs.[1] In the last few years, a large body of studies has been devoted in order to determine the likely conformational consequences of the presence of dehydro residues, especially α,β-dehydrophenylalanine (ΔPhe). Incorporation of ΔPhe in bioactive peptides confers increased resistance to enzymatic degradation[2] as well as altered bioactivity.[3] Determination of the crystal and molecular structure of many ΔPhe containing peptides has provided evidence that ΔPhe is strong inducer of β-bend[4] in short sequences and 3_{10}-helical conformation in long sequences.[5] A great deal of NMR studies has also been performed, which confirms the presence of ordered structure for dehydro peptides[5b,m,t] in solution as well. In this regard the behavior of ΔPhe is somewhat similar to that of α-amino isobutyric acid (Aib), a highly helicogenic non-protein amino acid residue.[6]

The versatility of ΔPhe residues was demonstrated in a dehydropentapeptide containing two ΔPhe residues wherein a novel β-bend ribbon conformation was observed[5o] as well as in another pentapeptide having a single ΔPhe residue exhibiting an α-helical conformation.[5s] However, there is relatively less information on peptide containing consecutive ΔPhe residues; there are only three crystal structure reports of dehydrotripeptides containing two consecutive ΔPhe residues.[5h−j] In these peptides either an extended structure or 3_{10}-helical conformations with both left-handed and right-handed screw sense are observed. It is of much interest to determine the behavior of more than two consecutive ΔPhe residues in peptides. Here, we report for the first time the crystal and solution structure of a peptide containing three consecutive ΔPhe residues *viz.* Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe (**1**). The peptide exhibits a left-handed 3_{10}-helical conformation in spite of the presence of two

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L-Val residues. This demonstrates the remarkable conformational consequences produced by consecutive occurrence of ΔPhe residues in peptides.

Experimental Procedures

Synthesis. Boc-Val-DL-Phe(β-OH)-OH (2). To a solution of Boc-Val-OH (4.0 g, 18.43 mmol) in tetrahydrofuran (20 mL) at −10 °C, *N*-methylmorpholine (2.02 mL, 18.43 mmol) and isobutylchloroformate (2.4 mL, 18.43 mmol) were added. The reaction mixture was stirred for 10 min. A precooled solution of DL-Phe(β-OH)-OH (3.34 g, 18.43 mmol) in aqueous NaOH (1 N, 18.4 mL, 18.43 mmol) was added to the reaction mixture. The mixture was stirred for 2 h at 0 °C and overnight at room temperature. After completion (checked by TLC) reaction mixture was concentrated in *vacuo*, and residue was taken in water. Maintaining the pH of the aqueous layer to 8−9, it was washed once with ethyl acetate. Aqueous layer was then acidified with solid citric acid to pH 3, and the resulting oil was extracted three to four times with ethyl acetate. Combined organic extract was washed with saturated NaCl, dried over sodium sulfate, and evaporated to give **2** as an oily product (yield = 80%). R_f [CHCl$_3$−CH$_3$OH (9:1)] = 0.5; ^{1}H NMR (270 MHz, CDCl$_3$) δ (ppm): 7.2 (5H, m, aromatic protons); 6.6 (1H, br, NH DL-Phe(β-OH)); 5.2 (1H, br, NH Val); 4.8 (1H, br, C$^\alpha$H DL-Phe(β−OH)); 4.1 (1H, m, C$^\alpha$H Val); 1.4 (9H, s, 3 × CH$_3$ Boc); 1.0 (6H, d, 2 × CH$_3$ Val).

Boc-Val-ΔPhe-Azlactone (3). A solution of dipeptide **2** (6.20 g, 16.31 mmol) in acetic anhydride (40 mL) and anhydrous sodium acetate (1.734 g, 21.15 mmol) was stirred for 20 h at room temperature. The reaction mixture was then poured over crushed ice, and the precipitate was filtered, washed with 5% NaHCO$_3$ and water, and dried under vacuum. The azlactone was crystallized from acetone/water to get the pure compound (yield = 75%). Mp = 114−115 °C; R_f [CHCl$_3$−CH$_3$-OH (9:1)] = 0.8, R_f [butanol−acetic acid−H$_2$O (4:1:1)]= 0.9; R_f [CHCl$_3$−CH$_3$OH (9:1)] = 0.5; ^{1}HNMR (270 MHz, CDCl$_3$) δ (ppm): 7.3−7.1 (5H, m, aromatic protons); 5.1 (1H, br, NH Val); 4.1 (1H, br, C$^\alpha$H Val); 2.1 (1H, m, C$^\beta$H Val); 1.35 (9H, s, 3 × CH$_3$ Boc); 1.0 (6H, d, 2 × CH$_3$ Val).

Boc-Val-ΔPhe-ΔPhe-ΔPhe-Val-OMe (1). DL-Phe(β-OH)-OH (1.4 g, 7.54 mmol) dissolved in 1 N NaOH (12 mL) and acetone (20 mL) was added to a solution of azlactone **3** (2 g, 5.8 mmol) in acetone, and the reaction mixture was stirred at room temperature. After 24 h, the reaction mixture was neutralized by adding 1 N HCl (12 mL). Solvent was removed under vacuum, and the residue was dissolved in ethyl acetate, washed with water, dried over sodium sulfate, and evaporated to yield Boc-Val-ΔPhe-DL-Phe(β-OH)-OH (**4**) (single spot on TLC). Tripeptide **4** was used in the next step with no further purification and (2.8 g, 5.3 mmol) reacted with anhydrous sodium acetate (0.48 g, 5.88 mmol) and acetic anhydride (25 mL) for 48 h at room temperature. The reaction was worked up as before to yield Boc-Val-ΔPhe-ΔPhe-azlactone (**5**) in pure form. Peptide **5** (2 g, 4.1 mmol) was also reacted in acetone (20 mL) with DL-Phe(β-OH)-OH (0.817 g, 4.5 mmol) dissolved in 1 N NaOH (10 mL, 0.018 g, 4.5 mmol) for 50 h at room temperature. Boc-Val-ΔPhe-ΔPhe-DL-Phe(β-OH)-OH (**6**) was obtained after usual workup and was azlactonized using acetic anhydride (20 mL) and sodium acetate (0.345 g, 4.2 mmol) to yield Boc-Val-ΔPhe-ΔPhe-ΔPhe-azlactone (**7**), which showed a single spot on TLC.

To a solution of peptide **7** (0.914 g, 1.44 mmol) in dichloromethane was added HCl·Val-OMe (0.365 g, 2.2 mmol) and triethylamine (0.3 mL, 2.2 mmol). The reaction mixture was stirred for 80 h. The reaction mixture was then washed with NaHCO$_3$ solution, 5% citric acid solution, and water and dried over sodium sulfate. The solvent was removed under reduced pressure to give pentapeptide **1**, which was recrystallized from methanol and water. Yield = 60%. Mp = 212−214 °C; R_f [CHCl$_3$−CH$_3$OH (9:1)] 0.63, R_f [butanol−acetic acid−H$_2$O (4:1:1)] 0.97; the molecular mass of the pentapeptide determined by ES-MS was 766.4 (calculated molecular mass = 765.904); ^{1}H NMR (270 MHz, CDCl$_3$) δ (ppm): 9.1 (1H, s, NH ΔPhe4); 8.79 (1H, s NH ΔPhe3); 7.75 (1H, s, NH ΔPhe2); 7.65 (1H,d, NH Val5); 7.55−7.2 (18 H, m, aromatic + C$^\beta$H protons of ΔPhe2, ΔPhe 3 and ΔPhe4); 4.81 (1H, d, NH Val1); 4.55 (1H, m, C$^\alpha$H Val5); 4.12 (1H, m, C$^\alpha$H Val1); 2.3 (1H, m, C$^\beta$H Val1); 1.95 (1H, m, C$^\beta$H Val5); 1.2 (9H, s, 3 × CH$_3$ Boc); 1.1 (6H, dd, C$^\gamma$H Val1); 0.9 (6H, dd, C$^\gamma$H Val1).

X-ray Diffraction. Single crystals of peptide **1** were grown by controlled evaporation of the peptide (C$_{43}$H$_{51}$N$_5$O$_8$, M_w = 765.9) in aqueous methanol solution at 4 °C. A colorless crystal mounted on a glass fiber was used for X-ray diffraction experiments. The crystals belong to orthorhombic space group $P2_12_12_1$, a = 11.624(2), b = 17.248(2), c = 21.532(2)Å, V = 4216(1) Å^3, Z = 4, d_c = 1.18 g cm^{-3}. Three-dimensional X-ray intensity data were collected on an Enraf-Nonius CAD4 diffractometer with Ni filtered Cu K$_\alpha$ radiation (λ = 1.5418 Å) up to a Bragg angle of 65° using ω-2θ scan method. A total of 4083 unique reflections were collected of which 3213 had $|F_o|$ > 4σ$|F_o|$. No significant variation was observed in the intensities of three standard reflections monitored at regular intervals during data collection implying the electronic and crystal stability. Lorentz and polarization corrections were applied to the data, and no absorption correction was made (μ = 6.3 cm^{-1}). The structure was solved by direct methods using the computer program SHELXS86[7] and refined on $|F|^2$ using all 4083 reflections by full-matrix least-squares procedures using the computer program SHELXL93.[7] All the hydrogen atoms were fixed using stereochemical criteria and used only for structure factor calculations. The conventional R-factor R1 based on $|F|$'s for 3213 reflections with $|F_o|$ > 4σ$|F_o|$ is 3.64% and 5.45% for all 4083 data. The weighted R-factor wR2 based on $|F_o|^2$ is 9.79% for all 4083 data $\{w = 1/[\sigma^2 |F_o|^2 + (0.0482 + P)^2 + 0.7 + P]$, $P = (\max(|F_o|^2, 0) + 2 + |F_c|^2)/3\}$. The maximum and minimum residual electron density in the final difference fourier map are 0.15 and −0.16 eÅ^{-3}, respectively.

Spectroscopic Studies. ^{1}H NMR spectra were recorded on a Bruker 400 MHz FT NMR at Sophisticated Instruments Facility, Indian Institute of Science. Spectral width of 10 ppm was used for both one- and two-dimensional spectra. All chemical shifts are expressed as δ(ppm) downfield from internal reference tetramethylsilane. Spectra were recorded at concentration of 5 mg/mL. Two-dimensional COSY[8a] and ROESY[8b] spectra were recorded in CDCl$_3$ at room temperature using standard procedures. Short mixing time (200−300 ms) was used in the ROESY experiment in order to minimize spin-diffusion effects. Data block sizes were 1024 addresses in t_2 and 512 equidistant t_1 values. Circular dichroism (CD) measurements were carried out on a JASCO 500 spectropolarimeter equipped with a data processor 500 N. A 1 mm path length cell was used. The spectra were recorded in three different solvents—chloroform, methanol, and trifluoroethanol. The spectra were normalized for concentration and path length to obtain the mean residue ellipticity after base line correction. The theoretical CD calculations were carried out on the basis of the exciton chirality method. For ΔPhe chromophore, only the low energy π-π* transition at 280 nm was considered, and the calculations were carried out as reported earlier.[9]

Results

Crystal Structure. The bond lengths and bond angles are listed in Tables 1 and 2, respectively. All bond lengths and bond angles are normal except those corresponding to the three ΔPhe residues. The C$^\alpha$=C$^\beta$ bond length in the three ΔPhe residues are 1.331(4), 1.324(5), and 1.326(5) Å, respectively, which corresponds to classical C=C double bond. The N−C$^\alpha$ [1.418(4), 1.419(3), and 1.421(4) Å] and C$^\alpha$−C′ [1.490(5), 1.500(5), and 1.501(5) Å] bond distances in ΔPhe residues are slightly shorter than the corresponding bonds in saturated residues (1.45 and 1.53 Å, respectively[10]), as seen in other ΔPhe peptides.[5g−t] This shortening is probably due to the extended conjugation of the ΔPhe ring electrons and the remaining part of the residue.

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Table 1. Bond Distances (in Å Units) Involving the Non-Hydrogen Atoms of the Pentapeptide Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe

atom1−atom2	distance (Å)	atom1−atom2	distance (Å)	atom1−atom2	distance (Å)
C1−C4	1.511(6)	C2−C4	1.504(7)	C3−C4	1.513(8)
C4−O1	1.481(5)	O1−C5	1.344(4)	C5−O2	1.217(4)
C5−N1	1.336(3)	N1−C1A	1.451(4)	C1A−C1′	1.517(4)
C1A−C1B	1.540(4)	C1′−O1′	1.223(4)	C1′−N2	1.344(3)
C1B−C1G1	1.508(6)	C1B−C1G2	1.519(6)	N2−C2A	1.418(4)
C2A−C2′	1.490(5)	C2A−C2B	1.331(4)	C2′−O2′	1.229(4)
C2′−N3	1.360(4)	C2B−C2G	1.467(6)	C2G−C2D1	1.386(6)
C2G−C2D2	1.370(5)	C2D1−C2E1	1.389(6)	C2D2−C2E2	1.377(6)
C2E1−C2Z	1.360(7)	C2E2−C2Z	1.362(6)	N3−C3A	1.419(3)
C3A−C3′	1.500(5)	C3A−C3B	1.324(5)	C3′−O3′	1.216(3)
C3′−N4	1.360(3)	C3B−C3G	1.480(5)	C3G−C3D1	1.371(7)
C3G−C3D2	1.385(5)	C3D1−C3E1	1.380(9)	C3D2−C3E2	1.400(9)
C3E1−C3Z	1.349(10)	C3E2−C3Z	1.343(13)	N4−C4A	1.421(4)
C4A−C4′	1.500(5)	C4A−C4B	1.326(5)	C4′−O4′	1.226(4)
C4′−N5	1.342(4)	C4B−C4G	1.459(5)	C4G−C4D1	1.391(5)
C4G−C4D2	1.392(5)	C4D1−C4E1	1.383(5)	C4D2−C4E2	1.365(6)
C4E1−C4Z	1.376(6)	C4E2−C4Z	1.361(7)	N5−C5A	1.445(4)
C5A−C5′	1.502(6)	C5A−C5B	1.534(6)	C5′−O5′	1.173(6)
C5′−O3	1.299(5)	C5B−C5G1	1.508(7)	C5B−C5G2	1.521(7)
O3−C6	1.457(10)				

Table 2. Bond Angles (in deg) Involving the Non-Hydrogen Atoms of the Pentapeptide Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe

atom1−atom2−atom3	angle (deg)	atom1−atom2−atom3	angle (deg)	atom1−atom2−atom3	angle (deg)
C2−C4−C3	111.3(4)	C1−C4−C3	111.8(4)	C1−C4−C2	112.6(4)
C3−C4−O1	101.6(3)	C2−C4−O1	110.2(3)	C1−C4−O1	108.8(3)
C4−O1−C5	121.7(3)	O1−C5−N1	109.8(3)	O1−C5−O2	125.5(2)
O2−C5−N1	124.7(3)	C5−N1−C1A	120.1(3)	N1−C1A−C1B	111.6(3)
N1−C1A−C1′	110.7(2)	C1′−C1A−C1B	110.3(3)	C1A−C1′−N2	114.2(3)
C1A−C1′−O1′	123.4(3)	O1′−C1′−N2	122.4(3)	C1A−C1B−C1G2	110.7(3)
C1A−C1B−C1G1	113.4(2)	C1G1−C1B−C1G2	112.2(3)	C1′−N2−C2A	123.9(2)
N2−C2A−C2B	122.5(3)	N2−C2A−C2′	116.9(2)	C2′−C2A−C2B	120.3(3)
C2A−C2′−N3	116.3(3)	C2A−C2′−O2′	122.3(3)	O2′−C2′−N3	121.4(3)
C2A−C2B−C2G	128.8(3)	C2B−C2G−C2D2	119.3(4)	C2B−C2G−C2D1	123.6(3)
C2D1−C2G−C2D2	117.0(4)	C2G−C2D1−C2E1	121.3(4)	C2G−C2D2−C2E2	121.7(4)
C2D1−C2E1−C2Z	120.0(4)	C2D2−C2E2−C2Z	120.5(4)	C2E1−C2Z−C2E2	119.4(4)
C2′−N3−C3A	122.0(2)	N3−C3A−C3B	122.4(3)	N3−C3A−C3′	118.0(2)
C3′−C3A−C3B	119.5(3)	C3A−C3′−N4	115.5(2)	C3A−C3′−O3′	121.8(3)
O3′−C3′−N4	122.7(3)	C3A−C3B−C3G	126.3(4)	C3B−C3G−C3D2	121.1(4)
C3B−C3G−C3D1	120.9(4)	C3D1−C3G−C3D2	118.0(4)	C3G−C3D1C3E1	121.0(5)
C3G−C3D2−C3E2	119.7(5)	C3D1−C3E1−C3Z	120.5(7)	C3D2−C3E2−C3Z	120.6(5)
C3E1−C3Z−C3E2	120.1(7)	C3′−N4−C4A	121.0(2)	N4−C4A−C4B	123.5(3)
N4−C4A−C4′	116.8(3)	C4′−C4A−C4B	119.6(3)	C4A−C4′−N5	116.4(3)
C4A−C4′−O4′	121.7(3)	O4′−C4′−N5	121.8(3)	C4A−C4B−C4G	130.9(3)
C4B−C4G−C4D2	118.3(4)	C4B−C4G−C4D1	124.2(3)	C4D1−C4G−C4D2	117.4(3)
C4G−C4D1−C4E1	120.6(3)	C4G−C4D2−C4E2	121.8(4)	C4D1−C4E1−C4Z	119.9(4)
C4D2−C4E2−C4Z	119.8(4)	C4E1−C4Z−C4E2	120.4(4)	C4′−N5−C5A	121.8(3)
N5−C5A−C5B	113.7(3)	N5−C5A−C5′	111.6(3)	C5′−C5A−C5B	110.2(3)
C5A−C5′−O3	115.3(4)	C5A−C5′−O5′	122.1(4)	O5′−C5′−O3	122.6(4)
C5A−C5B−C5G2	112.9(4)	C5A−C5B−C5G1	111.0(4)	C5G1−C5B−C5G2	110.9(4)
C5−O3−C6	115.9(5)				

The shortening of the bond length $C^\alpha{=}C^\beta$ because of the double bond and increased planarity of the dehydro residue as a whole because of sp^2 hybridized α and β carbon atoms leads to certain unfavorable steric contacts between the side-chain and main-chain atoms of the ΔPhe residue. These steric contacts are partly released by rearrangement of bond angles at α and β carbon atoms. For example, the bond angle N−C$^\alpha$−C′ [116.9(2), 118.0(2), and 116.8(3)° in ΔPhe^2, ΔPhe^3, and ΔPhe^4, respectively] is reduced from the standard trigonal value of 120°, while the angles N−C$^\alpha{=}$C$^\beta$ [122.5(3), 122.4(3), and 123.5(3)°] and C$^\alpha{=}$C$^\beta$−C$^\gamma$ [128.8(3), 126.3(4) and 130.9(3)°] are increased.

The torsion angles that characterize the Boc group, ω^0 and θ^1, assume values of −172.3(2)° and −179.0(3)°, respectively, which corresponds to a *trans−trans* conformation.[11] This particular conformation of the Boc group makes it possible for O2(Boc) atom to participate in the first 4 → 1 intramolecular hydrogen bond. The values of ω^0 and θ^1 represent a generally

preferred[11] planar urethane moiety [between C1(Boc) and C1$^\alpha$]. The dihedral angle C1−O1−C5−O2 ($\theta^{1\prime}$) has a value of 0.4-(4)°, indicating that C5−O2 bond is *syn* planar with C1−O1 bond, as seen for urethane in general.[12] The three methyl carbon atoms of the Boc group assume energetically favorable staggered positions with respect to the O1−C5 bond [$\theta^2_1 = 62.0(4)$, $\theta^2_2 = 180.0(3)$, and $\theta^2_3 = -62.0(4)°$].

The peptide molecule is characterized by a left-handed 3$_{10}$-helical conformation (Figures 1, 5, and 6) composed of three consecutive, overlapping β-bends stabilized by appropriate 4 → 1 intramolecular N−H···O hydrogen bond (Table 3). The first β-bend is a type II β-bend (-L-Val^1-ΔPhe^2-) which is nonhelical, while the other two are type III′ (ΔPhe^2-ΔPhe^3-) and type I′ β-bends(ΔPhe^3-ΔPhe^4-). The average main chain

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Table 3. Main Chain Torsion Angles in the Solid State Conformation of the Pentapeptide Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe

residue	i	ϕ_i	ψ_i	ω_i	$\chi_i^{1,1}$	$\chi_i^{1,2}$	$\chi_i^{2,1}$	$\chi_i^{2,2}$
Boc	0			−172.3(2)				
Val	1	−56.0(3)	131.2(3)	179.8(2)	52.3(4)	−74.8(4)		
ΔPhe	2	56.0(4)	18.4(4)	−168.3(3)	5.2(6)		28.3(6)	−150.3(4)
ΔPhe	3	58.1(4)	13.0(4)	−171.3(3)	9.2(4)		57.3(6)	−125.9(5)
ΔPhe	4	78.2(4)	4.5(4)	178.0(3)	1.6(6)		−32.7(7)	−148.5(4)
Val	5	−122.5(3)			−60.5(5)	64.8(5)		
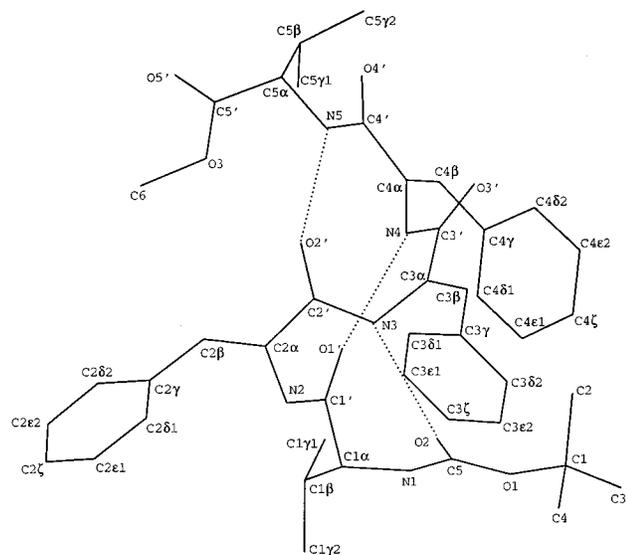
Figure 1. Molecular structure of Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe showing left-handed helical conformation. Dotted lines indicate the intramolecular 4 → 1 hydrogen bonds.

dihedral angles for the three ΔPhe residues are ⟨ϕ⟩ = 64.1° and ⟨ψ⟩ = 12.0°; a slight deviation in ψ from the observed 3$_{10}$-helical value in peptides[13a,b] (30°) and proteins[13c](18°). As noted by others[14] the presence of a type I′ β-bend in a 3$_{10}$-helix does not disturb the helicity.

The two Val residues (Val[1] and Val[5]) in the pentapeptide assume similar side-chain conformations (Table 3) and so do the side-chain torsion angle χ^1 of the three ΔPhe residues. However, the signs of the side-chain torsion angles $\chi^{2,1}$ and $\chi^{2,2}$ for ΔPhe[4] residue are opposite to those for ΔPhe[2] and ΔPhe[3] residues. Model building suggests that this is probably to release unfavorable steric interactions between the bulky side chains of ΔPhe residues which arise because of increase in ϕ torsion angle in ΔPhe[4] approximately by 20° (Table 3). In solid state each peptide molecule interacts with four other molecules through one hydrogen bond each (Figure 2). N1 donates a hydrogen bond to O4ó of a symmetry related molecule, while N2 donates a hydrogen bond to O5ó of another symmetry related molecule (Table 4). No helical rods are observed in the solid state; a frequent feature observed in the crystal structure of helical peptide molecules.[5n,6a]

Solution Conformation. Well-resolved ^{1}H NMR spectra were obtained for pentapeptide **1** in CDCl$_3$. Assignments were made by standard two-dimensional NMR techniques.[8] The relevant NMR parameters for the NH group resonances in the pentapeptide are given in Table 5. Solvent titration experiments in CDCl$_3$−(CD$_3$)$_2$SO mixtures establish that only two NH group resonances, assigned to Val1 and ΔPhe2, move appreciably
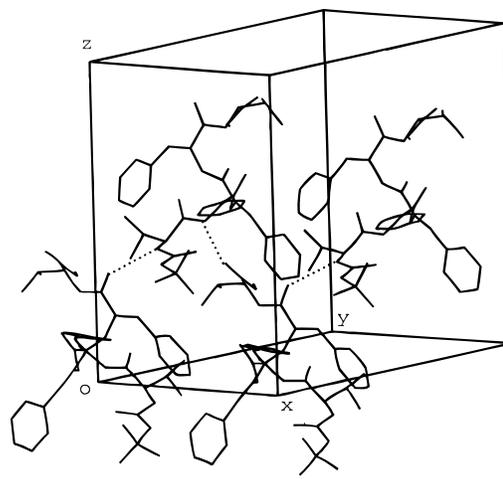
Figure 2. Crystal packing of Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe. View down the crystallographic *b* axis.

downfield on addition of the strongly hydrogen-bonding solvent, (CD$_3$)$_2$SO, to solution in the apolar solvent, CDCl$_3$. Also, both Val1 and ΔPhe2 NH resonances exhibit high temperature coefficient (dδ/dt > 0.004 ppm/K) in (CD$_3$)$_2$SO (Table 5), indicating that these NHs are not intramolecularly hydrogen bonded as they are exposed to the solvent for intermolecular hydrogen bonding.[15] On the other hand the remaining NH resonances (ΔPhe3, ΔPhe4, and Val5) show characteristics of solvent-shielded (intramolecular hydrogen bond) NH groups.[15a] These observation together with the known stereochemical preferences of ΔPhe residues to favor β-turn conformations[1] stabilized by intramolecular 4 → 1 hydrogen bond suggest that consecutive β-turn conformations are populated in solvents like chloroform. Both type III-III-III ($\phi_1 = \phi_2 = \phi_3 = \phi_4 \sim -60°$ and $\psi_1 = \psi_2 = \psi_3 = \psi_4 \sim -30°$) and type II-III′-III′ ($\phi_1 \sim -60°$, $\psi_1 \sim 120$, $\phi_2 = \phi_3 = \phi_4 = 60°$, and $\psi_1 = \psi_2 = \psi_3 \sim 30°$) structures[4] are compatible with the pentapeptide sequence. In the former arrangement, L-Val would have torsion angle $\phi \sim -60°$, $\psi \sim -30°$, and the three ΔPhe residues at positions 2, 3, and 4 would lie in the right-handed helical region of the ϕ, ψ map. In the latter arrangement, L-Val would have $\phi_1 \sim 60°$, $\psi_1 \sim 120°$, and the ΔPhe2, ΔPhe3, and ΔPhe4 would lie in the left-handed region.[16] A distinction between type II and type III β-turn conformation may be readily made by NMR method using NOEs[16] between C$_i^\alpha$H and N$_{i+1}$H protons in the ROESY[17] spectrum (Figure 3). A strong cross peak is observed between C$^\alpha$H Val(1) and NH ΔPhe(2) suggesting close approach (<3 Å) of the Val C$^\alpha$H and ΔPhe NH groups,[18] supporting a type II β-turn conformation for the Val(1)-ΔPhe(2) segment which in fact is the case in crystal structure also. Another evidence in

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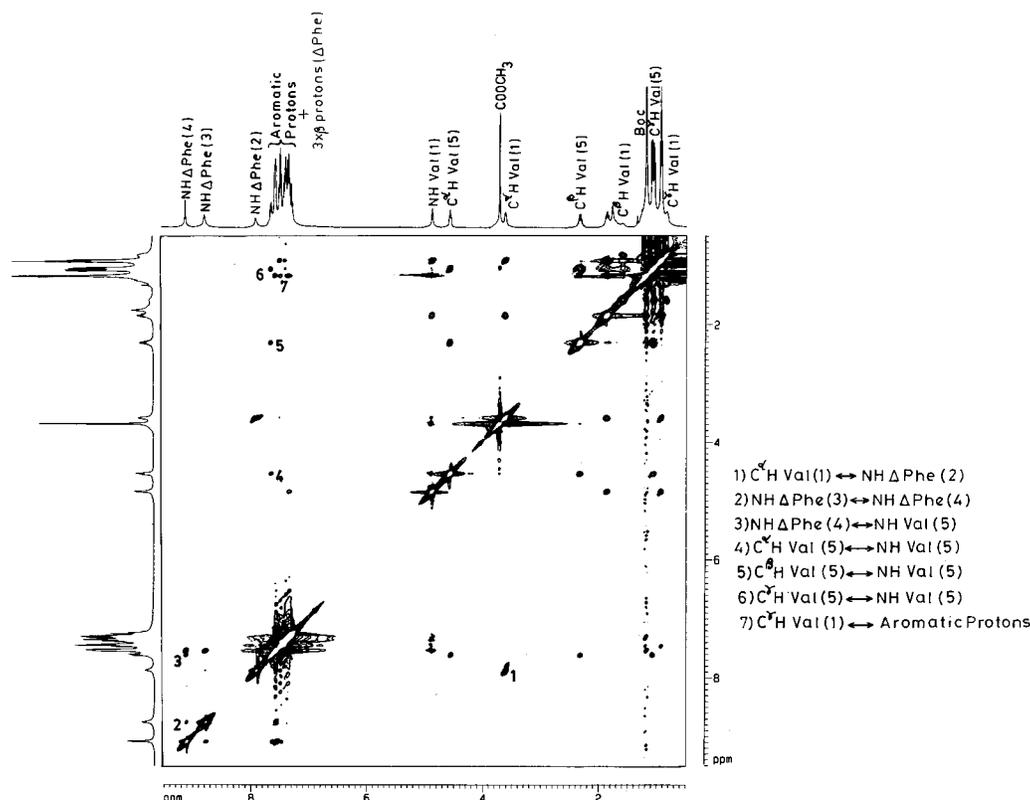
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Figure 3. 400 MHz ROESY spectrum of Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe in CDCl₃ at room temperature. The cross peaks are represented by the numbers.

Table 4. Intermolecular and Intramolecular Hydrogen Bonds Observed in the Crystal Structure of the Pentapeptide Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe

donor D	acceptor A	distance D—A (Å)	distance H—A (Å)	angle D—H—A (deg)	symmetry
N_1	O'_4	2.880(3)	2.115(3)	148.0(2)	$-x, y + 0.5, -z + 1.5$
N_2	O'_5	2.737(4)	1.929(4)	155.6(6)	$-x + 1, y + 0.5, -z + 1.5$
N_3	$O_2(Boc)$	2.913(3)	2.165(3)	145.1(3)	x, y, z
N_4	O'_1	2.890(3)	2.041(3)	168.6(3)	x, y, z
N_5	O'_2	2.964(3)	2.141(3)	160.3(2)	x, y, z

Table 5. NMR Parameters for NH Protons in the Pentapeptide Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe

residues	CDCl₃ (ppm)	(CD₃)₂SO (ppm)	Δ (ppm)	dδ/dT [(CD₃)₂SO] (10^{-3} ppm/K)	$^3J_{NH\alpha}$ value (in Hz)
Val(1)	4.80	7.25	2.45	10.00	4.00
ΔPhe(2)	7.71	10.30	2.59	4.57	
ΔPhe(3)	8.79	10.00	1.21	2.67	
ΔPhe(4)	9.17	9.45	0.28	3.50	
Val(5)	7.65	7.80	0.15	1.00	8.75

support of the type II β-turn is the absence of NOE between NH Val(1) and NH ΔPhe(2), since such $N_{i+1}H \leftrightarrow N_{i+2}H$ NOEs are expected only in a type I β-turn, where an interproton distance of 2.6 Å is estimated[18b.] The corresponding distance in a type II β-turn is 4.5 Å, and therefore no NOE was observed between NH Val(1) and NH ΔPhe(2) as expected.

Apart from this, NOEs between ΔPhe(3) NH ↔ ΔPhe(4) NH ↔ Val(5) NH are also observed in the ROESY spectrum (Figure 3). The observation of successive $N_{i+1} \leftrightarrow N_{i+2}$ NOE connectivities is diagnostic of a 3_{10} or α-helical conformation over the three residues.[18b] To differentiate between a 3_{10} and α-helical conformation medium range NOEs of the type $d_{\alpha N}$ (i, i+2 or i, i+3) have been used.[19] However, since successive ΔPhe residues, which lack CαH proton, are present in the pentapeptide, medium range $d_{\alpha N}$ NOEs were not observed, and

therefore unambiguous assignment of the type of helical conformation could not be made. But in the light of other NOE data (d_{NN} and $d_{\alpha N}$ cross peaks) and temperature and solvent dependence studies, it is clear that the pentapeptide adopts consecutive β-turn (helical) conformation in solvent like CDCl₃. The observed $^3J_{NH\alpha}$ values in CDCl₃ were 4.0 Hz [Val(1)] and 8.75 Hz [Val(5)], and the corresponding ϕ angles obtained with Karplus like equation[8,20] were ϕ[Val(1)] = 105°, 15°, −174°, −66° and ϕ[Val(5)] = −142°, −98°. The torsional angles −66° [Val(1)] and −142° [Val(5)] are in agreement with the conformation proposed for the pentapeptide.

Circular dichroism studies were carried out in three different solvents—chloroform, methanol, and trifluoroethanol—to probe the screw sense of the peptide chain conformation. In all the solvents, the peptide displays a couplet (+ −) of intense bands with opposite signs at 285 and 260 nm and a crossover at 275 nm (Figure 4). This kind of splitting pattern originates from the rigid disposition of the ΔPhe residues involved in a system of consecutive β-turns, such as a 3_{10}-helix.[51,9,21] The sign of the couplet (+ −) in case of **1** is opposite to that observed for dehydro peptides having right-handed screw sense.[51] The most likely explanation for the different signs is the presence of a preferred conformation having opposite chirality, i.e., left-handed 3_{10}-helix. The theoretical CD calculations were also carried

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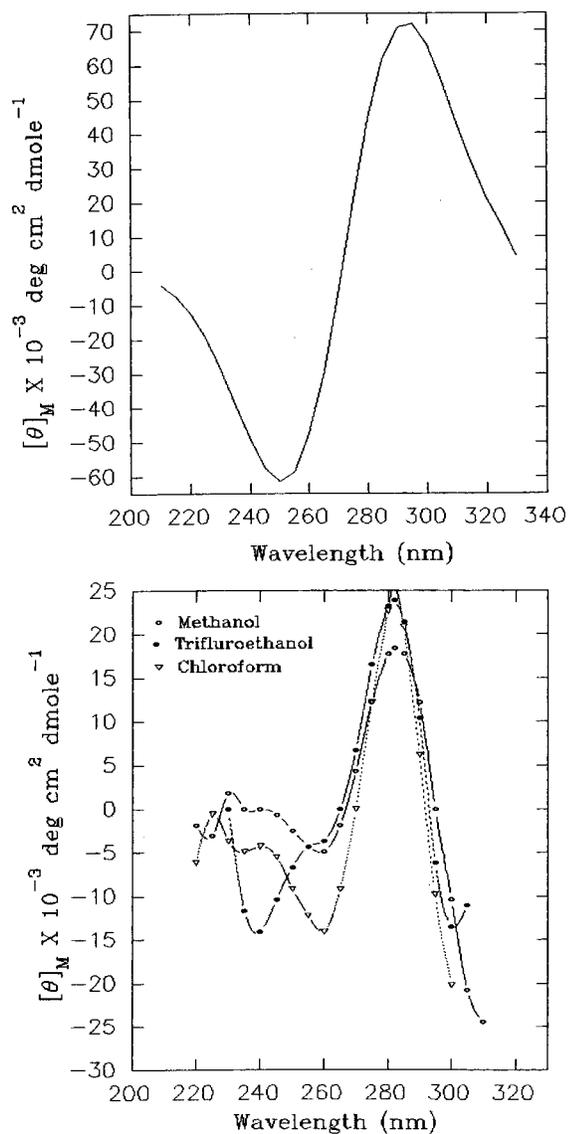3210 *J. Am. Chem. Soc., Vol. 119, No. 14, 1997*

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Figure 4. Theoretical (top) and experimental (bottom) CD spectra of Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe.

out with several 3_{10}-type helix main-chain torsion angles including the angles from the crystallographic data. The calculations are based on the spatial array of dehydro Phe residues, of 280 nm transition moment by MO calculation, the planarity of $NC^{\alpha}-C^{\beta}-C=O$ in dehydro Phe residue was assumed, and thus $\chi^{1,1}$ of dehydro Phe was fixed to 0°. The theoretical CD spectra obtained for the pentapeptide is similar to the experimental CD spectra giving a positive exciton couplet (positive peak at longer wavelength) as shown in Figure 4. This supports that the pentapeptide forms a left-handed helix.

Discussion

Structure elucidation of pentapeptide **1** is the first report of a dehydropeptide containing three consecutive ΔPhe residues, which may reflect on the conformational preference of poly (ΔPhe) system; this was the main purpose of the present study. The peptide molecule exhibits preference for left-handed 3_{10}-helical conformation, in spite of the presence of two L-Val residues (Figure 1). The terminal L-Val residues (L-Val[1] and L-Val[5]) are not strictly helical as indicated by their main chain torsion angles (Table 4). As mentioned earlier the segment centered around -L-Val[1]-ΔPhe[2]- exhibits a type II β-bend; a nonhelical turn conformation and L-Val[1] residue adopts a

semiextended conformation typical of *i*+1 position of type II β-turn. In Aib rich peptides it is observed that when the N-terminal -X-Aib sequence is of type II β-bend conformation, the adjacent segment of the peptide chain is forced to adopt the left-handed 3_{10}-helical conformation even if the peptide sequence contains some L residues.[22am] Also from model building it can be seen that if a type II β-bend is followed by a helix, this helix would tend to be left-handed, because of steric reasons. The deviation of terminal L-Val residues from helical conformation is not surprising considering the fact that L residues are not usually part of left-handed helices and instead accommodated in right-handed helices. In a peptide containing two consecutive ΔPhe residues (Boc-L-Ala-ΔPhe-ΔPhe-NHMe)[5j] both left-handed and right-handed helical structures were observed. Interestingly, although the L-Ala residue was part of the helix in both cases, it was found to be more distorted in the left-handed conformer.

Left-handed helical conformation is also observed in a few peptides containing Aib residues.[22] Fully blocked (Aib)$_n$ (n = 3−8,10) homopeptides crystallize in centrosymmetric space groups exhibiting both left, and right-handed 3_{10}-helical conformation.[22e−l] Remarkably, left-handed 3_{10}-helices are observed in Aib rich peptides *viz.*, Boc-Pro-Aib-Ala-Aib-Ala-OH, Z(Cl)-Pro-Aib-Ala-Aib-Ala-OMe and Ac-(Aib)$_2$-S-Iva-(Aib)$_2$-OMe,[22a−d] despite the presence of residues of L chirality. In none of the former peptides is L-Pro a part of the helix. Moreover both peptides start with a type II β-bend at the N-terminus. Surprisingly Ala[3] and S-Iva residues in these peptides thought to have L chirality adopt left-handed helical backbone torsion angles. However in the present peptide the terminal L-Val residues do not adopt left-handed helical conformation.

Results of NMR experiments suggest that the pentapeptide tends to maintain 3_{10}-helical conformation in chloroform. However, the handedness of the helical structure cannot be ascertained by NMR experiments. For this theoretical and experimental CD calculations were carried out.[9] In fact, CD has been used as a method of choice to monitor the interconversion of a right-handed helix into a left-handed helix in a model dehydrophenylalanine containing peptide, as a function of change in solvent and temperature.[23] In ΔPhe containing peptides which adopt right-handed 3_{10}-helical structures, very similar CD pattern have been observed;[9,21] the CD spectrum in most cases displays a couplet of intense bands with opposite signs (− +) at ~300 nm and ~270 nm and a crossover at ~275−285 nm. This CD pattern is a typical exciton splitting

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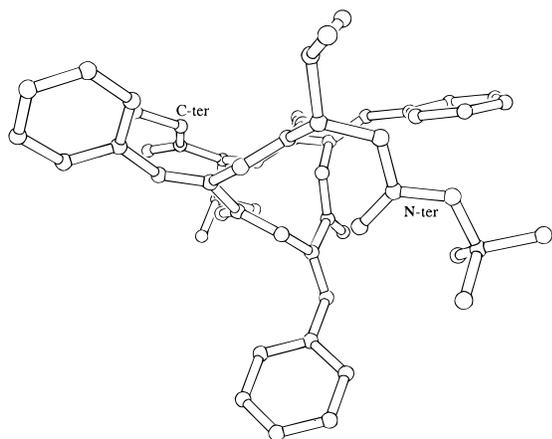
Boc-L-Val-ΔPhe-ΔPhe-ΔPhe-L-Val-OMe

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Figure 5. Pentapeptide molecule showing helical sense with side chains.

due to dipole−dipole interaction between charge-transfer electronic transitions of the dehydro chromophores and is a strong indication that the two ΔPhe residues are placed in mutual fixed disposition within the molecule. This arrangement is possible when at least two dehydro residues are involved in a system of consecutive β-turns, i.e., a 3_{10}-helix.[9] The fact that the sign of the couplet (+ −) in case of the present pentapeptide is opposite in both calculated CD spectrum and experimental CD spectra provides the evidence for the existence of large ensemble of left-handed 3_{10}-helical conformations in solution.

Conclusion

Given the conformation restricting ability of dehydroamino acids in peptides, the structure determination of polydehydro amino acids is of obvious interest. The present study highlights the conformational consequence of consecutive ΔPhe residues in peptides. Both crystal studies and solution studies suggest that pentapeptide exclusively adopts a left-handed helical conformation, despite the presence of two L-amino acid residues. Helical structures for poly (ΔAla)[24] have been predicted, which however have yet to be confirmed experimentally. Although no studies are performed on the poly (ΔPhe) system, the present study suggests that a left-handed helical conformation may be more stable in a peptide containing (ΔPhe)$_n$ motif. It is however not clear as to why a left-handed helix is preferred despite the presence of two L-amino acids in the sequence. It will therefore be of considerable interest to carry out conformational studies on peptides containing three or more consecutive ΔPhe residues.
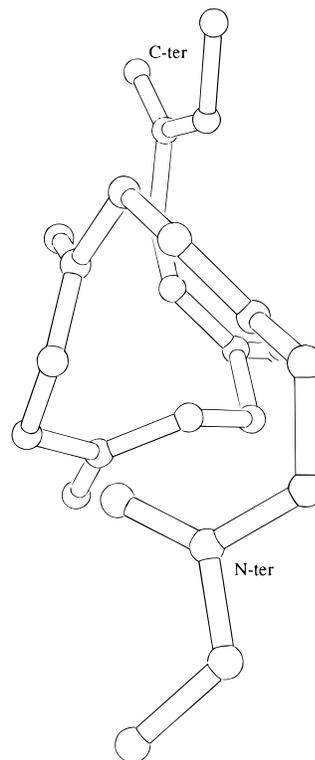

Figure 6. Pentapeptide molecule showing helical sense without side chains.

These results might be of relevance to chemists and biochemists working in fields such as *denovo* peptide/protein design, conformational energy calculations, and design of peptide based drugs which are resistant to enzymatic degradation *in vivo*[25] using amino acid residues such as ΔPhe.

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