Synthesis, crystal and molecular structure of the 310-helical α,β-dehydro pentapeptide Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe

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Synthesis, and Crystal and Molecular Structure of the 3_{10}-Helical α,β-Dehydro Pentapeptide Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe

α,β-Dehydro amino acid residues are known to constrain the peptide backbone to the β-bend conformation. A pentapeptide containing only one α,β-dehydrophenylalanine (ΔPhe) residue has been synthesized and crystallized, and its solid state conformation has been determined. The pentapeptide Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe ($C_{39}H_{55}N_5O_8$, M_w = 721.9) was crystallized from aqueous methanol. Monoclinic space group was $P2_1$, a = 10.290(2)°, b = 17.149(2)°, c = 12.179(2) Å, β = 96.64(1)° with two molecules in the unit cell. The x-ray (Mo K_α, λ = 0.7107A) intensity data were collected using a CAD4 diffractometer. The crystal structure was determined by direct methods and refined using least-squares technique. R = *4.4% and* R_w = *5.4% for 4403 reflections having* $|F_0| \geq 3\sigma(|F_0|)$. *All the peptide links are* trans *and the pentapeptide molecule assumes 3_{10}-helical conformation. The mean ϕ,ψ values, averaged over the first four residues, are −64.4°, −22.4° respectively. There are three 4 → 1 intramolecular hydrogen bonds, characteristic of 3_{10}-helix. In the crystal, the peptide helices interact through two head-to-tail, N—H—O intermolecular hydrogen bonds. The peptide molecules related by 2_1 screw symmetry form a skewed assembly of helices.* © *1995 John Wiley & Sons, Inc.*

INTRODUCTION

α,β-Unsaturated (or α,β-dehydro) amino acid residues have been found to occur naturally in many peptides from microbial origin and in some proteins.[1-3] It has been noted that the presence of α,β-dehydro residues (mainly α,β-dehydrophenylalanine or ΔPhe) in bioactive peptide sequences confers increased resistance to enzymatic degradation[4] as well as highly altered biological activity.[5,6] It has been shown on the basis of conformational energy calculations[7] and solution studies[8,9] that model linear peptides containing ΔPhe have a strong tendency to adopt β-bend[10] structures. The x-ray diffraction studies have confirmed this observation.[11-14] Further, 3_{10}-helical structures are observed in longer peptides containing more than one ΔPhe residue.[15-17] These results demonstrate the

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utility of the ΔPhe residue in peptide design. However, the conformational consequence of the number and positioning of ΔPhe residues in peptide sequences is not well understood. As a part of our continuing research program on α,β-dehydro oligopeptides, we report here the crystal and molecular structure of the dehydro pentapeptide Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe, containing only one ΔPhe residue. The peptide molecule, as a result of three consecutive type III β-bends, adopts a 3_{10}-helical conformation. This result shows that a pentapeptide with mainly bulky residues may adopt 3_{10}-helical conformation if it contains a single ΔPhe residue.

EXPERIMENTAL PROCEDURE

Synthesis

Amino acid derivatives were obtained from Nova Biochem (Switzerland). The ΔPhe moiety was introduced as a dipeptide block, obtained through azlactonization and dehydration of suitable dipeptides containing DL-β-phenylserine at the carboxyl end. All the intermediates were checked for purity by thin layer chromatography (tlc) and nmr spectroscopy. Solvent systems used for tlc were (1) $CHCl_3$:CH_3OH-9:1 and (2) *n*-butanol:CH_3COOH:H_2O-3:1:1.

Boc-Ala-DL-Phe(β-OH)OH (1). To a precooled solution (−10°C) of Boc-Ala-OH (5.6 g, 30 mmol) in dry tetrahydrofuran (30 mL), N-methylmorpholine (3.3 mL, 30 mmol), and isobutylchloroformate (3.9 mL, 30 mmol) were added. After 10 min of stirring, a solution of DL-Phe(β-OH)-OH (5.97 g, 30 mmol) in NaOH (1N, 33 mL) was added and the mixture stirred at 0°C for 2 h and at room temperature overnight. The organic solvent was removed under reduced pressure, and the aqueous phase was washed once with ether and acidified with solid citric acid to pH 3.0. The oily product thus obtained was extracted in ethyl acetate (3 × 20 mL). Ethyl acetate layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to yield **1** as an oily compound. Yield: 9.0 g (86%), $R_f(1) = 0.5$, $R_f(2) = 0.74$; ^{1}H-nmr (60 MHz, $CDCl_3$): 7.2 (5H, m, Aromatic Protons); 6.2 [1H, br, Phe(β-OH)—OH]; 5.02 (1H, d, Ala NH); 4.2 [2H, m, Ala $C^\alpha H$ and Phe(β-OH)—OH $C^\beta H$]; 1.4 (9H, s, 3 × CH_3 of Boc); 1.3 (3H, d, Ala $C^\beta H_3$).

Boc-Ala-ΔPhe Azlactone (2). To a solution of **1** (8.0 g, 22.7 mmol) in acetic anhydride (70 mL) was added freshly fused sodium acetate and the mixture was left overnight at room temperature. The reaction mixture was poured over crushed ice and stirred. The resultant solid was filtered, washed with 10% $NaHCO_3$, and then water, and dried under vacuum. Crystallization from acetone/water gave **2** as crystalline solid. Yield: 6.8 g (90%), $R_f(1) = 0.83$, $R_f(2) = 0.96$, mp = 103–105°C; ^{1}H-nmr (60 MHz, $CDCl_3$): 7.5–7.2 (5H, m, aromatic protons of ΔPhe); 7.15 (1H, s, $C^\beta H$ of ΔPhe); 4.2 (1H, br, $C^\beta H$ Ala); 1.4 (9H, s, 3 × CH_3 of Boc); 1.3 (3H, d, $C^\beta H_3$ Ala).

Boc-Ala-ΔPhe-Leu-OMe (3). To a solution of **2** (7.0 g, 22 mmol) in dichloromethane (40 mL), Leu-OMe.HCl (4.5 g, 25.0 mmol) was added, followed by triethylamine (3.5 mL, 25.0 mmol), and the mixture was stirred at room temperature for 30 h. Solvent was removed under reduced pressure and the residue was worked up by the usual procedure reported earlier to give **3.** Yield: 7.0 g (72%), $R_f(1) = 0.79$, $R_f(2) = 0.82$, mp = 136–138°C; $[\alpha]_D^{25} = -64.7°$ (c, 0.85 g/dL, MeOH); ^{1}H-nmr (60 MHz, $CDCl_3$): 7.9 (1H, s, NH ΔPhe); 7.4–7.1 (7H, m, aromatic protons, $C^\beta H$ ΔPhe and NH Leu); 5.2 (1H, d, NH Ala); 4.8 (1H, m, $C^\alpha H$ Leu); 4.2 (1H, m, $C^\alpha H$ Ala); 3.8 (3H, s, OMe); 1.9–1.5 (3H, m, $C^\beta H_2$ Leu and $C^\gamma H$ Leu); 1.4 (9H, s, 3 × CH_3 Boc); 1.35 (3H, d, $C^\beta H_3$ Ala); 0.9 (6H, d, 2 × CH_3 Leu).

Boc-Phe-Ala-ΔPhe-Leu-OMe (4). Tripeptide **3** (5.0 g, 10.4 mmol) was deprotected at its N-terminal using a mixture of trifluoroacetic acid in DCM (TFA:DCM; 1:1 v/v; 20 mL) at room temperature for 30 min. The excess acid was removed in vacuo and the residue triturated with dry ether and filtered. To a precooled (0°C) solution of Boc-Phe-OH (3.31 g, 12.5 mmol) in dimethylformamide (DMF; 30 mL) was added dicyclohexylcarbodiimide (DCC; 2.57 g, 12.5 mmol) and hydroxybenzatriazole (HOBT; 1.9 g, 12.5 mmol) and the mixture stirred for 30 min. The TFA salt of **3** in DMF (15 mL) and triethylamine (1.45 mL, 10.4 mmol) were added to the above mixture and stirred at room temperature for 16 h. The precipitated dicyclohexylurea was filtered off and the solvent was removed in vacuo. The reaction was worked up as in the case of **3** to give tetrapeptide **4.** Yield: 5.5 g (83%), $R_f(1) = 0.76$, $R_f(2) = 0.94$, mp = 70–72°C; ^{1}H-nmr ($CDCl_3$): 8.1 (1H, s, ΔPhe NH); 7.5–7.2 (10H, aromatic protons Phe, ΔPhe); 7.15 (1H, br, NH Leu); 6.65 (1H, br, NH Ala); 5.06 (1H, d, NH Phe); 4.5–4.0 (3H, m, $C^\alpha H$ Ala, Leu, Phe); 3.6 (3H, s, OMe); 3.02 (2H, d, $C^\beta H_2$ Phe); 1.9 (2H, m, $C^\beta H_2$ Leu); 1.6 (1H, q, $C^\gamma H$ Leu); 1.44 (9H, s, 3 × CH_3 Boc); 1.4 (3H, d, $C^\beta H_3$ Ala); 0.96 (6H, d, $C^\delta H_3$ Leu).

Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe (5). The Boc group of the tetrapeptide **4** (4 g, 6.5 mmol) was deprotected using TFA in dichloromethane as usual and the deprotected **4** was coupled with Boc-Leu-OH (1.9 g, 7.8 mmol) using DCC (1.6 g, 7.8 mmol) and HOBT (1.19 g, 7.8 mmol) as in case of **4.** Yield: 4.0 g (85%), $R_f(1) = 0.81$, $R_f(2) = 0.83$; mp = 178–180°C; $[\alpha]_D^{25} = -56°$ (c, 0.5g/dL, MeOH); ^{1}H-nmr: ($CDCl_3$, 270 MHz): 8.12 (1H, s, ΔPhe NH); 7.46 [1H, br, Leu (5) NH]; 7.36 [1H, br, Phe (2) NH]; 7.4–7.1 [10H, m, aromatic protons Phe (2) and ΔPhe (4)]; 7.06 [1H, br, Ala (3) NH]; 6.61 [1H, s, ΔPhe

Table 1 Final Atomic Fractional Coordinates and Equivalent Isotropic Thermal Parameters with Estimated Standard Deviations in the Parentheses

Atom	x	y	z	B_{eq} (Å^2)
C_1	0.2746 (4)	0.4002 (3)	0.3741 (4)	7.36 (16)
C_2	0.e334 (5)	0.4778 (4)	0.4163 (5)	10.15 (23)
C_3	0.2020 (6)	0.3604 (5)	0.4586 (5)	13.25 (27)
C_4	0.1905 (6)	0.4086 (6)	0.2662 (6)	10.19 (26)
O_1	0.3789 (3)	0.3424 (2)	0.3607 (2)	6.45 (08)
C_5	0.4675 (3)	0.3553 (2)	0.2898 (3)	4.60 (09)
O_2	0.4875 (2)	0.4171 (2)	0.2454 (2)	5.34 (07)
N_1	0.5335 (3)	0.2893 (2)	0.2733 (2)	4.27 (07)
C_1^{α}	0.6491 (3)	0.2922 (2)	0.2151 (3)	3.45 (08)
C_1'	0.6203 (3)	0.3245 (2)	0.0981 (2)	3.19 (07)
O_1'	0.6997 (2)	0.3678 (1)	0.0606 (2)	3.96 (05)
C_1^{β}	0.7095 (3)	0.2108 (2)	0.2118 (3)	3.85 (08)
C_1^{γ}	0.7758 (4)	0.1812 (2)	0.3228 (3)	4.50 (09)
$C_1^{\delta 1}$	0.9063 (4)	0.2233 (3)	0.3546 (3)	6.19 (12)
$C_1^{\delta 2}$	0.7970 (6)	0.0931 (3)	0.3149 (5)	6.57 (15)
N_2	0.5080 (3)	0.3040 (1)	0.0397 (2)	3.48 (06)
C_2^{α}	0.4807 (3)	0.3266 (2)	−0.0775 (3)	3.64 (08)
C_2'	0.4698 (3)	0.4147 (2)	−0.0900 (3)	3.67 (07)
O_2'	0.4816 (2)	0.4442 (1)	−0.1800 (2)	4.79 (06)
C_2^{β}	0.3570 (3)	0.2877 (2)	−0.1334 (3)	4.09 (09)
C_2^{γ}	0.2309 (3)	0.3152 (2)	−0.0956 (3)	4.04 (07)
$C_2^{\delta 1}$	0.1680 (4)	0.3812 (2)	−0.1439 (4)	5.54 (12)
$C_2^{\delta 2}$	0.1749 (4)	0.2778 (2)	−0.0134 (4)	5.10 (11)
$C_2^{\epsilon 1}$	0.0526 (4)	0.4069 (3)	−0.1098 (5)	7.27 (16)
$C_2^{\epsilon 2}$	0.0579 (4)	0.3034 (3)	0.0199 (4)	6.48 (13)
C_2^{ζ}	−0.0026 (4)	0.3688 (3)	−0.0294 (5)	7.33 (17)
N_3	0.4467 (3)	0.4574 (2)	−0.0017 (2)	3.69 (07)
C_3^{α}	0.4402 (3)	0.5420 (2)	−0.0105 (3)	4.30 (09)
C_3'	0.5718 (3)	0.5770 (2)	−0.0281 (2)	3.44 (07)
O_3'	0.5758 (2)	0.6449 (1)	−0.0613 (2)	4.65 (06)
C_3^{β}	0.3890 (7)	0.5763 (3)	0.0911 (6)	7.74 (21)
N_4	0.6792 (2)	0.5337 (1)	−0.0088 (2)	3.22 (06)
C_4^{α}	0.8012 (3)	0.5615 (2)	−0.0372 (2)	3.41 (08)
C_4'	0.8125 (3)	0.5703 (2)	−0.1593 (3)	3.94 (07)
O_4'	0.9025 (3)	0.6061 (2)	−0.1921 (2)	6.12 (08)
C_4^{β}	0.9034 (3)	0.5797 (2)	0.3050 (3)	4.25 (09)
C_4^{γ}	0.9141 (3)	0.5780 (2)	0.1565 (3)	4.55 (09)
$C_4^{\delta 1}$	0.9980 (5)	0.6309 (3)	0.2146 (4)	7.42 (16)
$C_4^{\delta 2}$	0.8463 (4)	0.5259 (3)	0.2162 (3)	5.70 (11)
$C_4^{\epsilon 1}$	1.0111 (6)	0.6322 (4)	0.3288 (4)	9.75 (22)
$C_4^{\epsilon 2}$	0.8595 (5)	0.5294 (4)	0.3308 (4)	7.48 (17)
C_4^{ζ}	0.9423 (6)	0.5830 (4)	0.3858 (4)	8.37 (18)
N_5	0.7213 (3)	0.5334 (2)	−0.2265 (2)	3.95 (07)
C_5^{α}	0.7211 (4)	0.5332 (2)	−0.3452 (3)	4.29 (08)
C_5'	0.6367 (4)	0.5996 (2)	−0.3972 (3)	5.64 (12)
O_5'	0.5556 (3)	0.6340 (2)	−03562 (2)	7.30 (10)
C_5^{β}	0.6694 (4)	0.4554 (2)	−0.3931 (3)	4.67 (09)
C_5^{γ}	0.7529 (4)	0.3855 (2)	−0.3529 (4)	5.72 (12)
$C_5^{\delta 1}$	0.8849 (5)	0.3867 (3)	−0.3962 (4)	8.26 (15)
$C_5^{\delta 2}$	0.6741 (8)	0.3097 (3)	−0.3842 (7)	9.39 (25)
O_3	0.6632 (4)	0.6107 (2)	−0.5000 (2)	9.44 (13)
C_6	0.5854 (7)	0.6692 (4)	−0.5633 (4)	12.13 (27)

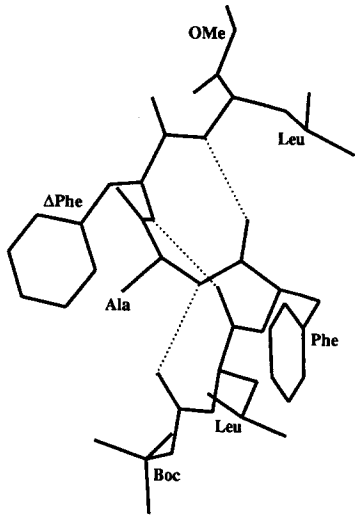

FIGURE 1 Molecular structure of Boc^0-Leu^1-Phe^2-Ala^3-ΔPhe^4-Leu^5-OMe, showing the 3_{10}-helical conformation. The dotted lines indicate the intramolecular 4 → 1 hydrogen bonds.

(4) $C^{\beta}H$]; 4.85 [1H, br, Leu (1) NH]; 4.72 [1H, m, Ala (1) $C^{\alpha}H$]; 4.45 [1H, m, Leu (5) $C^{\alpha}H$]; 4.40 [1H, m, Leu (1) $C^{\alpha}H$]; 3.767 [1H, m, Phe (2) $C^{\alpha}H$]; 3.7 (3H, s, OCH_3); 3.1 [2H, br, Phe (2) $C^{\beta}H$]; 3.1–3.2 [4H, m, $C^{\beta}H$ of Leu (1) and Leu (5)]; 1.63–1.9 [2H, br, $C^{\gamma}H$ of Leu (1) and Leu (5)]; 1.42 [3H, d, $C^{\beta}H$ Ala (3)]; 1.35 (9H, s, $3 \times CH_3$); 0.8–0.9 [12H, d, $C^{\delta}H_3$ of Leu (1) and Leu (5)].

X-Ray Structure Determination

Single crystals of the pentapeptide Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe ($C_{39}H_{55}N_5O_8$, M_w = 721.9) used in x-ray diffraction experiments were grown by controlled evaporation of the peptide solution in aqueous methanol. Even though crystals of the title compound were also obtained by slow evaporation of peptide solution in aqueous ethyl acetate at low temperature, no polymorphism was observed. A colorless crystal mounted on a glass fiber was used for determining the unit cell parameters and for measuring the three-dimensional x-ray intensity data. The cell constants were determined by setting the angles of 25 accurately measured high angle reflections on a Enraf-Nonius CAD4 diffractometer equipped with Mo K_{α} radiation (λ = 0.7107 Å). Monoclinic space group $P2_1$, a = 10.290(2)°, b = 17.149(2) Å, c = 12.179 Å β = 96.64(1)°, V = 2135 Å^3 and Z = 2 were obtained in the crystal. The x-ray intensity data were collected up to a Bragg angle of 28° using $\omega - 2\theta$ scan technique. A total of 4839 unique reflections were measured, of which 4403 reflections having $|F_0| \geq 3\sigma(|F_0|)$ were observed and used in crystal structure analysis. No significant variation was observed in the intensities of three standard reflections monitored at regular intervals of time during data collections, indicating the electronic and crystal stability. Lorentz and polarization corrections were applied to the data and no absorption correction was made. A partial structure was obtained using the direct methods employing MULTAN.[18] Partial structure expansion using SHELXS86[19] revealed the whole molecule. The structure was refined by full-matrix least-squares technique with anisotropic thermal factors for all nonhydrogen atoms. Most of the hydrogen atoms could be located in the difference Fourier map except those bonded to the terminal protecting group and C^{δ} atoms of Leu residues. The hydrogen atoms not observed in the difference Fourier map were fixed on the basis of stereochemistry and they were included only in structure factor calculation. The final agreement factors are R = 4.4% and R_w = 5.4%.

RESULTS AND DISCUSSION

The atomic parameters for all nonhydrogen atoms of the pentapeptide molecule are given in the Table I.

All bond lengths and bond angles are normal except those corresponding to the ΔPhe residue. The introduction of a double bond between C^{α} and C^{β} atoms in ΔPhe^4 affects the other bond lengths and angles in the same residue, as seen in other dehydro peptides containing ΔPhe residues.[11–17] The

Table II The Intramolecular and Intermoleculear Hydrogen Bonds Observed in the Solid State Structure of the Pentapeptide Boc^0-Leu^1-Phe^2-Ala^3-ΔPhe^4-Leu^5-OMe[a]

Donor (D)	Acceptor (A)	Distance D-A (Å)	Distance H-A (Å)	Angle D-H-A	Symmetry
N1	O'_5	3.028 (5)	2.19 (4)	170 (3)	1
N2	O'_3	2.883 (3)	2.32 (4)	142 (3)	1
N3	O_2	3.069 (4)	2.35 (3)	159 (3)	0
N4	O'_1	2.968 (3)	2.18 (3)	170 (3)	0
N5	O'_2	3.011 (4)	2.28 (3)	164 (3)	0

[a] Symmetry code: O: x, y, z; 1: $-x+1, y-\frac{1}{2}, -z$.

Table III Some of the Important Torsion Angles in the Molecular Structure of Boc^0-Leu^1-Phe^2-Ala^3-ΔPhe^4-Leu^5-OMe

Atoms A-B-C-D	Angle i=	Boc 0	Leu 1	Phe 2	Ala 3	ΔPhe 4	Leu 5
C_1-O_1-C_5-N_1	θ^1	−166.6 (3)					
C_1-O_1-C_5-O_2	$\theta^{1\prime}$	13.3 (6)					
O_1-C_5-N_1-$C_1\alpha$	ω^0	−169.4 (3)					
C'_{i-1}-N_i-C_i^α-C'_i	χ_i		−59.5 (4)	−64.5 (4)	−66.4 (4)	−67.3 (4)	−93.0 (4)
N_i-C_i^α-C'_i-N_{i+1}	ψ_i		−38.8 (4)	−19.5 (4)	−15.1 (4)	−16.1 (4)	−164.5 (3)[a]
C_i^α-C'_i-N_{i+1}-$C_{i+1}\alpha$	ω_i		−174.0 (3)	177.8 (3)	172.3 (3)	−177.4 (3)	
N_i-C_i^α-C_i^β-C_i^γ	χ_i^1		−71.0 (4)	69.1 (4)		−2.0 (6)	−62.6 (4)
C_i^α-C_i^β-C_i^γ-$C_i^{\delta 1}$	$\chi_i^{2,1}$		−73.6 (4)	86.3 (4)		31.0 (6)	−68.0 (5)
C_i^α-C_i^β-C_i^γ-$C_i^{\delta 2}$	$\chi_i^{2,2}$		164.4 (4)	−93.1 (4)		−149.8 (4)	166.6 (4)

[a] N_5-C_5^α-$C_{5'}$-$O_{3'}$.

C_4^α=C_4^β bond distance, in ΔPhe^4, is 1.328(4) Å, which corresponds to a classical C=C double bond. The N_4—C_4^α = 1.422(4) Å and C_4^α=C'_4 1.513(5) Å bond distances in ΔPhe^4 are slightly shorter than the corresponding bonds of saturated residues (1.45 and 1.53 Å, respectively).[20] The shortening of bonds N_4-C_4 and C_4^α—C'_4 is probably due to sp^2 hybridized C_4^α and C_4^β atoms and also might be a result of partial conjugation of ΔPhe^4 ring electrons and remaining atoms in the residue.

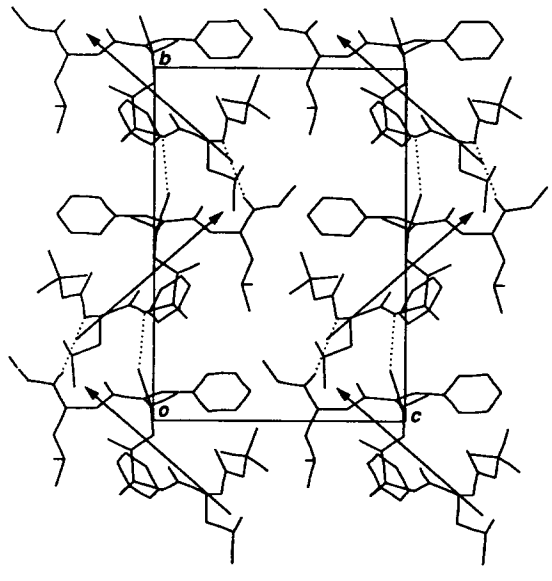

FIGURE 2 Crystal packing of Boc^0-Leu^1-Phe^2-Ala^3-ΔPhe^4-Leu^5-OMe. View down the crystallographic *a* axis. The intermolecular head-to-tail N—H—O hydrogen bonds are indicated by the dotted lines. The arrows represent the approximate helix axis with the arrow heads pointing toward the C-terminus.

Complete conjugation requires coplanarity of the ΔPhe ring with the peptide unit. However, in ΔPhe^4 complete conjugation is not observed, which may be due to steric reasons, as $\chi_4^{2,1}$ (31.0°) and $\chi 4_4^{2,2}$ (−149.8°) dihedral angles indicate a deviation from planarity of ΔPhe^4 residue.

The values of the bond angle N_4—C_4^α—C'_4 = 116.3(3)° is less than the standard trigonal value of 120°, while the bond angles N—C_4^α—C_4^β = 124.9(3)° and C_4^α—C_4^β—C_4^γ = 128.5(3)° are considerably larger. Due to the double bond between C_4^α,C_4^β atoms, the side-chain atoms approach the main-chain atoms, and the sp^2 hybridized C_4^α,C_4^β atoms make the residue more planar. These two factors lead to some unfavorable steric contacts between the side-chain and the main-chain atoms of the ΔPhe residue. To release these steric contacts, some rearrangement of the bond angles at the C_4^α and C_4^β atoms takes place, which is manifested as the above-mentioned deviations.

Conformation of the Peptide

A perspective view of the peptide molecule is given in Figure 1. The molecule is characterized by three consecutive type III β-turns that are stabilized by three intramolecular (4 → 1) hydrogen bonds (Table II). As a result of this, the pentapeptide molecule adopts a right-handed 3_{10}-helical conformation. The average backbone torsion angles are $\langle\phi\rangle$ = −64.4° and $\langle\psi\rangle$ = −22.4° (excluding C-terminal Leu^5, see Table III). These values are very close to the values reported for 3_{10}-helical peptides.[21,22] All the peptide links are of *trans* conformation. At the C-terminus residue Leu^5, the helix gets unwound,

a common feature observed in helical peptides[16,23] and supported by molecular dynamics simulations.[24]

The Boc group is characterized by the dihedral angles $C_1—O_1—C_5—N_1$ (θ^1) and $O_1—C_5—N_1—C_1^\alpha$ (ω_0), which assume values of −166.6(3)° and 169.4(3)° respectively[25]. These values correspond to a *trans–trans* conformation of the Boc group, which makes it possible for the O_2(Boc) atom to participate in the first 4 → 1 intramolecular hydrogen bond. The slight deviation of ω_0 and θ^1 from 180°, in the present case, indicate relatively a nonplanar urethane moiety [between C_1(Boc) and C_1^α] which generally prefers a planar conformation.[25] The dihedral angle $C_1—O_1—C_5—O_2$ ($\theta^{1'}$) has a value of 13.3(6)°, indicating that the $C_{5*}O_2$ bond is *syn* planar with the $C_1—O_1$ bond, as seen for esters in general.[26,27] The three methyl carbon atoms of the Boc group are staggered with respect to the $O_1=C_5$ bond [θ_1^2 = 62.3(6)°, θ_2^2 = 179.5(4)°, θ_3^2 = −61.6(5)°].

The two Leu residues (Leu[1] and Leu[5]) in the peptide molecule show similar side-chain conformations (Table III). The side chain conformation of Leu[1], Phe[2], and Leu[5] is consistent with the observed conformations in peptide crystal structures.[28] Viewed down the helix axis, the side chains assume the energetically favorable, slightly staggered arrangement as contrasted with the completely eclipsed arrangement in an ideal 3_{10}-helix.[21] Preliminary results obtained from ^{1}H-nmr spectroscopy, involving temperature and solvent dependence studies in a nonpolar solvent (like $CDCl_3$), indicate the involvement of three NH groups in intramolecular hydrogen bonding.[29] This suggests that a 3_{10}-helical arrangement observed in the solid state for the pentapeptide is also maintained in solution. This is in accordance with several earlier studies on ΔPhe containing peptides, where similar structures were observed in both solid and solution states.

Crystal Packing

The crystal packing of the pentapeptide molecules as observed normal to the crystallographic 2_1 screw axis is illustrated in Figure 2. It is frequently observed that the helical peptide molecules related by twofold screw symmetry form long rods by head-to-tail hydrogen bonding with the helix axes aligned in a straight line or zigzagged by a small amount.[30] It is noteworthy that in the present case the 3_{10}-helices related by twofold screw symmetry are approximately perpendicular (Figure 2). However, they interact through head-to-tail N-H–O hydrogen bonds (Table II) but they do not form long rods as the helix axes are not aligned in a straight line. Such a situation arises because the helix axis of the 3_{10}-helical pentapeptide molecule makes an angle of approximately 45° with the crystallographic 2_1 screw axis. The head-to-tail region does not meet in a good register, as O_4' does not participate in any intermolecular N-H–O type hydrogen bonds. The adjacent helices along the crystallographic *a* or *c* axis are related by translation, and hence are parallel.

CONCLUSION

The present results show the conformational consequence of the presence of a single ΔPhe residue in a pentapeptide sequence, Boc-Leu-Phe-Ala-ΔPhe-Leu-OMe, consisting mainly bulky residues like Leu and Phe. It is demonstrated that such a sequence with a single ΔPhe can adopt the 3_{10}-helical conformation. This would be of use when one wants to design a 3_{10}-helix with the least possible ΔPhe content. The effect of a single ΔPhe on longer peptide sequences, the role of positioning of ΔPhe in the sequence, and the influence of ΔPhe in peptide sequences with less bulky residues remains to be examined in detail.

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