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3_{10}-Helices, Helix Screw Sense and Screw Sense Reversal in the Dehydro-peptide Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe

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The pentapeptide Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe, containing two dehydro-phenylalanine (ΔPhe) residues, has been synthesized and its structure investigated. In the crystalline state, the molecule adopts a right-handed 3_{10}-helical conformation stabilized by two intramolecular hydrogen bonds between CO of Val[1] and NH of ΔPhe[4], and between CO of ΔPhe[2] and NH of Val[5], respectively. NMR measurements are consistent with the presence of 3_{10}-helical structures also in acetonitrile and dimethylsulphoxide solution: the distances between backbone protons estimated from NOE connectivities are in overall agreement with those observed in the solid state; the chemical shifts of the amide protons show the smaller temperature coefficients for the NHs that in solid state are involved in intramolecular hydrogen bonds. The CD spectra in acetonitrile, chloroform, methanol and dimethylsulphoxide display exciton couplets of bands corresponding to the ΔPhe electronic transition at 280 nm; the sign of the bands is consistent with the presence of helical structures having a prevalent left-handed screw sense. Addition of 1,1,1,3,3,3-hexafluoro-propan-2-ol gives rise to the gradual appearance of a couplet of opposite sign, suggesting the helix reversal from left-handed sense to right-handed sense. The conformational behaviour is discussed on the basis of the specific sequence of the peptide.

Keywords: Dehydro-peptides; 3_{10}-helix; helix reversal; crystal structure; circular dichroism

INTRODUCTION

Dehydro-peptides, that is peptides containing α,β-unsaturated amino acid residues, are currently being investigated in order to design compounds having well-defined secondary structures and to obtain structurally constrained analogues of natural peptides. α,β-Unsaturated residues, in fact, are characterized by peculiar structural features, such as the planarity of the C=C double bond in the side chain and the sp^2 hybridization of the C^α carbon atom. So, their incorporation in a peptide sequence produces remarkable conformational consequences and can induce structural motifs which are not available when the same residues are saturated.

Various types of folded structures, such as β-turns [1–8], 3_{10}-helices [9–17], α-helices [18, 19] and β-ribbon structures [20] have been found to occur in dehydro-peptides, depending on the specific sequence. The peptide Boc-Ala-ΔPhe-ΔPhe-NHMe, containing two consecutive dehydro-phenylalanine (ΔPhe) residues, was found to exist as 3_{10}-helices of both screw senses [12], in spite of the presence of the chiral Ala residue. The peptide Boc-Ala-ΔPhe-Gly-ΔPhe-Ala-OMe was found to give reversible screw sense reversal of the 3_{10}-helix, the right-handed or the left-handed sense depending on solvent conditions [21, 22].

The reversal of helicity experimentally observed in Boc-Ala-ΔPhe-Gly-ΔPhe-Ala-OMe is likely to be the result of a delicate balance between the conformational tendencies of the two chiral residues located in

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positions 1 and 5 of the sequence. It was therefore of interest to establish whether the helix reversal was a special property of the above pentapeptide or a general behaviour of dehydro-peptides having structure Boc-*Xxx*-ΔPhe-Gly-ΔPhe-*Xxx*-OMe. A better understanding of the phenomenon required the synthesis and investigation of new analogues in which Ala residues are replaced by other amino acid residues having different stereochemical and conformational parameters. In this report we describe the crystal structure and the conformational behaviour in solution of the β-branched valine derivative.

$$\text{Boc}-\text{Val}^1-\Delta\text{Phe}^2-\text{Gly}^3-\Delta\text{Phe}^4-\text{Val}^5-\text{OMe} \quad (1)$$

Structural and conformational features were found to be quite similar to those already reported for Boc-Ala-ΔPhe-Gly-ΔPhe-Ala-OMe [22]. In the solid state, X-ray diffraction analysis shows that the peptide adopts an incipient 3_{10}-helical structure having right-handed screw sense. NMR and CD measurements provide evidence that the helical structure is retained in solution. However, the prevalent helix sense depends on the solvent: the right-handed helix dominates in 1,1,1,3,3,3-hexafluoro-propan-2-ol, whereas the opposite left-handed helix prevails in chloroform, acetonitrile, methanol and dimethylsulphoxide.

MATERIALS AND METHODS

Synthesis of Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe (1)

Boc-Gly-ΔPhe Azlactone (2). This intermediate was obtained as previously reported [9]. $R_F = 0.93$ (chloroform/methanol = 9/1); m.p. = 120–121°C. ^{1}H-NMR ($CDCl_3$), δ: 8.05 (m, 2H, aromatic protons); 7.40 (m, 3H, aromatic protons); 7.13 (s, 1H, styryl proton); 5.18 (t, 1H, Gly NH); 4.28 (d, 2H, Gly CH_2); 1.45 (s, 9H, Boc CH_3). IR (KBr), ν: 3245 (urethane NH); 1810-1790 (azlactone CO); 1670, 1660 (urethane CO and azlactone CN); 1595 (CC); 1178, 1170 (azlactone CNO and COO); 770–690 cm^{-1} (aromatic CH).

Boc-Gly-ΔPhe-Val-OMe (3). Valine methylester hydrochloride (1.08 g, 6.50 mmol) was added to a stirred solution of the azlactone **2** (1.71 g, 5.65 mmol) and *N*-methylmorpholine (0.8 ml, 7.2 mmol) in tetrahydrofuran (35 ml), at 0°C. After 48 h, the reaction mixture was filtered and the solvent was evaporated. The residue was taken up in ethyl acetate and the organic solution was washed in the order with 10% $KHSO_4$, 5% $NaHCO_3$, water and finally dried over anhydrous sodium sulphate. Crystallization from ethyl acetate/petroleum ether gave **3** as crystalline white needles. Yield: 1.32 g (54%).

$R_F = 0.83$ (chloroform/methanol = 9/1); $R_F = 0.81$ (*n*-BuOH/AcOH/H_2O = 4/1/1); m.p. = 127°C; $[\alpha]_D^{20} = -44.7°$ ($c = 0.11$ g/dl, in MeOH). ^{1}H-NMR ($CDCl_3$), δ: 7.90 (s, 1H, ΔPhe NH); 7.40–7.20 (m, 6H, aromatic protons + styryl proton); 7.05 (d, 1H, Val NH); 5.35 (t, 1H, Gly NH); 4.55 (m, 1H, Val $C^{\alpha}H$); 3.88 (d, 2H, Gly CH_2); 3.68 (s, 3H, $COOCH_3$); 2.18 (m, 1H, Val $C^{\beta}H$); 1.42 (s, 9H, Boc CH_3); 0.95–0.90 (2d, 6H, Val CH_3). IR (KBr), ν: 3460–3250 (NH); 1735 (ester CO); 1690, 1670, 1660 (urethane and amide CO); 1630 (C=C); 1540 (amide II), 770–685 cm^{-1} (aromatic CH). UV absorption (MeOH): $\lambda_{max} = 279$ nm; $\varepsilon_{max} = 18{,}000$ mol^{-1} cm^{-1}.

TFA·H-Gly-ΔPhe-Val-OMe (4). The tripeptide **3** (1.32 g, 3.04 mmol) was treated with a 1/1 solution of trifluoroacetic acid (2 ml) and methylene chloride (2 ml) for 1 h at room temperature. The resulting peptide was precipitated with ethyl ether, washed repeatedly with the same solvent and the product recovered as trifluoroacetate salt. It was used in the successive reaction without any further purification.

Boc-Val-ΔPhe azlactone (5). D, L-β-Hydroxy-phenylalanine ethyl ester hydrochloride (14.73 g, 60 mmol), Boc-valine hydroxy-succinimide ester (18.84 g, 60 mmol) and *N*-methylmorpholine (60 mmol) were added to dry tetrahydrofuran. The mixture was stirred at room temperature overnight and then poured into ice-water. The product was extracted with ethyl acetate, washed with water and dried to give a diastereomeric mixture of Boc-Val-D,L-(β-OH)Phe-OEt (21 g, yield 92%).

The peptide ethyl ester (21 g, 55 mmol) was saponified by treatment with 1N NaOH (60 ml) in methanol (100 ml), for 3 h at room temperature. The solution was acidified to pH 2–3 with 10% $KHSO_4$ and extracted with ethyl acetate. Evaporation of the organic solvent gave Boc-Val-D,L-(β-OH)Phe-OH as an amorphous solid (17.48 g, yield 84%).

The product (16.28 g, 46 mmol) was reacted with anhydrous sodium acetate (3.95 g, 46 mmol) in 50 ml of freshly distilled acetic anhydride for 72 h at room temperature. The reaction mixture was poured over crushed ice, stirred, and the resultant precipitate was collected on a Buchner funnel, washed with 10% $NaHCO_3$, cool water and finally dried. Recrystallization from acetone/water at room

temperature gave **5** as a white crystalline product. Yield: 11.87 g (75%).

$R_F = 0.80$ ($CHCl_3$/MeOH = 9/1); $R_F = 0.90$ (*n*-BuOH/AcOH/H_2O = 4/1/1); m.p. = 114–115°C; $[\alpha]_D^{20} = -91.0°$ ($c = 0.10$ g/dl, in methanol).

^{1}H-NMR ($CDCl_3$) δ: 8.08 (m, 2H, aromatic protons); 7.42 (m, 3H, aromatic protons); 7.18 (s, 1H, styryl proton); 5.06 (broad, 1H, Val NH); 4.62 (m, 1H, Val $C^{\alpha}H$); 2.23 (m, 1H, Val $C^{\beta}H$); 1.45 (s, 9H, Boc CH_3); 1.02 (d, 3H, Val CH_3; 0.96 (d, 3H, Val CH_3). IR (KBr), ν: 3260 (NH); 1810–1790 (azlactone CO); 1670 (urethane CO); 1650 (azlactone CN); 1160 (azlactone CN–O); 770–690 cm^{-1} (aromatic CH).

Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe (1). The peptide trifluoroacetate **4** (1.32 g, 3.04 mmol) was dissolved in *N,N*-dimethylformamide (50 ml), then the azlactone **5** (0.98 g, 2.85 mmol) and *N*-methylmorpholine (0.36 ml, 0.33 mmol) were added to the solution. After 72 h at room temperature, the solvent was evaporated and the residue was taken up in ethyl acetate. The organic solution was washed with 10% $KHSO_4$, 5% $NaHCO_3$, water, and finally dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a solid which was purified by repeated crystallization from tetrahydrofuran/petroleum ether. Yield: 1.25 g (65%).

$R_F = 0.70$ ($CHCl_3$/benzene/MeOH = 8.5/0.5/1.0); m.p. = 210–212°C; $[\alpha]_D^{20} = +35.8°$ ($c = 0.95$ g/dl, in MeOH). ^{1}H-NMR ($CDCl_3$) δ: 8.58 (s, 1H, ΔPhe^4 NH); 7.86 (t, 1H, Gly^3 NH); 7.68 (s, 1H, ΔPhe^2 NH); 7.60–7.20 (m, 12H, aromatic and styryl protons); 7.13 (d, 1H, Val^5 NH); 5.04 (d, 1H, Val^1 NH); 4.55 and 3.95 (m, 4H, Val^1, Val^5 and Gly^3 $C^{\alpha}H$); 3.72 (s, 3H, $COOCH_3$); 2.20 (m, 2H, Val^1 and Val^5 $C^{\beta}H$); 1.43 (s, 9H, Boc CH_3); 0.94–0.92 (2d, 12H, Val^1 and Val^5 $4CH_3$). IR ($CHCl_3$), ν: 3435, 3391, 3338, 3296 (NH); 1747, 1736, 1691, 1670 (urethane and peptide CO); 1720 (CO ester group); 1633 cm^{-1} (C=C). UV absorption (MeOH): $\delta_{max} = 280$ nm; $\varepsilon_{max} = 35{,}600$ mol^{-1} cm^{-1}.

X-ray Diffraction

Colourless needle-shaped single crystals suitable for X-ray analysis were obtained by slow coiling from a methanol/water solution. Crystals were found to belong to orthorhombic space group $P2_12_12_1$ with $a = 10.462(5)$ Å, $b = 15.584(2)$ Å, $c = 22.998(7)$ Å, $V = 3749$ Å^3, $Z = 4$, $D_c = 1.218$ g cm^{-3}.

Unit cell parameters were obtained from least-squares refinement of the setting angles of 25 reflections in the range $15 < \theta < 22$ on an Enraf-Nonius Cad4 automatic single crystal diffractometer using graphite monocromated CuK_{α} radiation. Data collection was performed in the range $1 < \theta < 75$ with a maximum sen $\theta/\lambda = 0.6265$. Reflections were measured in the ranges $0 < h < 13$, $0 < k < 19$, $0 < l < 28$ in the ω–θ scan mode. Two standard reflections monitored periodically during data collections showed only random fluctuations. A collection of 4311 independent reflections was made, 3163 of which, having $I > 3\sigma(I)$ were considered for the refinement. Lorentz and polarization corrections were applied; no correction for absorption and extinction was made.

The stucture was solved by means of the program SIR92 [23] and completed by standard Fourier method. Coordinates and anisotropic thermal parameters of all non-H atoms were refined on F by full matrix least-squares method. Hydrogen atoms were placed in theoretical stereochemical positions with the exception of H atoms of peptide N, whose positions were defined on the basis of difference Fourier maps. The hydrogen atoms were included in the structure factor calculation but not refined. The final cycle of refinement converged ($\delta/\sigma = 0.03$) with the unweighted and weighted *R* indices as 0.053 and 0.048. The final Fourier map showed the highest peak of 0.22 e/Å^3. Unit weights were used throughout the refinement. All calculations were performed on a Digital MicroVMS V4.7 computer.

NMR and CD Measurements

NMR samples were prepared in the appropriate 99.9 + % deuterated solvents, at 2.6–3.5 mM concentration. NMR spectra were collected on a Bruker 400 MHz AM spectrometer. Two-dimensional NMR spectra were recorded in the phase-sensitive mode, using time-proportional phase incrementation (TPPI). COSY, TOCSY and NOESY (mixing times 300, 400, 500 and 750 ms) spectra were collected with 512 experiments of 96–160 scans each. Spectral widths were set to 4000 and 2000 Hz in ω_2 and ω_1 dimensions, respectively, with a time-domain in ω_2 of 2K points. The recycling delay in all experiments was set to 1 s. All NOE effects observed were in the positive NOE regime. ROESY spectra were collected with a 200 ms mixing pulse. Data were processed on an Aspect 3000 computer with Bruker DISR871 software. Apodization using Lorentzian to Gaussian transformation or shifted sine-bell window functions was applied in both dimensions, and data were zero filled to 1K points prior to Fourier transformation in t_1. Base plane correction was applied to the spectra.

Table 1 Atomic Coordinates and Equivalent Isotropic Parameters (Å^2) with ESDs in Parentheses

Atom	x	y	z	B_{eq}[a]
O_1	1.1183(4)	− 0.1886(2)	− 0.0740(2)	6.03(9)
O_2	0.9479(4)	− 0.2156(2)	− 0.1326(2)	7.0(1)
O_3	0.1634(3)	0.1547(2)	0.1861(2)	6.74(9)
O_1'	0.8320(3)	− 0.0115(2)	− 0.0083(1)	4.22(6)
O_2'	0.7364(3)	0.1119(2)	0.1259(1)	5.08(7)
O_3'	0.5147(3)	0.2364(2)	0.0139(2)	5.35(8)
O_4'	0.2794(3)	0.0427(2)	0.0376(1)	5.28(8)
O_5'	0.2567(4)	0.2217(3)	0.1120(2)	7.7(1)
N_1	0.9765(4)	− 0.0861(3)	− 0.0914(2)	5.16(9)
N_2	1.0049(3)	0.0317(2)	0.0440(1)	3.29(6)
N_3	0.8347(3)	0.1670(2)	0.0464(2)	3.74(7)
N_4	0.6057(3)	0.1047(2)	0.0070(2)	3.63(7)
N_5	0.4318(3)	0.0875(2)	0.0997(1)	3.89(7)
C_1	1.1757(6)	− 0.2762(4)	− 0.0801(3)	7.5(2)
C_2	1.2154(9)	− 0.2892(5)	− 0.1428(4)	11.4(2)
C_3	1.2909(7)	− 0.2681(5)	− 0.0389(4)	10.5(2)
C_4	1.0826(8)	− 0.3427(5)	− 0.0590(4)	11.9(3)
C_5	1.0101(5)	− 0.1683(3)	− 0.1012(2)	5.2(1)
C_6	0.0672(6)	0.2211(4)	0.1891(3)	8.8(2)
C_1'	0.9478(4)	− 0.0019(3)	− 0.0039(2)	3.51(8)
C_2'	0.8254(4)	0.1109(3)	0.0915(2)	3.67(8)
C_3'	0.6092(4)	0.1915(3)	0.0174(2)	3.93(9)
C_4'	0.3902(4)	0.0643(3)	0.0464(2)	4.00(9)
C_5'	0.2531(5)	0.1623(3)	0.1450(2)	5.0(1)
C_1^{α}	1.0400(4)	− 0.0255(3)	− 0.0524(2)	3.90(9)
C_2^{α}	0.9304(4)	0.0465(3)	0.0954(2)	3.51(8)
C_3^{α}	0.7349(4)	0.2292(3)	0.0352(2)	4.2(1)
C_4^{α}	0.4887(4)	0.0604(3)	− 0.0007(2)	3.79(8)
C_5^{α}	0.3438(4)	0.0878	0.1488(2)	4.4(1)
C_1^{β}	1.0883(5)	0.0547(4)	− 0.0849(2)	6.3(1)
C_2^{β}	0.9539(4)	0.0081(3)	0.1464(2)	4.03(9)
C_4^{β}	0.4666(4)	0.0066(3)	− 0.0454(2)	4.3(1)
C_5^{β}	0.4164(5)	0.0862(4)	0.2075(2)	4.9(1)
$C_1^{\gamma 1}$	1.1881(6)	0.0283(7)	− 0.1310(2)	10.9(2)
$C_5^{\gamma 1}$	0.5016(6)	0.0055(5)	0.2119(3)	8.0(2)
$C_1^{\gamma 2}$	0.9825(7)	0.1072(4)	− 0.1116(2)	7.7(2)
$C_5^{\gamma 2}$	0.4911(6)	0.1694(5)	0.2183(2)	7.6(2)
C_2^{γ}	1.0485(4)	− 0.0575(3)	0.1617(2)	3.94(9)
$C_2^{\delta 1}$	1.1091(5)	− 0.1112(30	0.1219(2)	5.0(1)
$C_2^{\varepsilon 1}$	1.2005(6)	− 0.1694(3)	0.1394(2)	6.3(1)
C_2^{ζ}	1.2317(6)	− 0.1771(3)	0.1980(3)	6.4(1)
$C_2^{\varepsilon 2}$	1.1699(6)	− 0.1264(4)	0.2378(2)	6.7(1)
$C_2^{\delta 2}$	1.0789(5)	− 0.0666(3)	0.2204(2)	5.5(1)
C_4^{γ}	0.5466(4)	− 0.0086(3)	− 0.0974(2)	4.4(1)
$C_4^{\delta 1}$	0.5414(8)	− 0.0896(4)	− 0.1221(3)	7.8(2)
$C_4^{\varepsilon 1}$	0.6107(9)	− 0.1080(4)	− 0.1715(3)	10.0(2)
C_4^{ζ}	0.6864(6)	− 0.0470(5)	− 0.1969(3)	8.6(2)
$C_4^{\varepsilon 2}$	0.6899(5)	0.0332(5)	− 0.1730(2)	7.7(2)
$C_4^{\delta 2}$	0.6214(5)	0.0543(4)	− 0.1229(2)	6.1(1)

[a] $B_{eq} = 4/3(a^2B_{11} + b^2B_{22} + c^2B_{33} + ab\cos\gamma B_{12} + ac\cos\beta B_{13} + bc\cos\alpha B_{23})$

Temperature coefficients in acetonitrile solution were measured from 1D spectra (256 scans each) taken at 298, 305, 310, 315 and 320K with 16K points and 4000 Hz spectral widths. Spectra were referenced to the residual solvent signal (acetonitrile-d_2, $\delta = 1.93$ p.p.m.; DMSO-d_5, $\delta = 2.50$ p.p.m.).

CD spectra were recorded on a Jasco J500A spectropolarimeter; data are expressed in term of total molar ellipticity (Θ), in deg cm^2 dmol^{-1}.

RESULTS AND DISCUSSION

Crystal and Molecular Structure

Molecular Conformation. The final positional parameters for all non-hydrogen atoms are listed in Table 1. Relevant bond lengths and bond angles are given in Tables 2 and 3. They are in general agreement with the standard values observed in peptides containing unsaturated residues [2–15]. According to the azlactone method of synthesis, the C=C double bonds show a *trans* configuration of phenyl ring with respect to CO group (*Z* configuration). NC^{α} and $C^{\alpha}C'$ distances in both the unsaturated residues are a little shorter than the corresponding bonds of saturated residues, as already observed in other dehydro-peptides. Such an effect could be attributed to a partial conjugation between the peptide chain and the lateral styryl group. A complete conjugation is not allowed, owing to the lack of planarity between the side-chain double bond and the adjacent peptide bonds. Actually, the plane of the C=C double bond and the plane of the preceding peptide bond form skew angles of 118.6(4)° and 130.5(5)° for ΔPhe^2 and ΔPhe^4, respectively. Lack of planarity is also found in the styryl groups: for ΔPhe^{2}the plane of the phenyl ring forms an angle of $-20°$ with the plane of the C=C double bond; for ΔPhe^4 the corresponding angle is $-32°$.

Table 3 Relevant Bond Angles

Angle	Deg.[a]
$C_5N_1C_1^{\alpha}$	126.7(4)
$C_1'N_2C_2^{\alpha}$	119.5(4)
$C_2'N_3C_3^{\alpha}$	121.0(4)
$C_3'N_4C_4^{\alpha}$	121.6(4)
$C_4'N_5C_5^{\alpha}$	120.1(4)
$N_2C_1'C_1^{\alpha}$	114.1(4)
$N_3C_2'C_2^{\alpha}$	115.2(4)
$N_4C_3'C_3^{\alpha}$	117.2(4)
$N_5C_4'C_4^{\alpha}$	116.5(4)
$N_1C_1^{\alpha}C_1'$	108.6(4)
$N_2C_2^{\alpha}C_2'$	117.4(4)
$N_2C_2^{\alpha}C_2^{\beta}$	123.4(4)
$C_2'C_2^{\alpha}C_2^{\beta}$	119.2(5)
$N_3C_3^{\alpha}C_3'$	114.8(4)
$N_4C_4^{\alpha}C_4'$	119.0(4)
$N_4C_4^{\alpha}C_4^{\beta}$	123.2(5)
$C_4'C_4^{\alpha}C_4^{\beta}$	117.4(5)
$N_5C_5^{\alpha}C_5'$	111.0(4)
$C_2^{\alpha}C_2^{\beta}C_2^{\gamma}$	130.2(5)
$C_4^{\alpha}C_4^{\beta}C_4^{\gamma}$	128.4(6)

[a]Numbers in parentheses are estimated standared deviations in the least significant digits.

The molecular conformation of the pentapeptide in the crystalline state is shown in Figure 1. Two intramolecular hydrogen bonds are present between CO of Val^1 and HN of ΔPhe^4, and between CO of ΔPhe^2 and NH of Val^5, respectively. Such hydrogen bonds stabilize two consecutive β-turns so that the molecule assumes, in correspondence of the segment ΔPhe-Gly-ΔPhe, a 3_{10}-helix conformation only slightly distorted. In fact the (φ, ψ) torsion angles that define such conformation for ΔPhe^2, Gly^3 and ΔPhe^4 (Table 4), are rather close to the (φ, ψ) values for type III β-turns ($\varphi = -60°$, $\psi = -30°$) with some deviations only in correspondence of Gly^3 residue ($\varphi = -65.6°$, $\psi = -7.4°$) that falls in the 'bridge' region of the conformational map

Table 2 Relevant Bond Lengths (Å) for the Dehydropeptide Boc-Val^1-ΔPhe^2-Gly^3-ΔPhe^4-Val^5-OMe[a]

Bond	Val^1	ΔPhe^2	Gly^3	ΔPhe^4	Val^5
NC^{α}	1.463(7)	1.433(6)	1.448(6)	1.417(6)	1.457(7)
$C^{\alpha}C'$	1.519(7)	1.492(6)	1.498(6)	1.496(6)	1.502(7)
C'O	1.225(5)	1.222(5)	1.214(6)	1.225(5)	1.198(7)
C'N[b]	1.358(6)	1.359(6)	1.374(6)	1.349(6)	-
$C^{\alpha}C^{\beta}$	-	1.348(7)	-	1.347(8)	-

[a]Numbers in parentheses are estimated standard deviations in the least significant digits.
[b]Peptide bond linking the successive residue in the sequence.

($\pm 120° < \varphi < \pm 60°$, $\pm 20° < \psi < \pm 20°$). The small ψ torsion angle ($-7.4°$) is paralleled by a fairly large value of τ ($NC^{\alpha}C'$) bond angle (114.8°) with respect to the regular tetrahedral value. So the known dependence of τ and ψ is confirmed also in the present study [24]. The two chiral residues, Val^1 and Val^5, are out of the spiralized fragment of the molecule.

In its main features, the molecular conformation of the present pentapeptide is similar to the pentapeptide Boc-D-Ala-ΔPhe-Gly-ΔPhe-D-Ala-OMe already studied [9], except for the helix sense. Because of the different configuration of the chiral amino acid residues, in fact, the pentapeptide Boc-D-Ala-ΔPhe-Gly-ΔPhe-D-Ala-OMe was found to assume a left-handed helix in the solid state, while the present pentapeptide Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe adopts a right-handed helix. The conformation of Val residues is such that the two methyl groups assume a staggered position respect to NH [25], as already found in other helical peptides containing Val residues, including dehydro [10] and Aib (α-amino isobutyric acid)-peptides [26].

Crystal Packing. Crystal packing is characterized by the formation of columns of helical molecules in a way very similar to pentapeptide Boc-D-Ala-ΔPhe-Gly-ΔPhe-D-Ala-OMe already studied [9]. Such columns of helices are formed by head-to-tail intermolecular hydrogen bonds between NH of ΔPhe^2 and CO of ΔPhe^4 which are positioned at the opposite ends of the 3_{10}-helix. The helical columns are linked two by two by means of intermolecular hydrogen bonds involving reciprocally the NH and CO functions of Gly^3 residue. Each double column of helices is then surrounded at van der Waals interaction by six double columns, two running in parallel and four in antiparallel sense with respect to the central one. The crystal packing as vieved along *a* axis is shown in Figure 2. Distances and angles of the various hydrogen bonds are reported in Table 5.

Conformation in Solution

NMR measurements. Proton resonance assignments followed from examination of COSY, TOCSY and NOESY spectra, which were well resolved with respect to the different spin systems, by the sequential resonance assignment method (Figure 3). Only

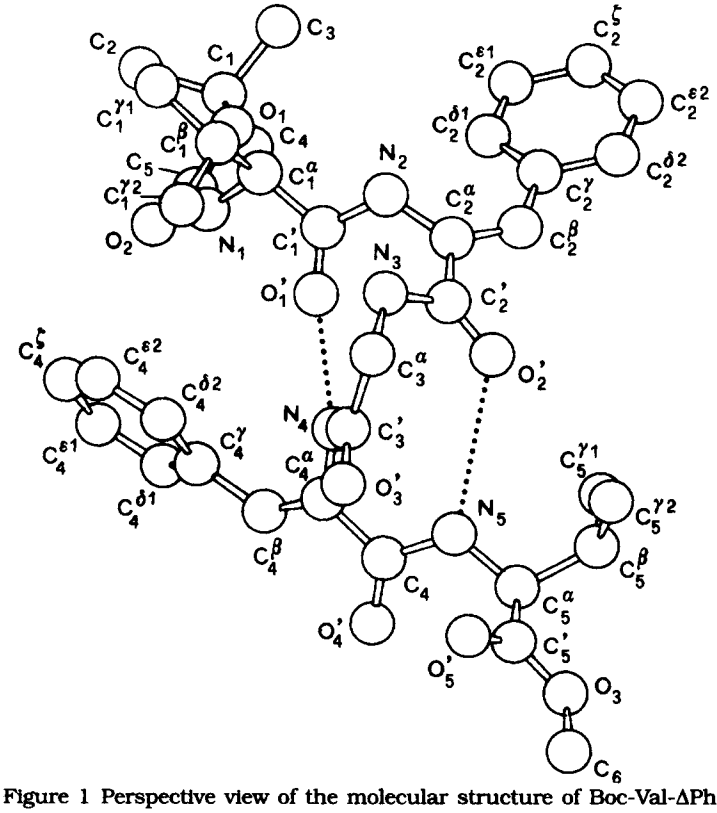


Figure 1 Perspective view of the molecular structure of Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe.

Table 4 Torsion Angles Around NC^{α} Bond (φ), $C^{\alpha}C'$ Bond (ψ) and C'N Bond (φ) for the Dehydro-peptide Boc-Val[1]-ΔPhe[2]-Gly[3]-ΔPhe[4]-Val[5]-OMe

Residue	φ	ψ	ω
Val[1]	− 115.5(0.5)	160.7(0.4)	− 172.2(0.4)
ΔPhe[2]	− 64.0(0.5)	− 19.1(0.5)	175.9(0.4)
Gly[3]	− 65.6(0.5)	− 7.4(0.6)	169.5(0.4)
ΔPhe[4]	− 57.3(0.5)	− 19.3(0.6)	− 176.2(0.4)
Val[5]	− 73.6(0.5)	171.9(0.4)	-

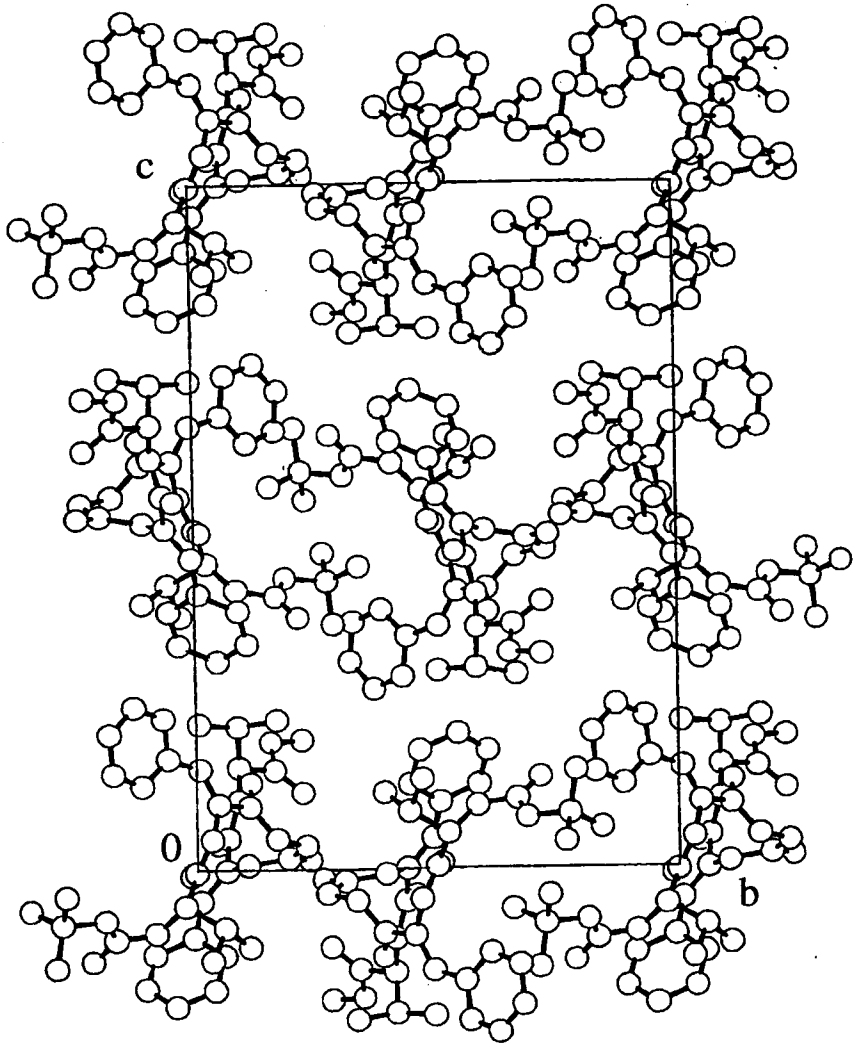


Figure 2 Molecular packing of the Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe molecules as viewed along the a-axis.

the ring protons of the two dehydro-Phe residues could not be completely assigned.

Both in acetonitrile and in dimethylsulphoxide (DMSO) solution, the presence of NH ↔ NH (i, $i+1$) and $C^{\alpha}H \leftrightarrow$ NH (i, $i+1$) NOE effects suggests that the peptide contains a significant population of conformers in the helical region of the (φ, ψ) space. In order to translate the NOEs into distance ranges, a reference distance was needed. As no suitably resolved cross-peak between protons in a known geometrical relationship could be detected, we resorted to assigning to the strongest NOE peak (Val[1] $C^{\alpha}H \leftrightarrow$ ΔPhe[2] NH) the relevant distance derived from the X-ray structure (2.4 Å). Distance estimations for

Table 5 Hydrogen Bonds

Type	H-bond	Distance	Angle (deg) (CON)	Symmetry
Intramol.	$N_4H\cdots O_1'$	3.001(5)	134.1(3)	x,y,z
Intramol.	$N_5H\cdots O_2'$	3.265(5)	128.5(3)	x,yz
Intermol.	$N_2H\cdots O_4'$	2.881(5)	162.1(3)	$x+1,y,z$
Intermol.	$N_3H\cdots O_3'$	2.781(5)	153.7(3)	$½+x,½-y,\bar{z}$

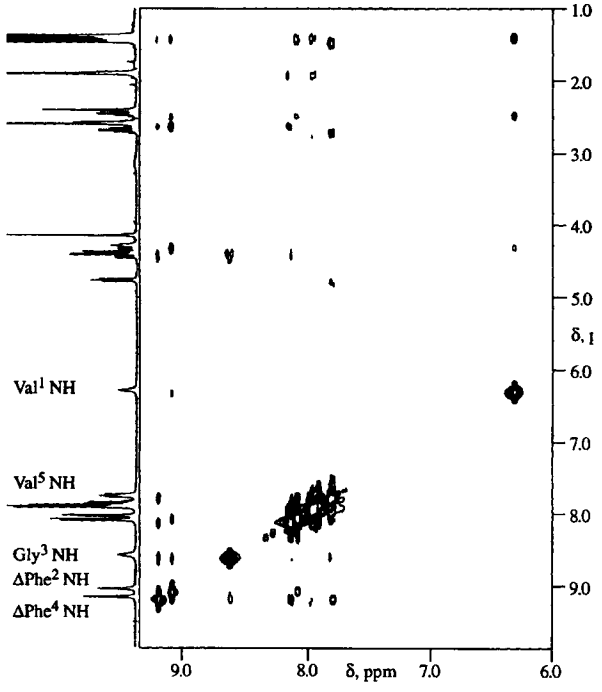


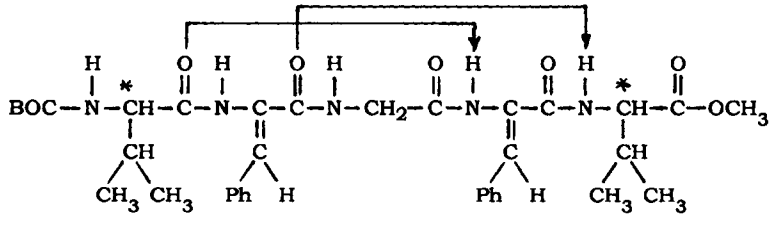
Figure 3 Partial 400-MHz ^{1}H-NOESY spectrum of Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe in CD_3CN at 300 K (mixing time 500 ms), showing the assignments for the NH resonances.

all other couples of backbone protons connected by measurable NOEs were then calculated by the method of Esposito and Pastore [27].

Table 6 summarizes the distance values calculated from the backbone NOEs in the two solvents with the corresponding crystal state distances. A *vis-à-vis* comparison with the crystal-state structure shows a satisfactory overall agreement, except for some deviations in the N-terminal region, which could be accounted for in terms of an increased rotational freedom in solution of the Boc-protected valine. The results for the N-terminal Val residue, both in acetonitrile and in DMSO, could also be quite comparable with the extension of the 3_{10}-helix all the way through the N^{α}-blocking group. Indeed, the strong NOE detected between $C^{\alpha}H$ of Val^1 and NH of ΔPhe^2 may be a probe for the presence of an L-residue included in a left-handed helical structure, which is characterized by short (< 3 Å) $C_i^{\alpha}H \leftrightarrow N_{i+1}H$ distance [28]. On the other hand, it may also suggest an extended or semi-extended conformation of the Boc-protected Val^1.

The temperature dependence of the chemical shifts of the amide protons in acetonitrile solution between 298 and 320 K was also measured. All NH protons show a linear behaviour, the temperature coefficients being − 5.9 (Val^1 NH), − 4.8 (ΔPhe^2 NH), − 5.9 (Gly^3 NH), − 2.9 (ΔPhe^4 NH) and − 2.6 (Val^5 NH) p.p.b./K. The smaller coefficients, indicative of shielding from the solvent and/or involvement in stable intramolecular hydrogen bonding, correspond to Val^5 NH (− 2.6) and ΔPhe^4 NH (− 2.9). Interestingly, in the X-ray structure both of these amide protons are involved in intramolecular hydrogen bonding with the CO of ΔPhe^2 and the CO of Val^1, respectively. In conclusion, NMR measurements are consistent with the presence of 3_{10}-helical structures both in acetonitrile and in DMSO, characterized by a H-bonding pattern as illustrated in Scheme 1.

More extended helical structures also involving Val^1, suggested by backbone NOEs but not confirmed by temperature dependence of NH chemical shifts, may be present as a smaller population of confor-



Scheme 1

mers coexisting in equilibrium with the main conformer, as occurring for the analogue Boc-Ala-ΔPhe-Gly-ΔPhe-Ala-OMe [22].

Circular Dichroism Measurements. Like all peptides containing dehydro-Phe residues, peptide **1** shows an intense absorption band in the near-UV region having maximum in the range 275–280 nm, depending on the solvent. This band has been assigned to a charge-transfer transition from the styrene moiety to the electron-accepting carbonyl group of the $C_6H_5-C=C-C=O$ chromophore [29]. Other bands are observed in the far-UV region at 216 and 203 nm. These bands cannot be assigned with confidence, because of overlapping of aromatic and peptide electronic transitions. For this reason, discussion of CD spectra and their structural implications will be limited only to the near-UV region.

The CD spectra in various solvents having different polarity and different proton-donor/acceptor properties are reported in Figure 4. The spectra display typical couplets of bands originating from the exciton splitting of the electronic transition at 280 nm, thus indicating that the two styryl side chains are placed in a rigidly fixed disposition within the molecule. This can be well achieved if the peptide adopts the 3_{10}-helical structure and the hydrogen-bonding pattern observed in the solid state and suggested by the NMR measurements. In fact, at least two intramolecular hydrogen bonds, forming two consecutive β-bends, are necessary for maintaining the two ΔPhe residues in a mutually fixed disposition.

The most striking feature of the CD spectra is the sign of the couplet, which is opposite to that expected for a peptide containing an alternate sequence of ΔPhe residues and adopting a right-handed 3_{10}-helical structure [22, 30, 31]. In other words, X-ray diffraction analysis shows that peptide **1** adopts a *right-handed* screw sense in a crystal obtained from a methanol/water solution. By contrast, circular dichroism indicates that it adopts a prevalent *left-handed* screw sense in chloroform, acetonitrile, methanol and DMSO solution. For the analogous dehydro-peptide Boc-Ala-ΔPhe-Gly-ΔPhe-Ala-OMe, the presence of a left-handed 3_{10}-helical structure in chloroform solution was also confirmed by vibrational circular dichroism measurements [32].

In order to have a more detailed picture of the conformational behaviour in solution, the CD spectra were measured either in the presence of increasing amounts of DMSO (strong H-bonding acceptor solvent), or in the presence of 1,1,1,3,3,3-hexafluoro-propan-2-ol (HFiP) (strong H-bonding donor solvent).

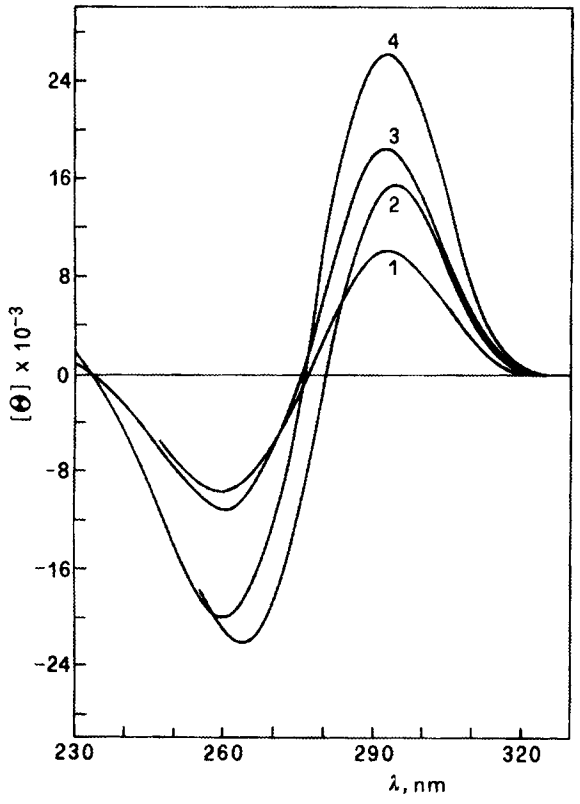


Figure 4 CD spectra of Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe (**1**) in: (1) chloroform; (2) DMSO; (3) methanol; (4) acetonitrile.

Addition of progressive amounts of DMSO to the chloroform solution produces, as a first step (up to 10% DMSO concentration), a strong increase of the CD bands ($[\Theta]_{295} = +28{,}000$; $[\Theta]_{265} = -34{,}000$). Then, higher concentrations of DMSO (up to 50% DMSO) cause a decrease in ellipticity. However, even in pure DMSO, CD bands are not cancelled, as they should be as a consequence of possible conformational disruption. Indeed, their intensity is higher than in pure chloroform (Figure 4). The behaviour is unexpected, since intramolecularly H-bonded peptide structures are usually more stable in a relatively non-polar solvent like chloroform than in the strong solvating medium DMSO [18, 19, 33]. So, both CD and NMR measurements are in good agreement and indicate that the peptide **1** still retains a considerable proportion of folded helical structures even in DMSO solution.

The spectra in chloroform/HFiP solvent mixtures are shown in Figure 5. Increasing amounts of HFiP to the chloroform solution give rise to the gradual appearance of a couplet, the sign of which is opposite

to the original one and corresponds to the sign expected for a right-handed 3_{10}-helix [22, 30, 31]. Therefore, addition of HFIP induces the reversal of the helix from left-handed to right-handed sense.

Since the single crystals for X-ray analysis were grown from a methanol/water solution, CD spectra were measured in MeOH/H_2O mixtures, with the aim of also observing a helix screw sense reversal under these conditions. Addition of water to methanol solutions at percentages low enough to prevent peptide precipitation produced only a strong decrease in intensity of the CD couplet, thus indicating almost complete unfolding of the ordered structures.

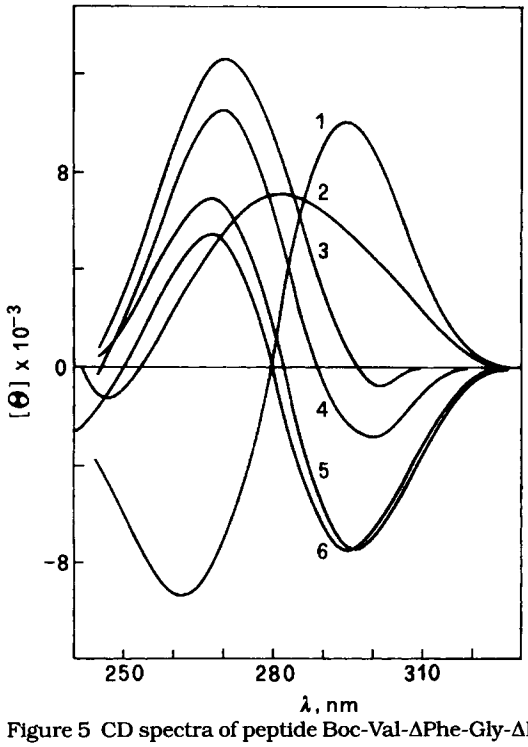


Figure 5 CD spectra of peptide Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe (**1**) in various chloroform/HFIP solvent mixtures. HFIP concentration: (1) 0%; (2) 2.4%; (3) 4.8%; (4) 13%; (5) 20%; (6) 100%.

CONCLUSIONS

Several factors may be responsible for the conformational behaviour above described.

(1) The sequence contains two valine residues positioned at the opposite ends of the chain. Interestingly, the two valine residues have a contradictory influence on backbone conformation of the helical peptide. In fact, the preferential handedness imposed by the chiral residue located at the C-terminal position is known to be opposite to the handedness imposed by a chiral residue located at the N-terminal or in an internal position of the sequence. This observation is well supported by the crystal-state structures of helical peptides containing Aib residues, in which the C-terminal residue usually adopts a conformation having opposite handedness with respect to the preceding ones [34–37]. Also in proteins, Schellman observed that many right-handed helical segments ended with a residue in the left-handed conformation [38].

(2) The actual conformation adopted by the peptide molecule is the result of intramolecular and intermolecular forces. In HFIP, the molecules of the strong H-bonding donor solvent are likely to replace the intermolecular hydrogen bonding of the crystal packing, so the peptide is able to maintain the right-handed structure assumed in the crystalline state. In the other less polar or non H-bonding donor solvents, the balance of the forces favours the opposite screw sense. The conformational structure

Table 6 Comparison of Crystal-state Distances with the Ranges Calculated from the Backbone NOEs, in CH_3CN and DMSO

Contact	Distance (Å)		
	X-ray	NMR (CH_3CN)	NMR (DMSO)
$Val^1NH \leftrightarrow \Delta Phe^2NH$	4.5	2.6–3.6	2.7–3.8
ΔPhe^2 NH ↔ Val^1 $C^\alpha H$	2.4	2.0–2.8[a]	2.0–2.8[a]
ΔPhe^2 NH ↔ Gly^3 NH	2.7	2.5–3.6	2.7–3.7
Gly^3 NH ↔ Gly^3 $C^\alpha H/C^\alpha H'$	2.3, 2.9	2.1–3.0	–
Gly^3 NH ↔ ΔPhe^4 NH	2.8	2.3–3.2	2.9–4.0
ΔPhe^4 NH ↔ Gly^3 $C^\alpha H/C^\alpha H'$	3.3, 3.5	2.7–3.8	2.4–3.4
ΔPhe^4 NH ↔ Val^5 NH	2.9	2.2–3.1	2.6–3.6
Val^5 NH ↔ Gly^3 $C^\alpha H/C^\alpha H'$	3.3, 4.3	3.3–4.6	–

[a] Reference value (see text).

in solution, therefore, should be characterized by the presence of an equilibrium between right-handed helices and left-handed helices, the intensity of the CD bands reflecting the prevalence of one screw sense over the other one. Such equilibria were found to exist also for other peptides having high propensity to stay in helical structure, including dehydro- [12] and Aib-peptides [39].

(3) Comparison of results obtained for Boc-Val-ΔPhe-Gly-ΔPhe-Val-OMe studied in this work, with those previously reported for Boc-Ala-ΔPhe-Gly-ΔPhe-Ala-OMe [21, 22] indicates that a crucial role is surely played by the achiral fragment -ΔPhe-Gly-ΔPhe- common to the two peptides, in which two helicogenic dehydro-Phe residues are separated by the flexible glycine residue. In fact, while Ala and Val residues have substantially different stereochemical parameters, only minor differences in conformational behaviour have been detected between the Ala and β-branched Val derivatives.

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