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Role of a Two-residue Spacer in an α,β-Didehydrophenylalanine containing Hexapeptide: Crystal and Solution Structure of Boc-Val-ΔPhe-Leu-Ala-ΔPhe-Ala-OMe

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Abstract: The peptide Boc-Val[1]-ΔPhe[2]-Leu[3]-Ala[4]-ΔPhe[5]-Ala[6]-OMe has been examined for the structural consequence of placing a two-residue segment between the ΔPhe residues. The peptide is stabilized by four consecutive β-turns. The overall conformation of the molecule is a right-handed 3_{10}-helix, with average (φ, ψ) values (−67.7°, −22.7°), unwound at the *C*-terminus. The ^{1}H NMR results also suggest that the peptide maintains its 3_{10}-helical structure in solution as observed in the crystal state. The crystal structure is stabilized through head-to-tail hydrogen bonds and a repertoire of aromatic interactions laterally directed between adjacent helices, which are antiparallel to each other. The aromatic ring of ΔPhe[5] forms the hub of multicentred interactions, namely as a donor in aromatic C–H···π and aromatic C–H···O=C interactions and as an acceptor in a CH_3···π interaction. The present structure uniquely illustrates the unusual capability of a ΔPhe ring to host such concerted interactions and suggests its exploitation in introducing long-range interactions in the folding of supersecondary structures. Copyright © 2003 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: 3_{10}-helix; aromatic interactions; constrained peptides; crystal and solution structure; *de novo* design; didehydrophenylalanine

INTRODUCTION

The *de novo* design of peptides and proteins has assumed considerable interest in recent years [1–3]. α,β-Didehydro residues, in particular α,β-didehydrophenylalanine (ΔPhe) [4], are being considered as one of the important conformational constraints in *de novo* design. These residues have been found to occur naturally in peptides from microbial sources [5,6]. In addition, didehydropeptides show enhanced resistance to enzymatic degradation [7]. Thus, introduction of α,β-didehydroamino acid residues into bioactive peptide sequences has become a useful tool to study structure–function relationships and to provide analogues of peptide hormones with improved bioactivity [8]. The versatility of the ΔPhe residue in defining the conformation facilitates designing a wide variety of secondary structural motifs. Various examples of such model structural motifs, designed using ΔPhe residues, are available in the literature [9].

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As part of the continued systematic effort in designing structural motifs using ΔPhe, we have examined its role with a variety of rules. A novel, flat β-ribbon structure has been observed in a didehydropentapeptide [10]. An α-helix in a pentapeptide with a ΔPhe residue in the second position has also been observed, illustrating the context dependent design rules for ΔPhe [11]. The third principal structural element occurring in globular proteins, after classical α-helix and β-sheets, is the 3_{10}-helix [12]. The 3_{10}-helix, first predicted as a reasonably stable polypeptide secondary structure almost 50 years ago [13], has widely attracted the attention of structural biochemists and protein crystallographers [14]. A notable number of consecutive ΔPhe-containing structures has been shown to adopt the 3_{10}-helix conformation. 3_{10}-Helices of both screw senses [15–17], varying in length, content and position of ΔPhe have been designed. A recent achievement in our laboratory in this direction has been the design and crystallographic characterization of a supersecondary structural element: a 21-residue, monomeric, helical hairpin motif containing natural amino acids carefully juxtaposed between ΔPhe residues, which act as conformational restrictors [18]. Interestingly, it has been noted that the geometry of a 3_{10}-helix brings ΔPhe residues at i^{th} and $i+3^{rd}$ position into a stacking arrangement and the structurally planar ΔPhe side-chains interdigitate to assist the cooperative recognition of helices. The key element in the design of the helical hairpin motif has been to introduce protein amino acids in 'spacer' positions ($i+1$ and $i+2$) between ΔPhe residues so as to preserve the weakly interacting ΔPhe core to achieve the desired folding. However, it has not been evident whether long-range interactions were responsible for the formation of the 3_{10}-helix or the positioning of ΔPhe with two spacer residues were alone sufficient to maintain the helical geometry. Therefore, the present sequence with ΔPhe residues at i^{th} and $i+3^{rd}$ positions is expected to provide information regarding the stabilization of the 3_{10}-helix, with both ΔPhe residues at the same wedge of the helix, and their interactions with the aromatic side chains of the adjacent helices in the crystal space.

In a minimalistic approach towards examining the role of the two-residue spacer, we have synthesized an analogous hexapeptide, Boc-Val^1-ΔPhe^2-Leu^3-Ala^4-ΔPhe^5-Ala^6-OMe (Boc, *tert*-butoxycarbonyl; OMe, methoxy) and determined its crystal and solution structure. Remarkably, the peptide maintains the 3_{10}-helical structure both in solution and in the crystal state, thus demonstrating the role of the ΔPhe residues as stereochemical directors. Intermolecular interactions mediated by aromatic residues and directed laterally to the helical axis are other interesting observations extracted from this structural work.

MATERIALS AND METHODS

Peptide Synthesis

The hexapeptide was synthesized in solution by the fragment condensation method [10,16,17]. The ΔPhe moiety was introduced as part of a dipeptide block, obtained through azlactonization and dehydration of Boc-Val-D,L-βPheSer-OH (βPheSer, β-phenyl serine) and Boc-Ala-D,L-βPheSer-OH [11]. All the intermediates were checked for purity by thin-layer chromatography (TLC).

Boc-Leu-Ala-ΔPhe-Ala-OMe. To a solution of Boc-Leu-OH (1.1 g, 4.7 mmol) cooled to −10 °C in tetrahydrofuran (15 ml), *N*-methylmorpholine (0.6 ml, 4.7 mmol) and isobutylchloroformate (0.65 ml, 4.7 mmol) were gradually added. A precooled solution of TFA.H-Ala-ΔPhe-Ala-OMe [10] (2.0 g, 4.7 mmol) and triethylamine (0.65 ml, 4.7 mmol) in tetrahydrofuran was then added to the reaction mixture. The reaction mixture was stirred for 2 h at 0 °C and overnight at room temperature. The solvent was evaporated in *vacuo* and the residue taken in ethyl acetate, washed successively with a 5% citric acid solution, water, a saturated $NaHCO_3$ solution and water; dried over anhydrous Na_2SO_4 and evaporated in *vacuo* to obtain the tetrapeptide. Yield 73%; m.p. 108°–110 °C.

Boc-Val-ΔPhe-Leu-Ala-ΔPhe-Ala-OMe. The tetrapeptide Boc-Leu-Ala-ΔPhe-Ala-OMe (1.5 g, 2.9 mmol) was deprotected at its *N*-terminus using a mixture of trifluoroacetic acid in methylene chloride (1 : 1 *v*/*v*) using the procedure reported elsewhere [17]. To a solution of Boc-Val-ΔPhe-Azl (Azl, azlactone) [17] (1.0 g, 2.9 mmol) in methylene chloride (20 ml), TFA.H-Leu-Ala-ΔPhe-Ala-OMe (1.5 g, 2.9 mmol) was added, followed by triethylamine (0.4 ml, 2.9 mmol) and the reaction mixture was stirred at room temperature until TLC showed a complete absence of the azlactone. The work up procedure was similar to that of the tetrapeptide and a crystalline solid of the hexapeptide was obtained. Yield 65%; m.p. 130°–132 °C. The molecular mass of the hexapeptide, determined by ES-MS, was

773.0 (calculated molecular mass 774.0). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.37 (1H, s, NH ΔPhe^5), 7.85 (1H, d, NH Ala^6), 7.78 (1H, s, NH ΔPhe^2), 7.51 (1H, s, C$^\beta$H ΔPhe^5), 7.5–7.3 (10 H, m, aromatic protons ΔPhe^2 and ΔPhe^5), 7.36 (1H, d, NH Leu^3), 7.34 (1H, d, NH Ala^4), 7.01 (1H, s, C$^\beta$H ΔPhe^2), 5.05 (1H, d, NH Val^1), 4.64 (1H, m, C$^\alpha$H Ala^4), 4.36 (1H, m, C$^\alpha$H, Ala^6), 4.35 (1H, m, C$^\alpha$H Leu^3), 3.81 (1H, m, C$^\alpha$H Val^1), 3.71 (3H, s, OCH_3), 2.17 (1H, br, C$^\beta$H Val^1), 1.81 (2H, br, C$^\beta$H Leu^3), 1.69 (1H, br, C$^\gamma$H Leu^3), 1.44 (9H, s, Boc CH_3), 1.04–1.07 (6H, dd, C$^\gamma$H Val^1), 0.98–0.92 (6H, dd, C$^\delta$ H Leu^3).

X-Ray Diffraction

Single crystals were grown by the controlled evaporation of the hexapeptide solution in a methanol/water mixture at 4 °C. A colourless crystal mounted on a glass fibre was used for the determination of unit cell parameters and to measure the three-dimensional x-ray diffraction intensity data. The crystal structure was determined by direct methods and refined using the least-squares technique [19] to an *R*-factor better than 5.3% for 3027 reflections having $|F_o| \geq 4\sigma|F_o|$. The details of intensity data collection and refinement are given in Table 1.

Circular Dichroism Spectroscopy

CD studies were carried out on a Jasco J 720 spectropolarimeter. Path length used was 1 mm. The CD spectra were acquired in various solvents (chloroform, methanol, dichloromethane, 2,2,2-trifluoroethanol and acetonitrile). Data were expressed in terms of total molar ellipticity.

Table 1 Details of Intensity Data Collection for the Hexapeptide

Empirical formula	$C_{41}H_{56}N_6O_9$
Molecular weight (a.m.u.)	774.93
Crystal system	Monoclinic
Space group	$P2_1$
a	10.899(1)Å
b	10.070(1)Å
c	20.003(3)Å
β	94.40(1)°
Cell volume	2188.9(5)Å^3
Z	2
Density calculated (g/cm^3)	1.1788
Radiation	CuK_α (λ = 1.5418 Å)
μ (cm^{-1})	6.850
Temperature	295K
2θ (up to which data were collected)	136°
Resolution	0.82Å
Instrument used	Enraf-Nonius CAD4 diffractometer
Scan type	$\omega - 2\theta$ scan, varying scan speed
Total number of collected reflections	4453
Unique reflections	4214
Observed reflections [$\|F_o\| > 4\sigma(\|F_o\|)$]	3027
R(int)	0.0463
Limiting indices	$0 \leq h \leq 13$, $0 \leq k \leq 12$, $-24 \leq l \leq 23$
Structure solution	SHELXS97 [19]
Refinement procedure	Full-matrix least-squares refinement on $\|F_o\|^2$'s using SHELXL97 [19]
Number of parameters refined	570
Unique reflections/number of parameter ratio	7.4
Goodness of fit (Goof)	1.267
wR2-value (on all data)	0.1805
Residual electron density	Max: 0.24 e/Å^3 Min: −0.13 e/Å^3
R-factor	0.0528 [$\|F_o\| \geq 4\sigma\|F_o\|$]

Nuclear Magnetic Resonance

^{1}H NMR experiments were performed at 400 MHz (Bruker DRX 400 NMR spectrometer). The spectra were recorded in $CDCl_3$ and dimethylsulfoxide d_6 (DMSO) using a sample concentration of 1.2 mM in both solvents. Chemical shifts were expressed as δ (ppm) downfield from internal reference tetramethylsilane. Two dimensional DQF COSY, TOCSY and ROESY spectra were acquired at 298 K. Pulse programs of the standard Bruker software library were used. ROESY spectra [20] were recorded at three mixing times (100 ms, 250 ms and 400 ms). 256×1024 data points were collected for the 2D experiments and zero filled to 1024×1024 data points. In DQF COSY experiments, however, 512×2048 data points were acquired. The possible involvement of NH groups in intramolecular hydrogen bonding was investigated using temperature coefficients (-dδ/dT) in DMSO and solvent dependence of amide protons. The temperature was varied from 298K to 323K. In case of the peptide dissolved in chloroform, titration with DMSO was carried out.

RESULTS AND DISCUSSION

Geometry of the ΔPhe Residues

The introduction of a double bond between C^α and C^β atoms in ΔPhe^2 and ΔPhe^5 affects the bond lengths and angles in the same residues. The bond length of $C^\alpha{=}C^\beta$ in both the ΔPhe residues is 1.34 Å, which corresponds to a classical C=C double bond [21]. The N–C^α and C^α–C′ bond distances in both the ΔPhe residues have slightly shorter values [1.430(7) and 1.470(9) Å, respectively] than the corresponding bonds of saturated residues [1.450(8) and 1.519(8) Å, respectively] [22]. The shortening of the bonds is probably due to sp^2 hybridized C^α–C^β atoms and also might be a result of partial conjugation of ΔPhe ring electrons and remaining atoms in the residue. Complete conjugation requires coplanarity of the ΔPhe ring with the peptide unit. However, in both the ΔPhe residues complete conjugation is not observed; which may be due to steric reasons. Both the phenyl groups are in *trans* configuration with respect to the carbonyl group. The bond angles $C2^\alpha$–$C2^\beta$–$C2^\gamma$ and $C5^\alpha$–$C5^\beta$–$C5^\gamma$ are 129.0(5)° and 131.9(6)°, respectively, as a consequence of steric constraints imposed by the respective ΔPhe residues. The near zero value of the side-chain torsion angle of ΔPhe N_i–C_i^α–C_i^β–$C_i^{\gamma 1}$ ($\chi_i^{1,1}$) corresponds to the Z-isomer of ΔPhe (Table 2). Inspection of the torsion angles C_i^α–C_i^β–C_i^γ–$C_i^{\delta 1}$ ($\chi_i^{2,1}$) shows that the plane of the ring has a slight deviation from the plane formed by the atoms C^α–C^β–C^γ, in order to minimize the steric clash between $C_i^{\delta 1}$–H and N_i–H groups within a ΔPhe residue. The observed features are consistent with those seen in other oligopeptides containing ΔPhe residues [15–18].

Conformation of the Peptide

A perspective view of the peptide molecule is given in Figure 1. The molecule is characterized by four consecutive β-turns (three type-III followed by one type-I β turns) [23], each stabilized by a 1←4 intramolecular N–H···O=C hydrogen bond, and by

Table 2 Selected Torsion Angles in the Molecular Structure of the Hexapeptide

Atoms A-B-C-D	Angle	Boc	Val^1	ΔPhe^2	Leu^3	Ala^4	ΔPhe^5	Ala^6
C_1-O_0-C_5-N_1	θ^1	−172.7(2)						
C_1-O_0-C_5-O_0'	θ'^1	9.1(1)						
O_0-C_5-N_1-C_1^α	ω_0	−168.3(6)						
$C_i'^{-1}$-N_i-C_i^α-C_I'	ϕ_I	—	−61.4(7)	−72.0(7)	−55.8(8)	−68.5(8)	−81.8(7)	−67.2(8)
N_i-C_i^α-C'_i-N_{i+1}	ψ_I	—	−39.6(8)	−11.4(8)	−36.7(8)	−17.5(8)	−9.0(8)	149.5(6)[a]
C_i^α-C'_i-N_{i+1}-C_{i+1}^α	ω_I	—	−172.5(5)	169.8(6)	179.7(6)	178.6(5)	−179.7(6)	—
N_i-C_I^α-C_i^β-$C_I^{\gamma 1}$	$\chi_i^{1,1}$	—	−63.9(9)	2.9(9)	−178.8(7)	—	−1.8(8)	—
N_i-C_i^α-C_I^β-$C_I^{\gamma 2}$	$\chi_i^{1,2}$	—	170.5(9)	—	—	—	—	—
C_i^α-C_i^β-C_i^γ-$C_i^{\delta 1}$	$\chi_i^{2,1}$	—	—	34.3(9)	−170.5(9)	—	14.5(8)	—
C_i^α-C_i^β-C_i^γ-$C_i^{\delta 2}$	$\chi_i^{2,2}$	—	—	−145.1(8)	60.8(9)	—	−164.8(8)	—

[a] N6-C6A-C6′-O7.

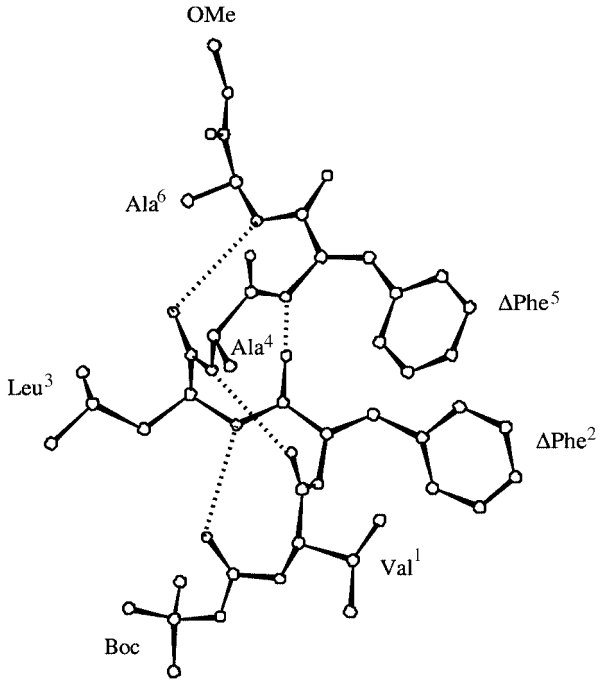

Figure 1 Molecular structure of the hexapeptide Boc-Val-ΔPhe-Leu-Ala-ΔPhe-Ala-OMe in the crystal state. The four intramolecular 1←4 hydrogen bonds are indicated by dotted lines.

a unique C–H···O=C hydrogen bond (Table 3). It has been noted that type I and type III β-turns are not very dissimilar and type I β-turn can be accommodated in a 3_{10}-helix without introducing much distortion in the helicity [23,24]. All the peptide links are in *trans* conformation. At the *C*-terminus, Ala^6, the helix gets unwound, a common feature observed in helical peptides and supported by molecular dynamics simulations [25]. No solvent molecules were located in the structure.

The Boc group assumes the frequently observed *trans-trans* conformation [26], the ω_0, θ^1 values being -168.1° and 172.6°, respectively. This disposition facilitates the carbonyl oxygen in the formation of intramolecular 1←4 N–H···O=C hydrogen bond. The hexapeptide molecule adapts a distorted 3_{10}-helical conformation [14]. The average backbone torsion angles are $\langle\phi\rangle = -67.7^\circ$ and $\langle\psi\rangle = -22.7^\circ$ (excluding the *C*-terminal Ala^6) (Table 3). These (ϕ, ψ) average values are close to the values reported for 3_{10}-helical peptides [14,27]. The (ϕ, ψ) values of Ala^6 residue are (-67.2°, 149.5°), showing the unwinding of the helix at the *C*-terminus, which is also a common feature observed in other peptides. In this context it is of general interest to note that designing structures with a proper number of residue spacers between ΔPhe residues has allowed several interesting observations. This is because the spacer residues relax the conformational restriction imposed by consecutive ΔPhe residues. Many examples in the literature show that a single residue spacer retains the 3_{10}-helical conformation in smaller peptides [28–30], whereas three-and-four residue spacers have brought in remarkable helix-turn configurations, such as helix termination by a π-turn [31] as seen in proteins [32] and helix termination [33] by Schellman motif [34]. However, a

Table 3 The Intramolecular and Intermolecular Hydrogen Bonds Observed in the Structure of the Hexapeptide

Type	Donor D	Acceptor A	Distance D···A (Å)	Distance H[a]···A (Å)	Angle D–H···A (°)	Symmetry
Intramolecular	N3	O0′	3.284(7)	2.28	164	x, y, z
4 → 1	N4	O1′	3.050(7)	2.12	152	x, y, z
	N5	O2′	3.219(4)	2.19	159	x, y, z
	N6	O3′	3.280(7)	2.32	157	x, y, z
Intermolecular	N1	O4′	2.882(7)	1.98	147	x+1, y, z
Head-to–tail	N2	O6′	3.026(8)	2.39	121	x+1, y, z[b]
	C2	O3′	3.591(9)	2.52	172	x+1, y, z[b]
Aromatic C-H···O	C5D1	O5′	3.28	2.43	152	$2-x, y+1/2, 1-z$

[a] Hydrogen atoms fixed on the donor atoms based on stereochemistry.
[b] May be a weak hydrogen bond.

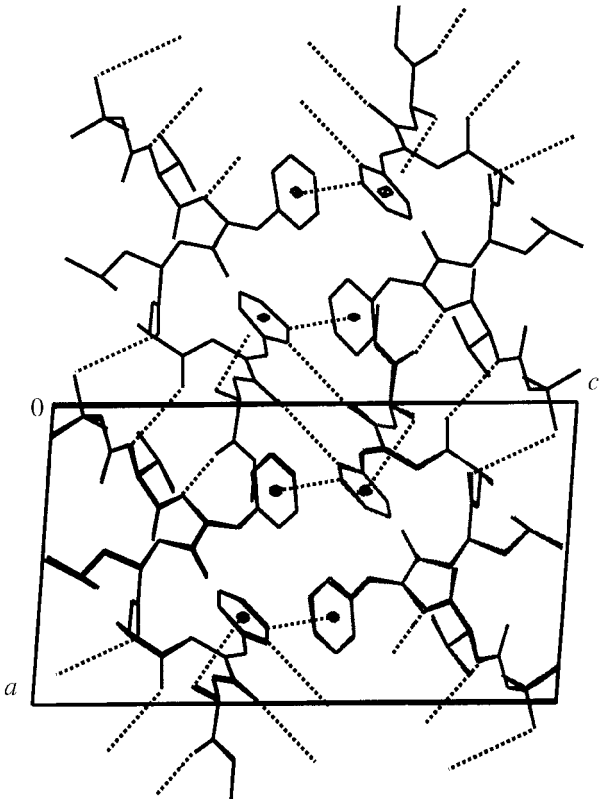

Figure 2 Crystal packing of the hexapeptide (view down the crystallographic *b* axis). The intermolecular head-to-tail N-H···O and C-H···O hydrogen bonds are represented by dotted lines.

two-residue spacer can retain the 3_{10}-helical structure as illustrated by present structure.

Crystal Packing

The crystal packing is in the form of long antiparallel helical rods along the crystallographic *a* axis (Figure 2). The packing shows two head-to-tail N–H···O=C and a weak C–H···O=C intermolecular hydrogen bonds (Table 3). This packing motif of the apolar helices in the present case is similar to many other apolar peptide helices whose crystal structures are known [35]. A predominantly aromatic slab consisting of ΔPhe^2 and ΔPhe^5 with Ala^6 residues can be observed parallel to the *ab*-plane. In addition to the hydrophobic forces being responsible for the stabilization, an ensemble of aromatic interactions within the aromatic slab appears to stabilize the helical rods in crystal.

Aromatic Interactions

Figure 3 illustrates the repertoire of aromatic interactions namely $CH_3\cdots\pi$ [36], aromatic C–H···π [37] and aromatic C–H···O=C [38] between the laterally adjacent helices. These helices are related to each other by crystallographic 2_1-screw symmetry. The ΔPhe^5 residue forms the hub of multicentred interactions acting both as a donor and an acceptor. The $C_5^{\delta 1}$-H of ΔPhe^5 makes a C–H···O=C interaction with the O5′ of backbone C5′. The hydrogen bonding potential of the carbonyl group C5′=O5′, which is not involved in regular N–H···O=C type of hydrogen bonding, is thus being effectively utilized. Also, $C_5^{\varepsilon 1}$-H of ΔPhe^5 makes an edge to face interaction with ΔPhe^2 (with $d_{\pi cX}$=3.60Å, $d_{\pi HX}$=2.76Å, $\alpha = 152^\circ$) [37]. This effect brings about the complete involvement of both the aromatic moieties within the crystal lattice. The above mentioned donor properties of the ΔPhe^5 residue presumably influence the acceptor potential of its π face. This fact is brought out as ΔPhe^5 is involved in a relatively weak $CH_3\cdots\pi$ (with $d_{C\pi c} = 3.78$Å, $d_{C-H\pi c} = 3.0$Å, $\alpha = 136^\circ$) [36] type of interaction with the methyl side chain of Ala^6. The capability of a ΔPhe aromatic moiety to act both as an acceptor and a donor is thus clearly brought out in the present structure and forms an example for the concerted involvement of a phenyl ring in such interactions. Aromatic interactions play a predominant role in stabilizing protein structural architecture as pointed out by the classical work of Burley and Petsko [39], more recently re-analysed by Steiner and Koellner [40]. The exploitation of such weakly polar interactions in *de novo* design has been increasingly attracting more attention in recent times [18,41].

Circular Dichroism Studies

The CD spectra of the hexapeptide display a negative couplet (−,+) in all solvents used. A negative band

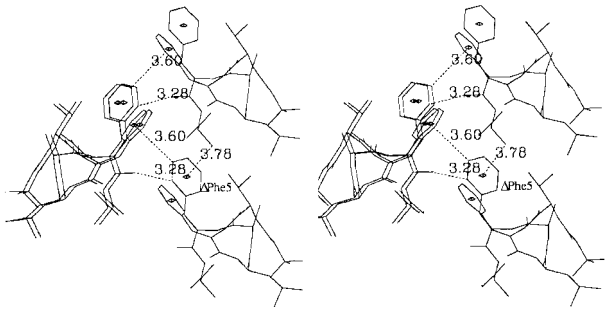

Figure 3 Stereodiagram of multicentred cooperative aromatic interactions in the crystal packing of the hexapeptide (view down the helical axis which coincides with crystallographic *a* axis). It may be seen that ΔPhe^5, acting as both donor and acceptor in hydrogen bonding, forms the hub of the interactions.

was observed at about 295 nm and an intense positive band at about 265 nm, with a crossover point at ~280 nm (Figure 4). This CD pattern corresponds to the absorption maximum at 270–280 nm and arises from dipole–dipole interactions between the charge transfer electric moments of the two didehydroamino acid chromophores placed in a mutual, fixed disposition within the molecule [42]. This pattern, as reported earlier [43], is typical of a right-handed 3_{10}-helix. The varying intensity of bands suggest a different content of the helical conformer in different solvents. The very low intensity of bands in the CD spectrum in methanol may be attributed to the high polarity of the solvent. It is known that folded peptide structures with stabilizing hydrogen bonds are more stable in apolar solvents than in polar ones [42]. Hence, the CD bands are found to be more intense in 2,2,2-trifluoroethanol and dichloromethane than in methanol and DMSO.

NMR Studies

Well resolved ^{1}H NMR spectra were obtained in both chloroform and dimethylsulfoxide. The NH proton signals of the two ΔPhe amino acids were recognized as singlet NH resonances. These and other protons were further assigned *via* ROESY and TOCSY bidimensional NMR experiments (Table 4). Chemical shift changes of amide resonances were probed by perturbation with temperature and solvent, which indicated potential hydrogen bonding interactions [44,45]. Figure 5 shows the variation of NH chemical shifts in $CDCl_3$ with increasing concentration of DMSO. An appreciable downfield shift with increasing concentration of DMSO for NH Val^1 and NH ΔPhe^2 indicates the absence of hydrogen bonding for these two NH groups. However, the remaining four amide protons (Leu^3, Ala^4, ΔPhe^5, Ala^6) show very little deviation in their chemical shift positions on addition of the strong hydrogen bond acceptor DMSO. This finding indicates that these NH groups are shielded from the solvent due to intramolecular hydrogen bonding. In DMSO, however, high values of the temperature coefficient (Table 4) clearly show the absence of any hydrogen bonding in the hexapeptide, suggesting an extended conformation.

Spatial proximity of the spin systems in the hexapeptide was investigated by means of ROESY. Both, intraresidue and interresidue NOEs were observed in the peptide in $CDCl_3$. Continuous d_{NN} NOE cross peaks were observed throughout the peptide sequence (NHVal^1-NHΔPhe^2-NHLeu^3,NHAla^4-NHΔPhe^5-NHAla^6). Such observations are characteristic of a helical conformation. Cross peaks of the type $d_{\alpha N}(i,i+2)$ between C$^\alpha$HVal^1-NHLeu^3 and C$^\alpha$HLeu^3-NHΔPhe^5 were also observed (Figure 6) which are diagnostic of 3_{10}-helical conformation [46,47], wherein consecutive type-III-β turns are present. Since no medium-range cross peak was observed between C$^\alpha$HAla^4-NHAla^6, it is possible that the 3_{10}-helical conformation does not continue

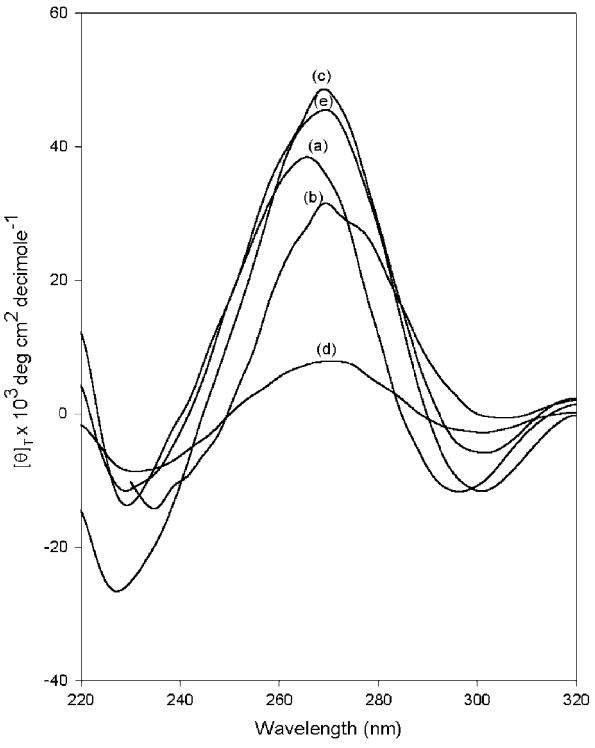

Figure 4 Near-UV CD spectra of the hexapeptide in various solvents: (a) acetonitrile, (b) chloroform, (c) dichloromethane, (d) methanol, (e) trifluoroethanol.

Table 4 NMR Parameters for NH Protons in the Hexapeptide

Residue NH	$CDCl_3$ (ppm)	DMSO (ppm)	$-d\delta/dT$ in DMSO (ppb/K)	$J_{NHC^\alpha H}$ (Hz)
Val^1	5.05	6.99	8.0	6.0
ΔPhe^2	7.79	9.73	6.48	—
Leu^3	7.36	7.99	4.0	5.88
Ala^4	7.34	8.20	4.40	7.95
ΔPhe^5	8.37	9.64	7.44	—
Ala^6	7.85	8.07	4.32	9.5

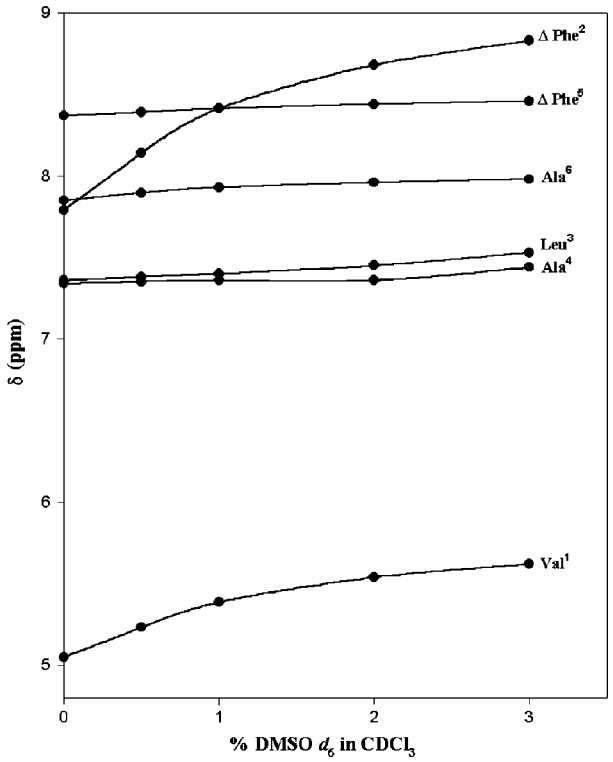

Figure 5 Solvent dependence of NH chemical shifts of the hexapeptide in $CDCl_3$-DMSO d_6.

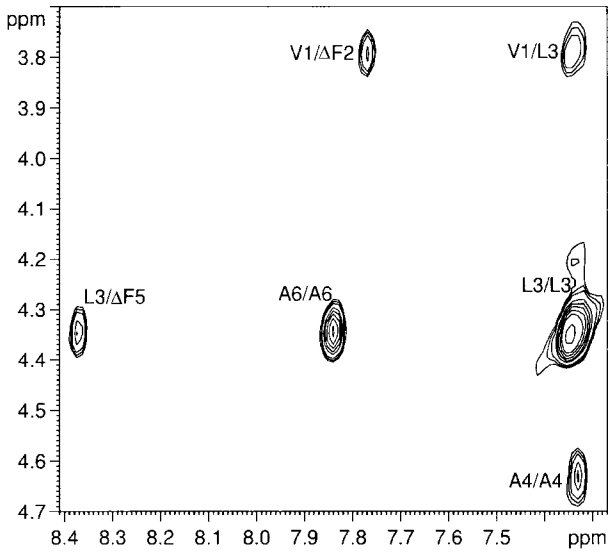

Figure 6 2D ROESY spectrum of the hexapeptide depicting the fingerprint region.

to the end of the sequence. A cross peak $d_{\alpha N}$ of moderate intensity from $C^{\alpha}HVal^1$ to $NH\Delta Phe^2$ was also observed. The simultaneous observation of $d_{\alpha N}$ and d_{NN} cross peaks is interpreted as the coexistence of both helical and extended conformers [48]. The vicinal coupling constants ($J_{NH-C\alpha H}$), showed high values (Table 4).

Both NMR and CD results suggest that the hexapeptide, Boc-Val-ΔPhe-Leu-Ala-ΔPhe-Ala-OMe, assumes a 3_{10}-helical conformation in solution, stabilized by four $1 \leftarrow 4$ intramolecular hydrogen bonds. The helix terminates at ΔPhe^5 and does not extend to the *C*-terminus. The lack of information obtained from the $J_{NHC^{\alpha}H}$ values is also expected in smaller peptides due to the coexistence of both helical and extended conformers in solution. However, in solvents like DMSO the peptide completely loses its helicity.

CONCLUSION

The original design strategy of examining the role a two-residue spacer between ΔPhe residues in a smaller peptide was to understand the preference of such a sequence for the 3_{10}-helical conformation. The 3D-structure described here shows that positioning of ΔPhe residues at i^{th} and $i+3^{rd}$ positions suffices to form the 3_{10}-helix. The 3_{10}-helical conformation in the present structure results in the stacking of ΔPhe residues one above the other, thus promoting the formation of aromatic slabs in the crystal packing. However, studies on more such designed peptides will certainly augment our understanding of the role of various natural amino acids in the spacer region and the consequence of their interactions on peptide conformation. Nevertheless, the observations extracted from the present structure can be incorporated during the design of longer peptides of desired fold.

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