Conformations of Dehydrophenylalanine Containing Peptides: NMR Studies of an Acyclic Hexapeptide with Two Δ^Z-Phe Residues

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Synopsis

The conformation of an acyclic dehydrophenylalanine (Δ^Z-Phe) containing hexapeptide, Boc-Phe-Δ^Z-Phe-Val-Phe-Δ^Z-Phe-Val-OMe, has been investigated in $CDCl_3$ and $(CD_3)_2SO$ by 270-MHz ^{1}H-nmr. Studies of NH group solvent accessibility and observation of interresidue nuclear Overhauser effects (NOEs) suggest a significant solvent-dependent conformational variability. In $CDCl_3$, a population of folded helical conformations is supported by the inaccessibility to solvent of the NH groups of residues 3–6 and the detection of several $N_iH \leftrightarrow N_{i+1}H$ NOEs. Evidence is also obtained for conformational heterogeneity from the detection of some $C_i^\alpha H \leftrightarrow N_{i+1}H$ NOEs characteristic of extended strands. In $(CD_3)_2SO$, the peptide largely favors an extended conformation, characterized by five solvent-exposed NH groups and successive $C_i^\alpha H \leftrightarrow N_{i+1}H$ NOEs for the L-residues and $C_i^\beta H \leftrightarrow N_{i+1}H$ NOEs for the Δ^Z-Phe residues. The results suggest that Δ^Z-Phe residues do not provide compelling conformational constraints.

INTRODUCTION

Recent interest in the conformational properties of dehydrophenylalanine containing peptides[1-7] has been stimulated by the possibility of using α,β-dehydroamino acids to introduce both side-chain and backbone conformational constraints into analogs of biologically active peptides.[8-10] Studies of model peptides, both cyclic[4,5] and acyclic,[6,7] containing a single Z-dehydrophenylalanine (Δ^Z-Phe) suggest that this residue shows a tendency to be readily accommodated into β-turn conformations, and evidence for its occurrence at the $i + 2$ position of type II β-turns has been presented.[4-7] As part of a continuing program, we have extended these conformational studies to longer oligopeptides containing more than one Δ^Z-Phe residue. In this report we describe nmr studies on the hexapeptide Boc-(Phe-Δ^Z-Phe-Val)$_2$-OMe (1) in organic solvents, using NH group accessibility and nuclear Overhauser effects (NOEs) as probes of molecular conformation.

EXPERIMENTAL

Peptide **1** was synthesized by conventional procedures and fully characterized by 270-MHz ^{1}H-nmr and elemental analysis. Representative procedures for the synthesis of Δ^Z-Phe containing peptides have been described in detail

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earlier.[6] Nuclear magnetic resonance studies were carried out on a WH-270 MHz Fourier transform nmr spectrometer equipped with an Aspect 2000 computer at the Sophisticated Instruments Facility, Indian Institute of Science. Difference NOE studies and two-dimensional correlated spectroscopy (COSY) were carried out as described earlier.[11] All chemical shifts are expressed as δ (ppm) downfield from internal $(Me)_4$ Si. Peptide concentrations of ~ 10 mg/mL were used in all the studies.

RESULTS AND DISCUSSION

A COSY spectrum of **1** in $CDCl_3$ is shown in Fig. 1. The spin systems of the two Phe and two Val NH groups are readily recognized. The Δ^Z-Phe NH resonances that appear as broad singlets in the one-dimensional spectrum display a weak long-range coupling to the corresponding Δ^Z-Phe $C^\beta H$ resonances. Specific assignments to Phe(1) and Phe(4) resonances could be made since the Phe(1) NH (urethane) appears at high field in $CDCl_3$ solutions.[6] Sequential assignments were obtained from difference NOE studies (discussed in a subsequent section), which permitted the observation of specific interresidue NOEs.[12] The derived assignments are marked in Fig. 1. Specific assignments were also made in $(CD_3)_2SO$ with the aid of COSY and NOE spectra. The chemical shifts of various NH resonances are summarized in Table I and the observed values are correlated with those obtained earlier[6] for a protected tripeptide, Boc-Phe-Δ^Z-Phe-Val-OMe (**2**), corresponding to residues 1–3 of **1** in Fig. 2. No significant concentration dependence of chemical shifts was observed for the NH and $C^\alpha H$ proton resonances of peptide **1** in $CDCl_3$ over the concentration range of 2–10 mg/mL, suggesting that aggregation effects are probably unimportant.

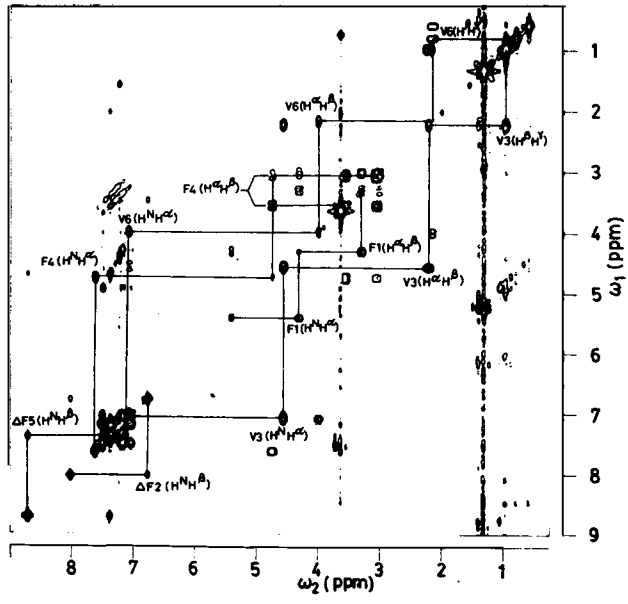

Fig. 1. COSY spectrum of **1** in $CDCl_3$ indicating connectivities of resonances corresponding to individual residues. One-letter code is used to indicate the amino acids (F, Phe; V, Val).

TABLE I
NMR Parameters for NH Groups in Peptide 1[a]

	Phe(1)	Δ^Z-Phe(2)	Val(3)	Phe(4)	Δ^Z-Phe(5)	Val(6)
$(CD_3)_2SO$						
δ (ppm)	7.13	9.84	7.66	8.28	9.71	7.86
$J_{HNC^{\alpha}H}$ (Hz)	b		b	7.3		7.7
$CDCl_3$						
δ (ppm)	5.45	8.05	7.07	7.65	8.75	7.07
$J_{HNC^{\alpha}H}$ (Hz)	3.9	—	4.7	7.8	—	2.7
$\Delta\delta$ (ppm)	1.68	1.79	0.59	0.63	0.96	0.79
	(2.24)	(2.16)	(0.89)			
	[1.99]	[1.44]	[2.04]			
$d\delta/dT$	7.3	6.3	2.7	4.3	5.3	5.7
(ppm $K^{-1} \times 10^{-3}$)	(8.1)	(5.6)	(4.4)			
	[6.0]	[3.0]	[6.0]			

[a] Values in parentheses are for Boc-Phe-Δ^Z-Phe-Val-OMe (1) and values in brackets for Boc-Phe-Phe-Val-OMe (2). Values under residue 2 for peptide 3 correspond to L-Phe(2).

[b] $J_{HNC^{\alpha}H}$ values could not be measured due to overlap with aromatic ring proton resonances.

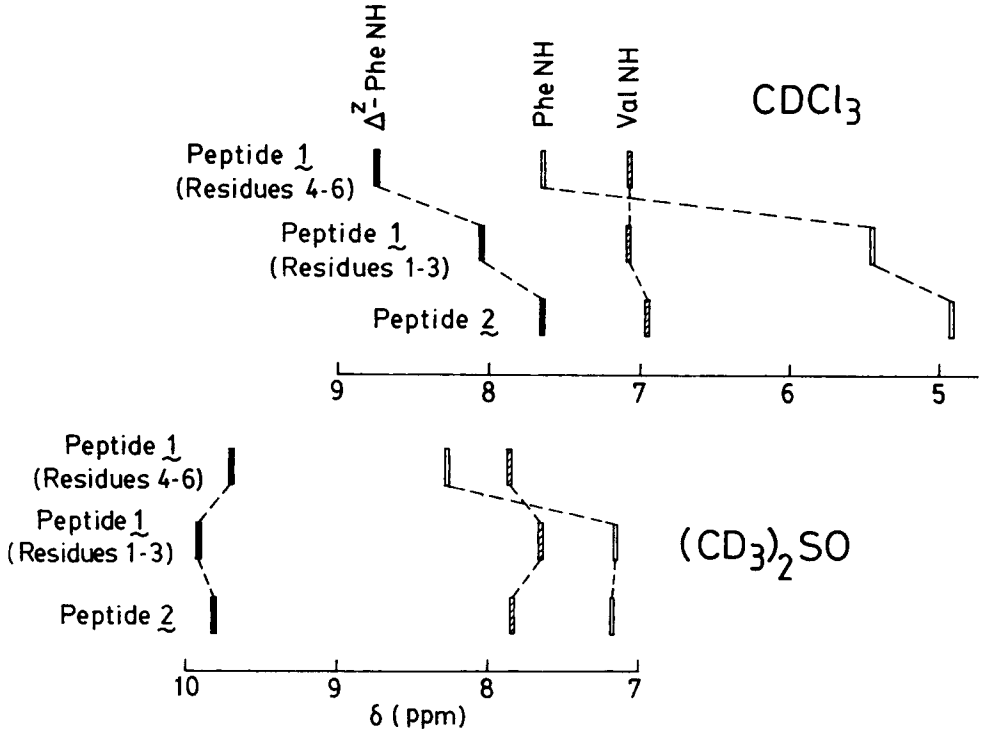

Fig. 2. Chemical shift correlation diagram for the peptides 1 and 2 in $CDCl_3$ and $(CD_3)_2SO$.

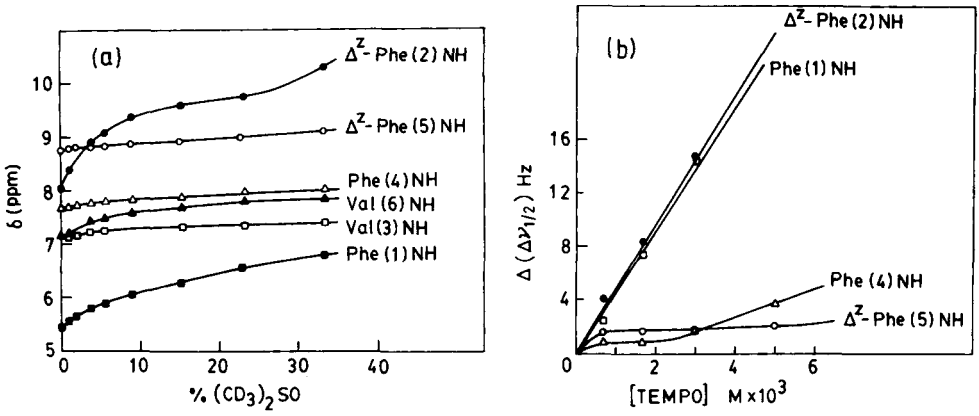

Fig. 3. (a) Solvent dependence of NH chemical shifts in peptide **1** in $CDCl_3/(CD_3)_2SO$ mixtures of varying composition. Peptide concentration ~10 mg/mL. (b) Dependence of NH resonance line widths in peptide **1** on the concentration of 2,2,6,6-tetramethylpiperidine-1-oxyl (M) in $CDCl_3$. Peptide concentration ~10 mg/mL.

Solvent Accessibility of NH Groups

Solvent accessibility of various NH groups in **1** was probed using solvent perturbation experiments in $CDCl_3$-$(CD_3)_2SO$ mixtures and paramagnetic radical induced broadening in $CDCl_3$ solutions.[11] The results are summarized in Fig. 3. Phe(1) and Δ^Z-Phe NH groups show an appreciable downfield shift with increasing concentration of the strongly hydrogen bond accepting solvent $(CD_3)_2SO$. The remaining four NH groups of residues 3–6 are largely unaffected by $(CD_3)_2SO$ concentrations upto 35 vol %. The results in Fig. 3(b) demonstrate that the Phe(1) and Δ^Z-Phe(2) NH groups are dramatically broadened upon addition of the free radical TEMPO; in contrast, the Phe(4) and Δ^Z-Phe(5) NH resonances are much less affected. The two Val NH resonances could not be monitored in this experiment due to overlap with aromatic proton resonances. Temperature dependence of the NH chemical shifts was measured in $(CD_3)_2SO$ and the results are summarized in Table I. The parameters obtained are compared with the corresponding values for the protected tripeptide Boc-Phe-Δ^Z-Phe-Val-OMe (**2**)[6] and a saturated analog Boc-Phe-Phe-Val-OMe (**3**).

It is clear that in $CDCl_3$, four NH groups corresponding to Val(3), Phe(4), Δ^Z-Phe(5), and Val(6) residues are relatively inaccessible to solvent, suggesting their involvement in intramolecular hydrogen bonding. However, in $(CD_3)_2SO$, only the Val(3) resonance exhibits a $d\delta/dT$ value characteristic of a solvent-shielded NH group. While the $d\delta/dT$ values for Phe(1) and Δ^Z-Phe(2) NH resonances are characteristic of fully solvent-exposed NH groups, relatively high values are obtained for Phe(4), Δ^Z-Phe(5) and Val(6) NH resonances. These results suggest that peptide **1** favors a folded conformation in $CDCl_3$, with the NH groups of residues 3–6 involved in intramolecular hydrogen bonding. However, in $(CD_3)_2SO$, a more extended conformation is likely, which permits appreciable solvation of five of the six NH groups. A comparison of the data for hexapeptide **1** with the results for tripeptide **2** and **3** (Table I) establishes that appreciably lower $\Delta\delta$ values for Val(3) NH are obtained for

the Δ^Z-Phe(2) containing sequences (**1**, **2**), as compared to the saturated analog **3**. This is clearly indicative of a greater tendency toward β-turn formation by the Phe-Δ^Z-Phe sequence, resulting in shielding of the Val(3) NH group from solvent, in contrast to peptide **3**. The significantly lower $d\delta/dT$ value in **1** as compared to **2** and **3** also suggests that folded conformations are stabilized to a greater extent in the longer peptide even in a strongly solvating medium like $(CD_3)_2SO$.

NOE Studies

NOE studies were carried out in $CDCl_3$ at 293 K and in $(CD_3)_2SO$ at 343 K. At ambient probe temperature (293 K) the observed NOEs in $CDCl_3$ were weak and positive, suggesting that the condition $\omega\tau_c < 1$ is satisfied.[13,14] In $(CD_3)_2SO$, the NOE experiments were conducted at the higher temperature of 343 K in order to remain in the positive NOE region. Figure 4 (inset) shows representative difference NOE spectra obtained for peptide **1** in $(CD_3)_2SO$. The NOEs observed on irradiation of various NH resonances in the two solvents are summarized in Table II. Typical NOE magnitudes for the tripeptides **2** and **3** are also listed for comparison.

In $CDCl_3$, $N_iH \leftrightarrow N_{i+1}H$ NOEs are observed between Phe(1) NH and Δ^Z-Phe(2) NH, and Phe(4) NH and Δ^Z-Phe(5) NH. Such NOEs are characteristic of helical conformations,[12] with $\phi \sim -50°$, $\psi \sim -50°$. NOEs involving Val(3) and Val(6) NH could not be observed due to overlap of these resonances with aromatic protons. A few interresidue NOEs of the type $C_i^\alpha H \leftrightarrow N_{i+1}H$ are also observed between Phe(1) $C^\alpha H$ and Δ^Z-Phe(2) NH, Val(3) $C^\alpha H$ and Phe(4) NH, and Phe(4) $C^\alpha H$ and Δ^Z-Phe(5) NH. Their magnitudes are

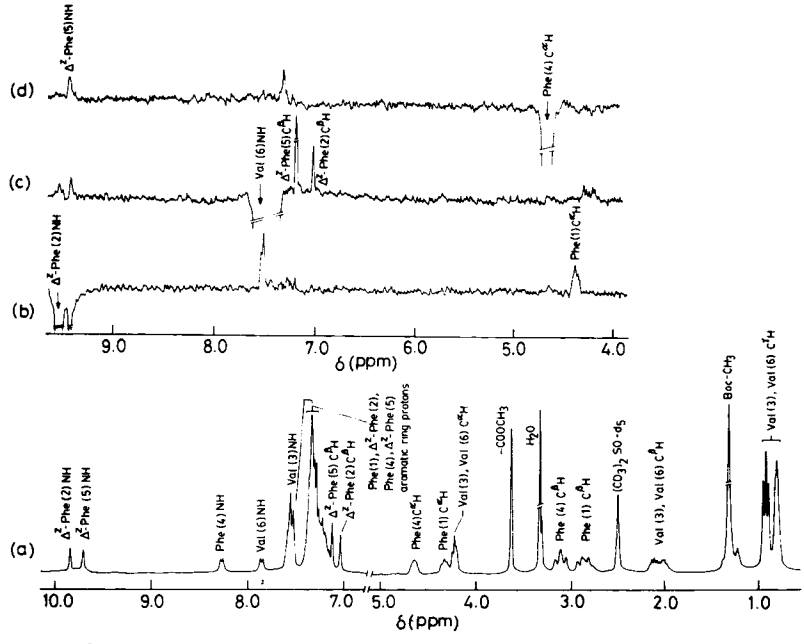

Fig. 4. (a) ^{1}H-nmr spectrum (270 MHz) of peptide **1** in $(CD_3)_2SO$ at 293 K. (b–d) Difference NOE spectra obtained by irradiation of the resonances, indicated by arrows, at 343 K.

TABLE II
NOEs[a] Observed in Peptide 1

Solvent	$(CD_3)_2SO$ at 343 K		$CDCl_3$ at 293 K	
Resonance irradiated	Resonance observed	NOE (%)	Resonance observed	NOE (%)
Phe(1) NH	Phe(1) $C^{\alpha}H$	3.4(b) [3.5]	Phe(1) $C^{\alpha}H$	3.3 (2.4)[b]
			Δ^Z-Phe(2) NH	2.2 (2.5) [2.9]
Δ^Z-Phe(2) NH	Phe(1) $C^{\alpha}H$	9.1 (1.8) [3.8]	Phe(1) $C^{\alpha}H$	4.2 (10.7) [10.3]
			Phe(1) NH	1.7 (2.6) [2.9]
Val(3) NH	Val(3) $C^{\alpha}H$	6.1(b) [4.8]	b	b
	Δ^Z-Phe(2) $C^{\beta}H$	6.7 (4.8) [6.3]		
Phe(4) NH	Val(3) $C^{\alpha}H$	8.0	Val(3) $C^{\alpha}H$	1.3
			Phe(4) $C^{\alpha}H$	5.8
			Δ^Z-Phe(5) NH	4.1
Δ^Z-Phe(5) NH	Phe(4) $C^{\alpha}H$	5.5	Phe(4) $C^{\alpha}H$	2.9
			Phe(4) NH	3.1
Val(6) NH	Val(6) $C^{\alpha}H$	5.2		
	Δ^Z-Phe(5) NH	3.3	b	b
	Δ^Z-Phe(5) $C^{\beta}H$	5.9		

[a] Values in parentheses are for tripeptides Boc-Phe-Δ^Z-Phe-Val-OMe (2) and in brackets for Boc-Phe-Phe-Val-OMe (3).
[b] NOEs not reported due to overlap of either irradiated or observed protons with other resonances.

comparable to those of the $N_iH \leftrightarrow N_{i+1}H$ NOEs. In $(CD_3)_2SO$, only a single $N_iH \leftrightarrow N_{i+1}H$ NOE could be detected between Δ^Z-Phe(5) NH and Val(6) NH. However, several strong interresidue $C_i^{\alpha}H \leftrightarrow N_{i+1}H$ NOEs were observed. These are Phe(1) $C^{\alpha}H \leftrightarrow \Delta^Z$-Phe(2) NH, Val(3) $C^{\alpha}H \leftrightarrow$ Phe(4) NH, and Phe(4) $C^{\alpha}H \leftrightarrow \Delta^Z$-Phe(5) NH NOEs. It may be noted that strong Δ^Z-Phe $C^{\beta}H \leftrightarrow$ Val NH NOEs are observed for both Δ^Z-Phe residues. This interproton distance is expected to be short only for $\psi_{\Delta^Z\text{-Phe}} \sim 120°$. These NOEs are absent in $CDCl_3$. A similar solvent dependence of NOEs is also observed for the tripeptides **2** and **3**, with $N_iH \leftrightarrow N_{i+1}H$ NOEs seen in $CDCl_3$ but largely undetectable in $(CD_3)_2SO$.

These results clearly demonstrate that the conformations of peptide **1** are solvent dependent, with largely extended conformations favored in $(CD_3)_2SO$, as supported by the observations of $C_i^{\alpha}H \leftrightarrow N_{i+1}H$ NOEs.[12] The simultaneous observation of $C_i^{\alpha}H \leftrightarrow N_{i+1}H$ and $N_iH \leftrightarrow N_{i+1}H$ NOEs in $CDCl_3$ may be interpreted in two possible ways:

1) A population of two distinct sets of conformers corresponding to a folded helical structure and a set of extended conformations. Under conditions where the residence time in these conformations is long enough compared to the rate of NOE buildup, the observed NOEs will be a weighted average reflecting the populations of different species.[15]

2) The occurrence of a largely left-handed helical conformation, which will place the L-residues in the right-hand upper quadrant of the Ramachandran ϕ, ψ map. Under these conditions, short (< 3 Å) $C^{\alpha}H—N_{i+1}H$ and $N_iH—N_{i+1}H$ distances are simultaneously observed.[16]

In the present situation, the interpretation involving conformational heterogeneity is reasonable since there does not appear to be any compelling

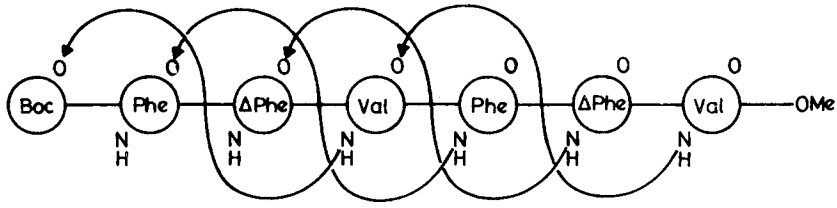

Fig. 5. Schematic hydrogen-bonding scheme proposed for peptide 1 in $CDCl_3$ consistent with the spectral data.

structural reason that will force the four L-residues into a less probable region of ϕ, ψ space. A direct distinction of left-handed helical conformations may be possible from CD studies. However, in the present case the high absorption at wavelengths below 230 nm, due to the presence of four aromatic chromophores, precludes a far-uv CD analysis.

Conformations of 1

The nmr studies described above provide evidence for solvent-dependent conformational variability in peptide **1**. In a relatively apolar, poorly hydrogen bond accepting solvent like $CDCl_3$, the nmr data are consistent with an appreciable population of folded conformations involving NH groups of residues 3–6 in intramolecular hydrogen bonding. Figure 5 shows a schematic hydrogen-bonding pattern that involves a succession of $4 \rightarrow 1$ hydrogen bonds and corresponds to a 3_{10}-helical conformation. Such a helical hydrogen-bonding scheme would result when the backbone conformational angles are $\phi \sim -60°$, $\psi \sim -30°$. In such a structure, short $N_iH—N_{i+1}H$ distances are expected,[12] compatible with observed NOEs. It may be noted that small changes in ϕ, ψ values can lead to the formation of mixed $3_{10}/\alpha$-helical structures, involving both $4 \rightarrow 1$ and $5 \rightarrow 1$ hydrogen bonds, and in some cases, bifurcated hydrogen bonds.[17] Subtle distinctions between various helical structures are not directly possible from the available nmr data. The observed $J_{HNC^{\alpha}H}$ values for Phe(1), Val(3), and Val(6) are less than 5 Hz, and are consistent with a largely helical conformation for these resonances.[18] The $J_{HNC^{\alpha}H}$ value for Phe(4) is appreciably larger (7.8 Hz) and may reflect a degree of conformational averaging involving this residue. Indeed, the observation of $C_i^{\alpha}H \leftrightarrow N_{i+1}H$ NOEs characteristic of $\psi \sim 120° \pm 60°$ in $CDCl_3$ also suggests conformational heterogeneity.[7]

Figure 6 is a schematic representation of a largely extended conformation for peptide **1** that is consistent with the observed NOEs in $(CD_3)_2SO$. Such a conformation would be characterized by ψ values of $\sim 120°$ at each residue. Since no specific information is available about the ϕ values at each residue, the fully extended representation is speculative. Nevertheless, it is clear that peptide **1** adopts extended or partially extended conformations in $(CD_3)_2SO$, which permit appreciable solvation of five of the six NH groups in the molecule. The only NH group that shows a degree of solvent inaccessibility as evidenced by a low $d\delta/dT$ (.0027 ppm K^{-1}) value is the Val(3) NH. It is not possible to distinguish at present whether this low $d\delta/dT$ value is diagnostic of a population of Phe(1)-Δ^Z-Phe(2) type II β-turns or whether it is a result of steric shielding by the relatively bulky proximal side chains.

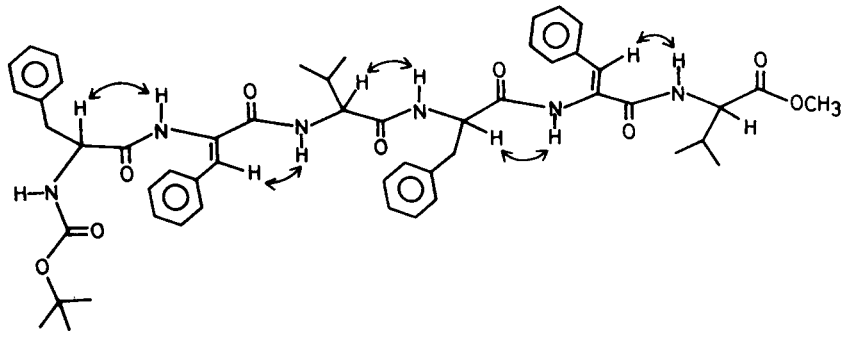

Fig. 6. Proposed extended conformation compatible with nmr data in $(CD_3)_2SO$ for peptide 1. Double-headed arrows indicate observed interresidue NOEs.

Although peptide **1** contains two Δ^Z-Phe residues, the available nmr evidence favors an appreciable conformational flexibility of the molecule. The presence of the Δ^Z-Phe residues does not lead to any overwhelming conformational preference that is independent of the nature of the solvent used. However, there is little doubt that incorporation of Δ^Z-Phe provides a measure of stabilization for folded, intramolecularly hydrogen-bonded conformations like β-turns and helical structures, in relatively apolar solvents like $CDCl_3$. In more strongly solvating media $[(CD_3)_2SO]$ more open, extended conformations are favored. The backbone conformational constraints introduced by introduction of an α, β double bond and the consequent flattening of the structure at the C^α atom appear to be relatively weak. These conclusions are in agreement with the presence of several comparable energy minima in the ϕ, ψ surface obtained from conformational energy calculations for a model Δ^Z-Phe residue.[1] The spatial restrictions imposed on the side-chain atoms are likely to be of much greater significance than backbone constraints when using Δ^Z-Phe residues in designing analogs of biologically active peptides.[10]

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