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Conformations of dehydrophenylalanine containing peptides

NMR studies on three tripeptides with a central dehydrophenylalanyl residue

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Three tripeptides containing a central Z-dehydrophenylalanine residue (Δ^Z-Phe), Boc-L-Phe-Δ^Z-Phe-X-OMe (X = L-Val **1**, L-Leu **2** and X = L-Ala **3**) have been synthesized and their solution conformations investigated by 270 MHz ^{1}H NMR spectroscopy. In all three peptides, conformations involving the X residue NH in an intramolecular hydrogen bond were favoured in $CDCl_3$ solutions. Studies of the nuclear Overhauser effect (NOE) provided support for a Type II β turn conformation in these peptides with Phe and Δ^Z-Phe occupying the i + 1 and i + 2 positions, respectively. Significantly different conformations lacking any intramolecular hydrogen bonds were observed for peptide **1** in $(CD_3)_2SO$. NOE results were consistent with a significant population of molecules having semi-extended conformations ($\psi > 100°$) at the Δ^Z-Phe residue.

Key words: dehydrophenylalanine peptides; β turns; nuclear Overhauser effects

The introduction of backbone conformational constraints by the incorporation of unusual amino acids into biologically active peptides is a useful approach to the study of structure-activity relationships (1). In the design of analogs of biologically important peptides, α,β-dehydroamino acids are potentially important residues (2). The presence of a double bond between the C^α and C^β atoms not only affects backbone structures, but can also dramatically reduce the conformational flexibility of the specific side chain. Despite their presence in several naturally occurring peptides (2, 3) and their potential utility in designing conformationally constrained synthetic model peptides (1, 2), relatively few studies have appeared on the conformational analysis of peptides containing dehydroamino acid residues (4–9). We describe in this report ^{1}H NMR studies on the conformations of 3 tripeptides (Fig. 1) containing a central Z-dehydrophenylalanine (Δ^Z-Phe) residue.

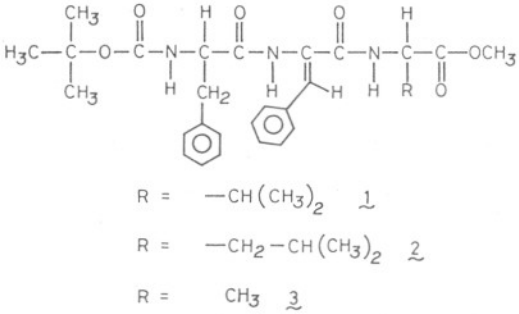

FIGURE 1
Structures of peptides **1**–**3**.

EXPERIMENTAL PROCEDURES

Synthesis of peptides

Boc amino acids and amino acid methyl esters were prepared by standard procedures. TLC was carried out on silica gel G in the following solvent systems: A) $CHCl_3$:MeOH (9:1) and B) nBuOH:AcOH:H_2O (4:1:5). The final peptides were each shown to yield a single peak by HPLC on a Lichrosorb RP-18 column (4 mm × 250 mm, particle size, 10 μm), using a methanol/water gradient (70–80% methanol in 20 min; flow rate 0.8 ml min^{-1}; detection 226 nm).

All ^{1}H NMR spectra were recorded on a Bruker WH-270 FT NMR spectrometer at the Sophisticated Instruments Facility, Indian Institute of Science. The solvent accessibility of NH groups in peptides was probed as described earlier (10, 11). Difference NOE experiments were carried out as described earlier (12).

Boc-Phe-DL-Phe(β-OH)-OH. A solution of Boc-L-Phe-OH (5.3 g, 20 mmol) and *N*-methylmorpholine (2.2 ml, 20 mmol) in THF (50 ml) was chilled, isobutylchloroformate (2.62 ml, 20 mmol) added and the mixture stirred for 20 min. A solution of DL-β-phenylserine (DL-Phe(β-OH)) (5.44 g, 30 mmol) in aqueous NaOH (1 M, 30 ml) was then added and the mixture stirred for 2 h at 0°C and overnight at room temperature. The reaction mixture was evaporated *in vacuo* to remove THF and the remaining aqueous solution washed with ethyl acetate, acidified with solid citric acid and extracted with ethyl acetate (3 × 30 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give an amorphous solid. Yield, 6.29 g (72%); m.p., 94–96°; R_f^B, 0.55.

Boc-Phe-Δ^z-Phe azlactone. A solution of Boc-Phe-DL-Phe(β-OH)-OH (4.27 g, 10 mmol) in acetic anhydride (30 ml) was stirred for 30 h at room temperature. The reaction mixture was poured over crushed ice, stirred and the resultant precipitate washed with $NaHCO_3$ solution (5%, 20 ml), water and dried. Recrystallization from ether/petroleum ether gave the azlactone in 70% yield (2.75 g); m.p., 132–133°; R_f^A, 0.90; R_f^B, 0.75.

Boc-PheΔ^z-Phe-Val-OMe **1**. A solution of H-Val-OMe (310 mg, 2.6 mmol) in THF (5 ml) was added to a THF solution of Boc-Phe-Δ^z-Phe-azlactone (1.0 g, 2.55 mmol) at 0°C. The mixture was stirred for 72 h at room temperature when TLC showed the absence of starting material. After evaporation *in vacuo*, the residue was taken in ethyl acetate (80 ml), washed successively with 5% $NaHCO_3$, 10% citric acid and water, dried over anhydrous sodium sulfate and evaporated to yield a solid. Recrystallization from ether/petroleum ether gave **1** as a white crystalline solid. Yield, 0.85 g (65%); m.p., 165–166°; $[\alpha]_D$, −37.5 (c = 0.33 in MeOH), $R_f(CHCl_3)$, 0.28; R_f^A, 0.9; R_f^B, 0.75, HPLC retention time, 13.3 min.

270 MHz ^{1}H NMR, δ ($CDCl_3$): 7.65 (Δ^z-Phe NH, 1H, s); 7.27 (Phe aromatic protons, Δ^z-Phe aromatic protons, Δ^z-Phe C^βH, 11H, m); 6.95 (Val NH, 1H, d); 4.95 (Phe NH, 1H, d); 4.65 (Val C^αH, 1H, q); 4.37 (Phe C^αH, 1H, q); 3.76 (−$COOCH_3$, 3H, s); 3.30 (Phe C^βH, 1H, q); 3.05 (Phe C^βH, 1H, q); 2.25 (Val C^βH, 1H, m); 1.4 (Boc CH_3, 9H, s); 1.03 (Val $C^\gamma H_3$, 3H, d); 0.98 (Val $C^\gamma H_3$, 3H, d).

Boc-Phe-Δ^z-Phe-Leu-OMe **2** was prepared by a procedure similar to that for **1**. Yield 62% m.p., 144–145°; R_f^A, 0.85; R_f^B, 0.80; $[\alpha]_D$, −48.0 (c = 0.33 in MeOH). HPLC retention time, 16 min.

270 MHz ^{1}H NMR, δ ($CDCl_3$): 7.50 (Δ^z-Phe NH, 1H, s); 7.25 (Phe aromatic protons, Δ^z-Phe aromatic protons, Δ^z-Phe C^βH, 11H, m); 7.08 (Leu NH, 1H, d); 4.87 (Phe NH, 1H, d); 4.65 (Leu C^αH, 1H, q); 4.20 (Phe C^αH, 1H, m); 3.71 (−$COOCH_3$, 3H, s); 3.25 (Phe C^βH, 1H, q); 2.98 (Phe C^βH, 1H, q); 1.65 (Leu $C^\beta H_2$, 2H, m); 1.22 (Boc CH_3, 9H, s); 0.92 (Leu $C^\delta H_3$, 6H, d).

Boc-Phe-Δ^z-Phe-Ala-OMe **3** was prepared by a procedure similar to that for **1**. Yield, 58%; m.p., 167–168°; $[\alpha]_D$, −34.5 (c = 0.33 in MeOH); R_f^A, 0.74; R_f^B, 0.85; HPLC retention time, 10.1 min.

270 MHz ^{1}H NMR, δ ($CDCl_3$): 7.65 (Δ^z-Phe NH, 1H, s); 7.28 (Phe aromatic protons, Δ^z-Phe ring protons, Δ^z-Phe C^βH, Ala NH, 12H, m); 4.97 (Phe NH, 1H, d); 4.67 (Ala C^αH, 1H, m); 4.33 (Phe C^αH, 1H, m); 3.75 (−$COOCH_3$, 3H,

s); 3.3 (Phe $C^{\beta}H$, 1H, q); 3.02 (Phe $C^{\beta}H$, 1H, q); 1.5 (Ala $C^{\beta}H_3$, 3H, d); 1.38 (Boc CH_3, 9H, s).

RESULTS AND DISCUSSION

The assignment of the NH resonances in all 3 peptides was straightforward. The Δ^z-Phe NH appeared as a singlet, while the Phe NH appeared at a relatively high field position in $CDCl_3$ (4.8–5.0 δ), characteristic of urethane groups (10). The remaining doublet NH resonance was then assigned to the X-NH group. The presence of intramolecularly hydrogen-bonded conformations in peptide **1** (X = L-Val) was probed using 3 criteria (13–15): temperature dependence of NH chemical shifts in $(CD_3)_2SO$; solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures; and paramagnetic radical-induced line broadening of NH resonances in $CDCl_3$. In the case of peptides **2** (X = L-Leu) and **3** (X = L-Ala) temperature coefficients of NH chemical shifts (dδ/dT) in $(CD_3)_2SO$ and the magnitude of chemical shift changes on going from $CDCl_3$ to $(CD_3)_2SO$ (Δδ) were measured. The relevant ^1H NMR parameters for the NH groups in peptides **1**–**3** are summarized in Table 1.

In **1**, the addition of a strong hydrogen bond-accepting solvent like $(CD_3)_2SO$ to a peptide solution in a poor hydrogen bond-accepting solvent like $CDCl_3$, resulted in appreciable downfield shifts of the Phe and Δ^z-Phe NH resonances, while the Val NH group was less affected (Fig. 2a). The changes in chemical shift were monotonic, up to a $(CD_3)_2SO$ concentration of 40% (v/v), suggesting the absence of any major conformational change in this solvent mixture (16). Addition of a paramagnetic radical, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) to $CDCl_3$ solutions of **1** resulted in a significant broadening of the Δ^z-Phe and Phe-NH groups, but left the Val NH unaffected (Fig. 2b). These results suggested that Val NH was relatively inaccessible to solvent in peptide **1**, whereas the other 2 NH groups were fully exposed (17). In pure $(CD_3)_2SO$, the observed dδ/dT value of 0.0044 ppm/K obtained for Val NH was higher than that expected for an appreciably solvent-shielded proton (<0.003 ppm/K) (18), suggesting that there may have been a difference in the conformations populated in $CDCl_3$ and $(CD_3)_2SO$. A similar pattern of dδ/dT values was also observed in peptides **2** and **3**, in $(CD_3)_2SO$ (Table 1). The δ values for the NH groups in **2** and **3** in both solvents were very similar to those observed for **1**, suggesting gross structural similarities between these peptides.

The NMR results for peptide **1** in $CDCl_3$

TABLE 1

NMR parameters for NH resonances in peptides Boc-Phe-Δ^z-Phe-X-OMe

Parameter	Residue	X = L-Val **1**	X = L-Leu **2**	X = L-Ala **3**
δ ($CDCl_3$)	Phe	4.94	4.85	4.96
	Δ^z-Phe	7.65	7.55	7.65
	X	6.95	7.09	7.27
δ ($(CD_3)_2SO$)	Phe	7.18	7.23	7.20
	Δ^z-Phe	9.81	9.81	9.79
	X	7.84	8.20	8.06
$J_{HNC^{\alpha}H}(CDCl_3)$[a]	Phe	6.7	6.2	6.2
(Hz)	X	7.3	6.8	b
$J_{HNC^{\alpha}H}((CD_3)_2SO)$[a]	Phe	6.8	b	5.6
(Hz)	X	8.0	5.0	6.6
dδ/dT($(CD_3)_2SO$)	Phe	0.0081	0.0075	0.0069
ppm/K	Δ^z-Phe	0.0056	0.0063	0.0063
	X	0.0044	0.0042	0.0038

[a]Errors in J values are ~ ± 0.4 Hz.

[b]J values could not be determined due to overlap of NH and aromatic proton resonances.

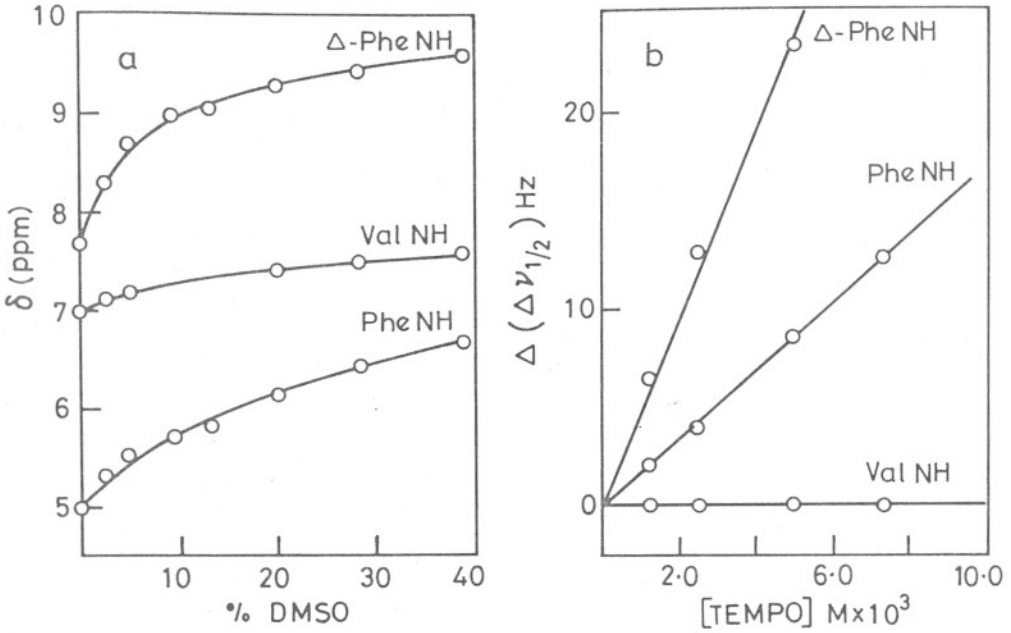

FIGURE 2

Left) Solvent dependence of NH chemical shifts in peptide **1** in $CDCl_3/(CD_3)_2SO$ mixtures of varying composition. Peptide concentration ~ 10 mg/ml. Right) Dependence of NH resonance linewidths in peptide **1** on concentration of 2,2,6,6,-tetra-methylpiperidine-1-oxyl (M). Solvent: $CDCl_3$. Peptide concentration ~ 10 mg/ml.

clearly supported a major population of conformations which possess a solvent-inaccessible Val NH group. This is consistent with a β turn conformation (19) having Δ^z-Phe at position i + 2, involving a $4 \rightarrow 1$ hydrogen bond between the Boc CO and Val NH groups (Fig. 3a). Recently, suggestions have indeed been made that Δ^z-Phe residues are readily accommodated, stereochemically, at position i + 2 of Type II β turns, with conformational angles of $\phi \sim 80°$, $\psi \sim 0°$ at Δ^z-Phe (6, 20). IR studies of peptide **1** in dilute $CHCl_3$ solutions establish the presence of a ν_{NH} band at ~3400 cm^{-1}, which is suggestive of only a weak, intramolecular hydrogen bond.

There was no significant change in the shape of the NH stretching bands over the concentration range 2.5 mM–20 mM in $CHCl_3$, suggesting that peptide association was insignificant at the concentrations used in the NMR studies. The NMR results favoured a structural change in $(CD_3)_2SO$ and supported a preponderance of nonintramolecularly hydrogen-bonded conformations in this solvent. The nuclear Overhauser effect (NOE) studies described below permitted further definition of the conformational features of peptides **1**–**3**.

NOE studies

The observation of interproton NOEs in small peptides can generally be used to identify pairs of protons which are $\leqslant 3$ Å apart and can serve as a useful diagnostic indication of the presence of specific conformations (12, 15). Representative difference NOE experiments on peptide **1** in $CDCl_3$ and $(CD_3)_2SO$ are illustrated in Figs. 4 and 5 and the NOE data are summarized in Tables 2 and 3. A large NOE of ~8–11% was observed on the Phe $C^{\alpha}H$ resonance when the Δ^z-Phe NH resonance was saturated in peptides **1**–**3**. The observation of $C_i^{\alpha}H-N_{i+1}H$ NOEs in peptides is indicative of $\psi_i \sim 120° \pm 30°$ (21, 22), a feature consistent with the presence of Phe at the i + 1 position of a Type II β turn (12, 19). Interestingly, irradiation of the Δ^z-Phe NH resulted in a small but clearly observed NOE on the Phe NH proton in all 3 peptides (Table 2). Such $N_iH-N_{i+1}H$ NOEs

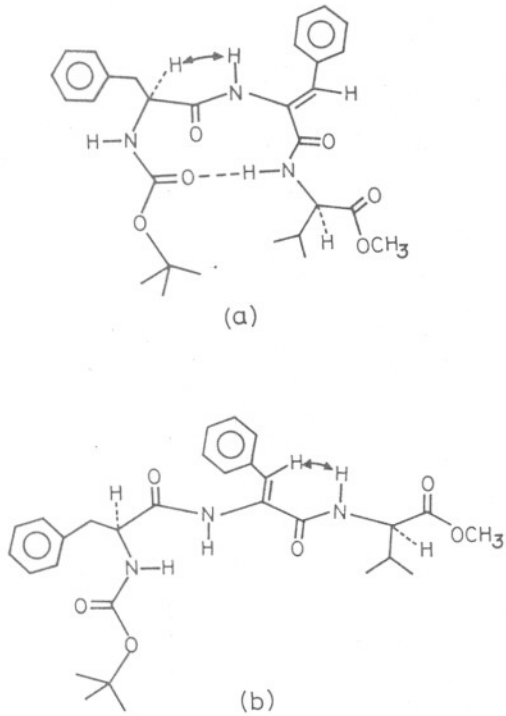

FIGURE 3

a) Proposed β turn conformation for **1** in $CDCl_3$ b) Proposed semi-extended conformation for **1** in $(CD_3)_2SO$. Double-edged arrow indicates short interproton distances which result in diagnostic NOEs.

TABLE 3

*Nuclear Overhauser effects observed in Boc-Phe-Δ^z-Phe-Val-OMe (**1**) in $(CD_3)_2SO$*

Resonance irradiated	Resonance observed	% NOE
Δ^z-Phe NH	Phe $C^\alpha H$	1.8
	Δ^z-Phe H_2, H_6 aromatic protons	2.8
Phe $C^\alpha H$	Δ^z-Phe NH	2.3
Δ^z-Phe H_2, H_6 aromatic protons	Δ^z-Phe NH	2.3
	Δ^z-Phe $C^\beta H$	4.2
Val NH	Δ^z-Phe $C^\beta H$	4.9

are not expected to occur between the i + 1 and i + 2 residues of a Type II β turn where the estimated interproton distance is 4.5 Å (22). Clearly, the observation of this NOE suggests the existence of a distinct set of conformations having $\psi_{Phe} \sim -30°$, $\phi_{\Delta^z\text{-Phe}} \sim -60°$ which would result in Phe NH – – – Δ^z-Phe NH distances of <3.0 Å (21). The NMR results, while favouring Type II β turns as a major conformational feature in peptides **1–3** in $CDCl_3$, also provided evidence for a population of distinctly different backbone conformations with rapid dynamic averaging between the various species.

The NOE between the Phe $C^\alpha H$ and Δ^z-Phe NH was dramatically reduced in $(CD_3)_2SO$ for peptides **1–3**, suggesting a significant

TABLE 2

Nuclear Overhauser effects observed in peptides **1–3** *in $CDCl_3$*

Resonance irradiated	Resonance observed	% NOE X = Val **1**	X = Leu **2**	X = Ala **3**
Phe $C^\alpha H$	Phe NH	a	2.1	1.8
	Δ^z-Phe NH	6.9	6.3	5.9
Δ^z-Phe NH	Phe NH	2.6	2.1	3.1
	Phe $C^\alpha H$	10.7	8.0	10.9
Δ^z-Phe $C^\beta H$ and aromatic protons of Phe, Δ^z-Phe[c]	X-NH	3.9	b	b
	X-$C^\alpha H$	1.6	4.6	5.8
	Phe $C^\alpha H$	4.5	4.4	3.9
	Phe NH	2.5	1.8	2.6
Phe NH	Δ^z-Phe NH	2.5	1.9	1.8
	Phe $C^\alpha H$	2.4	3.1	2.4

[a]No detectable NOE.
[b]Irradiated and observed frequencies were very close resulting in non observation of NOEs.
[c]Composite overlapping resonance.

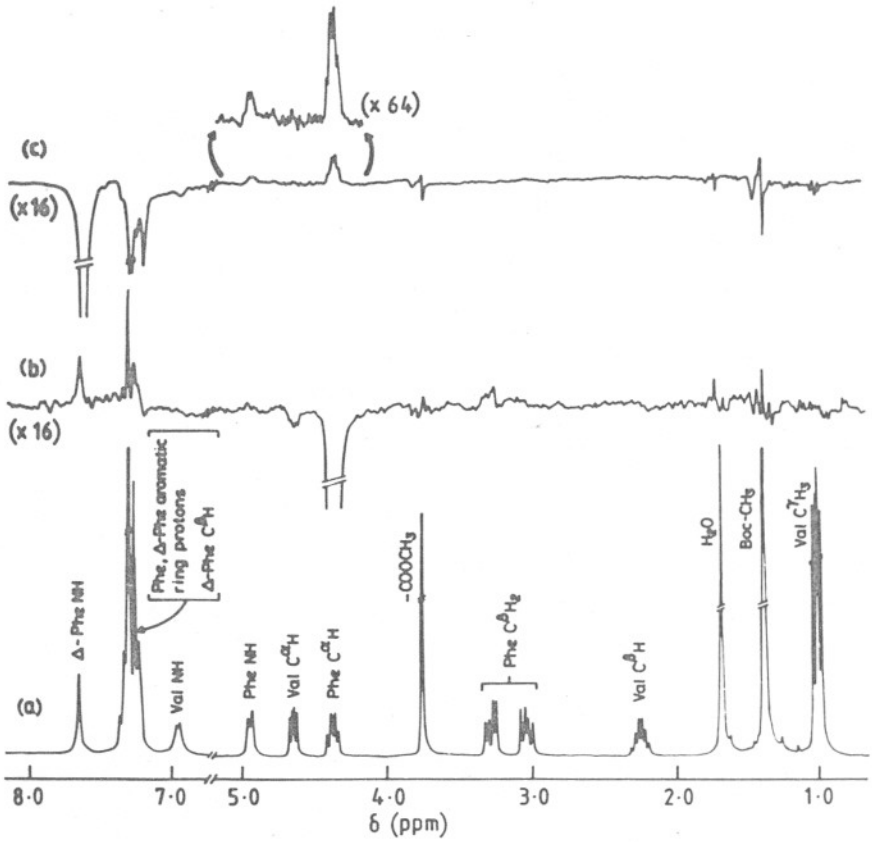

FIGURE 4

a) 270 MHz ^{1}H NMR spectrum of Boc-Phe-Δ^z-Phe-Val-OMe 1 in $CDCl_3$, b, c) Difference NOE spectra obtained by irradiation of b) Phe C^αH resonance, c) Δ^z-Phe NH resonance.

reduction in the population of Type II β turn conformations and further implying that the major conformations in $(CD_3)_2SO$ solutions had ψ_{Phe} values far removed from the semi-extended region ($\psi \sim 120° \pm 30°$).

In $(CD_3)_2SO$, appreciable NOEs were observed between the following pairs of protons: Val NH $\leftrightarrow$ Δ^z-Phe C^βH (4.9%) and Δ^z-Phe C^βH $\leftrightarrow$ Δ^z-Phe H_2, H_6 (aromatic ring) (4.2%). The Val NH – Δ^z-Phe C^βH distance is dependent only on $\psi_{\Delta z\text{-Phe}}$ and observation of a significant NOE was possible only for $\psi_{\Delta z\text{-Phe}}$ values of $\sim \pm 100° - \pm 180°$ (Fig. 6). Thus, the major conformations in $(CD_3)_2SO$ had distinctly different $\psi_{\Delta z\text{-Phe}}$ values from the structures suggested in $CDCl_3$ ($\psi_{\Delta z\text{-Phe}} \sim 0°$). The pronounced reduction in the magnitude of the Phe C^αH $\leftrightarrow$ Δ^z-Phe NH NOE in $(CD_3)_2SO$ as compared to $CDCl_3$, was consistent with a large change in ψ_{Phe} also, with change in solvent polarity. The unusually downfield position of the Δ^z-Phe NH proton in $(CD_3)_2SO$ ($\sim 9.8\ \delta$), relative to $CDCl_3$ ($\sim 7.55–7.65\ \delta$) was also consistent with significant differences in the conformations of 1 in the two solvents. The $J_{HNC^\alpha H}$ values for the Phe and X residues did not show a large solvent dependence suggesting that changes in ϕ_{Phe} and ϕ_x distributions were not appreciable. The major species in $(CD_3)_2SO$ clearly corresponded to a more extended backbone conformation lacking intramolecular hydrogen bonds (Fig. 3b), while in $CDCl_3$, folded, Type II β turn structures at the Phe-Δ^z-Phe segment were

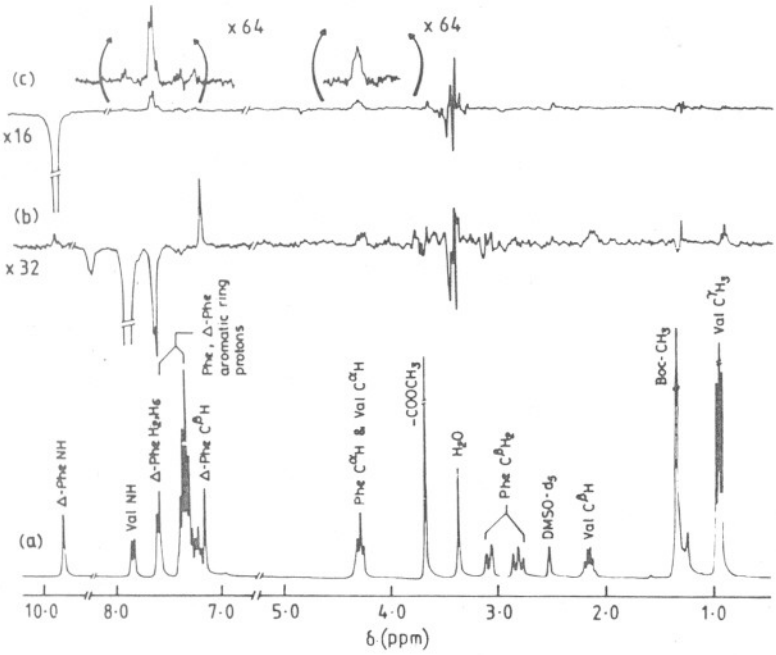

FIGURE 5

a) 270 MHz ^{1}H NMR spectrum of Boc-Phe-Δ^Z-Phe-Val-OMe **1** in $(CD_3)_2SO$. b, c) Difference NOE spectra obtained by irradiation of b) Val NH resonance, c) Δ^Z-Phe NH resonance.

significantly populated. The observation of semi-extended conformations at the Δ^Z-Phe residue suggested that ψ values $> 100°$ corresponded to favourable conformations. This implies that Δ^Z-Phe residues can also be accommodated at the i + 1 position of Type II β turns, which ideally require $\phi \sim -60°$, $\psi \sim +120°$ (19). Indeed, a few crystallographic observations of Δ^Z-Phe residues in this region of the ϕ, ψ map have been reported (5, 7). Examination of steric maps and conformational energy calculations (5, 9) suggest that stereochemically allowed and energetically favoured backbone conformations of Δ^Z-Phe residues are dependent on the value of χ^2 which influences the relative orientations of the aromatic ring and the peptide backbone (23). Further experimental studies of peptides

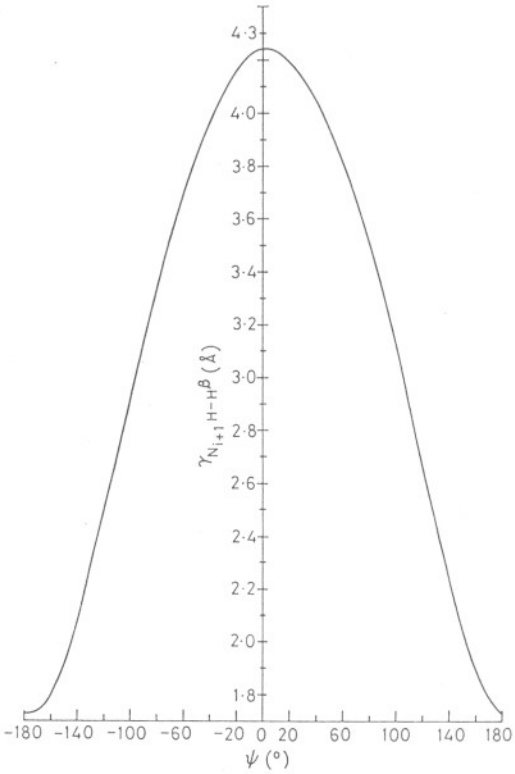

FIGURE 6

Dependence of the Δ^Z-Phe $C^\beta H$ – – – N*H*Me interproton distance in Ac-Δ^Z-Phe-NHMe as a function of the dihedral angle ψ. The geometry determined in the crystal structure analysis of Ac-Δ^Z-Phe-NHMe (7) has been assumed for purposes of calculation.

containing Δ^z-Phe are in progress, in order to define the nature of the preferred conformations for this residue.

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