Solution Conformation and Synthesis of a Linear Heptapeptide containing Two Dehydrophenylalanine Residues Separated by Three L-Amino Acids

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A heptapeptide containing two dehydrophenylalanine residues, Boc-Gly-Δ^zPhe-Ala-Phe-Leu-Δ^zPhe-Ala-NHMe (Boc = t-butoxycarbonyl) has been synthesised and its solution conformation investigated using 500 MHz ^{1}H NMR and IR spectroscopy. ^{1}H NMR studies of the solvent accessibility of NH resonances and observation of successive $N_iH \longleftrightarrow N_{i+1}H$ nuclear Overhauser effects (NOEs) suggest the presence of a significant population of folded, helical structures in $CDCl_3$. A 5→1 intramolecular hydrogen bonding pattern provides evidence in favour of an α-helix conformation. In $(CD_3)_2SO$, although the peptide largely favours the helical conformation, observation of a few $C_i^{\alpha}H \longleftrightarrow N_{i+1}H$ NOEs gives an indication of some conformational heterogeneity. IR studies in chloroform have provided supporting evidence in favour of these conclusions. Dehydrophenylalanine residues may be of potential use in designing peptides with preferred secondary structures.

Understanding the conformation of bioactive peptides important to receptor interactions is crucial to the rational design and development of potent agonists and antagonists. One of the most powerful tools for this is the design and synthesis of peptide analogues with conformational constraints and their structural and biological characterisation.[1,2] Several backbone modifications including introduction of unusual amino acids in peptides have been attempted to achieve this goal.[3] The use of dehydroamino acids in synthesising peptide analogues with preferred secondary structures is a promising new method.[4] Model peptides, cyclic[5] and acyclic,[6–12] containing a dehydrophenylalanine (Δ^zPhe) have shown a marked tendency to adopt a β-turn, with the ΔPhe residue occupying its $i + 2$ position. We have shown by ^{1}H NMR spectroscopy that in apolar solvents dehydroleucine (Δ^zLeu) also favours a β-turn conformation.[13] Crystal structure studies[7,9] on model peptides have firmly confirmed this suggestion. We have recently extended these studies to linear peptides containing more than one Δ^zPhe residue in the hope of obtaining peptides with a tendency to adopt helical conformations and have shown that, at least in apolar solvents, in a hexapeptide containing a -Δ^zPhe-X-X-Δ^zPhe- unit, a significant number of 3_{10} helical structures exists.[14]

In order to explore the possibilities of stabilising helical structures with different arrangements of dehydrophenylalanine units we have extended these studies to a synthetic peptide containing a -Δ^zPhe-X-X-X-Δ^zPhe- unit. We herein describe the synthesis and ^{1}H NMR studies of a heptapeptide, Boc-Gly-Δ^zPhe-Ala-Phe-Leu-Δ^zPhe-Ala-NHMe (Boc = t-butoxycarbonyl) in $CDCl_3$ and $(CD_3)_2SO$, using NH accessibility and nuclear Overhauser effects (NOEs) to probe the molecular conformation.

Experimental

Spectroscopy.—^{1}H NMR studies [in $CDCl_3$ and in $(CD_3)_2SO$] were carried out on a Bruker AM-500 MHz FT NMR spectrometer fitted with an ASPECT 3000 computer at the Tata Institute of Fundamental Research, Bombay. The peptide concentration ranged from 10 to 20 mg cm^{-3}. Difference NOE studies and one-dimensional ^{1}H NMR experiments for delineation of NH groups were carried out as reported earlier.[15] The COSY spectrum was recorded with 512 t_1 increments, accumulated into 1K data points with 80 scans for each t_1 value. A NOESY spectrum was recorded with 256 t_1 increments, with 80 scans per t_1 value and a mixing time of 500 ms.

A random variation of 3% in the mixing time was introduced to minimize contributions from coherent magnetization transfer between scalar coupled nuclei. The resulting 512 × 2048 data matrix was zero filled in the t_1 dimension and multiplied by the sine bell function in both dimensions. IR studies were carried out on a Perkin-Elmer 1710 FT IR spectrometer at the University Science Instrumentation Centre, Delhi University.

Synthesis.—The heptapeptide was synthesized by standard solution phase methodology. Amino acid couplings were performed by either mixed anhydride (MA) or dicyclohexylcarbodiimide (DCC)/hydroxybenztriazole (HOBT) procedures. The dehydrophenylalanine (Δ^zPhe) moiety was introduced using the literature procedure.[16] The synthetic scheme is outlined in Fig. 1. All reactions were monitored by TLC on precoated silica plates.

Solvents systems. (*A*) $CHCl_3$–MeOH, 9:1; (*B*) Bu^{n}OH–AcOH–H_2O, 4:1:1; (*C*) Bu^{n}OH–AcOH–pyridine–H_2O, 4:1:1:2. The final peptide was purified by HPLC using a gradient of methanol and water (50 to 95% MeOH in 30 min) on a Waters C_{18} column (3.9 × 300 mm); UV detection, 280 nm.

N$^{\alpha}$-*t-Butoxycarbonyl-*L*-alanine methylamide* **1**. To a chilled (−10 °C) solution of Boc-L-Ala-OH (5.0 g, 26.4 mmol) in dry tetrahydrofuran (THF) (30 cm^3), *N*-methylmorpholine (NMM) (2.91 cm^3, 26.4 mmol) and isobutylchloroformate (IBCF) (3.45 cm^3, 26.4 mmol) were added. After stirring for 10 min a precooled solution of methylamine hydrochloride (3.5 g, 52.8 mmol) and triethylamine (TEA) (7.3 cm^3, 52.8 mmol) in THF was added. The reaction mixture was stirred at 0 °C for 2 h and then overnight at room temperature. The solvent was evaporated *in vacuo* and the oily residue dissolved in ethyl acetate, washed successively with 5% citric acid solution, water, saturated $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to obtain **1** as a white crystalline solid (4.20 g, 80%), m.p. 148 °C, R_{FA} 0.65, R_{FB} 0.83 and R_{FC} 0.89, δ_H(60 MHz; $CDCl_3$; standard Me_4Si): 6.4 (1 H, br, N*H*Me), 5.1 (1 H, d, NHAla), 4.01 (1 H, br, C$^{\alpha}$H Ala), 2.8 (3 H, d, NMe), 1.44 (9 H, s, BocMe) and 1.4 (3 H, d, C$^{\beta}$H$_3$Ala).

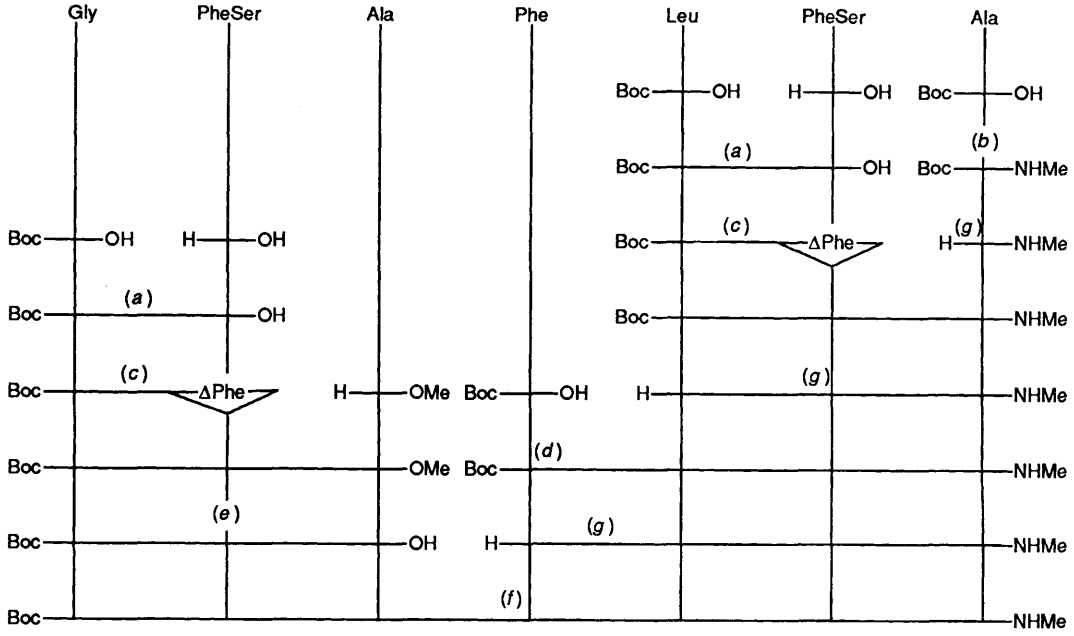

Fig. 1. The synthetic scheme for heptapeptide **8**. (*a*) MA/salt coupling; (*b*) MA/CH_3NH_2; (*c*) NaOAc/Ac_2O; (*d*) MA coupling; (*e*) alkaline hydrolysis; (*f*) DCC/HOBT coupling; (*g*) TFA/CH_2Cl_2.

*N$^{\alpha}$-t-Butoxycarbonyl-*L*-leucyl-α,β-dehydrophenylalanine azolactone* **2**. This was synthesized on a 19.6 mmol scale according to the procedure reported earlier.[11]

N$^{\alpha}$-*t-Butoxycarbonyl-*L*-leucyl-α,β-dehydrophenylalanyl-*L*-alanine methylamide* **3**. The t-butoxycarbonyl protection was removed from **1** by treatment (3.6 g, 18 mmol) with a mixture of trifluoroacetic acid (TFA) and dichloromethane (1:1 v/v) for 1 h. Excess acid was removed under reduced pressure and the trifluoroacetate salt was obtained as a colourless solid in quantitative yield. To a solution of azolactone **2** (6.4 g, 18 mmol) in dry THF (50 ml), was added a solution of the above TFA salt (3.9 g, 18 mmol) and TEA (2.5 cm^3, 18 mmol) in THF. The reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*, followed by work up as for **1**. Evaporation of the solvent gave **3** as a white solid. Recrystallization from ethyl acetate–light petroleum afforded **3** as colourless needle shaped crystals (6.15 g, 74.3%), m.p. 156 °C; R_{FA} 0.6, R_{FB} 0.68 and R_{FC} 0.65; $[\alpha]_D^{27}$ − 81.8° (*c* 1.0, MeOH); δ_H(60 MHz; $CDCl_3$; standard Me_4Si) 7.8 (1 H, s, NHΔPhe) 7.5–7.2 (6 H, m, aromatic H and C$^{\beta}$HΔPhe), 6.5 (1 H, br, N*H*Me), 5.02 (1 H, d, NHLeu), 4.2 (1 H, m, C$^{\alpha}$*H*Leu), 2.8 (3 H, d, NMe), 2.0 (2 H, m, C$^{\beta}H_2$Leu), 1.8 (1 H, m, C$^{\gamma}$HLeu), 1.44 (9 H, s, BocMe) and 0.96 (6 H, d, 2 × C$^{\delta}H_3$-Leu).

*N$^{\alpha}$-t-Butoxycarbonyl-*L*-phenylalanyl-*L*-leucyl-α,β-dehydrophenylalanyl-*L*-alanine methylamide* **4**. The tripeptide **3** (3.0 g, 6.6 mmol) was deprotected at its *N*-terminal using a similar procedure to that adopted for **1**. The TFA salt was obtained as a white solid in quantitative yield (3.12 g). To a chilled solution of Boc-L-Phe-OH (1.75 g, 6.6 mmol) in THF (40 cm^3), were added NMM (0.73 cm^3, 6.6 mmol) and IBCF (0.86 cm^3, 6.6 mmol). After stirring (10 min), a solution of the TFA salt and TEA (0.92 cm^3, 6.6 mmol) was added. The mixture was stirred at 0 °C for 2 h and then overnight at room temperature, followed by work up as for **1**. The tetrapeptide **4** was obtained as a white crystalline solid (2.52 g 63%), impure on TLC. Recrystallization from ethyl acetate–light petroleum afforded **4** in pure form: R_{FA} 0.64, R_{FB} 0.79 and R_{FC} 0.75; m.p. 174 °C; $[\alpha]_D^{27}$ −95.4° (C 0.99, MeOH).

N$^{\alpha}$-*t-Butyloxycarbonylglycyl-α,β-dehydrophenylalanine azolactone* **5**. Compound **5** was synthesized on a 14.0 mmol scale according to the literature procedure.[12]

N$^{\alpha}$-*t-Butyloxycarbonylglycyl-α,β-dehydrophenylalanyl-*L*-alanine methyl ester* **6**. To a solution of **5** (4.0 g, 13.2 mmol) in THF (50 cm^3) was added a solution of L-alanine methyl ester hydrochloride (2.8 g, 20.0 mmol) and TEA (2.8 cm^3, 20.0 mmol) in THF. The mixture was stirred at room temperature overnight and worked up using the procedure adopted for **3**. Evaporation of the solvent gave **6** as a white solid (4.12 g, 68%): R_{FA} 0.78 and R_{FB} 0.8; m.p. 148 °C; $[\alpha]_D^{27}$ + 68.9° (*c* 0.95, MeOH); δ_H(60 MHz, $CDCl_3$; standard Me_4Si) 8.0 (1 H, s, NHΔ^{z}Phe), 7.4–7.2 (6 H, m, aromatic H, ΔPhe and C$^{\beta}$HΔPhe), 6.4 (1 H, br, NHAla), 4.0 (2 H, m, C$^{\alpha}H_2$Gly), 3.8 (3 H, s, OMe). 1.44 (9 H, s, $BocMe_3$) and 1.4 (3 H, d, C$^{\beta}H_3$Ala).

N$^{\alpha}$-*t-Butyloxycarbonylglycyl-α,β-dehydrophenylalanyl-*L*-alanine* **7**. To a solution of tripeptide **6** (4.0 g, 10 mmol) in methanol (50 cm^3) was added 1 mol dm^{-3} NaOH (10 cm^3, 10 mmol). The mixture was stirred for 5 h at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in water, washed once with ethyl acetate, and acidified with solid citric acid to pH 3. The liberated peptide was extracted with ethyl acetate (3 × 20 cm^3). The combined organic extracts were washed with water, dried over anhydrous Na_2SO_4 and evaporated to yield **7** as a white solid (3.65, 95%), m.p. 162–165 °C; R_{FB} 0.92; $[\alpha]_D^{27}$ −99.8° (*c* 1.0, MeOH). ^{1}H NMR spectroscopy showed the absence of a methyl ester peak at δ 3.8.

N$^{\alpha}$-*t-Butoxycarbonylglycyl-α,β-dehydrophenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-leucyl-α,β-dehydrophenylanylalanine methylamide* **8**. The tetrapeptide **4** (2.42 g, 4.0 mmol) was deprotected at its N-terminal using the method adopted for **3**. The TFA salt was obtained in quantitative yield. To a solution of tripeptide **7** (1.5 g, 4.0 mmol) in dimethylformamide (DMF) (35 cm^3), cooled to 0 °C, were added HOBT (0.61 g, 4.0 mmol) and DCC (0.83 g, 4.0 mmol). After 30 min a pre-cooled solution of the TFA salt and TEA (0.56 cm^3, 4.0 mmol) in DMF (10 cm^3) was added. The reaction mixture was stirred at room temperature overnight and worked up using the procedure adopted for **1**. The desired heptapeptide was obtained as a white solid (3.51 g, 100%), m.p. 200 °C; R_{FB} 0.52, R_{FB} 0.94; $[\alpha]_D^{27}$ −103.4° (c 1.0, MeOH); HPLC retention time, 15.94 min.

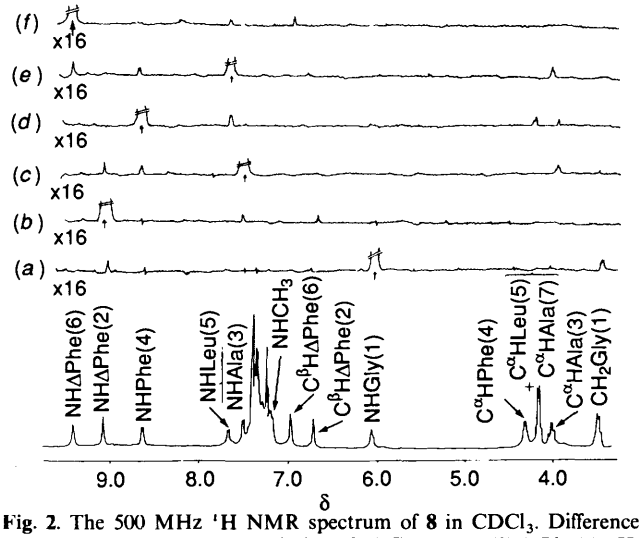

Fig. 2. The 500 MHz ^{1}H NMR spectrum of **8** in $CDCl_3$. Difference NOE spectra obtained on irradiation of (*a*) Gly(1)NH; (*b*) Δ^zPhe(2)NH; (*c*) Ala(3)NH; (*d*) Phe(4)NH; (*e*) Leu(5)NH; (*f*) Δ^zPhe(6)NH, respectively.

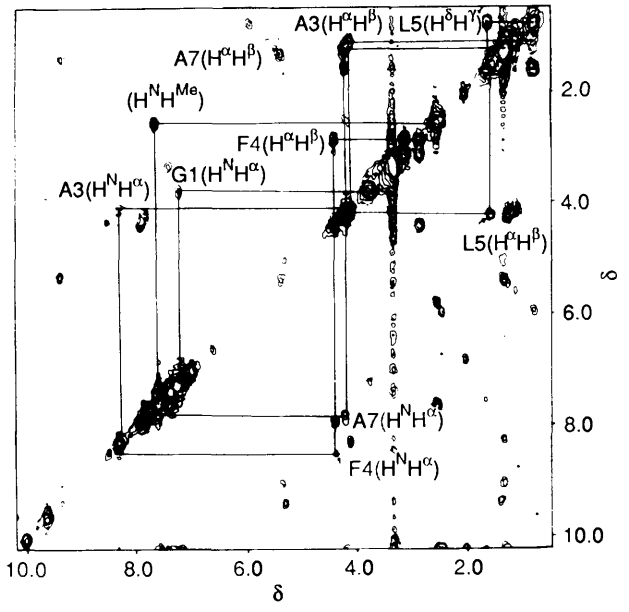

Fig. 3. The 500 MHz COSY spectrum of heptapeptide **8** in $(CD_3)_2SO$. G, Gly; F, Phe; A, Ala; L, Leu.

Results and Discussion

Spectral Assignments.—Fig. 2 shows the ^{1}H NMR spectrum of the heptapeptide **8** in $CDCl_3$. Assignment of the resonances to individual residues or groups other than NH and $C^\alpha H$ resonances was straightforward and was done on the basis of chemical shift positions. In $CDCl_3$, N_iH to $C^\alpha H$ connectivities were established with the aid of difference NOE spectra[17] (Fig. 2). A broad peak at δ 6.06 was assigned to urethane NH [Gly(1)NH] by virtue of its high field position in $CDCl_3$.[18] Two Δ^zPheNH resonances were readily assigned as two broad singlets (δ 9.42 and 9.06), downfield of all other signals.[14] Irradiation of Gly(1)NH at δ 6.06 resulted in an enhancement of the singlet at δ 9.06, which was therefore assigned to Δ^zPhe(2)NH. Subsequently the singlet at δ 9.42 was assigned to Δ^zPhe(6)NH. A distorted quartet at δ 7.2 was assigned to the methylamide NH since irradiation of this peak resulted in enhancement of the signal due to the NMe protons at δ 2.8. Specific assignments to Ala(3)NH, Phe(4)NH, Leu(5)NH and Ala(7)NH were also made from difference NOE spectra. Irradiation of the peak at δ 9.06 ascribed to Δ^zPhe(2)NH resulted in an NOE on the NH resonating at δ 7.45, which was therefore assigned to Ala(3)NH. Assignments to Phe(4)NH (δ 8.6) and Leu(5)NH (δ 7.68) were similarly made. The Ala(7)NH appeared to overlap with aromatic proton resonances and therefore it was not possible to determine its exact chemical shift. Fig. 2 shows the assignments, and the chemical shifts of various NH groups are noted in Table 1.

Table 1. ^{1}H NMR parameters for NH groups in heptapeptide **8**.

Residue	$CDCl_3$ δ (ppm)	$(CD_3)_2SO$ δ (ppm)	Δδ (ppm)	(dδ/d*T*) /10^3 ppm K^{-1}
Cly(1)	6.06	7.4	1.34	*a*
Δ^zPhe(2)	9.06	10.07	1.01	7.2
Ala(3)	7.45	8.48	1.03	6.8
Phe(4)	8.6	8.8	0.2	3.3
Leu(5)	7.68	7.9	0.22	3.5
Δ^zPhe(6)	9.42	9.60	0.18	3.8
Ala(7)	*a*	7.9	*a*	2.5
NHMe	7.2	7.64	0.44	2.0

[a] The parameter could not be determined due to the overlap of the specified protons with aromatic protons.

In these studies, two-dimensional COSY has been used to decipher N_iH–$C^\alpha H$–$C^\beta H$–$C^\gamma H$–$C^\delta H$ coupling connectivities[19] in $(CD_3)_2SO$. The assignments were based on unambiguous recognition of chemical shift positions of side chain protons such as $C^\beta H_2$Phe, $C^\beta H_2$Leu and $C^\beta H_3$Ala. Fig. 3 shows a COSY spectrum of the heptapeptide in $(CD_3)_2SO$. Based on $N_iH \longleftrightarrow C^\alpha H$ connectivities, chemical shift values assigned to various NH groups are summarized in Table 1. Specific assignments of the Δ^zPhe(2)NH and Δ^zPhe(6)NH resonances could not be made with the help of the COSY spectrum due to the lack of corresponding $C^\alpha H$ protons. Hence, the assignments were made by monitoring their chemical shift positions in $CDCl_3$–$(CD_3)_2SO$ mixtures, Fig. 4(*a*). Accordingly, the broad singlet at δ 10.07 was assigned to Δ^zPhe(2)NH and that at δ 9.6 to Δ^zPhe(6)NH.

Solvent Accessibility of NH Resonances.—The presence of intramolecularly hydrogen bonded NH resonances has usually been probed by accessing the degree of solvent exposure of various NH groups in a peptide.[20] In this study, three criteria were used: (*i*) the temperature dependence of NH chemical shifts;[23] (*ii*) the solvent dependence of NH chemicals shifts in $CDCl_3$–$(CD_3)_2SO$ mixtures,[22] and (*iii*) the paramagnetic radical, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), induced line broadening of NH resonances in $CDCl_3$.[23] Temperature coefficients (dδ/d*T*) of NH groups in the heptapeptide in $(CD_3)_2SO$ and Δδ values are listed in Table 1. The results of solvent and radical perturbation experiments are summarized in Figs. 4(*a*) and (*b*) respectively. Three NH groups from the *N*-terminal, Gly(1)NH, Δ^zPhe(2)NH and Ala(3)NH show appreciable downfield shift with increasing concentration of $(CD_3)_2SO$ [Fig. 4(*a*)], while the remaining five NH groups of residues Phe(4), Leu(5), Δ^zPhe(6) and the methylamide NH appear to be unaffected by the addition of a polar solvent and hence can be judged as solvent shielded NH groups. The results of radical perturbation experiments [Fig. 4(*b*)] show a remarkable broadening of the resonance lines of the first three NH groups while the remaining NH groups remain unaffected. Ala(7)NH could not be monitored in these experiments due to its overlap with aromatic proton resonances. High temperature coefficient, dδ/d*T*, values for Gly(1)NH, Δ^zPhe(2)NH and Ala(3)NH (Table 1) are above the range for solvent shielded protons ($\leq$0.003 ppm K^{-1}). However, dδ/d*T* values for the

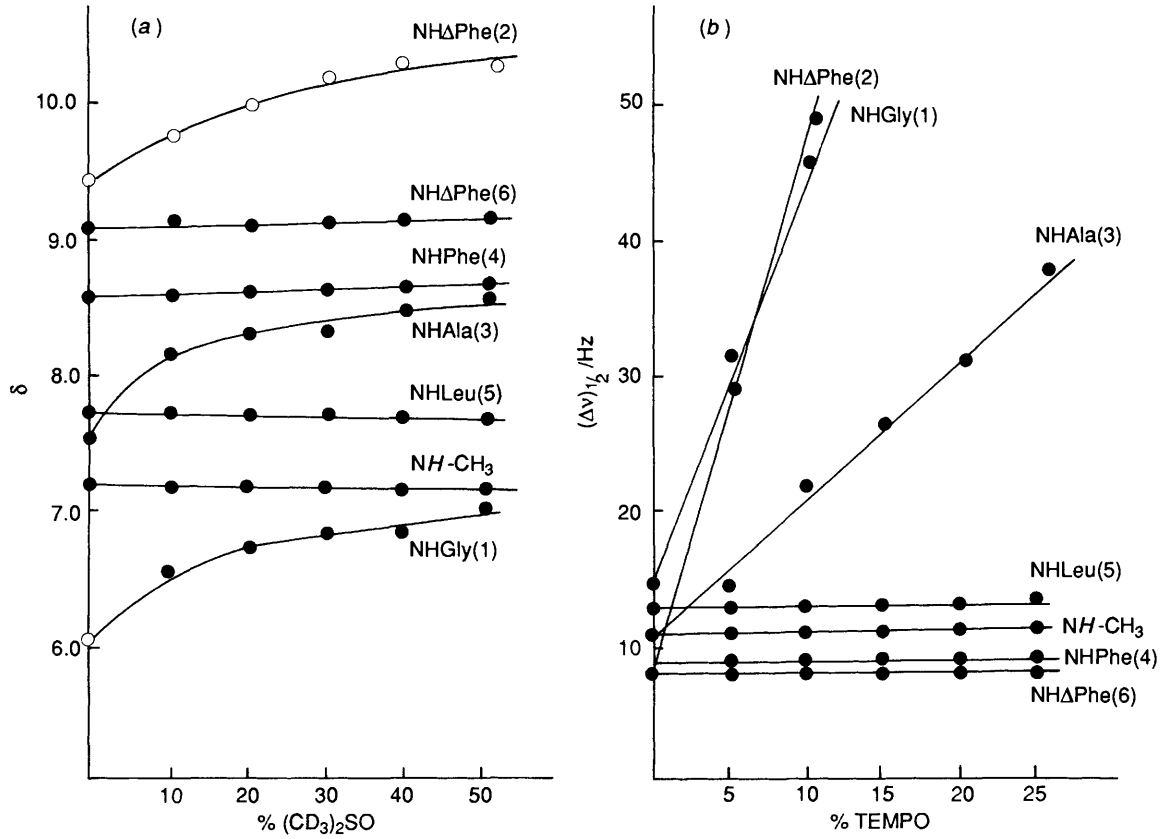

Fig. 4. (*a*) Solvent dependence of NH chemical shifts in heptapeptide **8** in $CDCl_3$–$(CD_3)_2SO$ mixtures of varying concentrations. (*b*) Dependence of NH resonance line widths in heptapeptide **8** on the concentration of 2,2,6,6-tetramethylpiperidine-1-oxyl in $CDCl_3$.

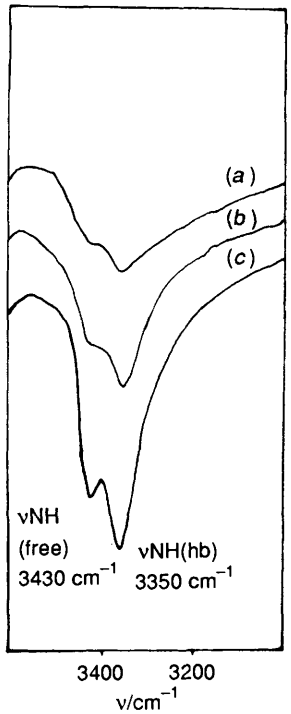

Fig. 5. IR spectra (NH stretching bands) of the heptapeptide in $CHCl_3$ at various peptide concentrations: (*a*) 0.23; (*b*) 5; (*c*) 30 mmol dm^{-3}.

other five NH groups ($\sim$0.002 ppm K^{-1}) suggest their solvent shielded nature. These NMR results clearly reflect upon the solvent exposed nature of Gly(1)NH, Δ^zPhe(2)NH and Ala(3)NH and the solvent shielded nature of Phe(4)NH, Leu(5)NH, Δ^zPhe(6)NH, ala(7)NH and methylamide NH due to their involvement in intramolecular hydrogen bonding in $CDCl_3$. The above results thus implicate that in $CDCl_3$ the heptapeptide favours folded, intramolecularly hydrogen bonded conformations. The involvement of the last five NH groups in intramolecular hydrogen bonding suggests a 5→1 hydrogen bonding pattern characteristic of α-helical structures.

Further support for intramolecularly hydrogen bonded NH groups is provided by IR studies. Fig. 5 shows the IR spectra of NH stretching bands for the heptapeptide in chloroform over the concentration range 0.23–30 mmol. Two well defined bands are observed at 30 mmol heptapeptide (3430 and 3350 cm^{-1}). A band at higher frequency (3430 cm^{-1}) was assigned to free NH groups and that at 3350 cm^{-1} to the hydrogen bonded NH group. The hydrogen bonded NH band is concentration independent, being observed even at 0.23 mmol heptapeptide, and therefore can be assigned to intramolecularly hydrogen bonded NH groups. A plot of the ratio $\nu_{NH}(hb)/\nu_{NH}(free)$ also shows no dependence on concentration (plot not shown).

Nuclear Overhauser Effects.—The spatial proximity of various spin systems in the heptapeptide was probed by means of nuclear Overhauser effect studies. Fig. 2 shows the representative difference NOE spectra for the heptapeptide in $CDCl_3$. The NOEs are positive, suggesting that the condition $\omega\tau c < 1$ is satisfied.[24] For NOEs in $(CD_3)_2SO$, the two-dimensional NOESY[25] spectrum was recorded (Fig. 6). Various NOEs observed in the heptapeptide are summarized in Table 2. In $CDCl_3$, appreciable $N_iH \longleftrightarrow N_{i+1}H$ NOEs were observed between the following pairs of protons; Gly(1)NH ⟷ Δ^zPhe(2)NH (2.4%); Δ^zPhe(2)NH ⟷ Ala(3)NH (2.82%);

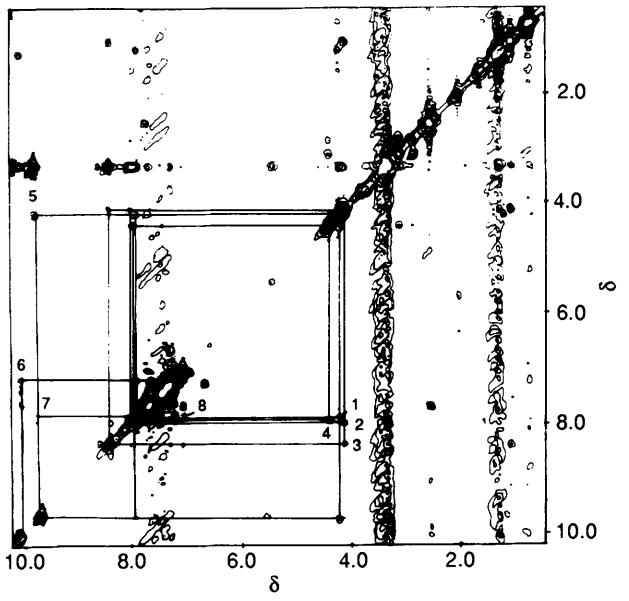

Fig. 6. The 500 MHz NOSEY spectrum of heptapeptide **8** in $(CD_3)_2SO$. G, Gly; F, Phe; A, Ala; L, Leu. (*a*) A(7)C$^{\alpha}$,H ⟷ NHMe; (*b*) A(7)C$^{\alpha}$H ⟷ A(7)NH; (*c*) A(3)C$^{\alpha}$H ⟷ A(3)NH; (*d*) F(4)C$^{\alpha}$H ⟷ L(5)NH; (*e*) L(5)C$^{\alpha}$H ⟷ Δ^zF(6)NH; (*f*) G(1)NH ⟷ Δ^zF(2)NH; (*g*) Δ^zF(6)NH ⟷ A(7)NH; (*h*) A(3)NH ⟷ F(4)NH.

Ala(3)NH ⟷ Phe(4)NH (4.1%); Phe(4)NH ⟷ Leu(5)NH (3.4%); and Leu(5)NH ⟷ Δ^zPhe(6)NH (5.5%). Such NOEs are characteristic of helical conformations[26] with an N_iH–$N_{i+1}H$ interproton distance of 2.6 Å (α-helix) or 2.8 Å (3_{10} helix) and backbone torsional angles ø *ca.* −50°, ψ *ca.* −50°. NOEs involving Ala(7)NH could not be monitored due to its overlap with aromatic protons. In addition, a few weak inter-residue NOEs of the type C^{α}_iH ⟷ $N_{i+1}H$ are also observed between Ala(3)C$^{\alpha}$H ⟷ Phe(4)NH (1.2%) and Leu(5)C$^{\alpha}$H ⟷ Δ^zPhe(6) NH (1.0%). These interproton distances have, however, been estimated to be *ca.* 3.5 Å in helical structures. This simultaneous observation of N_iH ⟷ $N_{i+1}H$ and C$^{\alpha}$H ⟷ $N_{i+1}H$ NOEs can be attributed to a small degree of conformational heterogeneity.[11] The observed NOEs are also compatible with the existence of a left handed helical structure which is characterized by short (< 3 Å) C$^{\alpha}$H–$N_{i+1}H$ distances.[27] This possibility, however, is not considered because such structures fall in a sterically disallowed region of space for L-residues and there appears to be no strong reason for such a structure in Δ^zPhe residues. In $(CD_3)_2SO$, NOESY cross peaks are observed between Ala(3)NH ⟷ Phe(4)NH and Δ^zPhe(6)NH ⟷ Ala(7)NH (Fig. 6, Table 2). Other N_iH ⟷ $N_{i+1}H$ NOEs that might be present are difficult to characterize in $(CD_3)_2SO$, since most of the NH resonances get crowded in the region of aromatic protons. However, appreciable C^{α}_iH ⟷ $N_{i+1}H$ type NOEs are noticeable in $(CD_3)_2SO$, between Phe(4)C$^{\alpha}$H ⟷ Leu(5)NH, Leu(5)C$^{\alpha}$H ⟷ Δ^z-Phe(6)NH, and Ala(7)C$^{\alpha}$H ⟷ NHMe. Such NOEs are characteristic of extended conformations.[26] Hence, in $(CD_3)_2SO$, there appears to be an equilibrium between folded (helical) and partially extended conformations.

Table 2. NOEs observed for heptapeptide **8**.

Resonance irradiated	$CDCl_3$ Resonance observed	% NOE	$(CD_3)_2SO$ Pairs of resonances observed	Volume of NOESY cross peaks
Gly(1)NH	Gly(1)C$^{\alpha}$H	3.8	Ala(3)NH,	1.1
	Δ^zPhe(2)NH	2.4	Phe(4)NH	
Δ^zPhe(2)NH	Ala(3)NH	2.82	Δ^zPhe(6)NH,	0.8
			Ala(7)NH	
Ala(3)NH	Δ^zPhe(2)NH	1.8		
	Phe(4)NH	4.1	Leu(5)C$^{\alpha}$H,	3.57
	Ala(3)C$^{\alpha}$H	4.2	Δ^zPhe(6)NH	
Phe(4)NH	Phe(4)C$^{\alpha}$H	5.8		
	Ala(3)C$^{\alpha}$H	1.2	Phe(4)C$^{\alpha}$H,	
	Leu(5)NH	3.4	Leu(5)NH	5.9
Leu(5)NH	Leu C$^{\alpha}$H	4.8		
	Phe(4)NH	2.7	Ala(7)C$^{\alpha}$H	
	Δ^zPhe(6)NH	5.5	N*H*CH_3	2.1
	Phe(4)C$^{\alpha}$H	1.0		
Δ^zPhe(6)NH	Leu(5)NH	1.9	Gly(1)NH,	
Ala(7)NH	*a*		Δ^zPhe(2)NH	
N*H*Me	*a*			

[a] NOEs could not be observed due to overlap with aromatic proton resonances.

Conformation of Heptapetide **8**.—In a relatively apolar solvent like $CDCl_3$, the spectroscopic data provide strong evidence for folded conformations of heptapeptide **8** involving NH groups of residues 4–7 and the NH of methylamide in intramolecular hydrogen bonding. A succession of 5→1 hydrogen bond patterns corresponding to an α-helical conformation, Fig. 7, is consistent with the observed data. This conformational arrangement is characterized by backbone conformational angles of ø *ca.* −50° and ψ *ca.* 50° and a short (2.6 Å) N_iH–$N_{i+1}H$ distance which is compatible with the observed NOEs.[26] However, since small changes in ø and ψ values can lead to the formation of mixed 3_{10}/α-helical structures, a clear distinction between the two types of helical structures may not be possible from the available data. The

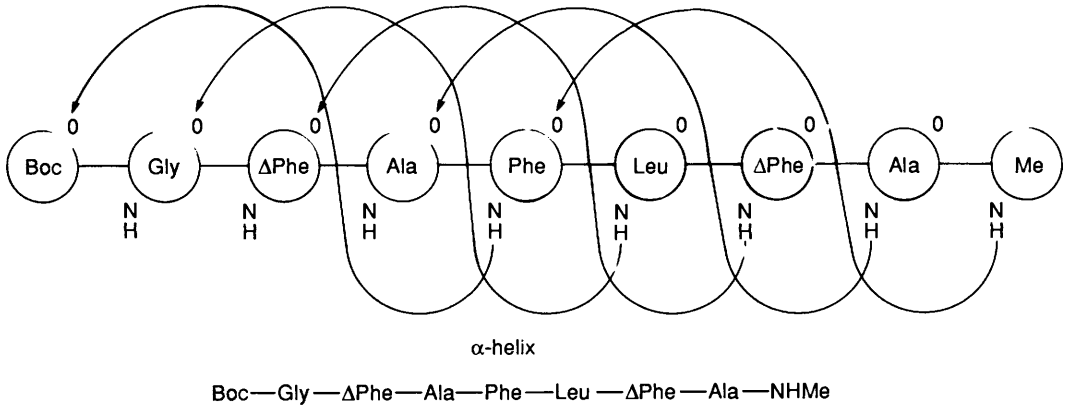

Fig. 7. The conformation compatible with NMR data for heptapeptide **8** in $CDCl_3$.

observed $J_{C^{\alpha}HNH}$ values (< 5 Hz) for L-residues Ala(3), Phe(4) and Leu(5) also support the existence of these residues in helical structures. In a highly polar, strongly hydrogen bond accepting solvent such as $(CD_3)_2SO$, helical conformations for peptide **8** appear to be rather unstable. Observed NOEs in this solvent suggest the existence of a population of partially extended conformations which are characterized by ψ values of 120° at least at residues Leu(5) and Δ^zPhe(6). These two NH groups also show some degree of solvent accessibility as evidenced by their moderate $d\delta/dT$ values (*ca.* 0.0035 ppm K^{-1}), which suggests a degree of conformational heterogeneity. In an earlier study [14] we have shown that two Δ^zPhe residues in -Δ^zPhe-X-X-Δ^zPhe- stabilize a 3_{10} helical structure, and in more polar solvents such as $(CD_3)_2SO$ the helical structure is not completely stable. The present results indicate that two Δ^zPhe residues separated by three L-amino acid residues, (-Δ^zPhe-X-X-X-Δ^zPhe-) also favour folded structures with α-helical conformation being most probable. While the conformational constraints brought about by the introduction of Δ^zPhe residues in the peptide backbone are not very severe it is clear that peptides with a tendency to favour β-turns and helical structures can be synthesized by introducing Δ^zPhe residues. This may be useful in the design and synthesis of bioactive peptides, and peptides with special structural features, *e.g.* amphiphilic peptides.

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