SOLUTION CONFORMATION OF δ-RECEPTOR AGONIST: [D-Ser²]-LEUCINE-ENKEPHALIN-THREONINE BY NMR

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ABSTRACT

The solution conformation of [D-Ser²]-leucine-enkephalin-threonine (H₂N-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH) in DMSO-d_6 has been studied by 500 MHz NMR spectroscopy. The resonances from the spin systems of different amino acid residues have been assigned from the 2D-COSY spectrum. The temperature variation of chemical shifts of the amide protons indicates that all the backbone –NH protons are exposed to the solvent and there is no evidence for any intramolecular hydrogen bonding. The absence of NOESY cross peaks between $N_i H$ and $N_{i+1} H$ and $C_i^\beta H$ and $N_{i+1} H$ in conjunction with the absence of intramolecular hydrogen bonding rules out the possibility of any long-range ordering in the molecule. The solution conformation of this hexapeptide has been compared with that of endogenous pentapeptides leucine- and methionine-enkephalins and other synthetic analogs. The structure-activity relationship is discussed vis-a-vis the structural modifications.

INTRODUCTION

THE hexapeptide, [D-Ser²]-leucine-enkephalin-threonine (H₂N-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH) (with residue numbers 1 2 3 4 5 6) abbreviated as DSLT, a more potent synthetic analog of endogenous peptide:leucine-enkephalin was first reported by Gacel et al[1]. This hexapeptide exhibits a very high streospecificity towards δ-receptor and is about 620 times more potent in Mouse Vas Deferens (MVD) than in Guinea Pig Ileum (GPI)[1-3]. Is the greater potency of this synthetic analog relative to that of the endogenous peptides due (i) to greater stability of Tyr-D-Ser peptide bond to brain enzymes (aminopeptidases) or (ii) to conformational differences between the endogenous peptides and the hexapeptide or (iii) to the increased chain length? In this paper we report the results of our investigations on the conformational behaviour of [D-Ser²]-leucine-enkephalin-threonine in solution using proton magnetic resonance spectroscopy with an aim to answer the above posed questions.

MATERIALS AND METHODS

The hexapeptide, [D-Ser²]-leucine-enkephalin-threonine as free acid was purchased from Sigma. Five mg of the hexapeptide were dissolved in 99.8% DMSO-d_6. 1D proton spectra in the temperature range of 300-360 K were recorded on a Bruker AM-500 MHz FT-NMR spectrometer. The chemical shifts were measured relative to DMSO-d_6 and then converted to TSP (sodium 3-trimethylsilyl-propionate-d_4) scale by adding 2.60 ppm to the observed chemical shift values. Proton spectrum of DSLT at 300 K is shown in figure 1.

2D COSY and NOESY experiments were performed with a data matrix of 512 × 2048 and 256 × 1024, respectively. J-resolved spectrum was recorded with 128 × 4096 data size. The time domain data for the NOESY spectrum were multiplied by phase-shifted sine and sine square bell functions in t_1 and t_2 dimensions, respectively before Fourier transformation.

RESULTS AND DISCUSSION

Assignment of resonances: Resonance assignment of exchangeable protons (amide protons) and non-exchangeable protons has been made from the shift-correlated spectrum (COSY) shown in figure 2. The scalar coupling between the protons is manifested as cross-peaks in the COSY spectrum and forms the basis of assignment and identification of individual spin systems of the amino acid residues of DSLT[4]. Although the solvent DMSO-d_6 has a trace of water (the peak marked with asterisk in figure 1) all the amide protons are clearly observable at 300 K. The protons from the –OH groups of Tyr, Ser and Thr are also observable. The serine –OH proton is assigned on the basis of its coupling with the β –CH₂ protons (figure 2). The tyrosine –OH is known to resonate at quite low filed. The complete assignment of the resonances is indicated in the 1D spectrum of DSLT in figure 1.
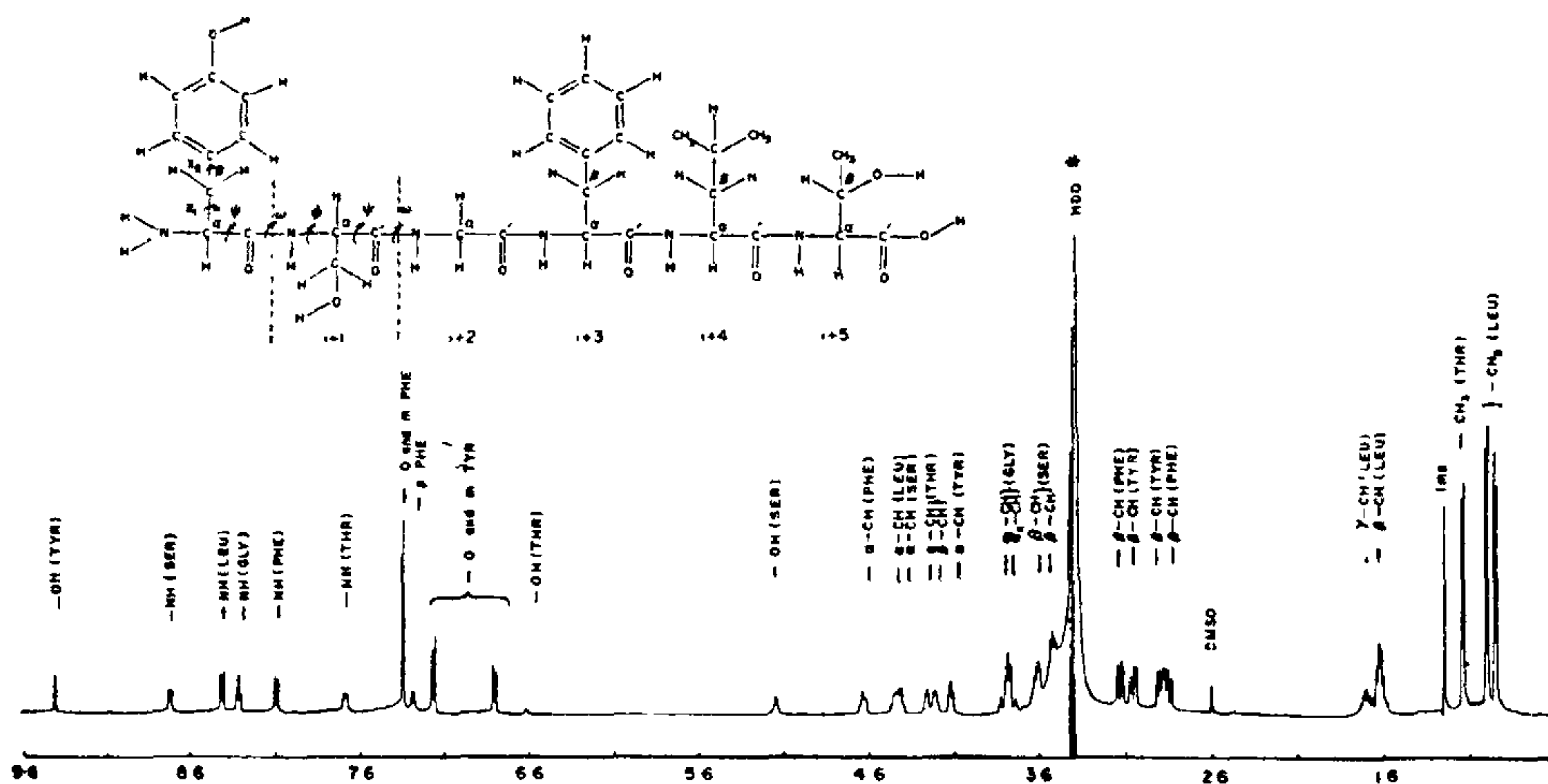
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Figure 1. 500 MHz proton magnetic resonance spectrum of [D-Ser^2]-leucine-enkephalin-threonine in DMSO-d_6 at 300 K. Peak positions are in ppm relative to TSP.

Temperautre dependence of amide proton shifts: The variation of chemical shifts of amide protons with temperature is shown in figure 3. The temperature coefficient for each proton is also indicated in the paranthesis. It is seen from figure 3 that the −NH proton of serine disappears beyond 340 K which implies that it starts exchanging with H$_2$O present in the solvent and the exchange rate increases with increasing temperature. This results in broadening of this resonance and finally its disappearance at high temperatures. This exchange is also reflected in the observed cross-peak in the NOESY spectrum at ω_2 = 8.7 ppm and ω_1 = 3.38 ppm (figure 4). Further, the continual shift to the high field with temperature (figure 3) of the amide proton resonances reveal that all the −NH protons are inter-molecularly hudrogen-bonded to the solvent. The similar magnitude of the temperature coefficients and their magnitude being 2 to 3 times larger than is expected for a solvent shielded hydrogen bonded amide proton ($\leqslant 2.0 \times 10^{-3}$ ppm/°C), indicate that none of the amide protons are intramolecularly hydrogen bonded[5,6]. In other words, they are all exposed to the solvent.

NOESY spectrum: The NOESY spectrum of DSLT with a mixing time of 600 msec is shown in figure 4. The strong cross-peak at ω_2 = 8.7 ppm, ω_1 = 3.38 ppm is between the serine amide proton and H$_2$O signal. This clearly indicates that only this proton is

exchanging with H$_2$O while none of the remaining amide protons exchange with H$_2$O at 300 K. The NOESY cross-peaks at (ω_2 = 9.47 ppm, ω_1 = 3.38 ppm), (ω_2 = 6.50 ppm, ω_1 = 3.38 ppm) and (ω_2 = 4.80 ppm, ω_1 = 3.38 ppm) are due to exchange of −OH protons of Tyr, Ser and Thr, respectively with H$_2$O.

The expansion of the dotted area in figure 4 shown in figure 5 indicates the NOESY cross-peaks between the amide −NH protons and −C$^\alpha$H and −C$^\beta$H protons of different amino acid residues. The self cross-peaks between −C$^\alpha$H and −C$^\beta$H protons and −C$^\beta$H and −C$^\gamma$H$_3$ are also observed for some of the amino acid residues. The variation of mixing time from 300 to 900 msec did not reveal any new cross-peaks.

The solution conformation of leucine- and methione-enkephalins in DMSO-d_6 has been demonstrated to be dependent on the nature of the ionic state of these molecules[7-13]. Leu/Met-enkephalins in their zwitter ionic form have been shown to exist in a folded conformation (i.e. Gly-Phe, β-bend) while the carboxyamide derivative of methionine-enkephalin which exists in the cationic form adopts an unfolded conformation in solution[12,14]. The folding of chain is probably due to the electrostatic interaction between the positively charged NH$_3^+$ and the negatively charged COO$^-$ groups at the terminii of these pentapeptides[12].
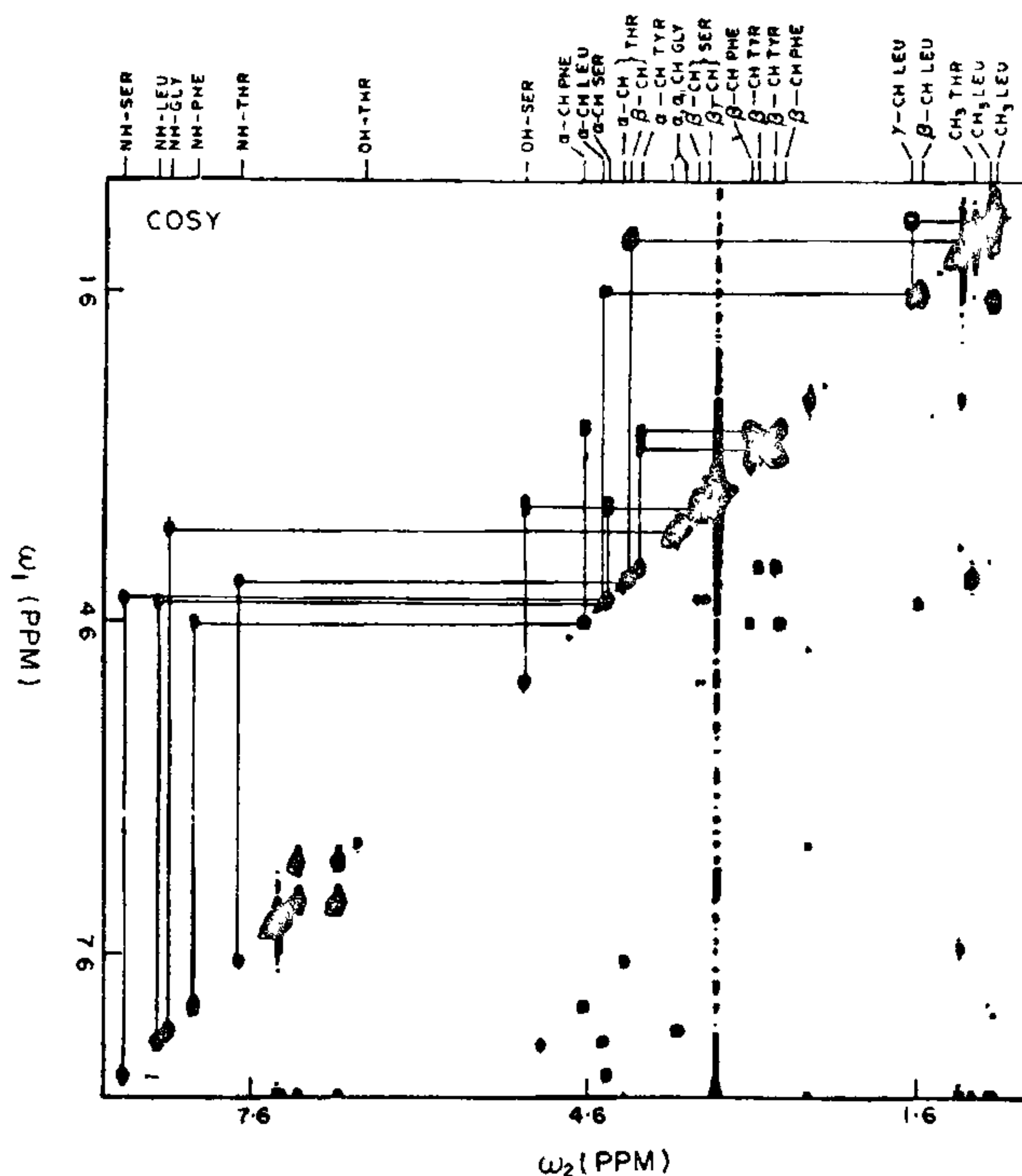
Figure 2. Shift-correlated (COSY) spectrum of [D-Ser²]-leucine-enkephalin-threonine at 300 K.

The backbone conformation of DSLT depends on the preferred values of Φ and Ψ torsion angles of the constituent amino acid residues. Since only four degenerate Φ values (table 1) for the individual amino acid residues are available, these are not sufficient to determine the backbone conformation. However, the absence of evidence for an intra-molecular hydrogen bonding (table 1) clearly rules out the possibility of a folded conformation for DSLT in solution. Although, the hexapeptide exists in the zwitter ionic form in DMSO-d_6 solution, there is no evidence for the retention of folded conformation which has been observed for the zwitter ionic forms of leu/met-enkephalins[9,12].

Is [D-Ser²] in position 2 responsible for forcing DSLT into an unfolded conformation? The influence of D-amino acid residues in position 2 does not seem to perturb the characteristic folded conformation of endogenous peptides as is reflected in the folded geometry of [D-Ala²]-leucine-enkephalin in its dipolar form[15]. It is, thus, clear from the present results that the introduction of the sixth residue to

the C-terminal leads to an alteration in the characteristic conformation (Gly-Phe, β-bend) of endogenous peptides in spite of its being present in dipolar form. The present result is also at variance with that of Gacel et al[16] who have demonstrated that despite the addition of one more amino acid residue to the N-terminal of methionine-enkephalin, the characteristic folded conformation (β-bend) of methionine-enkephalin, is retained in the hexapeptides (X-Tyr-Gly-Gly-Phe-Met-OH, X = Tyr, Phe, Lys and Gly)[12].

The observation of NOESY cross-peaks which reflect the spatial disposition of various hydrogen atoms[17] can, in principle, provide insight into the three-dimensional structure of the molecules in solution[18]. The observation of NOESY cross-peak between NH and C^αH of the preceding residue (i.e C_i^αH and N_{i+1}H) in conjunction with COSY assignment helps in the sequential assignment of resonances from various amino acid residues in the chain. The distance d_1 between C_iH and N_{i+1}H varies between 2.2 and 3.5 Å as a consequence o
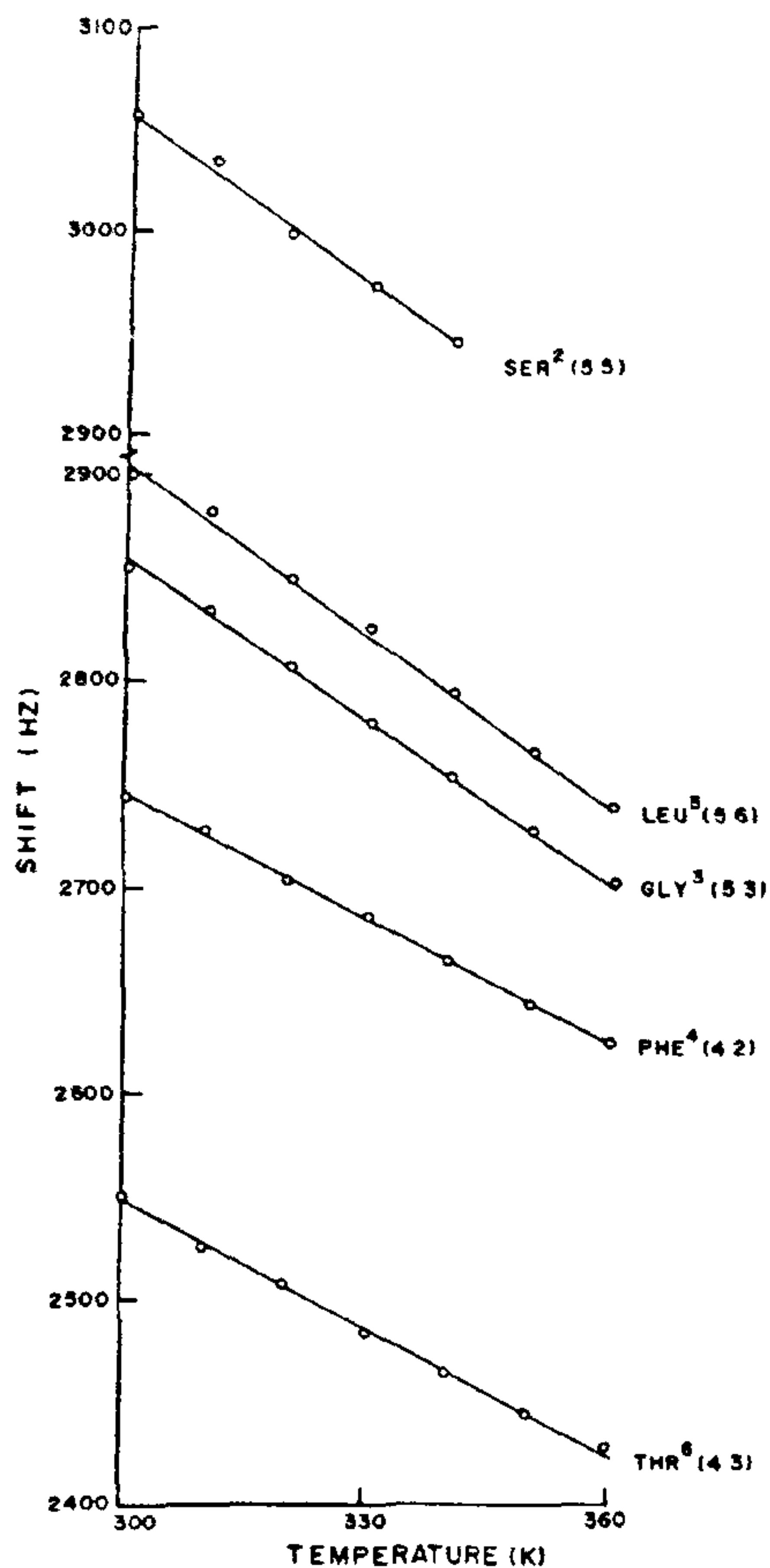
Figure 3. Effect of temperature on the amide proton chemical shifts. The temperature coefficients given in parenthesis are in ppm/°C.

rotation about bonds $N-C^\alpha(\Phi)$ and $C^\alpha-C'(\Psi)$. Large NOEs observed in DSLT (figure 5) indicate that the $C_i^\alpha H$ and N_{i+1} H distances are quite small and less than 3.0 Å, the maximum distance between the $C^\alpha H$ and NH of the same residue. This is due to the fact that self-NOEs are either not observed or if observed, they are very weak except in the case of glycine where large self-NOEs are observed between $C^\alpha H$ and NH. The absence of NOESY cross-peaks between $C_i^\beta H$ and N_{i+1} H and $N_i H$ and N_{i+1} H suggests the absence of a long range ordering
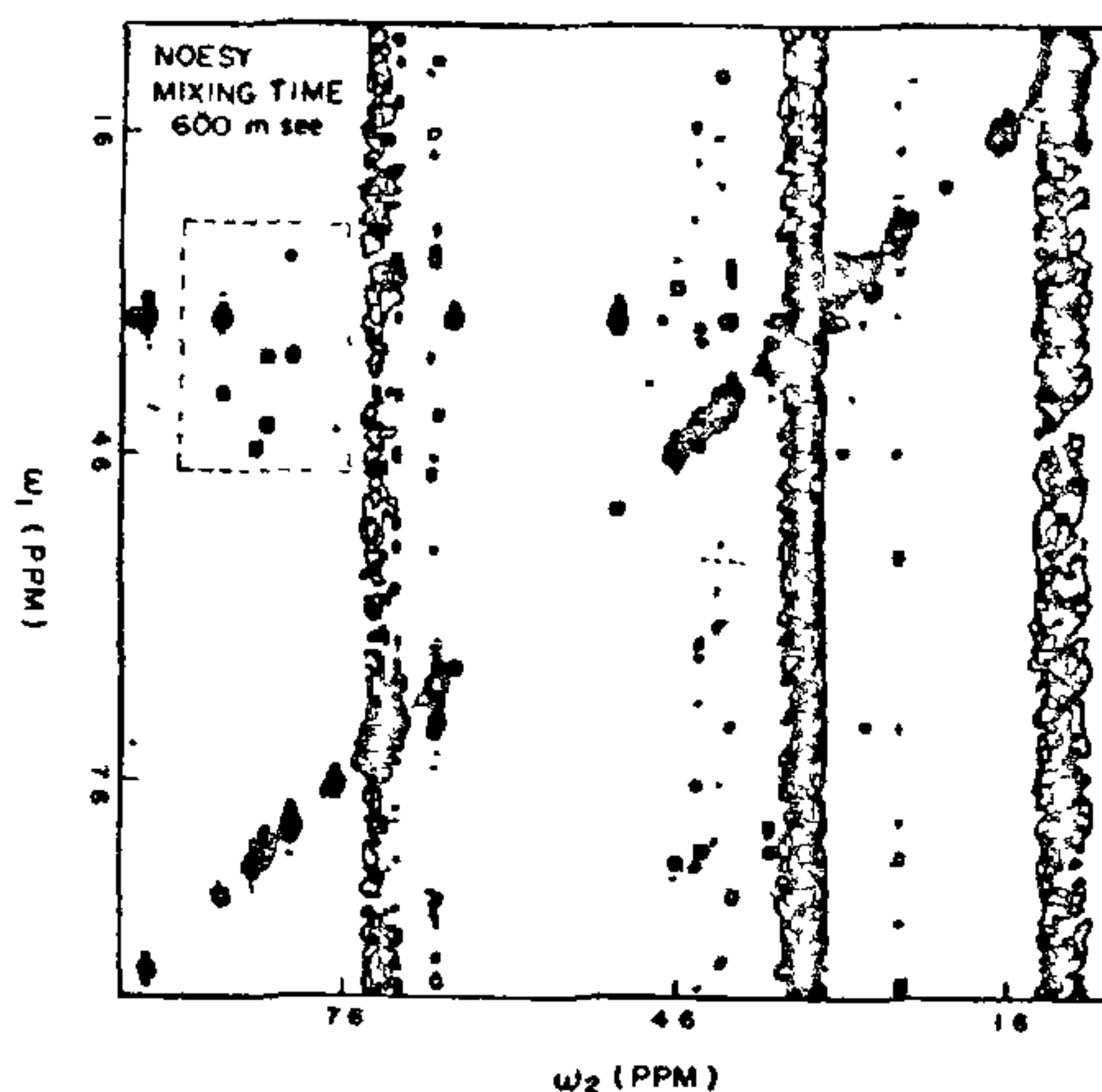
Figure 4. NOESY spectrum with a mixing time of 600 msec at 300 K.

Table 1 *Vicinal ^1H-^1H coupling constants $J_{HN-C^\alpha H}$, conformational parameter Φ, temperature coefficients in [D-Ser^2]-leucine-enkephalin-threonine*

Residue	$J_{HN-C^\alpha H}$ in HZ	$\Phi(°)$	$-\delta\Delta/dt^*$
Tyr^1	–	–	–
D-Ser^2	8.1	-150	5.5
		-85	
		$+45$	
		$+80$	
Gly^3	6.0	-165	5.3
		-70	
		$+30$	
		$+90$	
Phe^4	8.4	-145	4.2
		-90	
		$+45$	
		$+70$	
Leu^5	8.7	-140	5.6
		-95	
		$+50$	
		$+55$	
Thr^6	7.7	-155	4.3
		-80	
		$+35$	
		$+100$	

$^*10^{-3}$ ppm/°C.

Figure 5. Expansion of the dotted region of figure 4, showing inter-residue (C_i^α–H and N_{i+1}H) intra-residue (C_i^β–H) and N_iH) exchange (Ser-NH and H_2O) cross peaks.

in structure for the hexapeptide, and DSLT is essentially a random coil and adopts predominantly extended conformation.

Since DSLT is more potent than the native enkephalins which in solution adopt a Gly-Phe β-bend folded conformation and DSLT is bereft of such a folded conformation, it is logical to conclude that there is no clear-cut relationship between the conformation and potency of the synthetic analogs of enkephalins. The fact that DSLT does not retain the folded conformation characteristic of native enkephalins but is more potent, suggests that for a synthetic analog of enkephalin to be potent, it need not exist in a folded conformation. This is true for methionine-enkephalinamide which prefers an un-folded conformation in solution[12] but is relatively more potent than the endogeneous methionine-enkephalin. It is quite likely that the changes in the position of the chemical groups or the relative orientation of two aromatic rings critical for the interaction with the receptor are more important than the overall conformation of the enkephalins.

CONCLUSION

DSLT, although exists in dipolar form in DMSO-d_6-solution, does not retain the β-bend conformation characteristic of endogenous peptides. The existence of folded conformation does not seem to be directly responsible for the opioid activity of enkephalins.

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