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Synthetic and conformational studies on dehydrovaline-containing model peptides

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Abstract. To explore the conformational preferences of α,β-dehydrovaline (ΔVal) residue, three model dipeptide N-methylamides containing ΔVal were synthesized. Conformational investigations using 300 MHz NMR spectroscopy were based on delineation of intramolecularly hydrogen-bonded NH groups and Nuclear Overhauser Effect (NOE) studies. Temperature and solvent dependence studies in $(CD_3)_2SO$ and $CDCl_3$–$(CD_3)_2SO$ mixtures showed the absence of any intramolecular hydrogen bonding which suggests that all the three peptides have an extended conformation in solution. Dilution studies in $CHCl_3$ conducted using IR spectroscopy further supported the above conclusions. NOE studies also ruled out the existence of any type of discernible secondary structure for these peptides. Conformational behaviour of these dehydrovaline peptides is in contrast with corresponding peptides containing Δ^zPhe and Δ^zLeu, both of which stabilize β-turn (type-II) structure. These results highlight the importance of steric factors in deciding the conformational properties of dehydropeptides.

Keywords. Solution conformation; dehydroaminoacid; NMR; constrained peptides.

1. Introduction

For the past two decades α,β-dehydroamino acids are being used to increase the potency of various biologically important peptides (Stammer 1982; Spatola 1983; Nitz *et al* 1986; Pieroni *et al* 1986). It is now clear that the introduction of an α,β-dehydroamino acid residue limits the range of available conformations as compared to the saturated analogues. Efforts have also been made to explore the conformational preferences of α,β-dehydroamino acids, in particular, in model peptides containing dehydrophenylalanine residues. It is now established that in synthetic linear peptides, α,β-dehydrophenylalanine inherently nucleates β-turn structures (Chauhan *et al* 1987). Few reports have suggested that α,β-dehydroleucine also behaves in a similar manner (Uma *et al* 1989; Narula *et al* 1990). We have recently shown that dehydroalanine, unlike Δ^zPhe an Δ^zLeu, does not stabilize a β-turn structure (Gupta and Chauhan 1990). It appears from these studies that the β-substituent plays an important role in determining the conformational behaviour of an α,β-dehydroamino acid. It was therefore thought worthwhile to study conformational characteristics of model peptides containing other α,β-dehydroamino acids. As a part of a continuing programme, we have extended these conformational studies to peptides containing dehydrovaline (ΔVal) residues. There are two reasons for choosing protected dipeptide methylamides

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of the type, Boc–X–ΔVal–$NHCH_3$, as suitable models for studying the conformational behaviour of ΔVal. First, this peptide unit (Boc–X–ΔVal–$NHCH_3$) is the minimum necessary structure that would allow an intramolecular, $4 \rightarrow 1$ hydrogen bond required to stabilize a β-turn. This is relevant in the light of the fact that both Δ^zPhe and Δ^zLeu tend to nucleate a type II β-turn structure in model peptides (Chauhan *et al* 1987; Uma *et al* 1989). Secondly, a direct comparison of ΔVal residue with Δ^zPhe, Δ^zLeu and ΔAla would be possible from our earlier published work on similar model peptides (Chauhan *et al* 1987; Uma *et al* 1989; Gupta and Chauhan 1990) containing these dehydroresidues. The present report describes synthesis and solution conformational studies, using ^{1}H NMR and IR spectroscopy, of the model peptides containing ΔVal, of the type Boc-X-ΔVal-$NHCH_3$ [X = Phe, 1; Val, 2 and Ala, 3). A comparison with the corresponding Δ^zPhe and ΔAla containing peptides is also presented.

2. Experimental

Boc amino acids and the C-terminal methyl esters of amino acids were prepared by standard procedures (Brenner and Huber 1953; Carpino 1957). Peptides were synthesized by solution phase methodology. Amino acid couplings were performed by mixed anhydride coupling method (Makowski *et al* 1986). The dehydrovaline moiety was generated by N-chlorination-dehydrochlorination method described by Schmidt and Ohler (1977), starting from valinemethyl ester. Thin-layer chromatography (tlc) was carried out on silica gel G plates in the following solvent systems. A) $CHCl_3$:MeOH (9:1), B) *n*-BuOH:AcOH:H_2O(4:1:1) and C) *n*-BuOH:AcOH:Pyridine:H_2O (4:1:1:2). Peptides were first purified on silica gel columns using $CHCl_3$–MeOH as eluent and finally by HPLC using gradient of acetonitrile-water (40% acetonitrile to 100% acetonitrile in 45 min) on Zorbax ODS column (9.4 mm × 250 mm, particle size 5 mm, detection at 226 nm). All the intermediates were characterized by NMR spectroscopy. ^{1}H NMR studies of final compounds were carried out on a Bruker (300 MHz) FT NMR spectrometer at the Centre for Cellular and Molecular Biology, Hyderabad. IR spectra were recorded on a Perkin Elmer 1710 FT–IR spectrophotometer.

2.1 *Synthesis*

2.1a *Boc–Ala–ΔVal–OMe*: Val–OMe.HCl (2·0 g, 11·9 mmol) was dissolved in $CHCl_3$ (30 ml) and treated with saturated Na_2CO_3 (30 ml). The chloroform layer was washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The residue was taken in dry ether.(30 ml) and *t*-butylhypochlorite (1·4 ml, 11·9 mmol) was added dropwise over a period of 1 h in ice bath in dark. The reaction mixture was concentrated *in vacuo*, residue taken up in $CHCl_3$, washed successively with 0·5 N HCl and water, dried over anhydrous Na_2SO_4 and evaporated to yield N-chlorovaline methyl ester which was dissolved in dry ether (30 ml). 1,8-Diazobicycloundec-7-ene (DBU) (1·8 ml, 11·9 mmol) was added to the solution obtained and DBU–HCl was filtered off from the reaction mixture after 3 h.

To a chilled solution of Boc–Ala–OH (2·24 g, 11·9 mmol) and N-methylmorpholine (1·4 ml, 11·9 mmol) was added isobutylchloroformate (1·4 ml, 11·9 mmol) and the reaction mixture stirred for 10 min at -10°C. Dehydrovaline methyl ester obtained

as above was then added and stirring was continued for 2 h in an ice bath and overnight at room temperature. The solvent was removed *in vacuo*, the residue was dissolved in ethylacetate and was washed successively with saturated $NaHCO_3$ solution, water and dried over anhydrous Na_2SO_4. The solvent was evaporated to dryness and Boc–Ala–ΔVal–OMe was obtained as an oil. Yield 2·3 g(65%); R_f^A0·75; R_f^B0·80; R_f^C0·82;. δH(60 MHz, $CDCl_3$): 7·5(ΔVal NH, 1 H, *br*); 5·2(Ala NH, 1 H, *br*); 4·2 (Ala $C^{\alpha}H$, 1 H, *m*), 3·7(−$COOCH_3$, 3 H, *s*), 2·12 (ΔVal $C_a^{\gamma}H_3$, 3 H, *s*); 1·8 (ΔVal $C_b^{\gamma}H_3$, 3 H, *s*); 1·49 (Boc CH_3, 9H, *s*); 1·3 (Ala $C^{\beta}H_3$, 3 H, *d*).

2.1b *Boc–Ala–ΔVal–OH*: Boc–Ala–ΔVal–OMe (1·55 g, 5·2 mmol) was dissolved in MeOH (15 ml) and treated with 1 N NaOH (7·7 ml, 1·5 equivalents) at room temperature. After stirring for 6 h, MeOH was removed *in vacuo*, residue dissolved in water and washed with ethylacetate. Aqueous layer was acidified with solid citric acid upto *p*H 3 and extracted with ethylacetate (2 × 20 ml). The combined ethylacetate extracts were washed with water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give Boc–Ala–ΔVal–OH as an oil. Yield, 0·85 g (58%); R_f^A0·27; R_f^B0·70, R_f^C0·78. 1 H NMR showed the absence of any peaks at 3·78δ corresponding to $-COOCH_3$ protons.

2.1c *Boc–Ala–ΔVal–NHCH₃(3)*: A solution of Boc–Ala–ΔVal–OH (0·78 g, 2·7 mmol) and N-methylmorpholine (0·3 ml, 2·7 mmol) in THF (15 ml) was stirred at −10°C, isobutylchloroformate (0·4 ml, 2·7 mmol) was added and the mixture stirred for 10 min. A pre-cooled solution of $CH_3NH_2 \cdot HCl$ (0·40 g, 5·5 mmol) and Et_3N (0·6 ml, 5·5 mmol) in THF:water (3·2, 5 ml) was added, the reaction mixture stirred for 2 h at 0°C and overnight at room temperature. The solvent was removed *in vacuo*, residue taken up in ethyl acetate, washed successively with saturated $NaHCO_3$ solution, 5% citric acid solution, and water, dried over anhydrous Na_2SO_4 and evaporated to dryness to yield 3 as white solid. It was purified by chromatography over silica gel column using $CHCl_3$–MeOH as eluent. Yield 0·25 g (32%), m.p. 138–140°C; R_f^A0·50; R_f^B0·79; R_f^C0·89; $[\alpha]_D^{20}$−42·72° (C, 0·03, MeOH); HPLC retention time, 13·8 min. δH (300 MHz, $CDCl_3$): 7·8(ΔVal NH, 1 H, *s*); 6·76(NH–CH_3, 1 H, *br*); 5·05 (Ala NH, 1 H, *d*); 4·13(Ala $C^{\alpha}H$, 1 H, *m*); 2·8 (NH–CH_3, 3 H, *d*); 2·02 (ΔVal $C_a^{\gamma}H_3$, 3 H, *s*); 1·75 (ΔVal $C_b^{\gamma}H_3$, 3 H, *s*); 1·42 (Boc CH_3, 9 H, *s*); 1·39 (Ala $C^{\beta}H_3$, 3 H, *d*).

Boc–Phe–ΔVal–$NHCH_3$ 1 and Boc–Val–ΔVal–$NHCH_3$ 2 were synthesised using similar procedures as described above for Boc–Ala–ΔVal–$NHCH_3$. The physical parameters for peptides 1, 2 and 3 are summarized in table 1.

Table 1. Physical characteristics of peptides Boc–Phe–ΔVal–$NHCH_3$ 1. Boc–Val–ΔVal–$NHCH_3$ 2 and Boc–Ala–ΔVal–$NHCH_3$ 3.

Peptide	Yield %	R_f^A	R_f^B	R_f^C	m.p. (°C)	$[\alpha]_D^{25}$
Boc–Phe–ΔVal–$NHCH_3$ (1)	41·6	0·57	0·72	0·88	180–182	−8·33
Boc–Val–ΔVal–$NHCH_3$ (2)	46·5	0·60	0·82	0·85	128–130	−22·22
Boc–Ala–ΔVal–$NHCH_3$ (3)	32·0	0·50	0·79	0·89	138–140	−42·72

3. Results and discussion

^{1}H NMR spectra of 1, 2 and 3 were recorded in $CDCl_3$ and $(CD_3)_2SO$. Detailed ^{1}H NMR are described here on peptide 3. Figure 1 shows the representative ^{1}H NMR of 3 in $CDCl_3$. The relevant ^{1}H NMR parameters of peptide 3 are summarized in table 2. The assignment of NH resonances was straightforward. Doublet at $\sim 5{\cdot}0\delta$ was assigned to urethane NH belonging to the Ala residue by virtue of its high field position in $CDCl_3$ (Nagaraj and Balaram 1981). NH resonances belonging to α,β-dehydroamino acids have usually been observed quite downfield ($\sim 7{\cdot}0\delta$–$9{\cdot}0\delta$) (Chauhan *et al* 1989). The ΔVal NH was therefore recognized as a broad singlet at

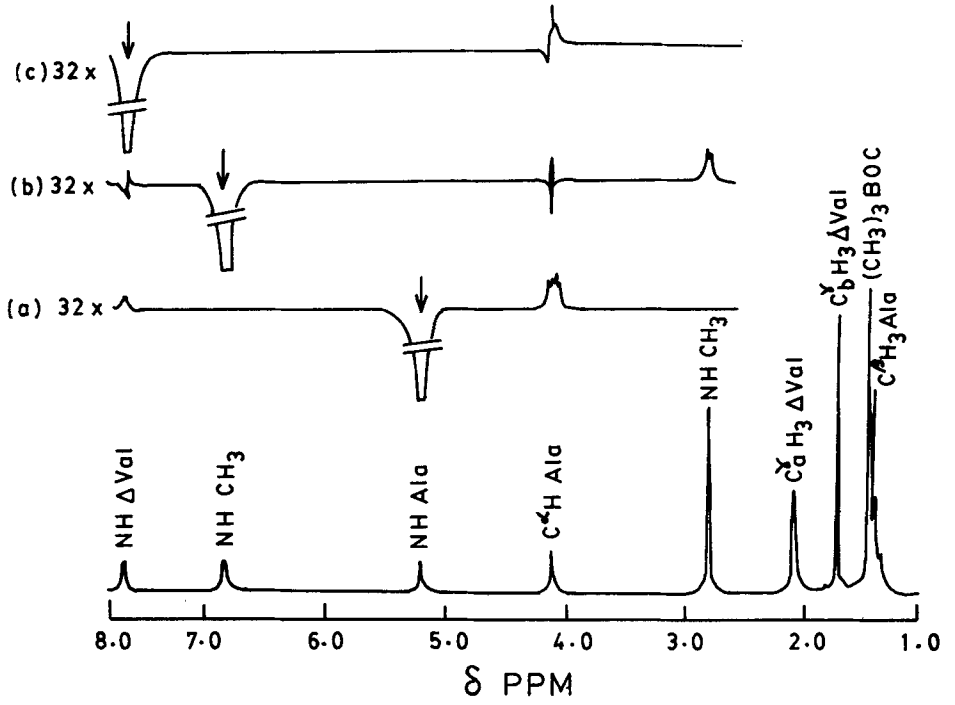
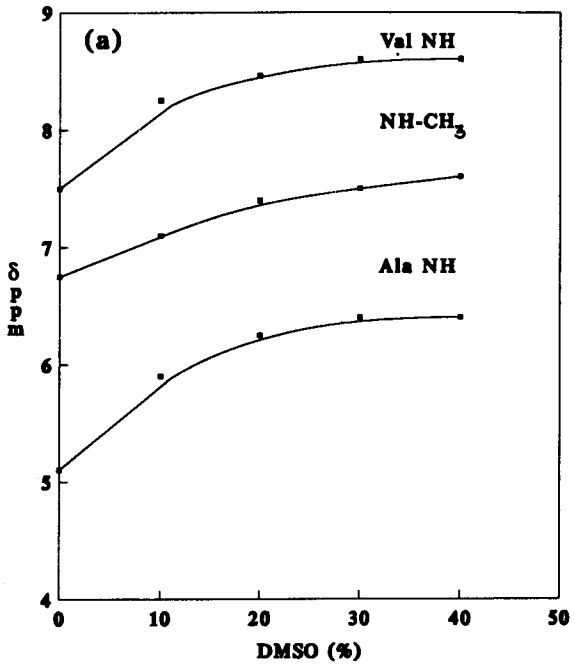


Figure 1. ^{1}H NMR spectrum (300 MHz) of peptide Boc–Ala–ΔVal–$NHCH_3$ in $CDCl_3$. (a–c) Difference NOE spectra obtained by irradiation of the NH resonances, indicated by arrows.

Table 2. NMR parameters for NH resonances in peptide Boc–Ala–ΔVal–$NHCH_3$.

	Residue		
Parameter	Ala	ΔVal	$-NHCH_3$
$CDCl_3$(δppm)	5·05	7·8	6·76
$(CD_3)_2SO$(δppm)	7·17	9·0	7·45
Δδ(ppm)	2·12	1·2	0·69
$d\delta/dT$ ppm $K^{-1} \times 10^3$	7·2	8·33	6·12
^3J HNC$^\alpha$H ($CDCl_3$) (Hz)	8·33		7·46
^3J HNC$^\alpha$H $(CD_3)_2SO$ (Hz)	8·77		6·57



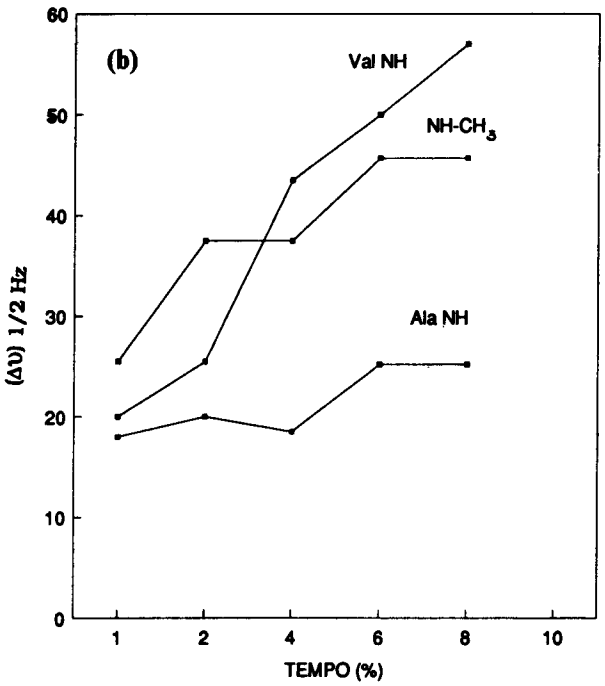


Figure 2. (**a**) Solvent dependence of NH chemical shifts in peptide Boc–Ala–ΔVal–$NHCH_3$ in $CDCl_3/(CD_3)_2SO$ mixtures of varying composition. (**b**) Line broadening of NH resonances as a function of 2,2,6,6-tetramethylpiperdine-1-oxyl concentration for Boc–Ala–ΔVal–$NHCH_3$.

$\sim 7{\cdot}58\,\delta$, most downfield of all signals. The broad quartet at $\sim 6{\cdot}78\,\delta$ was assigned to methylamide NH group.

The assignments of NH resonances in $(CD_3)_2SO$ were made by monitoring their chemical shift positions in $CDCl_3$–$(CD_3)_2SO$ titration experiments (figure 2a). In peptide 3, the addition of $(CD_3)_2SO$ to the peptide solution in a relatively non-polar solvent such as $CDCl_3$ resulted in appreciable downfield shifts of all the three NH resonances. As the concentration of $(CD_3)_2SO$ is increased up to 40%, the NH belonging to the dehydrovaline residue shifts by $\sim 1{\cdot}2\,\delta$ and urethane NH by $\sim 1{\cdot}5\,\delta$. Although methylamide NH exhibits analogous titration curve, the change in chemical shift is not so large ($0{\cdot}8\,\delta$). Figure 2a shows the solvent titration curves for peptides 3. Results of solvent perturbation and large differences in chemical shifts ($\sim 2\,\delta$) in the two solvents (table 3) clearly signify that all the NH groups are appreciably solvent exposed. The broadening of NH resonance lines on the addition of paramagnetic radical 2,2,6,6-tetramethyl-piperdine-1-ocyl (TEMPO) in peptide 3 is illustrated in figure 2b. Although the shapes of the curves clearly suggest that all the NH groups are solvent-exposed, the methylamide NH group appears to be least affected. The intermediate behaviour of the methylamide NH group indicates that there is some possibility of its involvement in an intramolecular hydrogen bond although no definite evidence is provided for the existence of an intramolecularly hydrogen bonded NH group.

The above results are supported by the temperature dependent studies for peptide 3 in $(CD_3)_2SO$. High temperature coefficient values ($d\delta/dT$) of the order of $\sim 7 \times 10^{-3}$ ppm K^{-1} are characteristic of fully exposed NH groups in peptide 3. These results are in marked contrast to those reported for analogous dehydrophenylalanine and dehydroalanine containing model peptides. In corresponding Δ^zPhe containing peptides (Kaur *et al* 1989), methylamide NH group and ΔAla NH group in corresponding ΔAla containing peptides (Gupta and Chauhan 1990) remained solvent shielded and were inferred to be intramolecularly bonded to the Boc carbonyl group through a hydrogen bond. Thus, it appears from the above studies that unlike Δ^zPhe and ΔAla, ΔVal does not stabilize any intramolecularly hydrogen bonded conformations in solution. It can therefore be suggested that peptides 1, 2 and 3 exist largely in fully extended conformations in solutions.

3.1 *NOE studies*

Nuclear Overhauser Effect studies for the peptide 3 were conducted in $CDCl_3$ (figure 2) and $(CD_3)_2SO$. The observed NOEs are summarized in table 3. In $CDCl_3$ appreciable NOEs are observed between NH Ala↔C^{α}H Ala, C^{α}H Ala↔NH ΔVal and NH

Table 3. NOE's observed in peptide Boc–Ala–ΔVal–$NHCH_3$ in $CDCl_3$ and $(CD_3)_2SO$.

Resonance irradiated	$CDCl_3$ resonance observed	%NOE	$(CD_3)_2SO$ resonance observed	%NOE
Ala NH	Ala C^{α}H	1·04	Ala C^{α}H	0·83
$-N\underline{H}CH_3$	N–CH_3	1·53	N–CH_3	1·42
ΔVal NH	Ala C^{α}H	3·25	Ala C^{α}H	4·44

methylamide↔CH_3 methylamide. A single interresidue NOE of the type $C^{\alpha}_{i+1}H\leftrightarrow N_{i+2}H$ between Ala $C^{\alpha}H$ and ΔVal NH is suggestive of the absence of any intramolecular hydrogen-bonded structure. Isolated $C^{\alpha}_{i+1}H\leftrightarrow N_{i+2}$ NOE followed by a $N_{i+2}H\leftrightarrow N_{i+3}H$ NOE is diagnostic of a type II β-turn conformation (Kaur *et al* 1989). In the corresponding Δ^zPhe and Δ^zLeu containing model peptides, a $C^{\alpha}_{i+1}H\leftrightarrow N_{i+2}H$ NOE was accompanied by a $N_{i+2}H\leftrightarrow N_{i+3}H$ NOE suggesting a type II β-turn conformation. The crystal structure studies on Δ^zPhe and Δ^zLeu model compounds have also confirmed a strong tendency for these dehydroresidues to induce a type II β-turn structure. An isolated interresidue NOE of the type $C^{\alpha}_{i+1}H\leftrightarrow N_{i+2}H$ however would be compatible with an inverse γ-turn type structure since this ($C^{\alpha}_{i+1}H\leftrightarrow N_{i+2}H$) interproton distance is ~2·5 Å in such structures. It is relevant to point out that inverse γ-turn structure have been proposed for model peptides containing dehydroalanine (ΔAla) residues. However, since other NMR data do not clearly reveal the presence of any intramolecular hydrogen bonds it can be concluded that at least a major population of peptide 3 molecules acquires fully extended conformation in chloroform.

In $(CD_3)_2SO$ also a strong interresidue NOE between $C^{\alpha}H$ Ala and ΔVal NH is

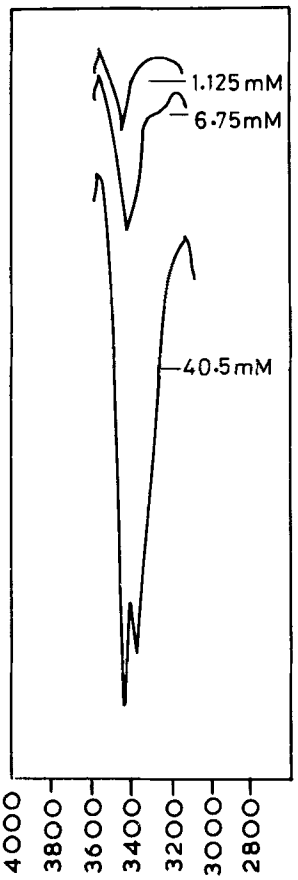


Figure 3. IR spectra (NH stretching bands) for peptide Boc–Ala–ΔVal–$NHCH_3$ in $CHCl_3$ at various peptide concentrations.

observed which is compatible with a large ψ value ($\sim 120°$) at the ΔAla residue. Thus, for peptide 3 extended structures dominate in both $(CD_3)_2SO$ and $CDCl_3$.

3.2 *IR studies*

Further support to above conclusions was provided by infrared studies in chloroform. These studies were carried out for peptides 1, 2 and 3 over a concentration range of about 40 mM to 1 mM in $CHCl_3$. Figure 3 shows the representative IR spectrum of 3 in the range 4000 cm^{-1} to 3200 cm^{-1} where the amide bands are primarily observed. Two bands at $\sim 3380\,cm^{-1}$ and $\sim 3440\,cm^{-1}$ were observed. The band at $\sim 3380\,cm^{-1}$ has usually been assigned to NH (hb) amide groups and the band at $\sim 3440\,cm^{-1}$ to free amide groups [νNH (free) (Aubry *et al* 1978; Rao *et al* 1980; Beneditti *et al* 1982). The band due to hydrogen-bonded NH groups (νNH(hb) $\sim 3380\,cm^{-1}$) disappears at further dilutions ($\sim 6{\cdot}8$ mM) suggesting that the hydrogen bonds contributing to this absorption are intermolecular rather than intramolecular ones. This may suggest that peptides 1, 2 and 3 exist as associated (intermolecular hydrogen bonded) species in higher concentration and random unassociated species at lower concentrations (Raj and Balaram 1985). IR studies thus also rule out the possibility of existence of intramolecularly hydrogen-bonded conformations for peptides 1, 2 and 3.

4. Conclusions

On the basis of spectroscopic evidences presented above, it can be suggested that peptides 1, 2 and 3 favour fully extended conformations in $CDCl_3$ and $(CD_3)_2SO$ solutions (figure 4). It emerges from these studies that ΔVal, unlike Δ^zPhe, Δ^zLeu and ΔAla, fails to encourage the formation of any specific type of folded structure. This highlights the importance of steric factors in deciding the conformational preferences of dehydroamino acids. Δ^zPhe and Δ^zLeu both having the large β-substituents, the phenyl ring in Δ^zPhe and the isopropyl group in Δ^zLeu, have the same conformational preferences, both inducing a type II β-turn structure in the peptide (Singh *et al* 1989; Uma *et al* 1989) backbone. In contrast ΔAla having the same $C^\alpha = C^\beta$ double bond but no β-substituent, induces a totally different type of conformational constraint in the backbone and stabilizes a C7 type of folded structure. The present studies indicate that ΔVal, with two methyl groups at the C^β position, behaves differently and although

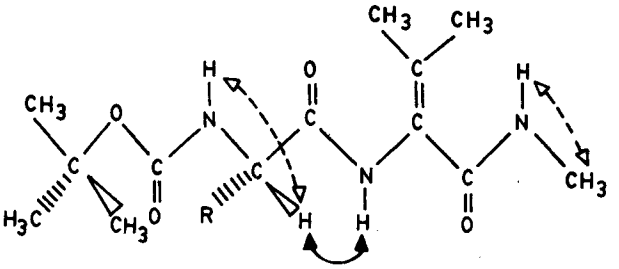


Figure 4. Proposed structure for Boc–X–ΔVal–$NHCH_3$ in $CDCl_3$ consistent with the spectral data.

it constrains the backbone on account of the $C^{\alpha} = C^{\beta}$ double bond, it does not induce a folded conformation. It becomes all the more clear that the steric factors play a dominant role along with the electronic factors, in deciding the conformational preferences of α,β-dehydroamino acids. It may be possible to design peptides with different conformational characteristics using α,β-dehydroamino acids.

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