

# Conformational variability in short acyclic peptides. Stabilization of multiple $\beta$ -turn structures in organic solvents

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The conformational characteristics of three hexapeptides Boc-Leu-Xxx-Val-Leu-Aib-Val-OMe (Xxx = Ala 1, D-Ala 2, Gly 3; Aib =  $\alpha$ -aminoisobutyryl) have been probed in CDCl<sub>3</sub> solution by NMR methods using solvent perturbation of chemical shifts and radical broadening of NH resonances to delineate intramolecularly hydrogen bonded NH groups. Nuclear Overhauser effects (NOEs) provide additional information on preferred backbone conformations. The substituent at position 2 acts as a major conformational determinant. While a continuous  $3_{10}$  helical conformation is favoured for the peptide with Xxx = Ala, a multiple  $\beta$ -turns conformation is supported by both NMR and CD data for the peptide with Xxx = D-Ala. In the peptide with Xxx = Gly CD and NMR data suggest that both  $3_{10}$  helical and multiple turns conformations are simultaneously populated. The results suggest that incorporation of D-amino acids and Aib residues into all L-sequences may prove useful in generating sequences containing multiple turns.

The stereochemical features of  $\beta$ -turns,<sup>1</sup> widely occurring structural elements in proteins, have been extensively investigated.<sup>2,3</sup>  $\beta$ -Turns have also been the focus of recent interest as nucleating elements for  $\beta$ -hairpin formation.<sup>4-7</sup>  $\beta$ -Turns have been classified into several types based on the conformational angles at the  $i + 1$  and  $i + 2$  residues.<sup>2,3</sup> Successive type III turns result in the generation of a  $3_{10}$  helical fold of the peptide chain. The occurrence of consecutive  $\beta$ -turns of other types is much less frequent in proteins.<sup>8</sup> Multiple  $\beta$ -turn conformations can be generated when a single residue simultaneously occupies the  $i + 2$  position of the first turn and the  $i + 1$  position of the second turn. The recent observation of novel multiple  $\beta$ -turns in an antigenic segment of the HIV V 3 loop<sup>9-12</sup> and the characterization of multiple turns in a short hydrophobic peptide containing both glycine and  $\alpha$ -aminoisobutyryl (Aib) residues<sup>13</sup> prompted an attempt to design stable multiple turns in short synthetic peptides. The conformational characteristics of three acyclic hexapeptides Boc-Leu-Xxx-Val-Leu-Aib-Val OMe (Xxx = L-Ala 1, D-Ala 2, Gly 3) are described in this report.

The choice of the D-Ala residue at position 2 in peptide 2 was made with the intention of stabilizing a type II Leu-D-Ala  $\beta$ -turn, a feature expected in heterochiral sequences.<sup>14,15</sup> The choice of the Aib residue at position 5 was expected to stabilize the  $\beta$ -turn at the C terminus of the peptide. The presence of the D and L residues in short segments may be expected to destabilize continuous helix formation, a feature often observed in Aib containing peptides.<sup>16-18</sup>

## Results and discussion

### NMR studies

The solvent accessibility of NH groups in peptides 1-3 was probed using chemical shift perturbation in CDCl<sub>3</sub>-(CD<sub>3</sub>)<sub>2</sub>SO<sup>19</sup> mixtures and free radical (TEMPO) induced line broadening in CDCl<sub>3</sub> solutions.<sup>20,21</sup> Resonance assignments were carried out using a combination of COSY and NOESY/ROESY spectra.<sup>22</sup> Table 1 lists relevant chemical shift parameters. Figs. 1 and 2 summarize the results of experiments designed to delineate the degree of solvent exposure of the peptide NH groups. Aggreg-

ation effects are minimal as evidenced by the absence of any pronounced chemical shift changes over the concentration range 9.36 to 0.39 mM for peptide 2.

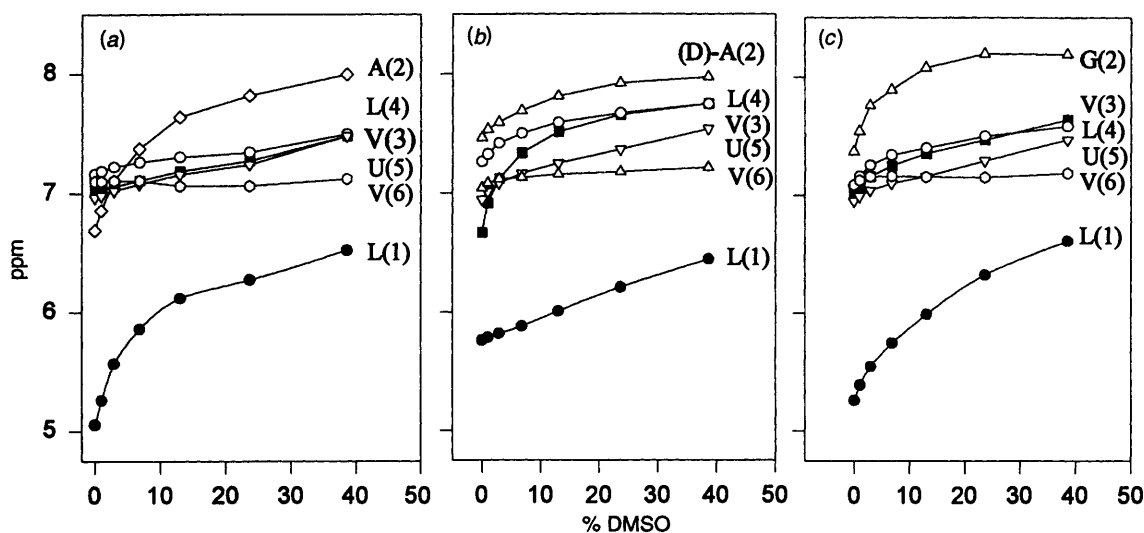
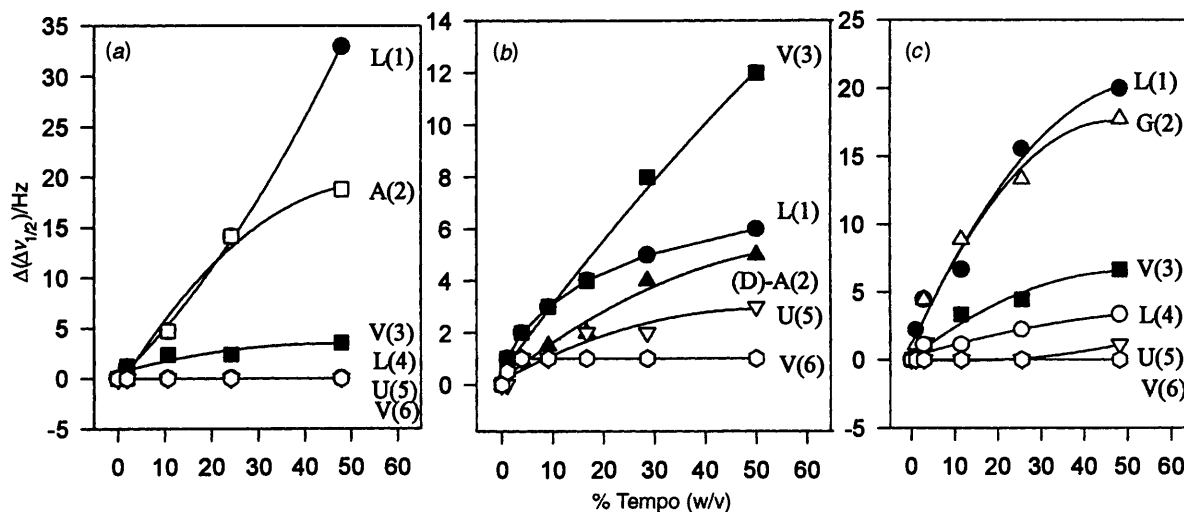
**Xxx = L-Ala 1.** The Leu(1) and Ala(2) NH groups are clearly solvent exposed as seen from the large effects in the solvent and radical perturbation experiments (Figs. 1, 2). The four remaining NH groups are inaccessible with Val(6) being the most solvent shielded. These observations are fully consistent with a  $3_{10}$ -helical conformation [Fig. 3(a)] having four successive 4  $\rightarrow$  1 hydrogen bonds, involving the Val(3), Leu(4), Aib(5) and Val(6) NH groups. This structure is also in accordance with a large body of data that suggests that even a single Aib residue in oligopeptides of length 6-7 residues promotes helix formation.<sup>18</sup> In NOE experiments the characteristic  $N_iH \leftrightarrow N_{i+1}$  ( $d_{NN}$ ) connectivities were observed only between Leu(1)  $\leftrightarrow$  Ala(2) and Leu(4)  $\leftrightarrow$  Aib(5) [Fig. 4(a)]. Limited chemical shift dispersion resulting in overlap of resonances and low NOE magnitudes are probably responsible for the non-observation of the continuous set of short  $d_{NN}$  connectivities. Several moderate to strong  $C_i^{\alpha}H \leftrightarrow N_{i+1}H$  NOEs are also observed [ $d_{\alpha N}$ , Fig. 4(a)]. In an ideal  $3_{10}$ -helix the  $C_i^{\alpha}H \leftrightarrow N_{i+1}$  distance is 3.4 Å while the  $N_iH \leftrightarrow N_{i+1}H$  distance is 2 Å.<sup>22</sup> In short peptides NOEs are likely to be observed to distances of ca. 3.0-3.5 Å under the conditions used. The observation of strong  $d_{\alpha N}$  NOEs characteristic of extended/semi-extended residue conformations ( $\psi$  ca. 120  $\pm$  60°) does suggest that multiple states may be populated, although the pattern of NH group accessibilities support a significant population of helical conformers.

**Xxx = D-Ala, 2.** Peptide 2 differs dramatically from 1 in its NMR behaviour. The data in Fig. 1(b) establish that Val(6) NH is strongly solvent shielded, while D-Ala(2) NH, Leu(4) NH and Aib(5) NH are moderately inaccessible. Val(3) NH is surprisingly accessible to solvent, showing the highest degree of solvent and radical induced perturbation [Fig. 2(b)]. In NOE experiments only a single  $d_{NN}$  connectivity, Leu(1)  $\leftrightarrow$  Ala(2) is observed. However, several  $d_{NN}$  NOEs [Fig. 4(b)] are observed characteristic of extended/semi-extended conformations at individual residues. The NMR data are largely consistent with a major conformation shown in Fig. 3(b), which features a C<sub>7</sub> ( $\gamma$ -turn) at Leu(1), a type II'  $\beta$ -turn at the D-Ala-Val segment and a type II  $\beta$ -turn at the Leu(4)-Aib(5) segment. The Aib(5) NH is exposed in this model, an inconsistency which may be

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**Table 1** NMR parameters<sup>a</sup> for peptides Boc-Leu-Xxx-Val-Leu-Aib-Val-OMe

Residue	Chemical shifts $\delta$ (ppm)															$^3J_{\text{NH-C}^{\alpha}\text{H}}$ /Hz		
	NH			C <sup><math>\alpha</math></sup> H			C <sup><math>\beta</math></sup> H			C <sup><math>\gamma</math></sup> H			C <sup><math>\delta</math></sup> H					
Xxx	Ala	D-Ala	Gly	Ala	D-Ala	Gly	Ala	D-Ala	Gly	Ala	D-Ala	Gly	Ala	D-Ala	Gly	Ala	D-Ala	Gly
Leu	5.08	5.76	5.41	3.95	4.08	4.03	1.5	1.61	1.52	1.61	1.60	1.60	0.90	0.94	0.94	5.4	6.6	5.5
Xxx	6.70	7.47	7.42	4.25	4.28	3.97	1.45	1.40	—	—	—	—	—	—	—	2.8	5.6	—
Val	7.05	6.67	7.09	4.18	4.15	4.16	2.37	2.33	2.28	1.0	0.93	1.0	—	—	—	5.6	6.3	6.9
Leu	7.17	7.24	7.11	4.39	4.33	4.38	1.69	1.69	1.68	1.69	1.69	1.72	0.94	0.93	0.94	7.5	7.4	7.9
Aib	6.97	6.95	7.04	—	—	—	1.49	1.49	1.49	—	—	—	—	—	—	—	—	—
Val	7.10	7.04	7.11	4.42	4.48	4.44	2.13	2.15	2.16	0.94	0.96	0.94	—	—	—	8.7	7.9	8.4

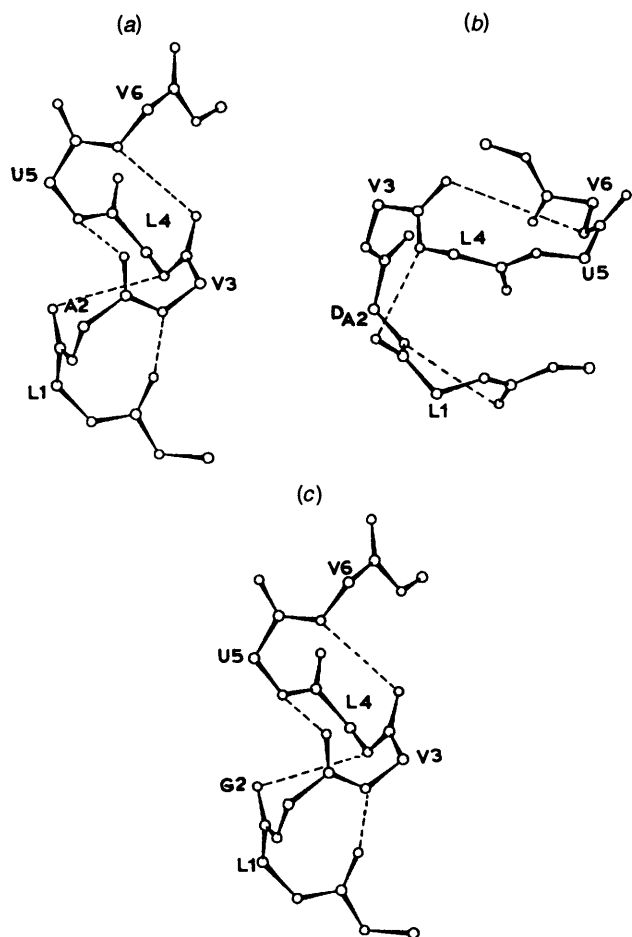
<sup>a</sup> Chemical shifts and coupling constants in CDCl<sub>3</sub>.**Fig. 1** Dependence of NH chemical shifts on the concentration of dimethyl sulfoxide (DMSO) (v/v) in CDCl<sub>3</sub> in peptides Boc-Leu-Xxx-Val-Leu-Aib-Val-OMe (1–3). Assignments are indicated using the one letter code (U = Aib). (a) Peptide 1 (Xxx = Ala), (b) peptide 2 (Xxx = D-Ala), (c) peptide 3 (Xxx = Gly).**Fig. 2** Changes in line width of NH resonances in peptides Boc-Leu-Xxx-Val-Leu-Aib-Val-OMe (1–3) with increasing concentration of TEMPO in CDCl<sub>3</sub> solution. (a) Peptide 1 (Xxx = Ala), (b) peptide 2 (Xxx = D-Ala), (c) peptide 3 (Xxx = Gly).

rationalized by invoking a population of type III Leu(4)-Aib(5)  $\beta$ -turns, which in turn would result in a D-Ala CO $\cdots$ HN-Leu(5) 4  $\rightarrow$  1 hydrogen bond. The large downfield shift of the Xxx(2) NH group in **2** as compared to **1** is consistent with its involvement in a 3  $\rightarrow$  1 hydrogen bond in the D-Ala (2) peptide [Fig. 3(b)].

Interconversions between Type II–III  $\beta$ -turn conformations are readily possible by a flip of the central peptide unit, corres-

ponding to a concerted rotation about the single bonds C <sup>$\alpha$</sup> <sub>*i*+1</sub>–C <sup>$\alpha$</sup> <sub>*i*+1</sub>O ( $\psi_{i+1}$ ) and N<sub>*i*+2</sub>–C <sup>$\alpha$</sup> <sub>*i*+2</sub> ( $\phi_{i+2}$ ). Indeed theoretical calculations within an AMI framework suggest an almost barrierless interconversion between type II and type III  $\beta$ -turns (L. Gomathi and J. Chandrasekhar, unpublished results).

Xxx = Gly, **3**. The Leu(1) and Gly(2) NH groups are exposed to solvent, with the remaining four NH resonances being shielded [Figs. 1(c) and 2(c)]. Successive  $d_{\text{NN}}$  and  $d_{\text{uN}}$  connecti-



**Fig. 3** Computer generated ball and stick structures for the peptide backbones. The idealized models were constructed using standard values of backbone dihedral angles. Structures were refined by energy minimization using the program INSIGHTII. Amino acid residues are indicated using the one letter code. Intramolecular hydrogen bonds are indicated by broken lines. (a) Peptide 1 (Xxx = L-Ala), (b) peptide 2 (Xxx = D-Ala), (c) peptide 3 (Xxx = Gly).

ties are also observed over the Leu(1)-Aib(5) segment [Fig. 4(c)]. The simultaneous observation of both types of inter-residue NOEs is suggestive of a conformational equilibrium, although the NH solvent exposure data are broadly consistent with a major  $3_{10}$ -helical conformation [Fig. 3(c)]. Interestingly, while peptide 3 resembles 1 in the pattern of NH accessibility, the low field position of the Gly(2) NH in 3 is similar to that observed in 2.

#### Circular dichroism

CD spectra of 1–3 are shown in Fig. 5. Peptide 1 (Xxx = L-Ala) exhibits two negative bands at 223–225 and 203 nm, in both methanol and 2,2,2-trifluoroethanol (TFE), characteristic of small helical peptides [Fig. 5(a) and (b)]. Similar spectra with widely differing intensities of the  $n-\pi^*$  and  $\pi-\pi^*$  bands have been observed in earlier studies of short Aib containing helical sequences.<sup>23</sup> The absence of solvent dependence is suggestive of a population of conformationally stable structures. Peptide 2 (Xxx = D-Ala) has a dramatically different CD spectrum, with a negative band at ca. 230 nm and a positive band at 210 nm [Fig. 5(b)]. In this case also, the absence of pronounced solvent dependence suggests that conformer populations are not influenced by changing the solvent from methanol to TFE, a known helix promoter in helicogenic sequences.<sup>24</sup> The positive band at 210 nm may be indicative of type II  $\beta$ -turn conformations,<sup>15,25–27</sup> while the long wavelength, 230 nm, band

**Table 2** IR band position in Boc-Leu-Xxx-Val-Leu-Aib-Val-OMe; Xxx = Ala, D-Ala, Gly

$\nu_{\text{NH}}/\text{cm}^{-1}$		
Ala	D-Ala	Gly
3286.2	3323.0	3287.4
3336.2	—	3330.1
3418.3	3419.7	3426.2

$\nu_{\text{CO}}/\text{cm}^{-1}$		
Ala	D-Ala	Gly
1666.9	1668.9	1667.7
1700.2	1732.7	1730.6

has indeed been observed in small peptides earlier, and sometimes ascribed to  $\gamma$ -turns.<sup>28</sup>

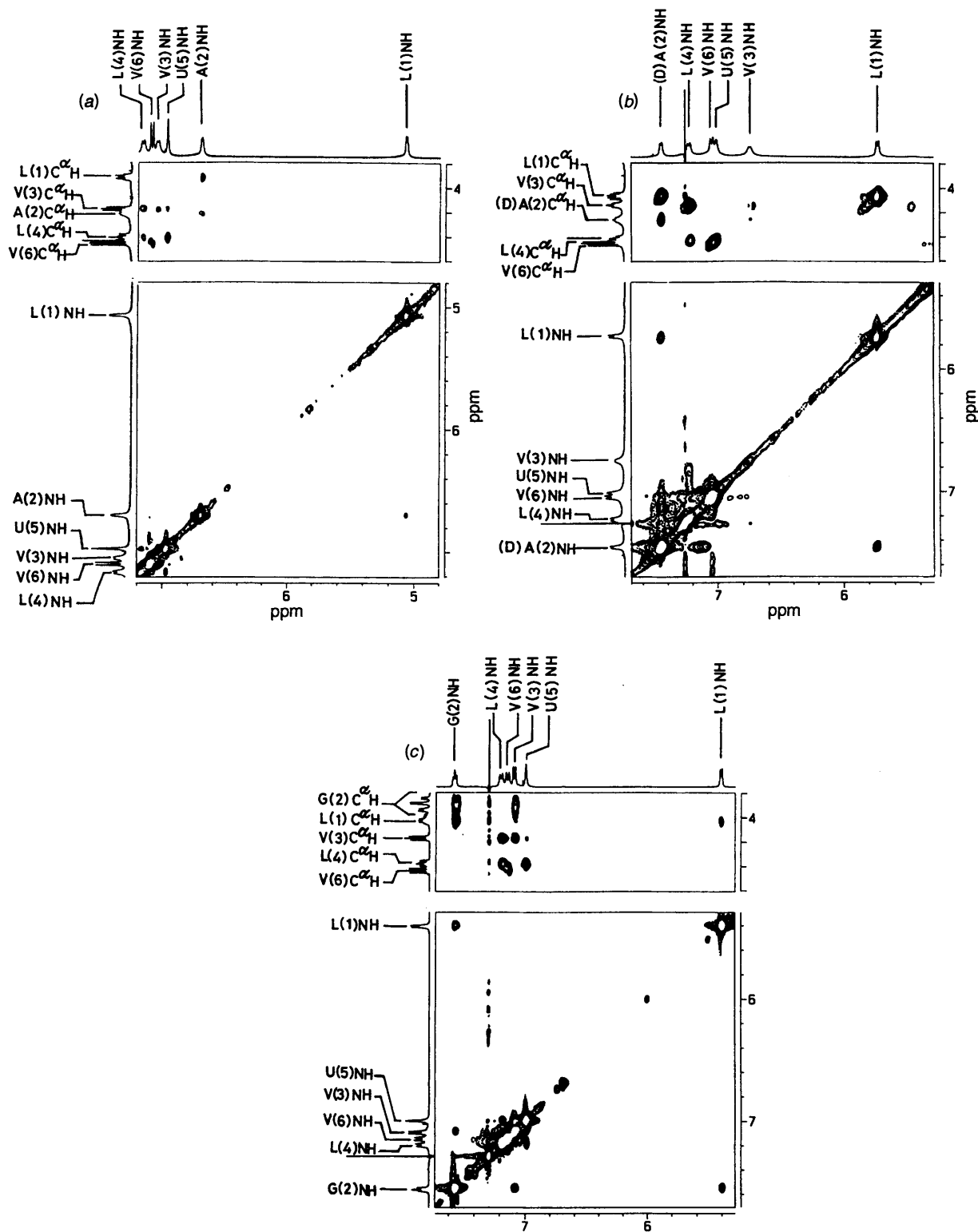
Peptide 3 (Xxx = Gly) exhibits considerable variation in CD spectra on going from methanol to TFE [Fig. 5(c)]. In the former, two negative bands are seen at 230 and 206 nm, while a weak positive band is observed at 215 nm. In the latter, only two negative bands are observed at 224 and 204 nm. Inspection of the spectra for peptides 1–3 in Fig. 5 suggests that the observed CD spectra for the Gly peptide 3 may arise from contributions of the spectral types observed for peptides 1 and 2. Thus, in 3, conformational states characterized for both 1 and 2 coexist, with populations varying appreciably with solvent. As already noted peptide 3 resembles 1 in the pattern of NH group accessibility [Fig. 1(a)] but the Gly(2) NH chemical shift is anomalously low, a feature also seen in peptide 2.

#### Infrared studies

IR spectra were recorded for peptides 1–3 in dilute chloroform solutions (3 mM). The positions of the NH ( $\nu_{\text{NH}}$ ) and CO ( $\nu_{\text{CO}}$ ) stretching bands are summarized in Table 2. Intense  $\nu_{\text{NH}}$  bands in the region 3300–3340  $\text{cm}^{-1}$  were observed in all cases, characteristics of intramolecularly hydrogen bonded conformations. In chloroform solution, the ratio of the intensity of the  $\nu_{\text{NH}}$  (H bonded) to  $\nu_{\text{NH}}$  (free) band follows the order L-Ala > Gly > D-Ala, confirming that the L-Ala peptide 1 has the largest population of intramolecularly hydrogen bonded species while the D-Ala peptide 2 has the lowest. This conclusion is clearly consistent with NMR results.

#### Conclusions

Spectroscopic studies on the three acyclic hexapeptide Boc-Leu-Xxx(L-Ala, D-Ala, Gly)-Val-Leu-Aib-Val-OMe reveal that a single substitution at position 2 has a dramatic influence on the nature of the conformational distribution in solution. In a poorly solvating medium like  $\text{CDCl}_3$ , the NMR results support a major population of  $3_{10}$  helical conformations for peptide 1 (Xxx = L-Ala). This conclusion is supported by the observed CD spectra which are similar to those frequently observed for short Aib containing peptides. NOE data for peptide 1 suggest that extended conformations are also populated, a feature which is consistent with the inherent flexibility in short sequences. NMR and CD results for peptide 2 (Xxx = D-Ala) strongly support a non-helical conformation with a  $\gamma$ -turn favoured at Leu(1), a type II'  $\beta$ -turn centred at the D-Ala(2)-Val(3) segment and a type II  $\beta$ -turn at the Leu(4)-Aib(5) segment. The tendency of the Leu-Aib segment to adopt type II  $\beta$ -turns has been earlier established in both short and long peptide sequences in this laboratory.<sup>29,30</sup> The type II'  $\beta$ -turn for the D-Ala-Val segment is in accord with the known stability of such conformation in a heterochiral sequences.<sup>14,16</sup> The spectroscopic data for peptide 3 (Xxx = Gly) are clearly consistent with



**Fig. 4** Partial ROESY spectra (400 MHz) of peptides 1,2,3 in  $\text{CDCl}_3$ . Top panel shows  $\text{C}^\alpha\text{H} \leftrightarrow \text{NH}$  NOEs and lower panel shows  $\text{NH} \leftrightarrow \text{NH}$  NOEs. Assignments are marked on the 1D spectra using the one letter code. Peptide concentration *ca.* 7–8 mM. (a) Peptide 1 (Xxx = L-Ala), (b) peptide 2 (Xxx = D-Ala), (c) peptide 3 (Xxx = Gly).

a significantly greater degree of conformational flexibility. Analysis of CD and NMR results suggests that the  $3_{10}$  helical conformational state characterized for 1 and the multiple  $\beta$ -turn conformation suggested for 2 are simultaneously populated in the case of peptide 3. This conclusion is stereochemically reasonable since the Gly 2 residue can indeed adopt conformations accessible for both L-Ala and D-Ala residues. The results of the present study demonstrate that folded multiple conformations

can be appreciably populated in short acyclic sequences. Stabilization of a specific conformation may be achieved by appropriate placement of D-Ala and Aib residues. Specific diagnostic spectral parameters may be used to establish conformational heterogeneity. The characterization of distinct conformational states in equilibrium may prove valuable in computer simulations which seek to explore the nature of conformational states accessible to short peptides.

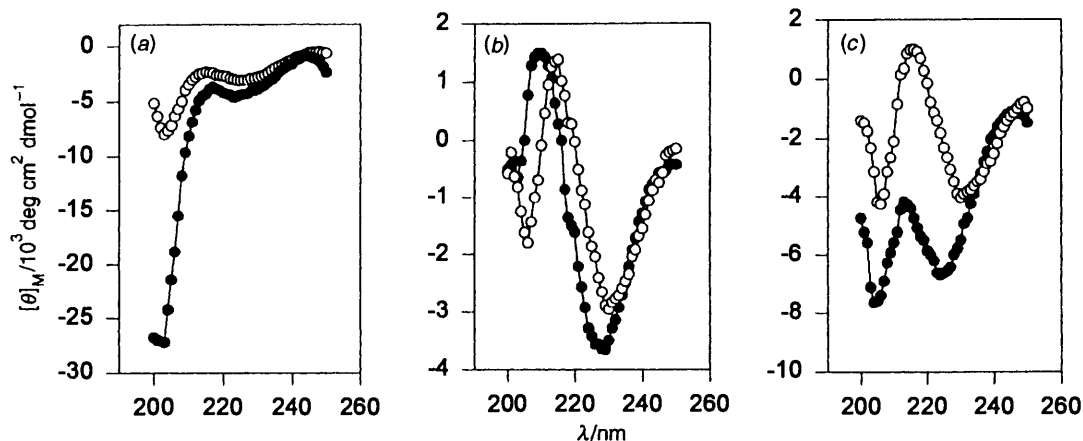


Fig. 5 CD spectra of peptides in MeOH (○) and TFE (●). Peptide concentration  $1.43 \times 10^{-3}$  M for 1, 2 and  $1.4 \times 10^{-3}$  M for 3. (a) Peptide 1 (Xxx = L-Ala), (b) Peptide 2 (Xxx = D-Ala), (c) Peptide 3 (Xxx = Gly).

## Experimental

### Materials and methods

The peptide was synthesized by conventional solution phase methods using a racemisation free fragment condensation strategy. The *tert*-butoxycarbonyl (Boc) group was used for N-terminal protection and the C-terminal was protected as a methyl ester (OMe). Deprotection was achieved by 98% formic acid or saponification, respectively. All intermediates were characterized by  $^1\text{H}$  NMR (80 MHz) and thin layer chromatography (TLC) on silica gel and used without further purification. The final peptides were purified by medium pressure liquid chromatography (MPLC) on a  $\text{C}_{18}$  (40–60  $\mu$ ) column followed by HPLC purification on a  $\text{C}_{18}$  (10  $\mu$ ) column using methanol–water gradient elution. Peptide homogeneity was demonstrated by analytical HPLC ( $\text{C}_{18}$  5  $\mu$ ) and complete assignment of 400 MHz  $^1\text{H}$  NMR spectra.

All NMR studies were carried out on a Bruker AMX-400 spectrometer. Resonance assignments were done using DFQ COSY and ROESY spectra.<sup>18</sup> All 2D experiments were recorded in phase sensitive mode by using the time proportional phase incrementation. 1024 and 512 data points were used in  $t_2$  and  $t_1$  dimensions, respectively. The resultant data set was zero filled to finally yield  $1\text{K} \times 1\text{K}$  data points. A shifted square sine bell window was used in both dimensions. Spectral widths were in the region of 4500 Hz. Peptide concentration was 7–8 mM and the probe temperature was maintained at 298 K. CD spectra were recorded on a JASCO J-500 spectropolarimeter using a 1 mm path-length cell.

### Peptide synthesis

**Boc-Ala-Val-OMe (1).** Boc Ala-OH (1.32 g, 6.98 mmol) was dissolved in 10 ml of dichloromethane (DCM) and cooled in an ice bath. H-Val-OMe, isolated from 2.33 g (13.96 mmol) of the hydrochloride by neutralization, subsequent extraction with ethyl acetate and concentration (5 ml), was added followed by 1.43 g (6.98 mmol) of dicyclohexylcarbodiimide (DCC). The reaction mixture was allowed to warm to room temp. and stirred for 12 h. Dichloromethane was evaporated, the residue redissolved in ethyl acetate and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 M  $\text{Na}_2\text{CO}_3$  ( $3 \times 30$  ml), 1 M HCl ( $3 \times 30$  ml) and brine ( $3 \times 30$  ml), dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated to yield 2.42 g (96%) of white solid, which was directly used in further steps.

**Boc-Leu-Ala-Val-OMe (2).** To 2.40 g (7.94 mmol) of 1, formic acid (18 ml) was added and the removal of the Boc group was monitored by TLC. After complete deprotection, the formic acid was removed *in vacuo*. The residue was taken in water (25 ml) and washed with diethyl ether ( $2 \times 30$  ml). The pH of the aqueous solution was then adjusted to 8 with  $\text{Na}_2\text{CO}_3$  and

extracted with EtOAc ( $4 \times 30$  ml). The EtOAc was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated (5 ml). The dipeptide free base was added to an ice cold solution of Boc-Leu-OH (0.88 g, 3.81 mmol) in 10 ml DMF followed by DCC (0.784 g, 3.81 mmol) and HOBT (2-hydroxybenzothiazole) (0.52 g, 3.81 mmol) and stirred for 48 h. The reaction was worked up as described for 1. Yield 1.32 g (38%).

**Boc-Leu-Ala-Val-OH (3).** 1.3 g (4.13 mmol) of 2 was dissolved in methanol (40 ml) and 2 M NaOH (5 ml) was added. The mixture was stirred at room temp. and the progress of the reaction was followed by TLC. After complete saponification, methanol was evaporated. The reaction mixture was diluted with water and washed with diethyl ether ( $2 \times 30$  ml). The aqueous layer was acidified with 1 M HCl and extracted with EtOAc ( $3 \times 30$  ml). The combined ethyl acetate layer was dried over  $\text{Na}_2\text{SO}_4$  and evaporated to a solid which was used directly in further steps. Yield 1.2 g (92%).

**Boc-D-Ala-Val-OMe (4).** 1.89 g (10 mmol) of Boc-D-Ala-OH was dissolved in dichloromethane (15 ml) and cooled in an ice bath. H-Val-OMe, isolated from 3.35 g (20 mmol) of the hydrochloride was added, followed by DCC (2.06 g, 10 mmol). The reaction mixture was allowed to warm to room temp. and stirred for 24 h. Work up was as described for 1. Yield 2.5 g (70%).

**Boc-Leu-D-Ala-Val-OMe (5).** Boc-Leu-OH (2.15 g, 9.3 mmol) was coupled to the free dipeptide free base, isolated from 4 (2.5 g 18.27 mmol) as described in the case of 2 in DMF (10 ml), using DCC (1.91 g, 9.3 mmol) and HOBT (1.26 g, 9.3 mmol). The reaction mixture was stirred for 48 h. The reaction mixture was worked up as described for 2. Yield 1.75 g (45%).

**Boc-Leu-D-Ala-Val-OH (6).** 1.70 g (5.09 mmol) of 5 was dissolved in methanol (50 ml), 2 M NaOH (5 ml) was added and the solution was stirred at room temp. After complete saponification, the mixture worked up as for 3. Yield 1.6 g (94%).

**Boc-Leu-Gly-OH (7).** 2.25 g (6.85 mmol) of Boc-Leu-OSu (Su = succinimido) was dissolved in dry tetrahydrofuran (THF) (10 ml) and a solution of Gly-OH (0.6 g, 8 mmol) and triethylamine (0.98 ml) in  $\text{H}_2\text{O}$  (10 ml) was added. After stirring at room temp. for 12 h, THF was evaporated. The aqueous solution was acidified (pH *ca.* 2) with 1 M HCl and extracted with EtOAc ( $3 \times 30$  ml). The combined organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated to give a white solid. Yield 1.85 g (84%).

**Boc-Leu-Gly-Val-OMe (8).** Boc-Leu-Gly-OH (1.8 g, 6.33 mmol) was cooled in ice and coupled to H-Val-OMe, isolated from 2.6 g (15 mmol) hydrochloride in DMF using DCC (1.64 g, 7.28 mmol) and HOBT (0.99 g, 7.28 mmol). The reaction mixture was allowed to warm to room temp. and stirred for 48 h. The reaction mixture was worked up as described for 2. Yield 2.0 g (74%).

**Boc-Leu-Gly-Val-OH (9).** 2.0 g of 8 was dissolved in metha-

nol (50 ml) and 2 M NaOH (5 ml) was added. The reaction mixture was stirred at room temp. After complete saponification, work up was as mentioned for **3**. Yield 1.8 g (90%).

**Boc-Leu-Ala-Val-Leu-Aib-Val-OMe (10)**. To 0.98 g (2.28 mmol) of Boc-Leu-Aib-Val-OMe,<sup>25,26</sup> formic acid (9 ml) was added and removal of the Boc group was monitored by TLC. After complete deprotection, the formic acid was removed *in vacuo*. The residue was taken in water (10 ml) and washed with diethyl ether (2 × 20 ml). The pH of the aqueous layer was then adjusted to *ca.* 8 with Na<sub>2</sub>CO<sub>3</sub> and extracted with EtOAc (4 × 30 ml). The EtOAc extracts were pooled, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to about 5 ml; this sample was ninhydrin positive. This free tripeptide base was coupled to an ice-cooled solution of Boc-Leu-Ala-Val-OH (**3**) (1.18 g, 2.94 mmol) in DMF (10 ml) using DCC (0.6 g, 2.94 mmol) and HOBT (0.4 g, mmol). The reaction mixture was allowed to warm to room temp. and stirred for 4 d. After this period, EtOAc (15 ml) was added and the dicyclohexylurea (DCU) filtered off. The organic layer was washed with 2 M HCl (3 × 30 ml), 1 M sodium carbonate (3 × 30 ml) and brine (3 × 30 ml), dried over anhydrous sodium sulfate and evaporated to yield 1.49 g of white solid (76%). The final compound was purified and characterized <sup>1</sup>H NMR (Table 1). Mp 175–178 °C.

**Boc-Leu-D-Ala-Val-Leu-Aib-Val-OMe (11)**. 1.5 g (3.5 mmol) of Boc-Leu-Aib-Val-OMe<sup>22</sup> was dissolved in 98% formic acid (12 ml). The removal of the Boc group was monitored by TLC. After complete deprotection, the tripeptide free base was coupled to Boc-Leu-D-Ala-Val-OH (**6**) (1.0 g, 2.4 mmol) in DMF (10 ml) using DCC (0.494 g, 2.4 mmol) and HOBT (0.327 g, 2.4 mmol). The mixture was stirred for 4 d, DCU filtered off and the product worked up as described in case of **10**. Yield 1.11 g (51%). The title compound was purified and characterized by <sup>1</sup>H NMR (Table 1). Mp 129–130 °C.

**Boc-Leu-Gly-Val-Leu-Aib-Val-OMe (12)**. 1.75 g (5.07 mmol) of Boc-Leu-Aib-Val-OMe was dissolved in 98% formic acid (15 ml). The removal of the Boc group was monitored by TLC. The free base was isolated as described as in the case of **10** and was used without further purification. 2 g (5.15 mmol) of **9** was cooled in an ice bath and coupled with the tripeptide with free base in DMF (10 ml) using DCC (1.06 g, 5.07 mmol) and HOBT (0.7 g, 5.07 mmol). The reaction mixture was stirred for 4 d, DCU filtered off and the product worked up as described for **10** to yield 0.94 g (28%). The title peptide was purified and characterized by <sup>1</sup>H NMR (Table 1). Mp 125–130 °C.

### Acknowledgements

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