

Stereochemistry of α -Aminoisobutyric Acid Peptides in Solution: Conformations of Decapeptides with a Central Triplet of Contiguous L-Amino Acids

HEMALATHA BALARAM, M. SUKUMAR, and P. BALARAM,*
*Molecular Biophysics Unit, Indian Institute of Science,
Bangalore 560 012, India*

Synopsis

The decapeptides Boc-Aib-L-Val-Aib-Aib-(L-Val)₃-Aib-L-Val-Aib-OMe and Boc-Aib-L-Leu-Aib-Aib-(L-Leu)₃-Aib-L-Leu-Aib-OMe have been studied in CDCl₃ and (CD₃)₂SO solutions by 270-MHz¹H-nmr. In CDCl₃, the presence of eight intramolecularly hydrogen-bonded NH groups has been established, consistent with a 3_{10} -helical conformation, for both decapeptides. In (CD₃)₂SO, only seven solvent-shielded NH groups are observed, supporting either an α -helical conformation or a partially unfolded 3_{10} -helix. Ir studies provided supporting evidence for intramolecularly hydrogen-bonded structures in CHCl₃, while CD studies suggest helical conformation in both decapeptides in various solvents. CD studies also support helical folding in the C-terminal hexapeptides. The central triplet of L-amino acids appears to destabilize 3_{10} -helical conformations in polar solvents like (CD₃)₂SO.

INTRODUCTION

α -Aminoisobutyryl (Aib) residues have been shown to promote helical folding in peptides.¹⁻¹⁰ Some controversy exists in the literature as to whether 3_{10} - or α -helical structures are preferred.^{3,4,9,11-13} Conformational energy calculations on N-acetyl-Aib-N'-methylamide have suggested that only limited regions of ϕ , ψ space are energetically accessible to Aib residues.¹⁴⁻¹⁶ The predicted energy minima encompass both 3_{10} - ($\phi \sim \pm 60^\circ$, $\psi \sim \pm 30^\circ$) and α - ($\phi \sim \pm 55^\circ$, $\psi \sim \pm 45^\circ$) helical conformations.¹ These structures show only a small difference in ϕ , ψ values, but can be distinguished on the basis of their intramolecular hydrogen-bonding patterns [α -helix 5 \rightarrow 1 (C₁₃); 3_{10} -helix 4 \rightarrow 1 (C₁₀)].¹⁷ Single-crystal x-ray diffraction studies on numerous short Aib-containing peptides, up to the pentapeptide level, have clearly established the tendency of Aib residues to promote 3_{10} -helical conformations.^{1,5,7} These structures have generally been characterized by the occurrence of two or three consecutive type III β -turns¹⁸ stabilized by 4 \rightarrow 1 hydrogen bonds. Crystal structures of longer Aib-containing peptides have shown less definitive structural preferences, with both 3_{10} - and α -helical structures being observed. While the 20-residue natural peptide alamethicin (Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol)¹⁹ and the

*To whom correspondence should be addressed at Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India.

synthetic 11-residue peptide Boc-Ala-Aib-Ala-Aib-Ala-Glu(OBzl)-Ala-Aib-Ala-Aib-Ala-OMe^{11,12,20} (Boc, *t*-butyloxycarbonyl) adopt α -helical structures in the solid state, 3_{10} -helical conformations have been established in the octapeptide *p*-BrBz-(Aib)₈-OtBu^{21,22} the heptapeptide Boc-Val-Aib-Val-Aib-Val-Aib-Val-OMe,²³ and the decapeptide Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe.²⁴ The C-terminal nonapeptide of alamethicin, Boc-Leu-Aib-Pro-Val-Aib-Aib-Glu(OBzl)-Gln-Phol crystallizes in a mixed $3_{10}/\alpha$ -helical conformation possessing three $5 \rightarrow 1$ and three $4 \rightarrow 1$ hydrogen bonds.²⁵

The interpretation of spectroscopic results in solution has necessarily been more controversial. While ¹³C-nmr²⁶ and CD^{2,3,12} results on Aib-containing peptides have often been interpreted in terms of α -helical conformations, ¹H-nmr studies of solvent-shielded NH groups have led to the proposal of 3_{10} -helical conformations for peptides up to 16 residues in length.^{9,27-31} More recently, nmr data on the naturally occurring Aib-containing fungal peptide, trichorzianine A III, has been interpreted in terms of 3_{10} -helical conformations.³² Undoubtedly, subtle sequence effects and the nature of the solvent may influence the observed solution conformations, while the presence of multiple conformations may further complicate spectroscopic interpretation. It is also uncertain if CD data can, in fact, distinguish 3_{10} - and α -helical structures,^{25,33,34} while several ambiguities are implicit in the interpretation of both ¹³C- and ¹H-nmr data. As part of a continuing investigation on the conformational and spectroscopic properties of Aib-containing peptides, this report describes studies on the synthetic decapeptides Boc-Aib-X-Aib-Aib-X-X-X-Aib-X-Aib-OMe (X = L-Val 1, X = L-Leu 2). These sequences were chosen in order to examine the influence of a central triplet of non-Aib residues on helical folding. These studies are relevant in establishing the precise role of sequence, the proportion and positioning of Aib residues, and the effect of the X-residue side chain in modulating the conformational preferences of Aib-containing peptides. Earlier studies from this laboratory have considered strictly alternating sequences of the type (Aib-X)_{*n*}.^{9,31,34-37}

EXPERIMENTAL

The scheme followed for the synthesis of the decapeptides Boc-Aib-X-Aib-Aib-X-X-X-Aib-X-Aib-OMe (X = L-Val 1, X = L-Leu 2) is summarized in Fig. 1. Illustrative procedures are briefly described below.

Couplings involving activation of chiral amino acids can lead to racemization, even in the presence of additives like 1-hydroxybenzotriazole (HOBt) when the amine component is sterically hindered as in Aib derivatives.³⁸ In the present scheme only a single step involved activation of C-terminal L-Leu or L-Val, i.e., in the preparation of Boc-X-Aib-X-Aib-OMe by a 3 + 1 procedure. In the case of X = L-Leu, no evidence for the formation of diastereomeric species was obtained [high performance liquid chromatography (HPLC), ¹³C-nmr], while for X = L-Val diastereomeric products were detectable requiring HPLC purification of the final product. All intermediate peptides were checked for homogeneity by thin layer chromatography (tlc) on silica gel [*R_f*(A), CHCl₃:MeOH 9:1; *R_f*(B), nBuOH:CH₃COOH:H₂O 4:1:1] and fully characterized by ¹H-nmr (60 MHz or 270 MHz).

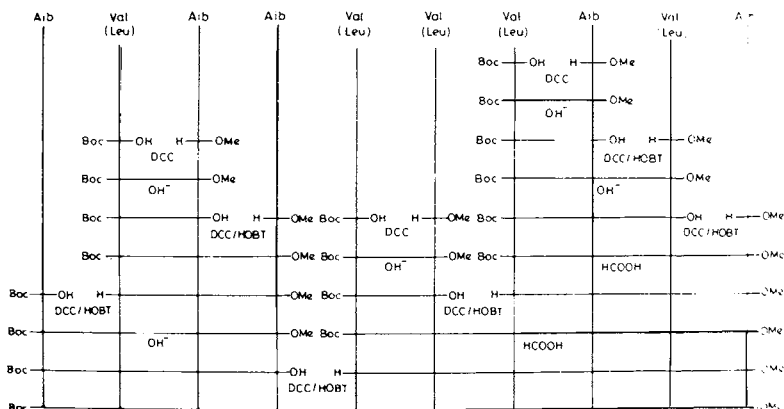


Fig. 1. The scheme for the synthesis of peptides Boc-Aib-Val-(Aib)₂-(Val)₃-Aib-Val-Aib-OMe and Boc-Aib-Leu-(Aib)₂-(Leu)₃-Aib-Leu-Aib-OMe.

SYNTHESIS OF PEPTIDES

Synthesis of Peptide 2

Boc-Leu-Aib-OMe

Boc-Leu-OH (1.61 g, 7 mmol) was dissolved in 7 mL of CH₂Cl₂ and cooled in an ice bath. Aib-OMe obtained from 1.5 g (10 mmol) of HCl · H-Aib-OMe was added, followed by 1.5 g (7.5 mmol) of N, N'-dicyclohexylcarbodiimide (DCC). The reaction mixture was stirred at 0°C for 4 h and at room temperature for 8 h. Dicyclohexyl urea (DCU) was filtered and the organic layer was washed successively with 1N HCl, 1N Na₂CO₃, and water. The organic layer was dried over anhydrous Na₂SO₄. Evaporation of CH₂Cl₂ yielded a white solid, homogeneous on tlc. Yield: 2.0 g (80%), mp = 132–134°C, R_f(A) = 0.78.

Boc-Leu-Aib-OH

Of Boc-Leu-Aib-OMe, 1.9 g (6 mmol) was dissolved in 10 mL of MeOH. Of 2N NaOH, 10 mL was added and the reaction mixture was stirred at room temperature. Conversion to the dipeptide acid was followed by tlc. After completion of the reaction, methanol was evaporated and the residue was diluted with water. The aqueous layer was washed with ether, acidified with 2N HCl, and extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to yield a solid, homogeneous on tlc. R_f(B) = 0.82. Yield: 1.8 g (95%), mp = 142–143°C [mp (lit) 142–145°C, 128–130°C⁴⁰] [α]_D²⁵ = -12.5 (c = 0.8 in MeOH) {[α]_D²⁵ (lit) -30.4°C³⁹, -23.3°C⁴⁰}.

Boc-Leu-Aib-Aib-OMe

Of Boc-Leu-Aib-OH, 0.9 g (2.2 mmol) was dissolved in 3 mL of dimethylformamide (DMF) and cooled in an ice bath. Aib-OMe obtained from 0.75 g

(5 mmol) of HCl · Aib-OMe was added, followed by 0.6 g (3 mmol) of DCC and 0.4 g (3 mmol) of 1-hydroxybenzotriazole (HOBT). The reaction mixture was stirred at 0°C for 4 h and at room temperature for 20 h. DCU was filtered and the reaction mixture worked up as described earlier to yield a white solid, homogeneous on tlc. Yield: 1.0 g (75%). $R_f(A) = 0.70$, mp = 120–122°C, $[\alpha]_D^{25} = -7.0$ (c = 2 in MeOH).

H-Leu-Aib-Aib-OMe

Of Boc-Leu-Aib-Aib-OMe, 0.82 g (2 mmol) was dissolved in 4 mL of 98% formic acid. Conversion to the formate salt was followed by tlc. After completion of the reaction, formic acid was evaporated and residue was dissolved in 10 mL of water. The aqueous layer was washed with ether, neutralized with Na₂CO₃, and extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to yield a gum, which was homogeneous and ninhydrin positive on tlc. Yield: 0.65 g (95%).

Boc-Aib-Leu-Aib-Aib-OMe

Of H-Leu-Aib-Aib-OMe, 0.63 g (2 mmol) was dissolved in 2 mL of DMF and coupled to 0.4 g (2 mmol) of Boc-Aib-OH using 0.4 g (2 mmol) of DCC and 0.27 g (2 mmol) of HOBT, as described earlier. Yield: 0.77 g (80%), mp = 163–165°C.

Boc-Aib-Leu-Aib-Aib-OH

Of Boc-Aib-Leu-Aib-Aib-OMe, 0.7 g (1.4 mmol) was saponified as described earlier. Yield: 0.45 g (60%). $R_f(A) = 0.42$, $R_f(B) = 0.76$, mp = 146–148°C, $[\alpha]_D^{25} = -6.5$ (c = 2 in MeOH).

Boc-Leu-Leu-OMe

Of Boc-Leu-OH, 1.15 g (5 mmol) was coupled to Leu-OMe derived from 1.3 g (7 mmol) of HCl-Leu-OMe as described earlier. Yield: 1.5 g (90%). $R_f(A) = 0.80$, mp = 120–122°C, [mp (lit) 131–134°C, 138–139°C⁴¹; 132–133°C⁴² $[\alpha]_D^{25} = -29.5$ (c = 2 in MeOH) ($[\alpha]_D$ (lit) = -50⁴²).

Boc-Leu-Leu-OH

Of Boc-Leu-Leu-OMe, 1.1 g (3 mmol) was saponified as described earlier. Yield: 1.0 g (95%), $R_f(A) = 0.64$, $R_f(B) = 0.80$, mp = 125–127°C.

Boc-Leu-Aib-Leu-OH

Of Boc-Leu-Aib-OH, 0.8 g (2.5 mmol) was coupled to Leu-OMe derived from 0.54 g (3 mmol) of HCl-Leu-OMe using equivalent amounts of DCC and HOBT as described earlier. Yield: 0.84 g (80%). Of the crude peptide ester, 0.8 g (1.8 mmol) was directly saponified as described in the case of Boc-Leu-Aib-OH. Yield: 0.7 g (92%), $R_f(A) = 0.43$, $R_f(B) = 0.80$, mp = 113–115°C, $[\alpha]_D^{25} = -16.5$ (c = 2.0 in MeOH).

Boc-Leu-Aib-Leu-Aib-OMe

Of Boc-Leu-Aib-Leu-OH, 0.65 g (1.5 mmol) was coupled to Aib-OMe derived from 0.4 g (2 mmol) of HCl-Aib-OMe as described in the case of Boc-Leu-Aib-Aib-OMe. Yield: 0.5 g (66%). $R_f(A) = 0.62$, $[\alpha]_D^{25} = -15.5$ ($c = 2.0$ in MeOH).

Boc-Leu-Leu-Leu-Aib-Leu-Aib-OMe

Of Boc-Leu-Aib-Leu-Aib-OMe, 1 g (1.9 mmol) was dissolved in 4 mL of 98% formic acid. The free base was isolated as described earlier. $R_f(A) = 0.45$, mp = 192–194°C, $[\alpha]_D^{25} = -20.5$ ($C = 2.0$ in MeOH). The tetrapeptide free base was coupled to an equivalent amount of Boc-Leu-Leu-OH and the reaction worked up as described earlier. Evaporation of EtOAc yielded a white solid. Yield: 1.05 g (75%), $R_f(A) = 0.62$, mp = 184–186°C, $[\alpha]_D^{25} = -29.5$ ($c = 2.0$ in MeOH).

Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe

Of Boc-Leu-Leu-Leu-Aib-Leu-Aib-OMe, 0.5 g (0.75 mmol) was deprotected using 98% formic acid as described earlier. A white solid, homogeneous on tlc and ninhydrin positive, was obtained.

Of Boc-Aib-Leu-Aib-Aib-OH, 0.24 g (0.5 mmol) was coupled to 0.35 g (0.5 mmol) of the hexapeptide free base using DCC and HOBT as described earlier. The crude decapeptide was purified on a silica-gel column using 2% MeOH in CHCl₃ as eluent. Yield: 0.09 g (20%), $R_f(A) = 0.58$, mp = 206–208°C.

TABLE I
Physical Characteristics of the Intermediates in the Synthesis of 1

Peptide	mp (°C)	R_f^a		$[\alpha]_D^{25b}$
		A	B	
Boc-Val-Aib-OMe	114–116 ^d (lit. 140 ³⁹ ; 115–118 ⁴³)	0.64	—	-29
Boc-Val-Aib-Aib-OMe	140–142 ^d (lit. 87–88 ³⁹ ; 140 ⁴³)	0.56	—	-10 (lit. -9.4 ³⁹ ; -17.5 ⁴³)
Boc-Aib-Val-Aib-Aib-OMe	163–165	0.59	—	-4 ^c
Boc-Val-Val-OMe	149–150 ^d (lit. 167–168 ⁴⁴ ; 165–166 ⁴⁵)	0.73	—	-42 (lit. -9 ⁴⁵)
Boc-Val-Val-OH	138–140	—	0.82	-22
Boc-Val-Aib-Val-OMe	110–112	0.58	—	-20
Boc-Val-Aib-Val-OH	95–96	—	0.70	-10
Boc-Val-Aib-Val-Aib-OMe	123–125	0.51	—	-17
Boc-(Val) ₃ -Aib-Val-Aib-OMe	167–168	0.50	—	—
Boc-Aib-Val-(Aib) ₂ -(Val) ₃ - Aib-Val-Aib-OMe	197–199	0.45	—	—

^a R_f : solvent systems—A, Chloroform: Methanol (9:1); B, *n*-Butanol: Acetic Acid: Water (4:1:1).

^b $[\alpha]_D^{25}$ ($c = 0.2$ in methanol).

^c $[\alpha]_D^{25}$ ($c = 0.27$ in methanol).

^dDi- and tripeptide intermediates in this study were not extensively purified. Differences in physical characteristics with reported data from the literature may also arise due to solvent incorporation in the solids, and solvent and temperature variations in the case of optical rotation values.

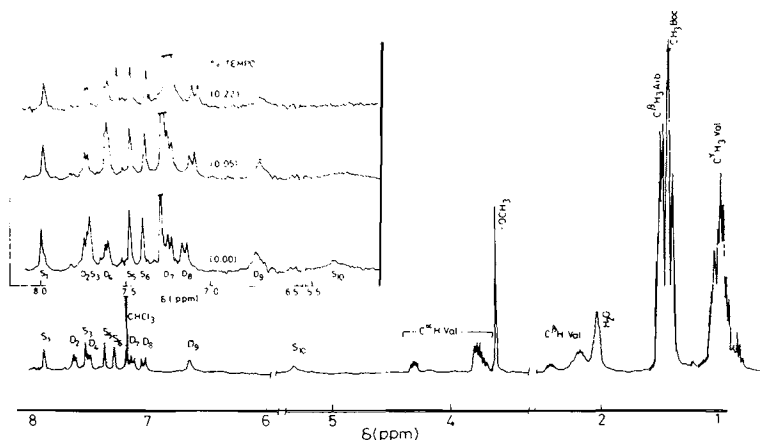


Fig. 2. The 270-MHz ^1H -nmr spectrum of Boc-Aib-Val-(Aib) $_2$ -(Val) $_3$ -Aib-Val-Aib-OMe, ($9.6 \times 10^{-3}M$) in CDCl_3 . Inset: NH proton resonances in the presence of varying concentrations (%w/v) of the nitroxide, TEMPO. Radical concentrations are indicated against the traces.

Synthesis of Peptide 1

Procedures entirely analogous to that described above for **2** were followed. The physical characteristics of key intermediates are listed in Table I. **1** was purified by silica-gel column chromatography to yield a white solid. HPLC analysis on a Lichrosorb RP-18 column, using a linear gradient of methanol in water (80–95% methanol in 20 min, flow rate 0.8 mL min^{-1} , detection 226 nm) showed the presence of an impurity, presumably a diastereomeric peptide due to racemization at Leu(9), as noted earlier. Peptide **1** was therefore further purified by HPLC under the above conditions to yield a homogeneous solid. $\text{mp} = 197\text{--}199^\circ\text{C}$, $R_f(\text{A}) = 0.45$.

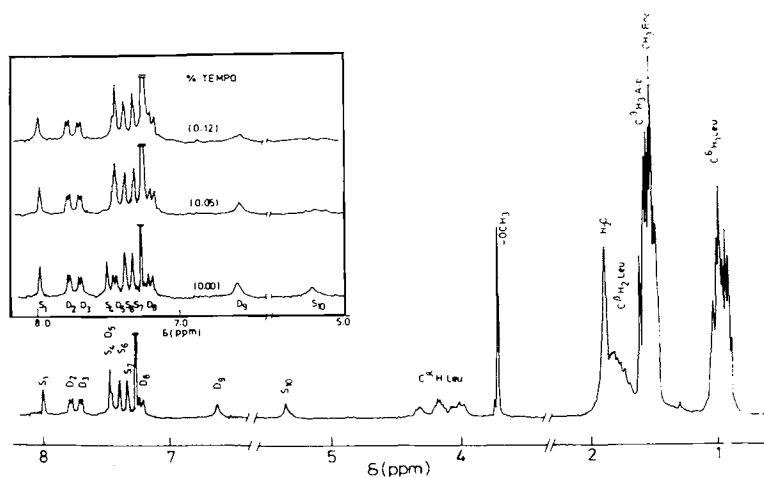


Fig. 3. The 270-MHz ^1H -nmr spectrum of Boc-Aib-Leu-(Aib) $_2$ -(Leu) $_3$ -Aib-Leu-Aib-OMe ($9.3 \times 10^{-3}M$) in CDCl_3 . Inset: NH proton resonances in the presence of varying concentrations (%w/v) of the nitroxide, TEMPO. Radical concentrations are indicated against the traces.

Characterization of Peptides 1 and 2

1 and 2 yielded 270-MHz ^1H -nmr spectra fully consistent with their structures (Figs. 2 and 3). The 67.89-MHz ^{13}C -nmr spectrum of 2 showed all expected resonances.

Spectroscopic Studies

Nmr spectra were recorded on a Bruker WH-270 spectrometer at an operating frequency of 270 MHz for ^1H -nmr and 67.89 MHz for ^{13}C -nmr at the Sophisticated Instruments Facility, Indian Institute of Science, Bangalore. Hydrogen-bonding studies were carried out as described earlier.^{27,30} Ir spectra were recorded on a Perkin-Elmer model 297 spectrometer in dry CHCl_3 solutions. CD spectra were recorded on a JASCO J-20 spectropolarimeter using 1-mm pathlength cells. Band intensities are expressed as molar ellipticities $[\theta]_{\text{M}}$, deg cm^2 decimol $^{-1}$.

RESULTS AND DISCUSSION

Of peptides 1 and 2 in CDCl_3 , 270-MHz ^1H -nmr spectra are shown in Figs. 2 and 3, respectively. All ten amide NH protons of both peptides are easily distinguished in CDCl_3 . In $(\text{CD}_3)_2\text{SO}$ there is greater overlap of the NH resonances. The NH resonances are designated as D_n (doublets, Val or Leu NH) and S_n (singlets, Aib NH), where the subscript n refers to the order of appearance of the resonance from low field in CDCl_3 . The corresponding assignment in $(\text{CD}_3)_2\text{SO}$ is based on spectra obtained in CDCl_3 - $(\text{CD}_3)_2\text{SO}$ mixtures. The chemical-shift values of the NH resonances in these solvents are summarized in Tables II and III, for peptides 1 and 2, respectively. The only resonance that can be unequivocally assigned is the urethane NH [Aib(1)NH, S_{10}] by virtue of its high-field position in CDCl_3 .^{9,27-29}

Intramolecularly hydrogen-bonded (solvent-shielded) NH groups in the peptides were identified⁴⁶ using the following criteria: (1) temperature coeffi-

TABLE II
NH Chemical Shifts and Temperature Coefficients
in Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe (1)

	NH (ppm) ^a		$d\delta/dT^b$ (ppm/ $^{\circ}\text{C}$)
	CDCl_3	$(\text{CD}_3)_2\text{SO}$	
S_1	7.96	8.35	0.0052
D_2	7.70	7.58	0.0017
S_3	7.61	7.72	0.0039
D_4	7.59	7.58	0.0017
S_5	7.45	7.81	0.0036
S_6	7.37	7.47	0.0022
D_7	7.21	7.29	0.0013
D_8	7.10	6.88	0.0015
D_9	6.69	7.72	0.0042
S_{10}	5.32	7.29	0.0058

^aConcentration of the peptide used was $9.6 \times 10^{-3}M$.

^bThese values were measured in $(\text{CD}_3)_2\text{SO}$.

TABLE III
NH Chemical Shifts and Temperature Coefficients
in Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe (**2**)

	NH (ppm) ^a		$d\delta/dT^b$ (ppm/°C)
	CDCl ₃	(CD ₃) ₂ SO	
S ₁	8.00	8.26	0.0030
D ₂	7.78	7.74	0.0025
D ₃	7.70	7.70	0.0024
S ₄	7.48	7.32	0.0016
D ₅	7.46	7.34	0.0016
S ₆	7.38	7.71	0.0032
S ₇	7.33	7.71	0.0049
D ₈	7.20	7.06	0.0013
D ₉	6.59	8.00	0.0046
S ₁₀	5.25	7.30	0.0047

^aConcentration of the peptide used was $9.3 \times 10^{-3}M$.

^bThese values were measured in (CD₃)₂SO.

coefficients of NH chemical shifts ($d\delta/dT$) in a polar hydrogen-bonding solvent, (CD₃)₂SO,⁴⁷ (2) solvent dependence of NH chemical shifts in CDCl₃-(CD₃)₂SO mixtures of varying composition,⁴⁸ and (3) paramagnetic radical-induced line broadening of NH resonances in CDCl₃.^{49,50} The $d\delta/dT$ values for peptides **1** and **2** are listed in Tables II and III, respectively. In **2** S₇, D₉, and S₁₀ have high $d\delta/dT$ values (> 0.004 ppm/K), indicating their solvent-exposed nature. Of the remaining seven NH resonances in **2**, five have low $d\delta/dT$ values (< 0.003 ppm/K) while two (S₁ and S₆) have moderately low values, suggestive of their relative inaccessibility to solvent molecules. Also, in the case of peptide **1** three NH resonances (S₁, D₉, and S₁₀) have high $d\delta/dT$ values. Of the remaining seven NH resonances, five have low $d\delta/dT$ values (D₂, D₄, S₆, D₇, D₈). Two NH groups, S₃ and S₅ exhibit intermediate temperature dependences ($d\delta/dT$ values of 0.0036 and 0.0039 ppm/K). Such intermediate $d\delta/dT$ values are indicative of partial exposure to solvent, which could arise in principle, due to involvement in relatively weak intermolecular hydrogen bonds. The dependence of NH chemical shifts on solvent composition in CDCl₃-(CD₃)₂SO mixtures is shown in Fig. 4. In both peptides **1** and **2**, only the D₉ and S₁₀ resonances show large downfield shifts with increasing (CD₃)₂SO concentration, up to about 40% vol., indicating the exposed nature of these NH groups. All the other eight NH groups in **1** and **2** display a marked insensitivity to solvent composition, establishing a high degree of shielding from the solvent. Further support for this conclusion is obtained from the results of paramagnetic radical-induced line broadening studies. Addition of the nitroxide radical, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) to a CDCl₃ solution of the peptides, causes a selective broadening of the D₉ and S₁₀ resonances in both **1** and **2** (see insets to Figs. 2 and 3). The dependence of NH resonance linewidths on radical concentration is summarized in Fig. 5, for a few well-resolved resonances. Quantitative measurements for all resonances were rendered difficult due to partial overlap.

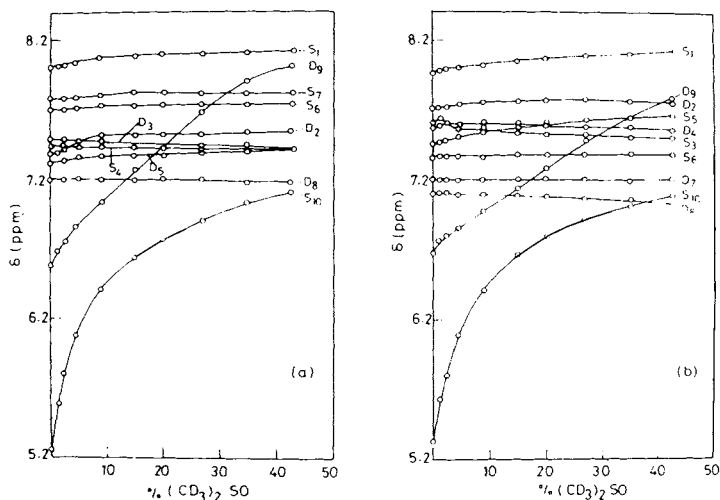


Fig. 4. Solvent dependence of NH proton chemical shifts of peptides in $CDCl_3$ - $(CD_3)_2SO$ mixture. Left: Boc-Aib-Leu-(Aib)₂-(Leu)₃-Aib-Leu-Aib-OMe; right: Boc-Aib-Val-(Aib)₂-(Val)₃-Aib-Val-Aib-OMe.

Nevertheless, it is clear that only two NH resonances, S₁₀ and D₉, in both peptides are appreciably perturbed by interactions with the paramagnetic species.

The above nmr experiments provide evidence for the solvent-shielded nature of *eight* NH groups in chloroform solutions in peptides 1 and 2. The inaccessibility of these groups is presumably due to their involvement in intramolecular hydrogen bonding. Ir studies in chloroform solutions provide additional support for the presence of intramolecularly hydrogen-bonded conformations in these peptides. Figure 6 shows the NH stretching bands (ν_{NH}) in peptides 1 and 2 over the concentration range 0.25 mM–4 mM. Two bands at $\sim 3440\text{ cm}^{-1}$ and $\sim 3330\text{ cm}^{-1}$, corresponding to free and intramolecularly hydrogen-bonded NH groups, respectively, are seen over the entire concentration range.⁵¹

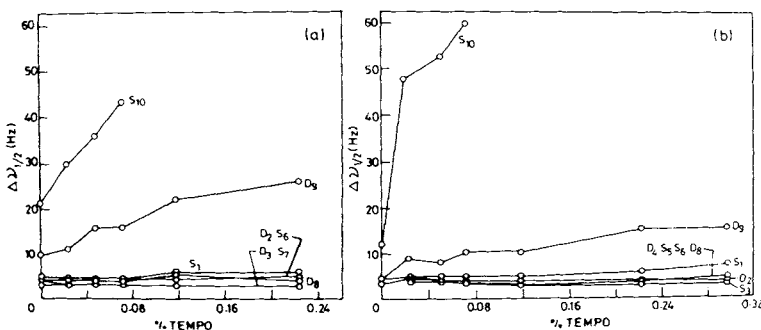


Fig. 5. Line widths ($\Delta\nu_{1/2}$) of NH proton resonances as a function of radical (TEMPO) concentration in $CDCl_3$: Left: Boc-Aib-Leu-(Aib)₂-(Leu)₃-Aib-Leu-Aib-OMe; right: Boc-Aib-Val-(Aib)₂-(Val)₃-Aib-Val-Aib-OMe.

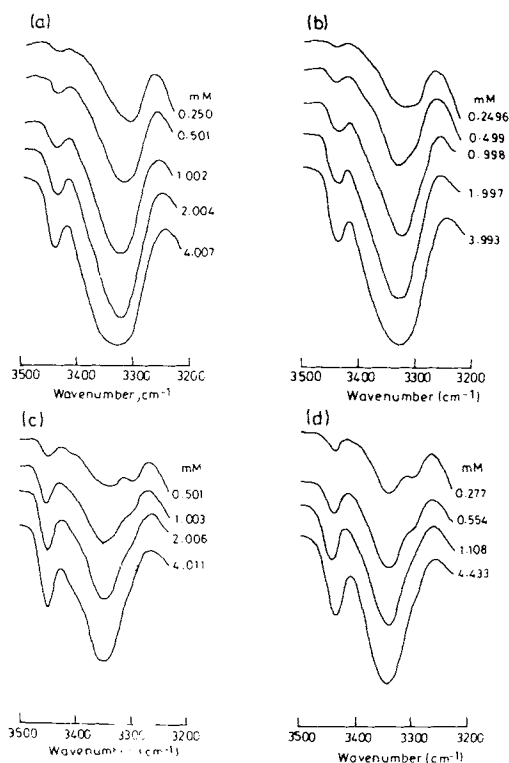


Fig. 6. Ir spectra (NH stretching bands) in CHCl_3 at various peptide concentrations as indicated against the traces. (a) Boc-Aib-Leu-(Aib)₂-(Leu)₃-Aib-Leu-Aib-OMe. (b) Boc-Aib-Val-(Aib)₂-(Val)₃-Aib-Val-Aib-OMe. (c) Boc-(Leu)₃-Aib-Leu-Aib-OMe. (d) Boc-(Val)₃-Aib-Val-Aib-OMe.

The nmr results establish the following points:

1. Peptides **1** and **2** adopt folded conformations in CDCl_3 , which are characterized by the presence of eight strongly solvent-shielded NH groups.
2. In $(\text{CD}_3)_2\text{SO}$ three NH resonances are solvent exposed in both peptides ($\text{D}_9, \text{S}_{10}, \text{S}_1$ in **1** and $\text{S}_7, \text{D}_9, \text{S}_{10}$ in **2**), suggesting that only seven NH groups are intramolecularly hydrogen bonded.

The results in CDCl_3 are consistent with the occurrence of fully 3_{10} -helical conformations stabilized by eight intramolecular $4 \rightarrow 1$ hydrogen bonds [Fig. 7(A)]. In such a structure the NH groups of the two amino terminal residues [Aib(1) and Leu(2) or Val(2)] are fully solvent exposed. The experimental results indeed establish that Aib(1) NH and a doublet NH resonance are solvent exposed in both peptides. The proposed 3_{10} -helical conformation is also consistent with the stereochemical preferences of Aib-rich sequences.^{1,4-7} Further, 3_{10} -helices have indeed been demonstrated in the solid state for sequences up to 10 residues in length.²⁴

The results in $(\text{CD}_3)_2\text{SO}$ suggest the presence of conformations having only seven intramolecular hydrogen bonds. This observation is compatible with the occurrence of conformations which are fully α -helical, where seven $5 \rightarrow 1$ hydrogen bonds are expected [Fig. 7(B)]. Three exposed NH groups in such a structure would give rise to two singlet [Aib(1) and Aib(3)] and one doublet

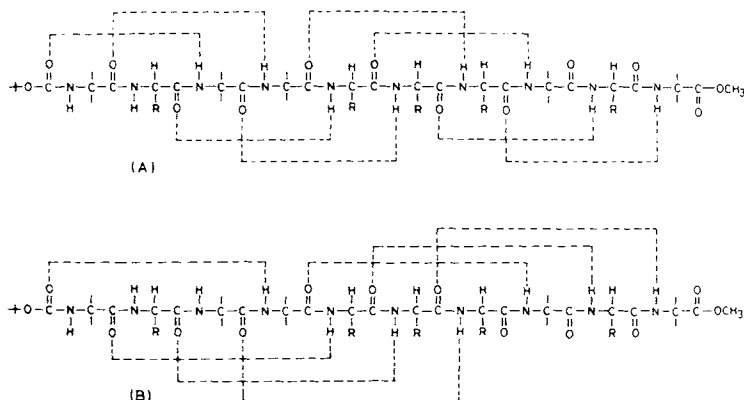


Fig. 7. Schematic hydrogen-bonding scheme proposed for the decapeptides, Boc-Aib-X-(Aib)₂-(X)₃-Aib-X-Aib-OMe (X = L-Val, L-Leu). (A) 3_{10} -helical conformation in CDCl_3 . (B) α -helical conformation in $(\text{CD}_3)_2\text{SO}$.

resonance. This is indeed the case. Alternative structures involving a partial opening of the 3_{10} -helical structure that result in the loss of one hydrogen bond cannot be ruled out. The crystallographic observation of an α -helix in an undecapeptide, with a central triplet of L-amino acid residues,^{11,12,20} provides support for the population of α -helical structures in the cases of peptides 1 and 2. An assumption implicit in these and earlier nmr studies is that only ideal 3_{10} - or α -helical structures are considered. The formation of bifurcated hydrogen bonds where a single CO group is bonded to two NH groups can result in mixed helical structures.

In the preceding analysis of the nmr results, peptide aggregation has been disregarded. Earlier studies on Aib-containing peptides have shown that association of such peptides is not significant in $(\text{CD}_3)_2\text{SO}$ at the concentrations used.^{9,52,53} Peptide association is appreciable in CDCl_3 , but intermolecular interactions are mediated preferentially by solvent-exposed CO and NH groups. Consequently, molecular association does not affect the intramolecular hydrogen-bonding pattern observed in the monomers.^{52,53} It may be stressed that in the present study temperature dependences of NH chemical shifts have not been determined in CDCl_3 since this parameter does not permit a clear distinction between solvent-exposed and inaccessible NH groups in this solvent.^{9,52,53}

CD

Additional support for the formation of helical structures in peptides 1 and 2 is obtained from the CD studies summarized in Fig. 8 and Table IV. CD results on the hexapeptides Boc-(X)₃-Aib-X-Aib-OMe (X = L-Val 3, X = L-Leu 4) are also shown for comparison. In methanol, 2,2,2-trifluoroethanol (TFE), and trimethylphosphate (TMP), both 1 and 2 show two strong negative bands at 203–205 nm (one component of the exciton split π - π^* band) and 218–220 nm (n - π^* band). These spectra are characteristic of helical polypeptides,⁵⁴ with the exception that the n - π^* band is significantly weaker than the π - π^* band (Table IV). Such CD spectra have been ascribed quite often to α -helical

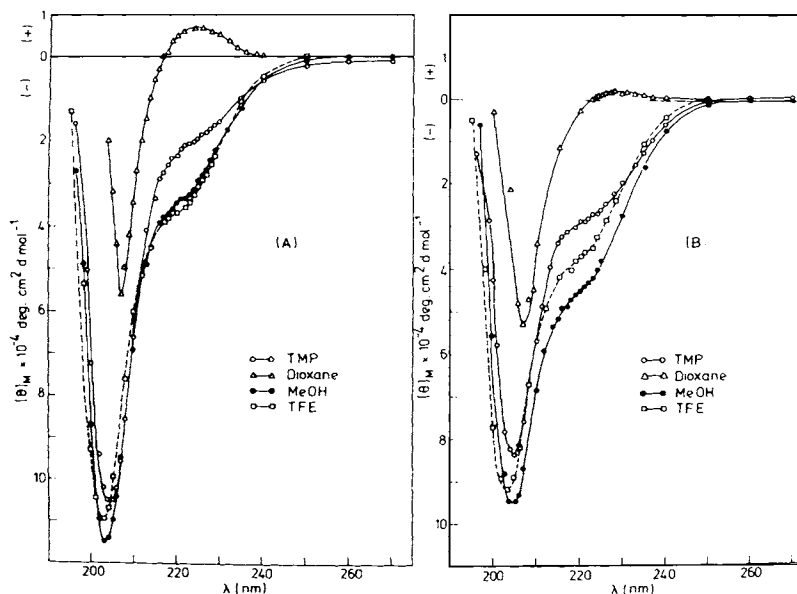


Fig. 8. CD spectra of peptides in various solvents. Left: Boc-Aib-Leu-(Aib)₂-(Leu)₃-Aib-Leu-Aib-OMe; right: Boc-Aib-Val-(Aib)₂-(Val)₃-Aib-Val-Aib-OMe. Peptide concentration ~ 1 mM.

conformations.^{2,3,12,55} However, 3_{10} -helical peptides have also been shown to yield similar spectra.^{33,34} A dramatically different CD pattern is observed in dioxane for **1** and **2**, characterized by a positive band of very low ellipticity at ~ 225 nm and a strong negative band at ~ 207 nm. This unusual pattern may be a consequence of peptide aggregation in this rather apolar, poorly hydrogen-bonding solvent (T. S. Sudha and P. Balaram, unpublished). Clear evidence for the aggregation of natural Aib-containing peptides, alamethicin⁵⁶ and trichotoxin A-40,⁵⁷ and synthetic (Ala-Aib)_{*n*} sequences⁵⁸ in dioxane and 1-octanol-dioxane mixtures, has been obtained from dielectric dispersion studies.

The hexapeptides Boc-(Val)₃-Aib-Val-Aib-OMe **3** and Boc-(Leu)₃-Aib-Leu-Aib-OMe **4** show CD spectra similar to that of the decapeptides in MeOH, TFE, and TMP. However, distinctly reduced ellipticities are observed for the shorter peptides (Table IV). The dependence of ellipticities on peptide chain length has been noted earlier in studies of Aib-rich sequences.^{3,34} The CD data suggest that folded conformations are indeed populated even at the hexapeptide level. Supporting evidence for the presence of intramolecularly hydrogen-bonded conformations in peptides **3** and **4** is obtained from ir studies in CHCl₃. A strong band corresponding to intramolecularly hydrogen-bonded NH groups is observed at ~ 3350 cm⁻¹ over the concentration range 0.50–4.0 mM for both **3** and **4** (Fig. 6). Peptide **3** (X = L-Val) exhibits a distinctive CD spectrum in TMP (single negative band at ~ 230 nm), which could arise from partially unfolded structures in this strongly hydrogen-bonding solvent. Conformations characterized by single β -turns may be expected to yield such a CD spectrum.^{59,60} The CD spectra of **3** and **4** in dioxane may be dominated by contributions from aggregated species. A comparison of the CD results on the hexapeptides and decapeptides suggests that folded conformations are stabi-

TABLE IV
CD Parameters for Peptides^a

Peptide	TFE		TMP		MeOH		Dioxane	
	(nm)	$[\theta]_M$	(nm)	$[\theta]_M$	(nm)	$[\theta]_M$	(nm)	$[\theta]_M$
Boc-Aib-Val-(Aib) ₂ -(Val) ₃ -Aib-Val-Aib-OMe (1)	219	-37870	220	-28930	222	-43130	226.5	1580
	203	-91520	205	-83630	204.5	-94680	207	-53130
Boc-Aib-Leu-(Aib) ₂ -(Leu) ₃ -Aib-Leu-Aib-OMe (2)	219	-36860	222	-21120	220	-34630	225	6700
	203	-109470	204.5	-105000	203	-102210	207	-56200
Boc-(Val) ₃ -Aib-Val-Aib-OMe (3)	217	-21987	230	-13610	222	-10890	221	-28270
	201	-45370			205	-12010		
Boc-(Leu) ₃ -Aib-Leu-Aib-OMe (4)	222	-13880	227.5	-15000	225	-10130	225	-26630
	200	-48750	200	32630	200	-37500		

^a $[\theta]_M$ expressed as deg cm² decimol⁻¹.

lized by the introduction of only two Aib residues in the C-terminal segment of the peptide chain.

CONCLUSIONS

The nmr studies on the peptides Boc-Aib-X-Aib-(X)₃-Aib-Aib-X-Aib-OMe (X = L-Val **1** and X = L-Leu **2**) provide compelling evidence for the presence of 3_{10} -helical conformations in chloroform solutions. In dimethylsulfoxide solutions the peptides appear to adopt different conformations. The presence of seven solvent-shielded NH groups in this solvent lends some support for α -helical structures. The observation of solvent-dependent structural changes in peptides **1** and **2** is in contrast to earlier results on the decapeptides, Boc(Aib-X)₅-OMe (X = L-Val, L-Ala), where a strict alternation of the Aib and X residues is maintained.⁹ Undoubtedly, the presence of the central triplet of L-residues in peptides **1** and **2** destabilizes the 3_{10} -helical conformation in polar solvents. This study emphasizes the role of a precise sequence and the nature of the solvent in modulating the preference of Aib-rich sequences for 3_{10} - or α -helical conformations. These helical conformations differ only slightly in the values of the backbone torsion angles (3_{10} , $\phi \sim \pm 60^\circ$, $\psi \sim \pm 30^\circ$; α , $\phi \sim \pm 55^\circ$, $\psi \sim \pm 45^\circ$) and can be interconverted with only small changes in molecular geometry.¹⁷ While there is little doubt that helical peptide conformations are important for transmembrane channel formation by Aib-rich peptides,^{2,6,19,20,52,61,62} categorical statements regarding the precise nature of the conformations in a lipid environment may be premature.

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