Conformations of the amino terminal tetrapeptide of emerimicins and antiamoebins in solution and in the solid state

R. Bardi, A. M. Piazzesi, C. Toniolo

Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padua, 35131 Padua, Italy

P. Antony Raj*, S. Raghothama† and P. Balaram*‡

**Molecular Biophysics Unit and †Sophisticated Instruments Facility, Indian Institute of Science, Bangalore 560 012, India*

(Received 19 November 1985; revised 18 February 1986)

The conformations of Boc-L-Phe-(Aib)$_3$-OH (**1**) *and Boc-L-Phe-(Aib)$_3$-OMe* (**2**) *which correspond to the amino terminal sequence of the emerimicins and antiamoebins have been studied in solution using 270 MHz ^{1}H n.m.r. In dimethyl sulphoxide solution both peptides show the presence of two strongly solvent shielded Aib NH groups, consistent with a consecutive β-turn conformation, involving the Aib(3) and Aib(4) NH groups in intramolecular 4 → 1 hydrogen bonds. This folded conformation is maintained for* **2** *in chloroform solution. Nuclear Overhauser effect studies provide evidence for a Type II Phe-Aib β-turn. An X-ray diffraction study of Boc-(D, L)-Phe-(Aib)$_3$-OH establishes a single type III(III′) β-turn conformation with Aib(2)-Aib(3) as the corner residues. A single intramolecular 4 → 1 hydrogen bond between Phe(1) CO and Aib(4) NH groups is observed in the crystal. The solution conformation may incorporate a consecutive type II–III′ structure for the Phe(1)-Aib(2)-Aib(3) segment, with the initial type II β-turn being destabilized by intermolecular interactions in the solid state.*

Keywords: Aib-containing peptides; emerimicins; antiamoebins; ^{1}H n.m.r.; β-turns; X-ray diffraction; β-turn conformation

Introduction

Acyclic peptides of microbial origin, which are rich in α-aminoisobutyric acid (Aib) have been shown to possess remarkable membrane modifying properties[1–4]. Considerable attention has therefore been centred on the structural chemistry of Aib-rich peptides[5–7], with major emphasis on studies of alamethicin [8–11]. These studies have led to detailed models for the voltage-gated transmembrane channels formed by alamethicin and related peptides[9,12–14]. The emerimicins[15] and antiamoebins[16,17] are relatively short Aib-rich peptides, which exhibit moderate antibiotic activity. No detailed analysis of their effects on membrane permeability is available as yet. The sequences of the major components of the emerimicins[18] and antiamoebin[19], determined by mass spectrometry are as follows:

Emerimicin III (IV): Ac-L-Phe-Aib-Aib-Aib-L-Val-Gly-L-Leu-Aib-Aib-L-Hyp-L-Gln-D-Iva-L-Hyp-L-Ala(Aib)-L-Phol.

Antiamoebin I (IV) (V): Ac-L-Phe-Aib-Aib-Aib-D-Iva(Aib)-Gly(L-Ala)-L-Leu-Aib-Aib-L-Hyp(L-Pro)-L-Gln-D-Iva-L-Hyp-Aib-L-Pro-L-Phol.

As part of a continuing programme to develop conformation–function relationships for Aib-containing membrane modifying peptides, we have undertaken the synthesis and conformational analysis of synthetic emerimicin fragments[20–22]. In this report we describe n.m.r. studies on the peptides Boc-L-Phe-(Aib)$_3$-OH (**1**) and Boc-L-Phe-(Aib)$_3$-OMe (**2**), the amino terminal fragments of the emerimicins and antiamoebins. The crystal structure of the peptide Boc-(D,L)-Phe-(Aib)$_3$-OH is also reported.

Experimental

The peptides Boc-L-Phe-(Aib)$_3$-OH (**1**) and Boc-L-Phe-(Aib)$_3$-OMe (**2**) were synthesized by conventional solution phase procedures and fully characterized by ^{1}H n.m.r. (270 MHz). Detailed synthetic procedures will be described elsewhere as part of the synthesis of emerimicin analogues. During attempts to crystallize peptide **1**, single crystals of the racemate Boc-(D,L)-Phe-(Aib)$_3$-OH were obtained, presumably due to minor contamination of the peptide. The identification of the racemate was made possible by the assignment of a *centrosymmetric* space group (Pcab) for the crystals. ^{1}H n.m.r. studies were carried out on a Bruker WH-270 FT n.m.r. spectrometer at the Sophisticated Instruments Facility, Bangalore as described earlier[23]. Peptide concentrations of ~5–10 mg/ml were used in all studies.

Single crystals of Boc-(D,L)-Phe-(Aib)$_3$-OH were obtained from methanol. X-ray diffraction data were

‡ To whom correspondence should be addressed.

0141–8130/86/040201–06$03.00
© 1986 Butterworth & Co. (Publishers) Ltd

collected on a Philips PW 1100 four circle diffractometer, using MoKα radiation, monochromatized by a graphite crystal. Intensities were corrected for Lorentz and polarization effects and put on an absolute scale by Wilson's method. No absorption corrections were applied. The crystallographic data for the peptide are summarized in *Table 1*.

The structure was solved by application of the direct methods program Multan 80[24]. The E-maps of the sets of phases with the best combined figure of merit revealed the position of 25 non-hydrogen atoms. The positions of the remaining non-hydrogen atoms were derived from subsequent difference Fourier maps. The structure was refined by the block-matrix least squares procedure. The function minimized was $\Sigma w\Delta^2$, where $\Delta = |F_0| - |F_c|$ and $w = [\sigma(F_0) + 0.0001F_0^2]^{-1}$. Weighting-scheme analysis showed no serious dependence of the mean $w\Delta^2$ as a function of either $|F_0|$ or $\lambda^{-1} \sin\theta$. The scattering factors were taken from the International Tables for X-ray Crystallography[25]. The refinement was carried out allowing all non-hydrogen atoms to vibrate anisotropically. The hydrogen atoms bound to C atoms were placed in idealized positions (C–H = 1.0 Å) but not varied, while the other H atoms were located from difference Fourier maps and included in the last cycle. Calculations were carred out using the Shelx-76 program[26]. The final conventional R value for the 2274 observed reflections $[I \geqslant 3\sigma(I)]$ was 0.060 ($R_w = 0.049$). The final positional parameters of the non-hydrogen atoms, along with equivalent isotropic thermal factors, are listed in *Table 2*. Anisotropic temperature factors, hydrogen positional parameters and structure factor Tables are available on request from Dr R. Bardi, Padua.

Results and discussion

N.m.r. studies

The peptides Boc-L-Phe-(Aib)$_3$-OH (**1**) and Boc-L-Phe-(Aib)$_3$-OMe (**2**) yielded well-resolved ^{1}H n.m.r. spectra in $(CD_3)_2SO$. The Phe NH group is readily assigned to the only *doublet* NH resonance, in both peptides. The remaining three Aib NH resonances are singlets and cannot be assigned unambiguously to specific residues. These resonances are labelled as S_n ($n = 1$–3), where the subscript refers to the order of appearance from low field in $(CD_3)_2SO$. The tetrapeptide ester **2** was also examined in $CDCl_3$. Peptide **1** is not readily soluble in the solvent.

Table 1 Crystal data for Boc-(D,L)-Phe-(Aib)$_3$-OH

Molecular formula	$C_{26}H_{40}N_4O_7$
MW (a.m.u.)	520.6
Density (calcd.) g/cm^3	1.159
Density (exptl.) g/cm^3	1.15
Space group	Pcab[a]
Z	8
a (Å)	16.149(3)
b (Å)	16.239(3)
c (Å)	22.754(3)
$\alpha = \beta = \gamma$ (°)	90
Reflections ($I \geqslant 3\sigma(I)$)	2274
R value	0.060
R_w value	0.049

[a] The space-group symbol *Pcab* is obtained by permutation of the standard symbol *Pbca* in accordance with the transformation of axes ($a\,b\,c \rightarrow b\,a\,\bar{c}$)

Table 2 Fractional coordinates ($\times 10^4$) and equivalent isotropic temperature factors (Å$^2 \times 10^3$) for Boc-(D,L)-Phe-(Aib)$_3$-OH (ESDs are given in parentheses)

Atom	$-x$	$-y$	$-z$	U_{eq}
O(1)	9361(2)	1228(2)	4851(1)	68(2)
O(2)	8310(2)	2035(2)	4542(1)	102(3)
O(3)	9306(2)	834(2)	3001(1)	57(2)
O(4)	10021(2)	2147(2)	1889(1)	64(2)
O(5)	11525(1)	3054(2)	2893(1)	60(2)
O(6)	12863(2)	1825(2)	3385(1)	85(2)
O(7)	11962(2)	2271(2)	4051(1)	68(2)
N(1)	8655(2)	859(2)	4082(1)	56(2)
N(2)	8191(2)	1476(2)	2605(1)	47(2)
N(3)	9369(2)	2697(2)	2674(1)	43(2)
N(4)	10752(2)	1994(2)	3192(2)	47(2)
C(1)	8857(3)	1824(5)	5770(2)	97(4)
C(2)	10247(3)	1209(5)	5643(2)	94(4)
C(3)	9932(5)	2547(4)	5154(3)	110(5)
C(4)	9580(3)	1735(3)	5359(2)	66(3)
C(5)	8743(3)	1435(2)	4492(2)	57(3)
C(6)	8090(2)	922(3)	3599(2)	48(3)
C(7)	7548(3)	153(3)	3548(2)	64(3)
C(8)	7120(3)	−44(3)	4119(2)	58(3)
C(9)	6579(3)	509(4)	4360(2)	90(4)
C(10)	6207(4)	349(5)	4894(3)	131(5)
C(11)	6382(5)	−348(5)	5181(3)	134(6)
C(12)	6901(5)	−904(5)	4954(3)	130(6)
C(13)	7277(4)	−743(3)	4417(3)	99(5)
C(14)	8583(3)	1067(2)	3034(2)	45(3)
C(15)	8614(2)	1706(3)	2062(2)	54(3)
C(16)	8822(3)	946(3)	1694(2)	80(4)
C(17)	8042(3)	2281(4)	1718(2)	78(4)
C(18)	9417(3)	2197(3)	2201(2)	47(3)
C(19)	10047(2)	3245(2)	2849(2)	44(2)
C(20)	9800(3)	3642(3)	3433(2)	61(3)
C(21)	10219(3)	3902(3)	2388(2)	69(3)
C(22)	10842(2)	2747(3)	2962(2)	47(3)
C(23)	11441(2)	1421(3)	3285(2)	52(3)
C(24)	11161(3)	751(3)	3713(2)	67(3)
C(25)	11691(3)	1052(4)	2695(2)	81(4)
C(26)	12167(3)	1875(3)	3562(2)	59(3)

The relevant n.m.r. parameters for the NH resonances in **1** and **2** are listed in *Table 3*. *Figures 1* and *2* summarize the results of free radical and solvent–perturbation experiments on **2**[27]. In both **1** and **2** two Aib NH resonances, S_2 and S_3, exhibit low temperature coefficients ($d\delta/dT$) in $(CD_3)_2SO$, characteristic of strongly solvent-shielded (intramolecularly hydrogen-bonded) NH groups. The Phe NH and the remaining Aib NH (S_1 in **1** and **2**) resonances in both peptides have high $d\delta/dT$ values (>0.005 ppm/K) indicative of their exposure to the solvent. The Phe and S_1 NH resonances also show large downfield shifts on addition of $(CD_3)_2SO$ to $CDCl_3$ solutions of **2**, while the S_2 and S_3 resonances are largely unaffected. The chemical shift changes are monotonic. The absence of sharp discontinuities suggests that *major* conformational perturbations do not occur on going from $CDCl_3$ to $(CD_3)_2SO$. Addition of the free radical TEMPO to $CDCl_3$ solutions of **2**, results in a dramatic broadening of the Phe and S_1 resonances, while S_3 is much less affected (*Figure 1*). The S_2 resonance is obscured by overlap with the aromatic proton resonances. These results are consistent with the involvement of two Aib NH groups in intramolecular hydrogen bonding in both **1** and **2** in $(CD_3)_2SO$, while this hydrogen bonding pattern is also maintained for **2** in

Table 3 ^{1}H n.m.r. parameters for NH groups in peptides **1** and **2**

NH resonance (Peptide)		($CDCl_3$) (ppm)	($CD_3)_2SO$ (ppm)	$\Delta\delta^c$ (ppm)	$d\delta/dT^d$ (ppm/K)
S_1	(1)	–	8.23	–	0.0056
S_2	(1)	–	7.31[a]	–	0.0016
S_3	(1)	–	7.18	–	0.0014
Phe	(1)[b]	–	7.03	–	0.0056
S_1	(2)	6.24	8.32	2.08	0.0064
S_2	(2)	7.38[a]	7.41	0.03	0.0020
S_3	(2)	6.92	7.18	0.26	0.0024
Phe	(2)[b]	5.01	7.06	2.05	0.0074

[a] Overlaps with aromatic proton resonances
[b] $J_{HNC^\alpha H}$ Phe: **1** ($(CD_3)_2SO$) 7.3 Hz, **2** ($CDCl_3$) 5.1 Hz, **2** ($(CD_3)_2SO$ 5.1 Hz
[c] $\Delta\delta = \delta(CD_3)_2SO - \delta CDCl_3$
[d] In $(CD_3)_2SO$

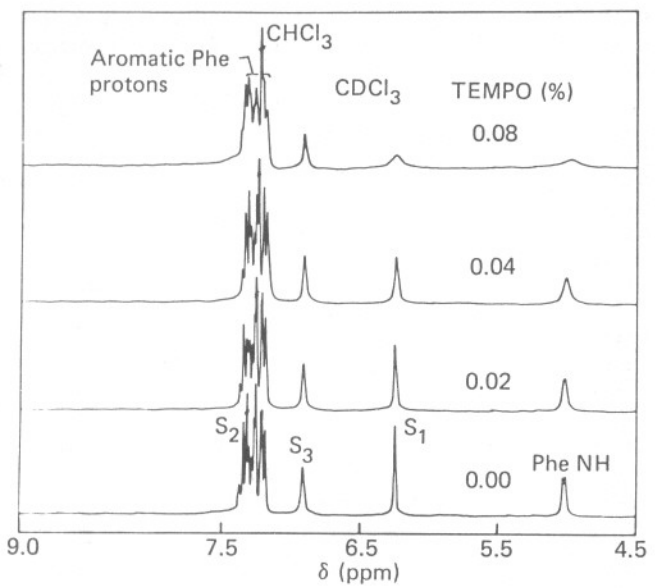


Figure 1 Effect of addition of the paramagnetic radical TEMPO on the low-field resonances (270 MHz, ^{1}H) of Boc-L-Phe-(Aib)$_3$-OMe (**2**) in $CDCl_3$. TEMPO concentration (w/v) are indicated against the traces

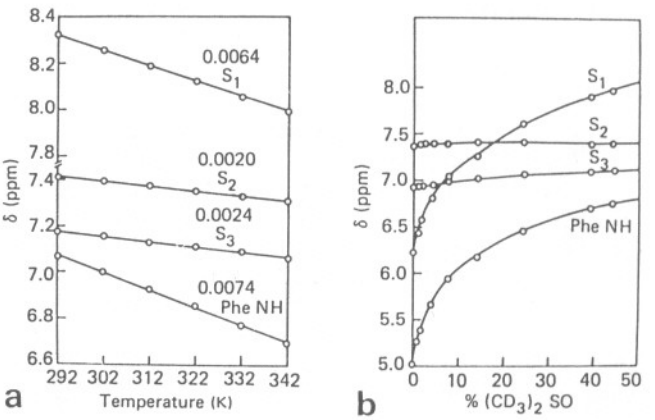
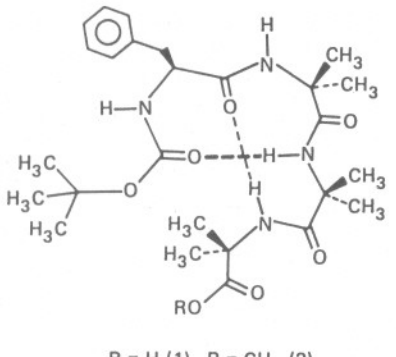


Figure 2 (a) Temperature dependence of NH chemical shifts in peptide **2** in $(CD_3)_2SO$. $d\delta/dT$ values are indicated against the traces. (b) Effect of addition of increasing concentrations of $(CD_3)_2SO$ to $CDCl_3$ solutions of peptide **2**

$CDCl_3$. Some evidence for small conformational differences for **2** in $CDCl_3$ and $(CD_3)_2SO$ is obtained from NOE studies and a comparison of $J_{HNC^\alpha H}$ values for Phe(1) (see below). At the concentrations used in these studies (5–10 mg/ml) aggregation effects are likely to be minimal in $(CD_3)_2SO$, as demonstrated earlier for Aib containing oligopeptides[28–30].

The above observations, together with the known stereochemical preferences of Aib residues to favour β-turn conformations stabilized by intramolecular $4\rightarrow1$ hydrogen bonds[5–7], suggest that consecutive β-turn conformations (*Figure 3*) are populated in **1** and **2** in solvents like chloroform and dimethyl sulphoxide. Both Type III–III ($\phi_1=\phi_2=\phi_3\sim-60°$ and $\psi_1=\psi_2=\psi_3\sim-30°$) and Type II–III′ ($\phi_1\sim-60°$, $\psi_1=120°$, $\phi_2\sim70°$, $\psi_2\sim20°$, $\phi_3\sim60°$, $\psi_3\sim30°$)[31] structures are compatible with the sequence. In the former, L-Phe would have torsion angles $\phi\sim-60°$, $\psi\sim-30°$ and the Aib residues at positions 2 and 3 would lie in the right-handed helical region of the ϕ, ψ map. In the latter, L-Phe would have $\phi\sim-60°$, $\psi\sim120°$ and Aib(2) and Aib(3) would lie in the left-handed helical region[5]. The observed $J_{HNC^\alpha H}$ value of 7.3 Hz for Phe(1) in **2** is consistent with a ϕ_{Phe} value of $\sim-60°$ (Ref. 32). Interestingly, a significantly lower value of 5.1 Hz is observed in $CDCl_3$. A distinction between Type II and Type I(III) β-turn conformations may be readily made by n.m.r. methods using nuclear Overhauser effects (NOEs) between the C^α_iH and $N_{i+1}H$ protons[23,33,34]. *Figure 4* illustrates representative difference NOE experiments carried out on peptide **2**. The results of NOE studies in $(CD_3)_2SO$ and $CDCl_3$ are summarized in *Table 4*. In $(CD_3)_2SO$ a significant NOE is observed on the Phe $C^\alpha H$ proton, when the S_1 NH resonance is saturated. S_1 corresponds to a solvent exposed NH proton and may be tentatively assigned to Aib(2) NH (*Figure 3*). Thus the observation of an NOE suggests close approach (<3.0 Å) of the Phe $C^\alpha H$ and Aib(2) NH groups[23,34], supporting a Type II conformation for the Phe(1)-Aib(2) segment. In the reverse experiment, smaller NOEs are observed on the S_1 NH resonance, when the Phe $C^\alpha H$ proton is irradiated. This is presumably due to the presence of alternative relaxation pathways for the Aib(2) NH group. Small NOEs ($\leqslant2\%$) are observed between the Phe NH and Aib(2) NH(S_1) protons. Such $N_{i+1}H$–$N_{i+2}H$ NOEs in β-turns are expected only in a Type I β-turn, where an interproton distance of 2.6 Å is estimated[34]. The corresponding distance in a Type II structure is 4.5 Å and



R = H (1), R = CH_3 (2)

Figure 3 Consecutive β-turn conformation of peptides **1** and **2** consistent with n.m.r. data

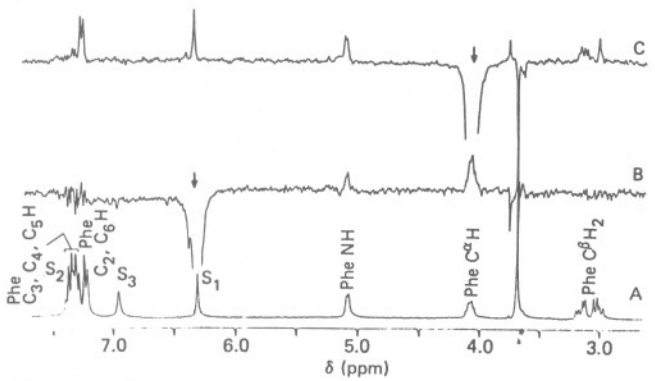


Figure 4 (A) Partial 270 MHz ^{1}H n.m.r. spectrum of Boc-L-Phe- (Aib)$_3$-OMe (**2**) in $CDCl_3$. (B,C) Difference NOE spectra ($\times 32$) obtained by irradiation of S_1 NH (B) and Phe C$^\alpha$H (C) resonances

no NOE is expected[34]. The observation of these NOEs, despite their low magnitudes suggests that Type I β-turn conformations at the Phe(1)-Aib(2) segment may also be populated in $(CD_3)_2SO$. The lack of additional lines in the ^{1}H n.m.r. spectrum and the absence of exchange broadening of resonances, suggests that these conformations may be interconverting rapidly on the n.m.r. timescale. The NOE between the S_1 NH (Aib(2)) and the S_3 NH resonance in $(CD_3)_2SO$, suggests that the latter may be assigned to the Aib(3) NH group. The observation of successive $N_{i+1}H$–$N_{i+2}H$ NOE connectivities is diagnostic of a 3_{10} or α-helical conformation over the three residues[34]. It may be noted that NOEs between NH protons are likely to have low magnitudes due to the existence of alternative relaxation pathways for such protons.

The interresidue NOE characteristic of a Phe(1)-Aib(2) Type II β-turn conformation (Phe C$^\alpha$H $\leftrightarrow$ S_1 NH) is also observed in $CDCl_3$. However, the observed magnitudes are significantly smaller (*Table 4*). Further, a large NOE is observed on the Phe NH when Phe C$^\alpha$H is irradiated. This NOE is absent in $(CD_3)_2SO$. These results suggest that the conformational distribution in $CDCl_3$ may be different from that in $(CD_3)_2SO$. Conformations having a significantly different ϕ_{Phe} value from that expected for the $i+1$ residue in Type II β-turns ($\phi \sim -60°$) are presumably populated. This is consistent with the change in $J_{HNC^\alpha H}$ Phe on going from $(CD_3)_2SO$ (7.3 Hz) to $CDCl_3$ (5.1 Hz). The large intraresidue NOE for Phe(1) is also consistent with a smaller NH–C$^\alpha$H distance, suggestive of a reduced magnitude of ϕ. In the region 0 to $-60°$, small changes in ϕ lead to appreciable changes in the $C_i^\alpha H$–N_iH interproton distance[35]. It is conceivable that peptide association in $CDCl_3$ may result in a perturbation of the conformational distributions, as compared to $(CD_3)_2SO$. Earlier studies have indeed established that aggregation effects are more pronounced in apolar solvents like $CDCl_3$, as compared to polar, hydrogen bond accepting solvents like $(CD_3)_2SO$[28–30].

The availability of single crystals of Boc-(D,L)-Phe-Aib-Aib-Aib-OH prompted an X-ray study, in order to examine the structural properties in the solid state.

Crystal structure of Boc-(D,L)-Phe-Aib-Aib-Aib-OMe

The molecular structure of the peptide Boc-(D,L)-Phe-(Aib)$_3$-OH, with the atomic numbering scheme is shown in *Figure 5*. Bond lengths and bond angles together with their estimated standard deviations are given in *Table 5*. Relevant torsional angles[36] are listed in *Table 6*. The geometry of the intra- and intermolecular hydrogen bonds is given in *Table 7*.

The observed structural parameters are largely unexceptional and do not merit special comment. The

Table 4 Nuclear Overhauser effects observed for Boc-L-Phe-(Aib)$_3$-OMe (**2**)

Proton irradiated	Proton observed	NOE (%)
Solvent $CDCl_3$		
Phe C$^\alpha$H	Phe NH	3.3
	S_1 NH	3.3
	Phe C_2H, C_6H	2.1
Phe NH	Phe C$^\alpha$H	6.2
	S_1 NH	1.7
S_1 NH	Phe C$^\alpha$H	5.5
	Phe NH	2.5
Phe C_2H, C_6H	Phe C$^\alpha$H	3.9
Solvent $(CD_3)_2SO$		
Phe C$^\alpha$H	S_1 NH	4.0
	Phe aromatic protons	2.0
S_1 NH	Phe C$^\alpha$H	10.9
	S_3 NH	2.2
	Phe NH	1.9

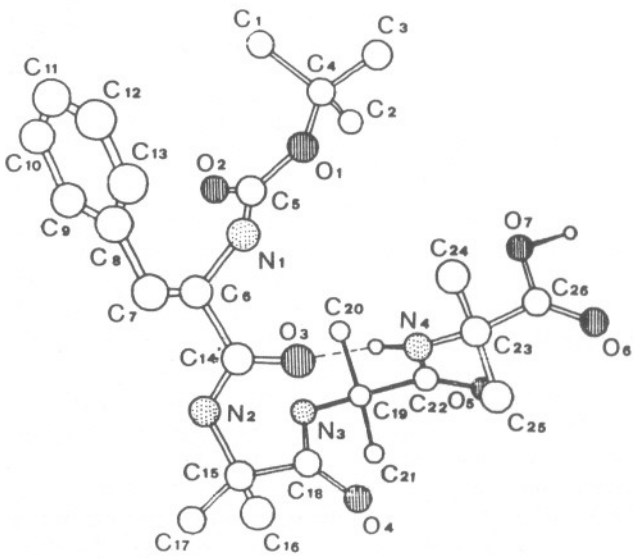


Figure 5 Molecular structure of Boc-(D,L)-Phe- (Aib)$_3$-OH in the solid state

Table 5 Bond lengths (Å) and bond angles (°) for Boc-(D,L)-Phe-$(Aib)_3$-OH (EDSs are given in parentheses)

Bond lengths		Bond angles	
C(1)–C(4)	1.503(7)	C(1)–C(4)–C(3)	113.6(5)
C(2)–C(4)	1.519(8)	C(1)–C(4)–C(2)	109.8(4)
C(3)–C(4)	1.510(8)	C(2)–C(4)–C(3)	110.7(5)
C(4)–O(1)	1.461(6)	C(3)–C(4)–O(1)	109.9(4)
O(1)–C(5)	1.333(5)	C(2)–C(4)–O(1)	101.1(4)
C(5)–O(2)	1.206(5)	C(1)–C(4)–O(1)	111.0(4)
C(5)–N(1)	1.328(5)	C(4)–O(1)–C(5)	121.6(3)
N(1)–C(6)	1.432(5)	O(1)–C(5)–O(2)	125.5(4)
C(6)–C(7)	1.530(6)	O(1)–C(5)–N(1)	109.5(3)
C(7)–C(8)	1.506(6)	O(2)–C(5)–N(1)	125.0(4)
C(8)–C(9)	1.369(7)	C(5)–N(1)–C(6)	123.9(4)
C(9)–C(10)	1.379(8)	N(1)–C(6)–C(7)	111.4(3)
C(10)–C(11)	1.337(10)	C(6)–C(7)–C(8)	111.7(4)
C(11)–C(12)	1.335(11)	C(7)–C(8)–C(9)	120.0(4)
C(12)–C(13)	1.390(10)	C(8)–C(9)–C(10)	120.5(5)
C(13)–C(8)	1.345(7)	C(9)–C(10)–C(11)	119.9(7)
C(6)–C(14)	1.529(5)	C(10)–C(11)–C(12)	121.1(7)
C(14)–O(3)	1.230(5)	C(11)–C(12)–C(13)	119.1(7)
C(14)–N(2)	1.340(5)	C(12)–C(13)–C(8)	121.3(5)
N(2)–C(15)	1.462(5)	C(13)–C(8)–C(9)	118.2(5)
C(15)–C(16)	1.528(7)	C(13)–C(8)–C(7)	121.8(4)
C(15)–C(17)	1.529(7)	C(7)–C(6)–C(14)	111.1(3)
C(15)–C(18)	1.554(6)	N(1)–C(6)–C(14)	108.9(3)
C(18)–O(4)	1.208(5)	C(6)–C(14)–N(2)	116.3(3)
C(18)–N(3)	1.350(5)	C(6)–C(14)–O(3)	119.9(3)
N(3)–C(19)	1.466(5)	O(3)–C(14)–N(2)	123.8(4)
C(19)–C(20)	1.531(6)	C(14)–N(2)–C(15)	121.5(3)
C(19)–C(21)	1.521(6)	N(2)–C(15)–C(18)	110.4(3)
C(19)–C(22)	1.538(6)	N(2)–C(15)–C(17)	107.8(3)
C(22)–O(5)	1.221(5)	N(2)–C(15)–C(16)	111.0(4)
C(22)–N(4)	1.338(6)	C(17)–C(15)–C(18)	107.2(4)
N(4)–C(23)	1.465(5)	C(16)–C(15)–C(18)	110.0(4)
C(23)–C(24)	1.528(7)	C(16)–C(15)–C(17)	110.2(4)
C(23)–C(25)	1.524(7)	C(15)–C(18)–N(3)	115.0(3)
C(23)–C(26)	1.522(6)	C(15)–C(18)–O(4)	121.3(4)
C(26)–O(6)	1.197(6)	N(3)–C(18)–O(4)	123.7(4)
C(26)–O(7)	1.327(6)	C(18)–N(3)–C(19)	122.6(3)
		N(3)–C(19)–C(22)	110.5(3)
		N(3)–C(19)–C(21)	112.0(3)
		N(3)–C(19)–C(20)	107.3(3)
		C(21)–C(19)–C(22)	109.4(3)
		C(20)–C(19)–C(22)	107.0(3)
		C(20)–C(19)–C(21)	110.5(4)
		C(19)–C(22)–N(4)	117.1(3)
		C(22)–N(4)–C(23)	123.7(3)
		O(5)–C(22)–C(19)	121.1(4)
		O(5)–C(22)–N(4)	121.4(4)
		N(4)–C(23)–C(26)	109.7(4)
		N(4)–C(23)–C(25)	108.9(4)
		N(4)–C(23)–C(24)	108.6(3)
		C(25)–C(23)–C(26)	110.5(4)
		C(24)–C(23)–C(26)	107.9(4)
		C(24)–C(23)–C(25)	111.1(4)
		O(7)–C(26)–C(23)	112.9(4)
		O(6)–C(26)–C(23)	123.5(4)
		O(6)–C(26)–O(7)	123.3(4)

geometries of the Boc group[37], Aib residues[5,38] and Phe ring[39] are in agreement with earlier results on related molecules. The urethane and peptide units are approximately *trans* planar (*Table 6*). The peptide backbone adopts a single β-turn conformation stabilized by an intramolecular hydrogen bond between the NH of the Aib(4) residue and the CO group of Phe(1). The N(4)–O(3) distance of 3.032 Å is within accepted limits for intramolecular hydrogen bonds in peptide structures[40,41]. The ϕ, ψ values for Aib(2) (53.9 (5)° and 35.1 (5)°) and Aib(3) (57.9 (5)° and 34.4 (5)°) are in excellent agreement with values expected for a Type III′ β-turn[31]. Interestingly, this compound does not adopt the conformation with the O(7)–H ... O(4) intramolecular hydrogen bond (oxy-analogues of the β-turn conformation) recently found in Z-$(Aib)_3$-OH[42]. In addition, the N(3)–H···O(2) intramolecular hydrogen bond is also lacking, thereby precluding the formation of the structure with two consecutive N–H···O=C intramolecular hydrogen bonds, typical of Aib-rich N-protected tetrapeptides[5,6].

Comparison of solution and solid state conformations

The n.m.r. results obtained on Boc-L-Phe-$(Aib)_3$-OH (**1**) and Boc-L-Phe-$(Aib)_3$-OMe (**2**) are consistent with consecutive β-turn conformations for both peptides, incorporating two intramolecular 4→1 hydrogen bonds namely, Boc CO···Aib(3) NH and Phe(1) CO···Aib(4) NH. In the solid state structure of Boc-(D,L)Phe-$(Aib)_3$-OH only a single Type III(III′) β-turn is observed, with Aib(2)-Aib(3) as the corner residues. This feature is stabilized by a lone intramolecular 4→1 hydrogen bond (Phe(1) CO···Aib(4) NH). The observed conformation of the Phe residue in the solid state ($\phi = \pm 108.2°$, $\psi = \pm 152.5°$) is not too far from that expected for Phe at the $i+1$ position of a Type II(II′) β-turn ($\phi = -60°$ ($+60°$), $\psi = 120°$ ($-120°$)). As noted earlier, one of the solution conformations consistent with the spectroscopic data for **1** and **2** is a consecutive Type II–III′ β-turn structure. An altered conformation of the amino terminal residue in the crystal results in the observed differences between the solid state and solution structures. Packing of enantiomeric peptides in the crystals of the racemate may contribute to the observed differences. It is noteworthy that in the crystal both the Boc CO and Aib(3) NH groups form intermolecular hydrogen bonds to the terminal carboxylic acid group of symmetry related molecules (*Table 7*, *Figure 6*). Thus, breaking of the *intramolecular* Boc CO···Aib(3) NH 4→1 hydrogen bond observed in solution is accompanied by formation of two new *intermolecular* hydrogen bonds in the solid state. This is accomplished with only moderate alterations in the conformation of the Phe residue.

Table 6 Torsion angles for Boc-(D,L)-Phe- $(Aib)_3$-OH [a,b]

ω_1		O(1)–C(5)–N(1)–C(6)	174.8(3)
ϕ_2	(Phe(1))	C(5)–N(1)–C(6)–C(14)	−108.2(4)
ψ_2	(Phe(1))	N(1)–C(6)–C(14)–N(2)	152.5(3)
ω_2		C(6)–C(14)–N(2)–C(15)	−175.4(3)
ϕ_3	(Aib(2))	C(14)–N(2)–C(15)–C(18)	53.9(5)
ψ_3	(Aib(2))	N(2)–C(15)–C(18)–N(3)	35.1(5)
ω_3		C(15)–C(18)–N(3)–C(19)	176.1(3)
ϕ_4	(Aib(3))	C(18)–N(3)–C(19)–C(22)	57.9(5)
ψ_4	(Aib(3))	N(3)–C(19)–C(22)–N(4)	34.4(5)
ω_4		C(19)–C(22)–N(4)–C(23)	−175.3(3)
ϕ_5	(Aib(4))	C(22)–N(4)–C(23)–C(26)	−45.3(5)
ψ_5	(Aib(4))	N(4)–C(23)–C(26)–O(7)	−54.2(5)
χ_2^1	(Phe(1))	N(1)–C(6)–C(7)–C(8)	−53.0(5)
χ_2^{21}	(Phe(1))	C(6)–C(7)–C(8)–C(9)	−61.3(6)
χ_2^{22}	(Phe(1))	C(6)–C(7)–C(8)–C(13)	116.1(5)

[a] ESDs are given in parentheses
[b] Torsion angles corresponding to one enantiomer are given

Table 7 Geometry of the hydrogen bonds in the crystal of Boc-(D,L)-Phe- $(Aib)_3$-OH

Donor D–H	Acceptor A	Symmetry equivalence of A	Distances (Å) D···A	Distances (Å) H···A	Angle (°) D–H···A
N(4)–H	O(3)	x, y, z	3.032(5)	2.265(5)	161.6(3)
N(3)–H	O(6)	x−1/2, 1/2−y, z	3.022(4)	2.074(4)	157.4(3)
N(2)–H	O(5)	x−1/2, 1/2−y, z	2.872(4)	1.881(4)	170.2(3)
O(7)–H	O(2)	x+1/2, 1/2−y, z	2.694(4)	1.686(4)	167.4(3)

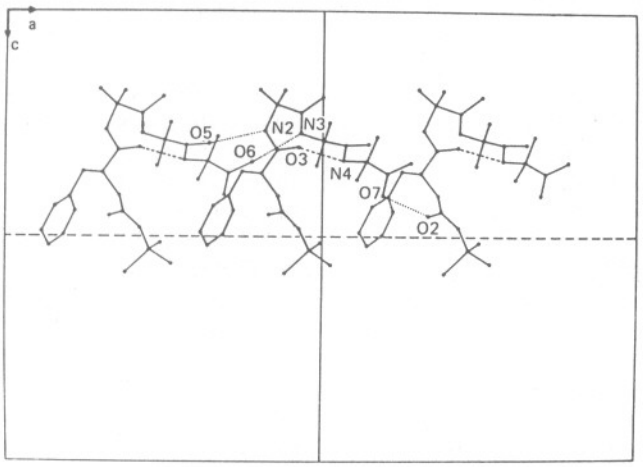


Figure 6 Intermolecular hydrogen bonding scheme observed in the crystal structure of Boc-(D,L)-Phe-$(Aib)_3$-OMe. Perspective view down the *b* axis. Only three symmetry related molecules are shown for clarity

Acknowledgements

This research was partially supported by a grant from the Department of Science and Technology, Government of India.

References

1 Mueller, P. and Rudin, D. O. *Nature* 1968, **217**, 713
2 Boheim, G., Janko, K., Leibfritz, D., Ooka, T., König, W. A. and Jung, G. *Biochim. Biophys. Acta* 1976, **433**, 182
3 Hall, J. E., Vodyanoy, I., Balasubramanian, T. M. and Marshall, G. R. *Biophys. J.* 1984, **45**, 233
4 Mathew, M. K. and Balaram, P. *Mol. Cell. Biochem.* 1983, **50**, 47
5 Prasad, B. V. V. and Balaram, P. *CRC Crit. Rev. Biochem.* 1984, **16**, 307
6 Toniolo, C., Bonora, G. M., Bavoso, A., Benedetti, E., DiBlasio, B., Pavone, V. and Pedone, C. *Biopolymers* 1983, **22**, 205
7 Jung, G., Bosch, R., Katz, E., Schmitt, H., Voges, K. P. and Winter, W. *Biopolymers* 1983, **22**, 241
8 Nagaraj, R. and Balaram, P. *Acc. Chem. Res.* 1981, **14**, 356
9 Fox, Jr., R. O. and Richards, F. M. *Nature* 1982, **300**, 325
10 Banerjee, U., Tsui, F. P., Balasubramanian, T. M., Marshall, G. R. and Chan, S. I. *J. Mol. Biol.* 1983, **165**, 757
11 Jung, G., Brückner, H. and Schmitt, H. in 'Structure and Activity of Natural Peptides', (Eds W. Voelter and G. Weitzel), Walter de Gruyter, Berlin, 1981, p. 75
12 Boheim, G., Hanke, W. and Jung, G. *Biophys. Struct. Mech.* 1983, **9**, 181
13 Mathew, M. K. and Balaram, P. *FEBS Lett.* 1983, **157**, 1
14 Hall, J. E., Balasubramanian, T. M., Hing, A. and Marshall, G. R. in 'Peptides Structure and Function', (Eds V. J. Hruby and D. H. Rich), Pierce Chemical Co., Rockford, Illinois, 1983, p. 487
15 Argoudelis, A. D. and Johnson, L. E. *J. Antibiot.* 1974, **27**, 274
16 Thirumalachar, M. J. *Hindustan Antibiotics Bull.* 1968, **10**, 287
17 Stroh, J. G., Carter Cook, J., Milberg, R. M., Brayton, L., Kihara, T., Huang, Z., Rinehart, Jr., K. L. and Lewis, I. A. S. *Anal. Chem.* 1985, **57**, 985
18 Pandey, R. C., Carter Cook, Jr., J. and Rinehart, Jr., K. L. *J. Am. Chem. Soc.* 1977, **99**, 5205
19 Pandey, R. C., Meng, H., Carter Cook, Jr., J. and Rinehart, Jr., K. L. *J. Am. Chem. Soc.* 1977, **99**, 5203
20 Benedetti, E., Bavoso, A., Di Blasio, B., Pavone, V., Pedone, C., Toniolo, C. and Bonora, G. M. *Proc. Natl. Acad. Sci. USA* 1982, **79**, 7951
21 Toniolo, C., Bonora, G. M., Benedetti, E., Bavoso, A., Di Blasio, B., Pavone, V. and Pedone, C. *Int. J. Biol. Macromol.* 1985, **7**, 357
22 Toniolo, C., Bonora, G. M., Bavoso, A., Benedetti, E., Di Blasio, B., Pavone, V. and Pedone, C. *J. Biomol. Struct. Dynamics* 1985, **3**, 585
23 Rao, B. N. N., Kumar, A., Balaram, H., Ravi, A. and Balaram, P. *J. Am. Chem. Soc.* 1983, **105**, 7423
24 Main, P., Fiske, S. J., Hull, S. E., Lessinger, L., Germain, G., Declercq, J. P. and Woolfson, M. M. 'Multan 80. A system of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data', University of York, England and University of Louvain, Belgium, 1980
25 'International Tables for X-Ray Crystallography', 2nd edn, Kynoch Press, Birmingham, 1974, Vol. IV
26 Sheldrick, G. M. 'Shelx 76. Program for Crystal Structure Determination', University of Cambridge, England, 1976
27 Wüthrich, K. 'NMR in Biological Research: Peptides and Proteins', North-Holland, Amsterdam, 1976
28 Iqbal, M. and Balaram, P. *Biochemistry* 1981, **20**, 7278
29 Iqbal, M. and Balaram, P. *Biopolymers* 1982, **21**, 1427
30 Raj, P. A. and Balaram, P. *Biopolymers* 1985, **24**, 1131
31 Venkatachalam, C. M. *Biopolymers* 1968, **6**, 1425
32 Bystrov, V. F. *Progr. NMR Spectroscopy* 1976, **10**, 41
33 Rose, G. D., Gierasch, L. M. and Smith, J. A. *Adv. Protein Chem.* 1985, **37**, 1
34 Wüthrich, K., Billeter, M. and Braun, W. *J. Mol. Biol.* 1984, **180**, 715
35 Shenderovich, M. D., Nikiforovich, G. V. and Chipens, G. I. *J. Magn. Res.* 1984, **59**, 1
36 IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry* 1970, **9**, 3471
37 Benedetti, E., Pedone, C., Toniolo, C., Némethy, G., Pottle, M. S. and Scheraga, H. A. *Int. J. Pept. Protein Res.* 1980, **16**, 156
38 Paterson, Y., Rumsey, S. M., Benedetti, E., Némethy, G. and Scheraga, H. A. *J. Am. Chem. Soc.* 1981, **103**, 2947
39 Gould, R. O., Gray, A. M., Taylor, P. and Walkinshaw, M. D. *J. Am. Chem. Soc.* 1985, **107**, 5921
40 Karle, I. L. in 'The Peptides', (Eds E. Gross and J. Meienhofer), Academic Press, New York, 1981, Vol. IV, p. 1
41 Taylor, R., Kennard, O. and Versichel, W. *Acta Cryst.* 1984, **B40**, 280
42 Toniolo, C., Valle, G., Bonora, G. M., Crisma, M., Formaggio, F., Benedetti, E., Di Blasio, B., Pavone, V. and Pedone, C. *J. Am. Chem. Soc.* 1986, submitted