Tetrahedron Vol 39, No. 16, pp 2725 to 2731, 1983
Printed in Great Britain.

0040-4020/83 $3.00 + 00
Pergamon Press Ltd

SOLUTION CONFORMATIONS OF PENTA AND HEPTAPEPTIDES CONTAINING REPETITIVE α-AMINOISOBUTYRYL-L-ALANYL AND α-AMINOISOBUTYRYL-L-VALYL SEQUENCES

E. K. S. VIJAYAKUMAR and P. BALARAM*
Molecular Biophysics Unit, Indian Institute of Science, Bangalore, 560 012, India

(*Received in UK 20 September* 1982)

Abstract—The presence of folded solution conformations in the peptides Boc-Ala-(Aib-Ala)$_2$-OMe, Boc-Val-(Aib-Val)$_2$-OMe, Boc-Ala-(Aib-Ala)$_3$-OMe and Boc-Val-(Aib-Val)$_3$-OMe has been established by 270 MHz ^{1}H NMR. Intramolecularly H-bonded NH groups have been identified using temperature and solvent dependence of NH chemical shifts and paramagnetic radical induced broadening of NH resonances. Both pentapeptides adopt 3_{10} helical conformations possessing 3 intramolecular H-bonds in $CDCl_3$ and $(CD_3)_2SO$. The heptapeptides favour helical structures with 5 H-bonds in $CDCl_3$. In $(CD_3)_2SO$ only 4 H-bonds are readily detected.

The tendency of α-aminoisobutyric acid (H_2N-$C(CH_3)_2$-COOH, Aib) residues to stabilize helical peptide backbone conformations was first predicted theoretically[1] and has been established experimentally by several spectroscopic[2-10] and X-ray diffraction studies.[10-17] For poly(Aib), fibre diffraction studies favour a 3_{10} helix,[18] while theoretical analyses support α-helical[19] or modified helical structures.[20] While 3_{10} helical conformations stabilized by intramolecular 4→1 H-bonds have been observed in crystal structures of a large number of Aib containing peptides,[10-16] α-helical conformations, incorporating 5→1 H-bonds, have been established in the crystal structures of Boc-Ala-(Aib-Ala)$_2$-Glu (OBzl)-(Ala-Aib)$_2$-Ala-OMe[17] and Boc-Aib-Pro-Val-Aib-Val-OMe.[11,21] The pentapeptide is distinguished by the presence of two β-branched Val residues, while the 11-peptide has a central tripeptide stretch, -Ala-Glu(OBzl)-Ala-, containing no Aib residues. Both peptide crystals contain solvent molecules. It was therefore of interest to establish the precise role of sequence and solvent effects in modulating the preference of Aib peptides for various helical conformations. As part of a continuing program to elucidate the conformational characteristics of Aib peptides,[10] we have chosen to examine (Aib-X)$_n$ sequences,[22,23] with a view to establishing the role of the X residue and chain length in determining peptide conformations. In this report we describe ^{1}H NMR studies on penta and heptapeptides containing L-Ala and L-Val as the X-residue. The choice of these residues was dictated by their different propensities for occurring in helical conformations.[24]

RESULTS AND DISCUSSION

Pentapeptides

In the peptides, Boc-X-(Aib-X)$_2$-OMe, where X = L-Ala or L-Val, five NH resonances could be clearly seen in both $CDCl_3$ and $(CD_3)_2SO$ at 270 MHz. The NH chemical shifts are listed in Table 1. Resonances are labelled as S_n (singlets) or D_n (doublets), where "n" designates the order of appearance from low field in $CDCl_3$. Of these the urethane NH signals could be unambiguously identified by virtue of their high field posi-

Table 1. NH chemical shifts and temperature coefficients in pentapeptides

Resonance	Boc-Val-(Aib-Val)$_2$-OMe			Boc-Ala-(Aib-Ala)$_2$OMe		
	δ_{NH}		$d\delta/dT^a$ $\times 10^3$ (ppm/°C)	δ_{NH}		$d\delta/dT^a$ $\times 10^3$ (ppm/°C)
	$CDCl_3$ 1.67×10^{-2} M	$(CD_3)_2SO$ 1.67×10^{-2} M		$CDCl_3$ 1.94×10^{-2} M	$(CD_3)_2SO$ 1.94×10^{-2} M	
D_1	7.33	7.34	2.05	7.54	7.66	2.86
S_2	7.29	7.89	3.40	7.28	7.68	2.77
D_3	6.97	7.15	1.85	7.26	7.47	2.29
S_4	6.55	8.14	4.67	6.67	8.26	6.14
D_5	4.96	6.72	7.04	5.35	7.10	7.05

[a]Solvent $(CD_3)_2SO$

tion in $CDCl_3$.[3,7-9] Unequivocal assignments of the remaining two doublets (Ala) and two singlets (Aib) to specific residues is not possible at present. However a tentative assignment of S_4 to Aib(2) NH may be considered later on the basis of conformational conclusions. The involvement of NH groups in intramolecular H-bonding was investigated using temperature[25] and solvent dependence[26] of NH chemical shifts. The temperature coefficient values ($d\delta/dT$) in $(CD_3)_2SO$ are listed in Table 1. The solvent titration curves obtained in $CDCl_3$–$(CD_3)_2SO$ mixtures are shown in Fig. 1.

In both pentapeptides three NH groups (2 × NH and 1 Aib NH) appear to be shielded from the solvent since they have $d\delta/dT \leq 3 \times 10^{-3}$ ppm/°C. The same resonances also show significantly lower chemical shift changes on increasing the composition of $(CD_3)_2SO$, a strongly H-bonding solvent (Fig. 1). The urethane NH (X(1)NH) and one Aib NH have high temperature and solvent dependence of chemical shifts, characteristic of solvent exposed NH groups. The NMR data thus favours conformations for the pentapeptides in which 3 NH groups are intramolecularly hydrogen bonded.

Heptapeptides

In both peptides Boc-X-(Aib-X)$_3$-OMe (X = L-Ala or L-Val), all seven NH resonances are clearly observable (Figs. 2, 3). The chemical shifts of the various resonances are listed in Tables 2 and 3. Again, only the urethane NH (X(1) NH) can be unambiguously assigned as the D_7 resonance in both peptides. The $d\delta/dT$ values for the various NH groups in $(CD_3)_2SO$ and the solvent dependence of chemical shifts are summarized in Tables 2, 3

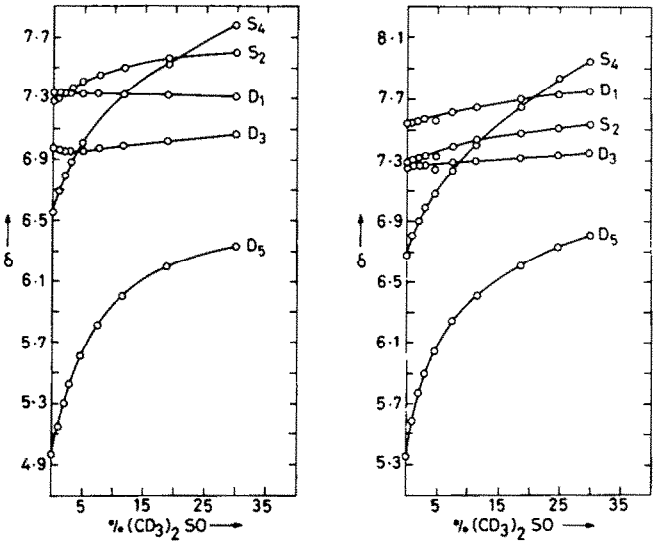

Fig. 1. Solvent dependence of NH chemical shifts in pentapeptides (left) Boc-Val-(Aib-Val)$_2$-OMe (right) Boc-Ala-(Aib-Ala)$_2$-OMe.

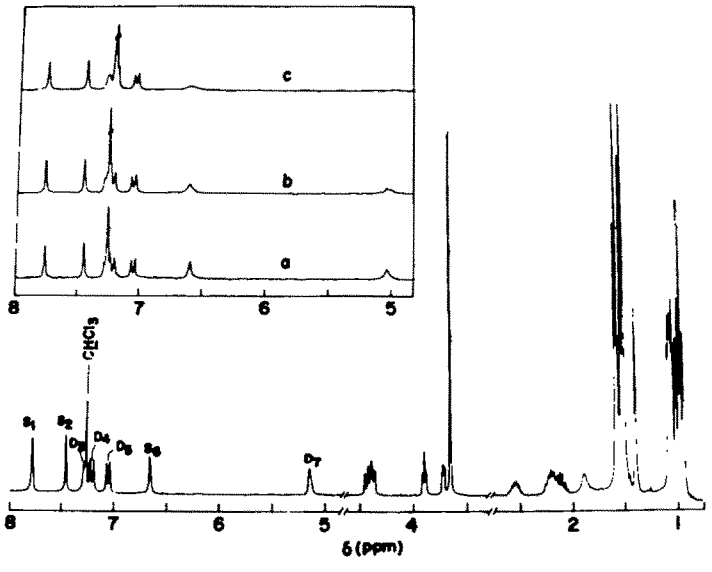

Fig. 2. 270 MHz ^{1}H NMR spectrum of Boc-Val-(Aib-Val)$_3$-OMe in $CDCl_3$, 1.28×10^{-2}M. (Inset) NH resonances observed in the presence of varying concentrations (wt %) of the nitroxide TEMPO (a) 0.025%; (b) 0.073%; (c) 0.23%.

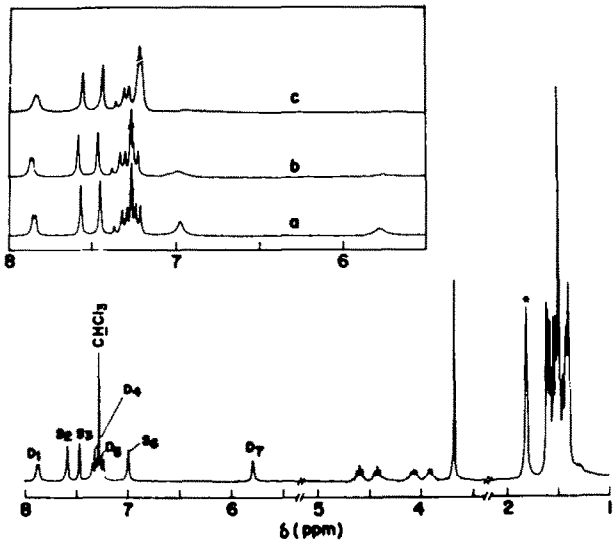

Fig. 3. 270 MHz ^{1}H NMR spectrum of Boc–Ala–(Aib–Ala)$_3$–OMe in $CDCl_3$, 1.49×10^{-2}M. (Inset) NH resonances observed in the presence of varying concentrations (wt %) of the nitroxide TEMPO (a) 0.025%; (b) 0.073%; (c) 0.23%.

Table 2. NH Chemical shifts and temperature coefficients in Boc–Val–(Aib–Val)$_3$–OMe

Resonance	δ_{NH}			$d\delta/dT^a$ $\times 10^3$ (ppm/°C)
	$CDCl_3$		$(CD_3)_2SO$	
	1.28×10^{-2} M	1.28×10^{-3} M	1.28×10^{-2} M	
S_1	7.81	7.75	7.95	3.29
S_2	7.48	7.44	7.56	1.66
D_3	7.31	7.27	7.53	4.53
D_4	7.22	7.23	7.15	0.66
D_5	7.07	7.05	7.12	1.34
S_6	6.67	6.53	8.27	5.04
D_7	5.16	4.89	6.95	7.05

[a] Solvent $(CD_3)_2SO$

Table 3. NH Chemical shifts and temperature coefficients in Boc–Ala–(Aib–Ala)$_3$–OMe

Resonance	δ_{NH}			$d\delta/dT^a$ $\times 10^3$ (ppm/°C)
	$CDCl_3$		$(CD_3)_2SO$	
	1.49×10^{-2} M	1.49×10^{-3} M	1.49×10^{-2} M	
D_1	7.88	7.70	7.96	3.98
S_2	7.61	7.47	7.81	2.79
S_3	7.49	7.36	7.53	2.21
D_4	7.32	7.25	7.44	1.33
D_5	7.23	7.19	7.33	1.56
S_6	7.13	6.55	8.37	5.81
D_7	6.07	5.10	7.19	7.28

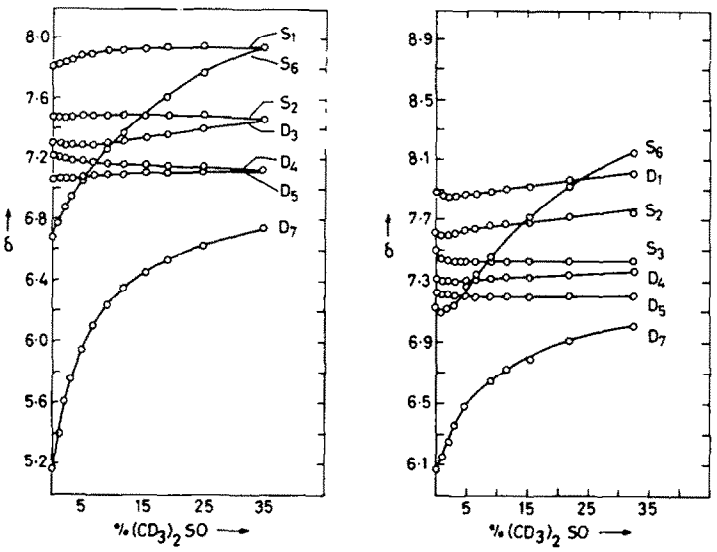

Fig. 4. Solvent dependence of NH chemical shifts in heptapeptides (left) Boc-Val-(Aib-Val)$_3$-OMe (right) Boc-Ala-(Aib-Ala)$_3$-OMe.

and Fig. 4, respectively. The dδ/dT values in $(CD_3)_2SO$ provide evidence for the involvement of 4 NH groups (2XNH and 2 Aib NH) in intramolecular H-bonding, while the remaining 3 NH groups have high dδ/dT values ($>4\times10^{-3}$ ppm/°C), indicative of exposure to solvent. However, the solvent titration experiments (Fig. 4) suggest that 5 NH groups in these peptides are relatively insensitive to changes in solvent composition. An examination of the data in Tables 2, 3 and Fig. 4 shows that resonances D_1 in the Ala-peptide and D_3 in the Val-peptide show only small $\Delta\delta$ values on going from $CDCl_3$ to $(CD_3)_2SO$, but have relatively high dδ/dT values in $(CD_3)_2SO$. These results suggest that while a four H-bond structure may be favoured in $(CD_3)_2SO$, there is a possibility of involvement of a fifth NH group in intramolecular H-bonding in $CDCl_3$.

Further support for this inference is obtained from examination of paramagnetic radical induced line broadening of NH resonances in $CDCl_3$.[27] Insets to Figs. 2 and 3 show the effect of addition of the free radical 2,2,6,6-tetramethyl piperidine-1-oxyl (TEMPO) on the Ala and Val heptapeptides, respectively. The dependence of the linewidths of the NH resonances on radical concentration are shown in Fig. 5. Quantitative linewidth determinations on all the NH resonances were rendered difficult due to overlap. It is clear from spectra in the insets to Figs. 2 and 3, that resonance D_1 in the Ala-heptapeptide and D_3 in the Val-heptapeptide are significantly less exposed to the radical than resonances S_6 and D_7 (urethane).

Conformations of penta and heptapeptides

The ^{1}H NMR results described above support the following conclusions:

(1) The pentapeptides, Boc-Ala-(Aib-Ala)$_2$-OMe and Boc-Val-(Aib-Val)$_2$-OMe favour folded conformations in solution, stabilized by *three* intramolecular H-bonds in both $CDCl_3$ and $(CD_3)_2SO$.

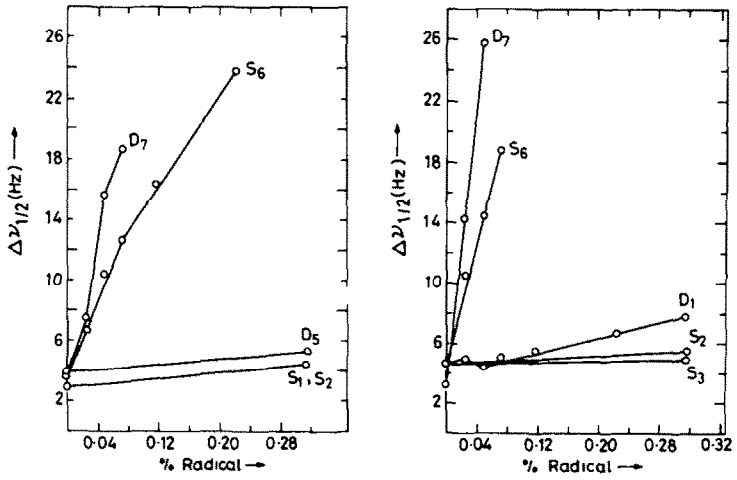

Fig. 5. Linewidths ($\Delta\nu_{1/2}$) of NH resonances as a function of radical (TEMPO) concentration in $CDCl_3$ (left) Boc-Val-(Aib-Val)$_3$-OMe (right) Boc-Ala-(Aib-Ala)$_3$-OMe. All NH resonances are not included due to spectral overlap.

(2) The heptapeptides Boc-Ala-(Aib-Ala)$_3$-OMe and Boc-Val-(Aib-Val)$_3$-OMe appear to adopt different conformations in $CDCl_3$ and $(CD_3)_2SO$. While a folded structure with five intramolecular H-bonds is favoured in $CDCl_3$, there appears to be a loss of one H-bond in $(CD_3)_2SO$.

In the analysis of the NMR results we have not explicitly considered the influence of peptide aggregation. Earlier studies from this laboratory of 10 and 11 residue Aib containing fragments of suzukacillin[28,29] and a model peptide benzyloxycarbonyl-(Aib-Pro)$_4$-OMe[23] have shown that aggregation of such peptides is not significant in $(CD_3)_2SO$ at the concentrations used. Peptide association is important in $CDCl_3$ but *intermolecular* H-bonding is preferentially mediated by NH and CO groups, which are free in the folded, monomeric peptide. Molecular association, thus, does not alter the secondary structure of the monomer.[23,28,29]

Together with the known stereochemical preferences of Aib residues,[10] the NMR data suggests that both pentapeptides favour 3_{10} helical conformations, stabilized by $4\rightarrow1$ H-bonding, in $CDCl_3$ and $(CD_3)_2SO$. A schematic representation of the H-bonding scheme is given in Fig. 6(a). X-Ray crystallographic studies have demonstrated such 3_{10} helical conformations in Aib oligopeptides[10,14,30] and two pentapeptide fragments of suzukacillin, Boc-Ala-Aib-Ala-Aib-Aib-OMe[11] and Boc-Leu-Aib-Pro-Val-Aib-OMe.[11,31] NMR evidence for the occurrence of 3_{10} helical conformations in fragments of alamethicin and suzukacillin has also been presented.[7-10]

The heptapeptides Boc-X-(Aib-X)$_3$-OMe, in contrast to the pentapeptides, show evidence of conformational changes on going from a relatively non-polar solvent like $CDCl_3$ to a polar, H-bonding solvent like $(CD_3)_2SO$. The presence of *five* H-bonded NH groups in $CDCl_3$ is consistent with a regular 3_{10} helical structure for both peptides in this solvent. Such a folded conformation would be stabilized by five $4\rightarrow1$ H-bonds (Fig. 6(b)). In $(CD_3)_2SO$ conformers, in which one of the $4\rightarrow1$ H-bonds involving an X NH group (Val or Ala) is loosened, are increasingly populated. Since unequivocal assignment of the X residue NH groups cannot be made, it is not possible to establish which Type III β-turn[32] is destabilized in $(CD_3)_2SO$. The fact that an X NH group is involved implies that an X-Aib β-turn is partially broken in $(CD_3)_2SO$. Evidence for 'loosening' of β-turns in incipient 3_{10} helical structures has been reported earlier in the peptide benzyloxycarbonyl-Aib-Pro-Aib-Ala-OMe.[3] While the NMR parameters in $(CD_3)_2SO$ for the resonances D_1 in the Ala-heptapeptide and D_3 in the Val-heptapeptide are indicative of partial exposure to solvent, it is noteworthy that $d\delta/dT$ values as high as 4.5×10^{-3} ppm/° have been accepted as diagnostic of hydrogen bonded NH groups.[33,34] An α-helical structure, stabilized by *four* $5\rightarrow1$ H-bonds, has not been considered for the heptapeptide in $(CD_3)_2SO$, since such conformations would result in three fully solvent exposed NH groups, corresponding to residues 1-3 in the sequence.

The studies described above suggested that the stereochemical constraints imposed by Aib residues dominate the folding of (Aib-X)$_n$ sequences. While the Ala and Val residues have substantially different tendencies to occur in helical conformations,[24] only minor differences in NMR parameters have been detected between the Val and Ala hepta and pentapeptides. A degree of conformational flexibility, albeit small, has been observed, leading to solvent dependent structural perturbations.

EXPERIMENTAL

All peptides were synthesized by soln phase procedures using Boc groups for amino protection, methyl esters for carboxyl protection and dicyclohexylcarbodiimide (DCC) or DCC-1-hydroxybenzotriazole as coupling agents.[35] Boc-Val and Boc-Ala were prepared by Schnabel's procedure,[36] while Boc-Aib was prepared by the procedure of Mayr and Jung.[37] All methyl esters of amino acids were prepared using the $SOCl_2$-MeOH procedure.[38] All peptides were checked for homogeneity by TLC on silica gel ($CHCl_3$:MeOH, 9.5:0.5) and yielded 60 MHz or 270 MHz ^{1}H NMR fully consistent with their structures.

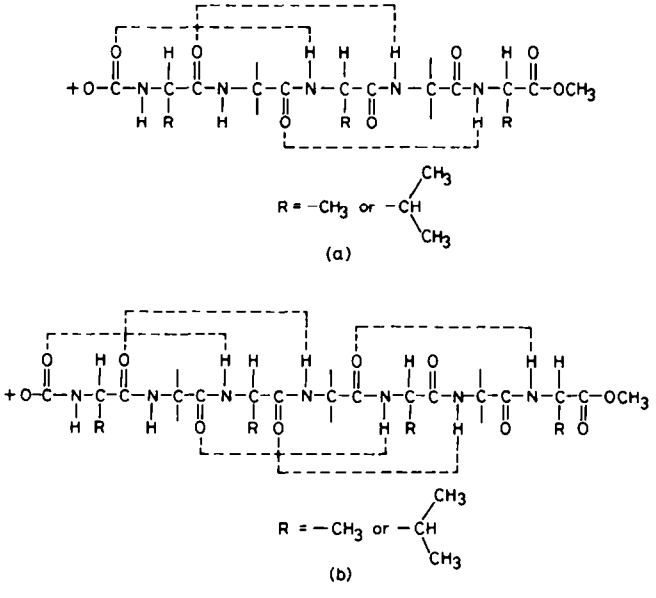

Fig. 6. Schematic hydrogen bonding pattern proposed for (a) Boc-X-(Aib-X)$_2$-OMe; (b) Boc-X-(Aib-X)$_3$-OMe. Note in $(CD_3)_2SO$ one $4\rightarrow1$ hydrogen bond is loosened.

Synthesis of Boc-Val-(Aib-Val)$_2$-OMe

Boc-Val-Aib-Val-OMe. Boc-Val (16 mmol, 3.3 g) was dissolved in CH_2Cl_2 (20 ml) and cooled to 0°. Aib-OMe (16 mmol, 1.9 g) was added followed by DCC (16 mmol, 3.3 g). The mixture was stirred for 4 hr at 0° and overnight at room temp. The precipitated dicyclohexylurea (DCU) was filtered off and the organic layer washed with 1N HCl (3 × 20 ml), 1N $NaHCO_3$ (3 × 20 ml) and finally with water (2 × 20 ml). The organic layer was dried over Na_2SO_4 and evaporated to yield Boc-Val-Aib-OMe as a solid, yield 4.2 g (81%). This was directly used without purification.

The dipeptide ester (12 mmol, 3.3 g) was dissolved in 10 ml MeOH and 2N NaOH (12 ml) was added and stirred for 12 hr. The mixture was washed with ether (2 × 15 ml) and the aqueous layer was acidified with 2N HCl, saturated with NaCl and extracted with EtOAc (4 × 15 ml). The EtOAc was dried and evaporated to give Boc-Val-Aib-OH as a white solid, yield 3.1 g (81%). This was directly converted to the tripeptide.

Boc-Val-Aib-OH (5 mmol, 1.5 g) was dissolved in DMF (5 ml) and cooled to 0°. Val-OMe (5 mmol, 0.7 g) was added followed by HOBt (5 mmol, 0.068 g) and DCC (5 mmol, 1.1 g). The mixture was maintained at 0° for 4 hr and at room temp for 20 hr. Work up as described above yielded the tripeptide as a white solid. 1.2 g (60%). M.p. = 160–162°. ^{1}H NMR (60 MHz, $CDCl_3$, δ): 7.18 (d, Val(3) NH), 6.78 (s, Aib NH), 5.18 (d, Val(1) NH), 4.51 (q, Val $C^\alpha H$), 3.89 (q, Val $C^\alpha H$), 3.76 (s, 3H, OMe), 2.07 (m, 2H, Val $C^\beta H$), 1.61, 1.58 (Aib $C^\beta H_3$), 1.48 (s, 9H, Boc CH_3), 0.95 (m, 12H, Val $C^\gamma H_3$).

Boc-Val-(Aib-Val)$_2$-OMe. Boc-Val-Aib-Val-OMe (2.5 mmol, 1.1 g) was dissolved in 99% formic acid (3 ml) and kept at room temp for 16 hr. The formic acid was removed *in vacuo*, the residue dissolved in water, neutralised with Na_2CO_3 and extracted with $CHCl_3$ (4 × 10 ml). The organic extract was dried and evaporated to give H_2N-Val-Aib-Val-OMe as a white solid (0.63 g, 80%), which was used directly.

Boc-Val-Aib-OH (1.7 mmol, 0.52 g) was dissolved in DMF (5 ml) and coupled to H_2N-Val-Aib-Val-OMe (1.7 mmol, 0.54 g) using DCC (1.7 mmol, 0.035 g) and HOBt (1.7 mmol, 0.023 g) as described earlier. The crude product was purified by column chromatography on silica gel, using $CHCl_3$ and $CHCl_3$–CH_3OH mixtures for elution. The pentapeptide was obtained as a white, crystalline solid, yield 0.8 g (80%) m.p. = 173–175°; $[\alpha]_D^{25} = -35°$ (c = 0.1, MeOH); R_f = 0.5 ($CHCl_3$–MeOH 9.5:0.5) (Found: C, 58.42; H, 8.99; N, 11.32. Calc. for $(C_{29}H_{53}O_8N_5)$: C, 58.10; H, 8.85; N, 11.69%). ^{1}H NMR (270 MHz, $CDCl_3$, δ): 7.33 (d, Val NH), 7.29 (s, Aib NH), 6.97 (d, Val NH), 6.55 (s, Aib NH), 4.96 (d, Val(1) NH), 4.46 (q, Val $C^\alpha H$), 4.18 (q, Val $C^\alpha H$), 3.76 (t, Val $C^\alpha H$), 3.71 (s, 3H, OCH_3), 2.44, 2.19, 1.95 (m, 3H, Val $C^\beta H$), 1.58, 1.56, 1.55, 1.45 (S, each 3H, Aib $C^\beta H_3$), 1.47 (9H, Boc CH_3), 0.93–1.04 (m, 18H, Val CH_3).

Synthesis of Boc-Val-(Aib-Val)$_3$-OMe

Boc-Val(Aib-Val)$_2$-OMe (1.2 mmol, 0.72 g) was dissolved in formic acid (2 ml). After deprotection of the Boc group the mixture was worked up as described earlier to yield H_2N-Val-(Aib-Val)$_2$-OMe as a white solid (0.55 g, 92%) which was directly used.

Boc-Val-Aib-OH (1 mmol, 0.3 g) in 5 ml DMF was cooled to 0°. H_2N-Val-(Aib-Val)$_2$-OMe (1 mmol, 0.5 g) was added followed by HOBt (1 mmol, 0.14 g) and DCC (1 mmol, 0.21 g). The reaction mixture was stirred at 0° for 6 hr and at room temp for 48 hr. Work up as described earlier, yielded a crude solid which was purified by column chromatography on silica gel ($CHCl_3$, $CHCl_3$–MeOH). The heptapeptide was obtained as a white solid, yield 0.68 g (86%); m.p. = 205–207°; $[\alpha]_D^{25} = -20°$ (c = 0.1, MeOH); R_f = 0.47 ($CHCl_3$:MeOH, 9.5:0.5). (Found: C, 58.06; H, 8.99; N, 12.41. Calc for C, 58.24; H, 8.81; N, 12.51%). ^{1}H NMR (270 MHz, $CDCl_3$) shown in Fig. 2. Single crystals suitable for X-ray diffraction were obtained from $CHCl_3$–ether soln.

Boc-Ala-(Aib-Ala)$_2$-OMe

The pentapeptide was prepared by procedures entirely analogous to those described for the corresponding Val analog. The peptide was obtained as a white solid after silica gel column chromatography, yield (2 + 3 coupling) = 32%; m.p. = 164–166° (m.p. (lit)[39] = 176°); $[\alpha]_D^{25} = -55°$ (c = 0.1, MeOH). The m.p. and $[\alpha]_D$ values differ from those reported in the lit.[39] It is possible that observations made on different crystalline polymorphs result in these differences. Preliminary X-ray evidence for such a phenomenon has been obtained for the Val peptides. R_f = 0.31 ($CHCl_3$:MeOH, 9.5:0.5). (Found: C, 53.75; H, 7.78; N, 13.04. Calc. for C, 53.59; H, 7.96; N, 13.59%). ^{1}H NMR (270 MHz, $CDCl_3$, δ): 7.54 (d, Ala NH), 7.28 (s, Aib NH), 7.26 (d, Ala NH), 6.67 (s, Aib NH), 5.35 (d, Ala(1) NH), 4.52, 4.19, 3.99 (m, 1H each, Ala $C^\alpha H$), 3.71 (s, 3H, OCH_3), 1.58, 1.56, 1.54, 1.44 (s, 3H each, Aib $C^\beta H_3$), 1.46 (s, 9H, Boc CH_3), 1.38–1.43 (m, 9H, Ala $C^\beta H_3$).

Boc-Ala-(Aib-Ala)$_3$-OMe

The heptapeptide was prepared by procedures described for the Val analog and purified by silica gel chromatography. Yield (2 + 5 coupling) = 65%, m.p. = 150–152°; $[\alpha]_D^{25} = -20°$ (c = 0.1, MeOH); R_f = 0.29 ($CHCl_3$:MeOH, 9.5:0.5). ^{1}H NMR (270 MHz, $CDCl_3$) shown in Fig. 3.

NMR Measurements. NMR studies were carried out on a Bruker WH-270 NMR spectrometer as described earlier.[7–9]

Acknowledgements—This research was supported by a grant from the Department of Science and Technology.

REFERENCES

[1]G. R. Marshall and H. E. Bosshard, *Circulation Res.* **30/31**, 143 (1972).
[2]G. Jung, N. Dubischar and D. Liebfritz, *Eur. J. Biochem.* **54**, 395 (1975).
[3]R. Nagaraj, N. Shamala and P. Balaram, *J. Am. Chem. Soc.* **101**, 16 (1979).
[4]Ch.P. Rao, R. Nagaraj, C. N. R. Rao and P. Balaram, *Biochemistry* **19**, 425 (1980).
[5]R. Oekonomopulos and G. Jung, *Biopolymers* **19**, 203 (1980).
[6]D. Liebfritz, W. Mayr, R. Oekonomopulos and G. Jung, *Tetrahedron* **34**, 2045 (1978).
[7]R. Nagaraj and P. Balaram, *Biochemistry* **20**, 2828 (1981).
[8]M. Iqbal and P. Balaram, *J. Am. Chem. Soc.* **103**, 5548 (1981).
[9]M. Iqbal and P. Balaram, *Biochemistry* **20**, 4866 (1981).
[10]R. Nagaraj and P. Balaram, *Acc. Chem. Res.* **14**, 356 (1981).
[11]A. K. Francis, Ch. P. Rao, M. Iqbal, R. Nagaraj, M. Vijayan and P. Balaram, *Biochem. Biophys. Res. Commun.* **106**, 1240 (1982).
[12]B. V. V. Prasad and P. Balaram, *Conformation in Biology* (Edited by R. Srinivasan and R. H. Sarma) pp. 133–140. Adenine Press, New York.
[13]G. D. Smith, V. Z. Pletnev, W. L. Duax, T. M. Balasubramanian, H. E. Bosshard, E. W. Czerwinski, N. E. Kendrick, F. S. Mathews and G. R. Marshall, *J. Am. Chem. Soc.* **103**, 1493 (1981).
[14]E. Benedetti, A. Bavoso, B. DiBlasio, V. Pavone, C. Pedone, M. Crisma, G. M. Bonora and C. Toniolo, *Ibid.* **104**, 2437 (1982).
[15]C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone and C. Pedone, *Biopolymers*, in press (1983).
[16]P. Van Roey, G. D. Smith, T. M. Balasubramanian, A. S. Redlinski and G. R. Marshall, *Int. J. Pept. Protein Res.* **19**, 499 (1982).
[17]T. Butters, P. Hutter, G. Jung, N. Pauls, N. Schmitt, G. M. Sheldrick and W. Winter, *Angew. Chem.* Int. Ed. Engl. **20**, 889 (1981).
[18]B. R. Malcolm, *Biopolymers* **16**, 2591 (1977).
[19]A. W. Burgess and S. J. Leach, *Ibid.* **12**, 2599 (1973).
[20]B. V. V. Prasad and V. Sasisekharan, *Macromolecules* **12**, 1107 (1979).
[21]A. K. Francis, M. Iqbal, P. Balaram and M. Vijayan, *J. Chem. Soc.* Perkin II 1235 (1982).
[22]Y. V. Venkatachalapathi, C. M. K. Nair, M. Vijayan and P. Balaram, *Biopolymers* **20**, 1123 (1981).
[23]Y. V. Venkatachalapathi and P. Balaram, *Ibid.* **20**, 1137 (1981).
[24]P. Y. Chou and G. D. Fasman, *Ann. Rev. Biochem.* **47**, 251 (1978).
[25]V. J. Hruby, *Chemistry and Biochemistry of Amino Acids,*

Peptides and Proteins (Edited by B. Weinstein), pp. 1–188. Dekker, New York (1974).
[26]T. P. Pitner and D. W. Urry, *J. Am. Chem. Soc.* **94**, 1399 (1972).
[27]K. D. Kopple, A. Go and D. R. Pilipauskas, *Ibid.* **97**, 6830 (1975).
[28]M. Iqbal and P. Balaram, *Biopolymers* **21**, 1427 (1982).
[29]M. Iqbal and P. Balaram, *Biochemistry* **20**, 7278 (1981).
[30]N. Shamala, R. Nagaraj and P. Balaram, *J. Chem. Soc.* Chem. Commun. 996 (1978).
[31]Ch. P. Rao and P. Balaram, *Biopolymers*, **21**, 2461 (1982).
[32]C. M. Venkatachalam, *Ibid.* **6**, 1425 (1968).
[33]Ch. P. Rao, R. Nagaraj, C. N. R. Rao and P. Balaram, *Biochemistry* **19**, 425 (1980).
[34]V. Renugopalakrishnan, M. A. Khaled, R. S. Rapaka and D. W. Urry, *Biochim. Biophys. Acta* **536**, 421 (1978).
[35]R. Nagaraj and P. Balaram, *Tetrahedron* **37**, 1263 (1981).
[36]E. Schnabel, *Liebigs Ann. Chem.* **702**, 188 (1967).
[37]W. Mayr, R. Oekonomopulos and G. Jung, *Biopolymers* **18**, 425 (1979).
[38]M. Brenner and W. Huber. *Helv. Chim Acta* **36**, 1109 (1953).
[39]R. Oekonomopulos and G. Jung, *Liebigs Ann. Chem.* 1151 (1979).