Stereochemically Constrained Linear Peptides. Conformations of Peptides Containing α -Aminoisobutyric Acid

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Abstract: ^1H NMR studies of the protected α -aminoisobutyric acid containing peptides Z-Aib-Pro-Aib-Ala-OMe and Z-Aib-Pro-Aib-OMe suggest that these molecules adopt well-defined conformations in solution. Evidence for type III β -bend structures is presented and an incipient 3_{10} helical conformation is proposed for the tetrapeptide. The interpretation of the NMR data is further substantiated by the crystal structure of the tetrapeptide, which shows two consecutive type III β bends in the solid state.

The determination of the conformation of peptides in solution has been the subject of considerable recent interest.^{1,2} The application of NMR methods to the conformational analysis of small linear peptides has been restricted by the fact that these molecules have a large number of conformational states of similar energies available, resulting in a dynamic averaging of the NMR spectral parameters. Cyclic peptides have therefore proved popular systems for investigation, not merely because of their biological relevance but also as a result of their comparatively restricted range of conformations, which renders them amenable to detailed analysis.³ Despite the difficulties associated with the study of flexible peptide systems, NMR studies of protected oligopeptides have been reported.⁴⁻⁸ Evidence for folded structures in linear peptides containing proline has been obtained by ^1H NMR.^{9,10} In principle, stereochemical constraints may be introduced into linear peptide sequences by the use of conformationally restricted amino acid residues. The steric hindrance introduced by α -alkylation of α -amino acids was first noted in synthetic investigations.^{11,12} Subsequent theoretical analysis showed that there is considerable restriction of conformational freedom in peptides derived from α,α -dialkylamino acids.¹³⁻¹⁵ The antibiotic alamethicin^{16,17} and related microbial peptides suzukacillin,¹⁸ emmerimicins,¹⁹ and antiamebics²⁰ contain a high proportion of α -aminoisobutyric acid. The amino acid sequences proposed for alamethicin on the basis of extensive ^1H NMR¹⁶ and mass spectrometric¹⁷ investigations differ only in the positioning of a phenylalaninol residue at the C-terminal end of the molecule. During the course of studies on the synthesis of alamethicin, we have prepared a number of oligopeptides containing Aib residues. This paper presents results of ^1H NMR studies of peptides incorporating Aib and describes a highly folded conformation in solution, for the tetrapeptide Z-Aib-Pro-Aib-Ala-OMe, which constitutes the amino terminal sequence of alamethicin.^{16,17} In a preliminary report²¹ we have described the incipient 3_{10} helical structure of the tetrapeptide in the solid state. The results of these x-ray diffraction studies are also discussed in terms of the 3_{10} helical conformation postulated on the basis of ^1H NMR experiments.

Experimental Section

Synthesis of Peptides. Amino acid methyl ester hydrochlorides were prepared by the thionyl chloride-methanol procedure.** Free esters were obtained by dissolving the hydrochloride in saturated Na_2CO_3 solution and extracting the ester into CH_2Cl_2 . Benzyloxycarbonyl- α -aminoisobutyric acid (Z-Aib) was prepared by the Schotten-Baumann procedure.** Melting points are uncorrected. Optical rotations were measured in methanol solutions at 25°C on a Jobin-Yvon polarimeter or a Jasco 5-20 spectropolarimeter. All compounds were checked for homogeneity by TLC on silica gel using the solvent

system 5% $\text{CH}_3\text{OH}/95\% \text{CHCl}_3$ for protected peptide esters and 85% $\text{CHCl}_3/10\% \text{CH}_3\text{OH}/5\% \text{CH}_3\text{COOH}$ for protected peptide acids.

Benzyloxycarbonyl- α -aminoisobutyrylprolyl Methyl Ester (Z-Aib-Pro-OMe).²³ Proline methyl ester (1.5 g) was added to a solution of Z-Aib (2.6 g) in 15 mL of CH_2Cl_2 at 0°C , followed by dicyclohexylcarbodiimide (DCC, 2.16 g) in 5 mL of CH_2Cl_2 . The mixture was stirred overnight at room temperature. After the precipitated dicyclohexylurea was filtered off, the filtrate was washed successively with 1 N HCl, H_2O , and 1 N NaHCO_3 . The organic layer was dried over Na_2SO_4 and evaporated to yield an oil (2.9 g, 80%): $[\alpha]^{25}_{\text{D}} -30.4^\circ$ (c 1.2, CH_3OH); ^1H NMR (CDCl_3) 1.47 (s, 3 H), 1.57 (s, 3 H), 1.82 (m, 4 H), 3.45 (m, 2 H), 3.62 (s, 3 H), 4.5 (m, 1 H), 5.1 (s, 2 H), 6.33 (s, 1 H), 7.2 (s, 5 H).

Benzyloxycarbonyl- α -aminoisobutyrylprolyl- α -aminoisobutyryl Methyl Ester (1, Z-Aib-Pro-Aib-OMe). Z-Aib-Pro-OMe (2.9 g) was dissolved in methanol (5 mL) and 5 mL of 2 N NaOH was added. After the solution had stood for 12 h at room temperature 30 mL of H_2O was added and the solution extracted with ethyl acetate. The aqueous layer was acidified with 2 N HCl and extracted with ethyl acetate (4 $\times$ 25 mL). The ethyl acetate layer was dried and evaporated to yield Z-Aib-Pro-OH as an oil (2.3 g, 88%). The oil was dissolved in 15 mL of CH_2Cl_2 and cooled to 0°C . A solution of Aib-OMe (0.8 g) in 3 mL of CH_2Cl_2 was added, followed by dicyclohexylcarbodiimide (1.45 g) in 4 mL of CH_2Cl_2 . The mixture was stirred at room temperature for 24 h and the precipitated urea was filtered. The filtrate was washed successively with 1 N HCl, H_2O , and 1 N NaHCO_3 and dried. Evaporation yielded an oily residue that solidified on addition of petroleum ether. The tripeptide ester was recrystallized from methanol-ether: yield 2.1 g (75%); mp 154°C ; $[\alpha]^{25}_{\text{D}} -5.0^\circ$ (c 0.4, CH_3OH). Anal. Calcd for $\text{C}_{22}\text{H}_{31}\text{O}_6\text{N}_3$: C, 60.96; H, 7.16; N, 9.7. Found: C, 61.32; H, 7.28; N, 9.4.

Benzyloxycarbonyl- α -aminoisobutyrylprolyl- α -aminoisobutyryl-alanyl Methyl Ester (2, Z-Aib-Pro-Aib-Ala-OMe). Z-Aib-Pro-Aib-OMe (2.1 g) was saponified using methanol-2 N NaOH as described for Z-Aib-Pro-OMe, yield 1.7 g (85%), mp 195°C .

Alanine methyl ester (Ala-OMe, 0.21 g) was added to a stirred suspension of Z-Aib-Pro-Aib-OH (0.77 g) in 10 mL of CH_2Cl_2 . A clear solution resulted after a few minutes. Dicyclohexylcarbodiimide (0.31 g) in 2 mL of CH_2Cl_2 was added and the mixture stirred for 36 h at room temperature. The dicyclohexylurea was filtered off and the filtrate washed with 1 N HCl, H_2O , and 1 N NaHCO_3 . Drying and evaporation of the organic layer yielded the tetrapeptide as a solid. Recrystallization from methanol-ether gave needle-shaped crystals: yield 0.63 g (70%); mp 145°C ; $[\alpha]^{25}_{\text{D}} -8.75^\circ$ (c 0.4, CH_3OH). Anal. Calcd for $\text{C}_{25}\text{H}_{36}\text{O}_7\text{N}_4$: C, 59.52; H, 7.14; N, 11.11. Found: C, 59.02; N, 11.27. Hydrogen analysis was not obtained. The molecular weight determined by X-ray methods²¹ was 503.3 (calcd, 504). ^1H NMR spectra were in full agreement with the structure.

The model peptides Z-Aib-Ala-OMe (mp 70°C , $[\alpha]^{25}_{\text{D}} -6.0^\circ$ (c 0.4, CH_3OH)), Z-Ala-Aib-Ala-OMe (mp 148°C , $[\alpha]^{25}_{\text{D}} -48.8^\circ$ (c 0.4, CH_3OH)), and Boc-Val-Aib-OMe (mp $115-118^\circ\text{C}$, $[\alpha]^{25}_{\text{D}} -27.5^\circ$ (c 0.4, CH_3OH)) were synthesized using the above procedures. The compounds were chromatographically homogeneous and yielded ^1H NMR spectra in full agreement with the expected structures.

Table I

compd ^d	proton	NH chemical shifts and $t_{1/2}$ ^a			
		CDCl ₃	(CD ₃) ₂ SO	$t_{1/2}$ CDCl ₃	$t_{1/2}$ (CD ₃) ₂ SO
Z-Aib-Pro-Aib-Ala-OMe (2)	Aib 1	5.83	7.93	48 min	16 min
	Aib 3	7.21	7.75	20 h	6.7 h
	Ala 4	7.52	7.49	20 h	12.1 h
Z-Aib-Pro-Aib-OMe ^b (1)	Aib 1	5.53	8.04		19 min
	Aib 3	7.39	7.56		24 h
Z-Aib-Pro-OMe	Aib 1	6.33	7.74	3.5 min	2 min
Z-Aib-Ala-OMe	Aib 1	5.28	7.23	31 min	18 min
	Ala 3	6.76	7.84	40 min	1 min
Z-Ala-Aib-Ala-OMe ^c	Ala 1	5.64	7.52	7.3 min	
	Aib 2	6.86	8.10	1.8 h	
	Ala 3	7.09		1.8 h	
Boc-Val-Aib-OMe	Val 1	5.14	6.44	10 min	
	Aib 2	6.66	8.15	1.5 h	

^a $t_{1/2}$ is the measured half-life for the first-order decay of the NH signals. ^b values are in parts per million from (CH₃)₄Si. ^c In (CD₃)₂SO only one Ala NH was observed, while the other was obscured by the phenyl resonance. The assignment of this resonance to Ala (1) NH is not unambiguous. ^d The $J_{\text{NH-C}^{\alpha}\text{H}}$ values for the Ala residues are as follows: Z-Aib-Pro-Aib-Ala-OMe, Ala (4), $J = 7.0 \pm 0.2$ (CDCl₃), 7.3 ± 0.2 Hz (CD₃)₂SO; Z-Aib-Ala-OMe, Ala (2), 7.2 ± 0.5 Hz; Z-Ala-Aib-Ala-OMe, Ala (1), Ala (3), $J = 7.0$ Hz (CDCl₃) for both residues.

Figure 1. Sequences of peptides

NMR Measurements. ¹H NMR spectra were recorded on a Varian HA-100 spectrometer at 28 °C. Deuterium exchange experiments in (CD₃)₂SO were done by addition of D₂O to a concentration of 10%. The exchange experiments in CDCl₃ were carried out by saturating the solvent with D₂O. In all experiments the peptide concentration was 50 mg/mL.

X-ray Diffraction. X-ray crystallographic studies were carried out as described elsewhere.²¹ A final *R* value of 0.031, using 2378 reflections, was obtained. The estimated standard deviation in the heavy atom coordinates is 0.003 Å.

Results and Discussion

Table I summarizes the chemical shifts of the NH protons in Z-Aib-Pro-Aib-Ala-OMe (**2**), Z-Aib-Pro-Aib-OMe (**1**) (Figure 1), and in model peptides containing Aib residues. The assignment of the resonances at δ 5.83 and 7.21 in the tetrapeptide, in CDCl₃, to the amide hydrogens of Aib (1) and Aib (3), respectively, follows from a comparison of the NH chemical shifts observed in the peptides Z-Aib-Ala-OMe, Z-Aib-Pro-OMe, and Z-Aib-Ala-Aib-OMe. In these peptides the assignment of the NH resonances is unequivocal, since the Aib NH appears as a singlet and the Ala NH as a doublet. The urethan NH appears consistently at higher field in CDCl₃, as compared to the peptide NH resonance, in all the compounds listed in Table I. The upfield shift of urethan NH peaks has also been noted in earlier studies.²⁴ The assignment of the doublet at δ 7.52 in the tetrapeptide spectrum to Ala (4) NH is unequivocal. The assignment of the Aib (1) and Aib (3) NH groups in the spectrum of **2** in (CD₃)₂SO was made by monitoring the change in chemical shifts in CDCl₃/(CD₃)₂SO mixtures as a function of (CD₃)₂SO concentration. The Aib (1) and Aib (3) NH resonances in the tripeptide Z-Aib-Pro-Aib-OMe (**1**) are assigned using similar comparisons.

Figure 2. Chemical shifts of the NH proton resonances of **1** and **2** in mixtures of CDCl₃ and (CD₃)₂SO.

The rates of exchange of the various amide and urethan hydrogens in these molecules were measured by monitoring the disappearance of the corresponding proton resonances on addition of D₂O. These results are presented in Table I. A comparison of the exchange half-lives ($t_{1/2}$) of the tetrapeptide (**2**) and tripeptide (**1**) with those of Z-Ala-Aib-Ala-OMe, Z-Aib-Pro-OMe, Z-Aib-Ala-OMe, and Boc-Val-Aib-OMe clearly shows that **2** contains two slowly exchanging amide hydrogens and that **1** has one slowly exchanging amide hydrogen. In all the compounds studied the urethan hydrogen underwent rapid exchange. It may be noted that, while both Aib (3) and Ala (4) NH resonances in **2** yielded $t_{1/2} > 20$ h in CDCl₃, the corresponding $t_{1/2}$ values in (CD₃)₂SO are significantly shorter. While a direct comparison of exchange rates in CDCl₃/D₂O and (CD₃)₂SO/D₂O systems is not valid, it is interesting that the $t_{1/2}$ value for Aib (3) NH in the tripeptide **1** in (CD₃)₂SO/D₂O is greater than 24 h. The corresponding value for **1** in CDCl₃/D₂O could not be determined owing to overlap of the NH peak with the phenyl resonances of the benzyloxycarbonyl protecting group.

Figure 2 shows the effect of altering the solvent on the chemical shifts of the NH resonances of **1** and **2**. Increasing the concentration of (CD₃)₂SO causes a large downfield shift of the urethan Aib (1) NH group of the tripeptide **1**, while the Aib (3) NH resonance is left almost unaffected. In the tetrapeptide **2**, the Aib (1) NH moves rapidly downfield with increasing (CD₃)₂SO concentration, while the Aib (3) and Ala

Figure 3. β -Turn conformation of Z-Aib-Pro-Aib-OMe (**1**)

(4) amide protons are less affected. The chemical shift values of the NH resonances, in the Aib-containing peptides being studied, in CDCl_3 and $(\text{CD}_3)_2\text{SO}$ are listed in Table I. Amide NH protons exposed to the solvent should show significant chemical shift variations on going from a poor hydrogen bond accepting solvent like CDCl_3 to a good hydrogen bond acceptor like $(\text{CD}_3)_2\text{SO}$. Solvent shifts may therefore be used to delineate exposed and shielded peptide hydrogens.²⁵ The results presented in Table I show that the Aib (3) and Ala (4) NH resonances in **2** and the Aib (3) NH resonance in **1** show significantly smaller chemical shift changes on going from CDCl_3 to $(\text{CD}_3)_2\text{SO}$ than the other NH resonances listed. Considered together with the deuterium exchange data, these results suggest that, in the tripeptide **1**, the Aib (3) NH hydrogen is shielded from the solvent, and that in the tetrapeptide **2**, the Aib (3) and Ala (4) NH hydrogens are shielded from the solvent.

The observation of solvent-shielded amide hydrogens in **1** and **2** argues for the presence of well-defined structures in solution. The ten-atom hydrogen bonded β turn,²⁶ involving the Aib (3) NH in a hydrogen bond with the urethane carbonyl group, is consistent with the data presented for the tripeptide **1** (Figure 3). For the tetrapeptide **2** structures involving both Aib (3) and Ala (4) amide hydrogens in intramolecular hydrogen bonds need to be considered. Figure 4 shows two possible conformations for the tetrapeptide involving two intramolecular hydrogen bonds. The structure shown in Figure 4b has been postulated for a tetrapeptide fragment of tropoelastin, Boc-Val-Pro-Gly-Gly-OMe in CDCl_3 .¹⁰ It has also been observed in the solid state for a collagenase substrate, *O*-bromocarboxybenzoxy-Gly-Pro-Leu-Gly-Pro.²⁷ However, for the tetrapeptide **2** the NH hydrogen of Aib (1) has been shown to be exposed to solvent, whereas the β structure would require Aib (1) NH to be involved in an intramolecular hydrogen bond. Further Aib (1) cannot be stereochemically accommodated in the β structure owing to unfavorable contacts of the geminal CH_3 groups of Aib (1) with the δ CH_2 group of Pro (2). A consideration of the conformational energy map reported for Aib residues confirms that the β structure ($\phi = -139^\circ$, $\psi = +135^\circ$) is energetically highly unfavorable.¹⁴ A structure that is compatible with the ^1H NMR data involving two consecutive β turns with Aib (1)-Pro (2) and Pro (2)-Aib (3) at the respective corners is shown in Figure 4a. While the NMR data provide evidence only for the degree of solvent exposure of amide hydrogens, their involvement in hydrogen bonds remains to be conclusively established. The postulation of ten-atom hydrogen bonded β -turn structures follows from the widespread occurrence of β turns in oligopeptides²⁸⁻³⁰ and proteins, demonstrated by X-ray crystallography.³¹⁻³³ Further, conformational energy calculations for acetyl α -aminoisobutyryl-*N*-methylamide (Ac-Aib-NHCH₃) indicate the presence

Figure 4. (a) Consecutive β turn or 3_{10} helical model for Z-Aib-Pro-Aib-Ala-OMe (**2**). (b) Antiparallel 6-sheet conformation for **2**.

of minima only in the right- and left-handed 3_{10} and α -helical regions.¹⁴ Consequently the β turns in the tetrapeptide, which contains two L amino acid residues, may fall into the type III category ($\phi = -60^\circ$, $\psi = -30^\circ$). This is reasonably close to the values for the right-handed α helix ($\phi \sim -50^\circ$, $\psi \sim -50^\circ$). The conformation shown in Figure 4b then forms an incipient 3_{10} helical structure.³⁴

The vicinal coupling constant between the amide hydrogen and the C^α proton has been extensively used in determining the conformational angle ϕ in peptides.² Alkylation at C^α in Aib residues removes this information from the spectrum. For the tetrapeptide **2** the only vicinal coupling constant obtainable is for Ala (4). The observed $^2J_{\text{HH}}$ of 7 Hz in CDCl_3 and 7.3 Hz in $(\text{CD}_3)_2\text{SO}$ suggests a conformationally averaged value for ϕ_4 in solution. This is consistent with the 3_{10} helical structure shown in Figure 4b but not with the β structure. It may be noted that $^3J_{\text{HH}}$ for both Ala residues in Z-Ala-Aib-Ala-OMe is -7 Hz. A consistent feature of the ^1H NMR spectra of the compounds listed in Table I is the nonequivalence of the benzylic $-\text{CH}_2-$ protons of the benzyloxycarbonyl group, in the peptides postulated to have a well-defined conformation in solution, involving the urethane carbonyl group as a hydrogen bond acceptor. The CH_2 protons (δ 5) appear as an AB quartet in the tripeptide **1** in CHCl_3 and $(\text{CD}_3)_2\text{SO}$ and in the tetrapeptide **2** in CDCl_3 . These protons, however, yielded a singlet in Z-Aib-Pro-OMe, Z-Aib-Ala-OMe, and Z-Ala-Aib-Ala-OMe. Among the related compounds examined benzyloxycarbonyl- α -aminoisobutyrylpropyl-*N*-methylamide (Z-Aib-Pro-NHCH₃) showed an AB quartet for the benzylic protons in CDCl_3 . The crystal structure of this molecule shows the presence of a type III β turn involving the urethane carbonyl and the methyl amide NH group in a hydrogen bond.³⁵ These observations suggest that enhanced chemical shift nonequivalence of the $-\text{CH}_2-$ protons follows the involvement of the urethane carbonyl group in stabilizing specific conformations. While the CH_2 protons are diastereotopic in these peptides and may be expected to show anisochrony in the absence of specific conformational effects,^{36,37} the observations support the view that conformational factors appear to determine the magnitude of nonequivalence. Figure 5 shows the effect of addition of trifluoroacetic acid (TFA) on the CH_2 quartet observed for the tetrapeptide **2** in CDCl_3 . Increasing the acid concentration leads to a collapse of the AB quartet to a broad singlet at about 4% (v/v) TFA in CDCl_3 . The structure breaking effect of TFA on polypeptides is well documented.³⁸ The results reported here further strengthen our contention that the tetrapeptide **2** adopts a folded conformation in CDCl_3 . Addition of TFA then leads to an unfolding of the tetrapeptide structure. The tetrapeptide **2** shows only a singlet for the benzylic CH_2 group in $(\text{CD}_3)_2\text{SO}$. The exchange rate of the Aib (3) amide hydrogen in **2** is significantly faster than the exchange rate of the corresponding NH group in the tripeptide **1** in $(\text{CD}_3)_2\text{SO}$. These results may

Figure 5. CH_2 proton resonances of the benzyloxycarbonyl group in Z-Aib-Pro-Aib-Ala-OMe (**2**). (a) CDCl_3 , (b) CDCl_3/TFA (40:1), (c) CDCl_3/TFA (26:1), (d) CDCl_3/TFA (16:1).

be tentatively interpreted, as resulting from a loosening of the Aib (1)–Pro (2) bend in the tetrapeptide in $(\text{CD}_3)_2\text{SO}$. A more rigorous comparison of the folded forms of **2** in CDCl_3 and $(\text{CD}_3)_2\text{SO}$ is not possible with the available experimental data.

The ^1H NMR results presented above strongly support the presence of defined conformations for the acyclic tri- and tetrapeptides **1** and **2** in CDCl_3 and $(\text{CD}_3)_2\text{SO}$. Theoretical calculations of the dipeptide conformational energy map for Aib residues show that type III β turns can accommodate these residues. The NMR data provide compelling evidence for the presence of intramolecular hydrogen bonds in **1** and **2** in solution, which are compatible with structures involving type III β bends. However, alternative hydrogen bonding schemes involving $1 \cdots 3$ (7 atom) and $1 \cdots 5$ (13 atom) hydrogen bonds cannot be ruled out from the ^1H NMR data alone. Indeed $1 \cdots 3$ hydrogen bonds have been postulated in solution for amino acid derivatives and peptides^{39,40} and have also been observed in the crystal structure of dihydrochlamydocin, a cyclic tetrapeptide containing one Aib residue.⁴¹ $1 \cdots 5$ hydrogen bonds are found extensively in α -helical segments in proteins.⁴² Since an unequivocal demonstration of the 3_{10} helical structure shown in Figure 4a did not appear to be feasible, exclusively on the basis of ^1H NMR studies, a single-crystal X-ray diffraction study of the tetrapeptide **2**, was carried out.

Crystal Structure of Z-Aib-Pro-Aib-Ala-OMe. A preliminary report of the structure at an earlier stage of refinement has been published.²¹ The projection of the molecule down the z axis is shown in Figure 6. The structure shows the presence of two intramolecular hydrogen bonds between the urethan CO and the NH group of Aib (3) and the CO group of Aib (1) and the NH group of Ala (4). The N $\cdots$ O distances are 3.17 and 3.06 Å, respectively. These distances compare well with reported values for hydrogen bond lengths in crystal structures of peptides.³⁰ The presence of a $1 \cdots 5$ hydrogen bond, corresponding to an α -helical conformation, is ruled out by the large separation of 4.12 Å between O(2) of the urethan group and N(4) of Ala (4). The crystal structure also does not provide any evidence for $1 \cdots 3$ hydrogen bonds. The conformational angles (ϕ , ψ , and ω) for the structure are listed in Table II. The ob-

Figure 6. Molecular structure of Z-Aib-Pro-Aib-Ala-OMe (**2**) viewed down the z axis

Table II. Conformational Angles^a in the Crystal Structure of Z-Aib-Pro-Aib-Ala-OMe

angle	Aib (1)	Pro (2)	Aib (3)	Ala (4)
ϕ	−51.2	−54.8	−72.0	−67.8
ψ	−45.3	−35.8	−11.2	155.6
ω ^b	−171.3	170.1	−173.2	

^a The convention followed is that proposed in ref 44. ^b The angle ω_0 defined by O(1)–C(8)–N(1)–C(9) is $−175.9^\circ$.

served values for ϕ and ψ are in fairly good agreement with the values expected for type III β turns. Full details of the crystal structure will be reported separately.

The structure observed for the tetrapeptide **2** in the crystalline state confirms that the incipient 3_{10} helical conformation is indeed an acceptable structure. The existence of two solvent-shielded amide hydrogens in **2** and one solvent-shielded amide hydrogen in **1** leads us to conclude that the tetrapeptide maintains the 3_{10} helical conformation in solution while the tripeptide adopts a type III β -bend structure. Further support for the type III β -bend structure in **1** comes from X-ray diffraction studies of Z-Aib-Pro-NHCH₃, which shows the type III β turn with Aib and Pro residues at the corners.³⁵ Our results substantiate earlier suggestions that Aib residues are sterically hindered^{13,14} and restrict conformational flexibility in small peptides. The property of Aib residues to occur in type III β -bend conformations has received further support from a single-crystal X-ray study of *p*-toluenesulfonyl-(Aib)₅-OMe, which shows three consecutive β bends in the solid state. We shall elaborate on these results elsewhere. Recently, fiber diffraction evidence has been presented for poly(α -aminoisobutyric acid) which suggests that the polypeptide adopts a 3_{10} helical conformation in the solid state.⁴³

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