X-Pro Peptides: Synthesis and Solution Conformation of Benzyloxycarbonyl-(Aib-Pro)₄-Methyl Ester. Evidence for a Novel Helical Structure

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Synopsis

The synthesis of the octapeptide, benzyloxycarbonyl-(α-aminoisobutyryl-L-prolyl)₄-methyl ester [Z-(Aib-Pro)₄-OMe] and an analysis of its solution conformation is reported. The octapeptide is shown to possess three strong intramolecular hydrogen bonds on the basis of studies of the solvent and temperature dependence of NH chemical shifts and rates of hydrogen–deuterium exchange. ^{13}C studies are consistent with a structure involving only *trans* Aib-Pro bonds, while ir experiments support a hydrogen-bonded conformation. The Aib 3, 5, and 7 NH groups are shown to participate in hydrogen bonding. A 3_{10} helical conformation compatible with the spectroscopic data is suggested. The proposed conformation consists of three type III β-turns with Aib and Pro at the corners and stabilized by 4 → 1 intramolecular hydrogen bonds.

INTRODUCTION

The conformational analysis of proline-containing peptides has been the subject of considerable attention. As part of a systematic program to examine the conformational characteristics of peptide sequences containing Pro at every alternate position, we have chosen to synthesize oligopeptide sequences of the type $(X\text{-Pro})_n$. The preceding paper described the solution and solid-state conformation of benzyloxycarbonyl-(Aib-Pro)₂-methyl ester.[1] In this report we describe the synthesis and conformation of benzyloxycarbonyl-(Aib-Pro)₄-methyl ester [Z-(Aib-Pro)₄-OMe] in organic solvents. α-Aminoisobutyric acid (Aib) was chosen as the X residue since the conformational freedom of peptides containing this amino acid is greatly restricted.[2,3] It was therefore expected that a sequential arrangement of Aib and Pro would lead to well-defined solution conformations even in short peptides. Small acyclic peptides containing Aib have been shown to adopt β-turn and 3_{10} helical conformations in solution and in the solid state.[2-7] Recent interest in the conformations of Aib-containing peptides has been stimulated by the widespread occurrence of the amino acid as a constituent of alamethicin and related membrane-active peptides.[8-14] The stereochemical constraints resulting from the gem dimethyl

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groups at the C^α atom make the introduction of Aib residues into peptides an attractive method for the generation of stereochemically defined acyclic peptides. Examples of this approach in generating biologically active analogs of enkephalins have been reported.[15,16] In this paper a novel helical conformation stabilized by three strong $4 \rightarrow 1$ intramolecular hydrogen bonds is established for the octapeptide Z-(Aib-Pro)$_4$-OMe in solution.

EXPERIMENTAL METHODS

Synthesis of Benzyloxycarbonyl-(α-aminoisobutyryl-L-prolyl)$_4$-methyl Ester [Z-(Aib-Pro)$_4$-OMe], 1

Z-Aib-Pro-Aib-Pro-OH

Z-Aib-Pro-Aib-Pro-OMe (1.35 g)[1] was dissolved in 10 ml CH_3OH and 5 ml of 2N NaOH was added. After standing for 8 hr at room temperature, 30 ml of water was added and the mixture extracted with ethyl acetate. The aqueous layer was acidified with 2N HCl and extracted with ethyl acetate (4 × 20 ml). The organic layer was dried and evaporated to yield the tetrapeptide acid as a chromatographically homogeneous (TLC, silica gel, 85% $CHCl_3$/10% CH_3OH/5% CH_3COOH) solid. Yield, 1.1 g (80%); mp 108–109°C. $[\alpha]_D^{25} = -43.6°$ ($c = 0.37$, CH_3OH).

H_2N-Aib-Pro-Aib-Pro-OMe

Z-Aib-Pro-Aib-Pro-OMe (1.4 g) was taken in 10 ml ethanol. Palladium black (0.1 g) and cyclohexene (6 ml) were added and the mixture refluxed for 6 hr on a water bath.[17] The catalyst was filtered and washed with ethanol. The filtrate and washings were combined and evaporated to yield an oil (0.8 g), which was used for further coupling without purification.

Z-(Aib-Pro)$_4$-OMe (1)

H_2N-Aib-Pro-Aib-Pro-OMe (0.8 g) was dissolved in 5 ml dimethylformamide, cooled in an ice bath, and Z-Aib-Pro-Aib-Pro-OH (1.1 g), 1-hydroxybenzotriazole (0.27 g), and DCC (0.41 g) were added successively. The mixture was stirred in an ice bath for 2 hr and overnight at room temperature. The dicyclohexylurea was filtered off and washed with ethyl acetate. The combined filtrate and washings were extracted with H_2O, 1N HCl, 1N NaHCO$_3$, and H_2O and dried. Evaporation of ethyl acetate yielded a solid which was dissolved in minimum volume of CH_3CN and filtered to remove insoluble material. Evaporation of CH_3CN resulted in a white shining solid, which was chromatographically homogeneous (TLC, silica gel, 5% CH_3OH/$CHCl_3$). Yield, 0.5 g (40%); mp 125–127°C. $[\alpha]_D^{25} = -27.9°$ ($c = 1.4$, CH_3OH).

ANAL.: *Calcd. for* $C_{44}H_{64}O_{11}N_8$: C, 60,0; H, 7.27; N, 12.72. *Found:* C, 59.57; H, 7.52; N, 12.66.

[1]H-nmr (270 MHz, CDCl$_3$):   7.80, s, 7.64, s, 7.64, s, 3H, Aib (3,5,7) NH; 7.36, m, 5H, phenyl; 5.48, s, 1H, Aib (1) NH; 5.12, q, 2H, benzyl CH$_2$; 4.59, 4.31, m, 4H, Pro (2,4,6,8) C$^\alpha$H; 3.72, m, 8H, Pro (2,4,6,8) C$^\delta$H$_2$; 3.69, s, 3H, OCH$_3$; 1.86, 1.92, m, 16H, Pro (2,4,6,8) C$^\beta$H$_2$, C$^\gamma$H$_2$; 1.56, 1.52, 1.50, 1.47, 1.45, 1.41, s, 24H, Aib (1,3,5,7) C$^\beta$H$_3$.

NMR and IR Studies

[1]H and ^{13}C-nmr spectra were recorded on a Bruker WH-270 FT nmr spectrometer at the Bangalore NMR Facility at 270 and 67.89 MHz, respectively, as described earlier.[1]   The ir spectra were recorded in CHCl$_3$ solution ($\sim$3–4 mM) on a Perkin-Elmer 580 spectrometer using a variable-path-length cell with NaCl windows.[3]

RESULTS

The low-field regions of the 270-MHz [1]H-nmr spectra of the octapeptide 1 in CDCl$_3$ and (CD$_3$)$_2$SO are shown in Fig. 1.   The high-field NH resonance at 5.48 δ in CDCl$_3$ may be unambiguously assigned to the urethane NH of Aib (1).[1,2,18]   The remaining three singlets at 7.67, 7.68, and 7.84 δ corre-
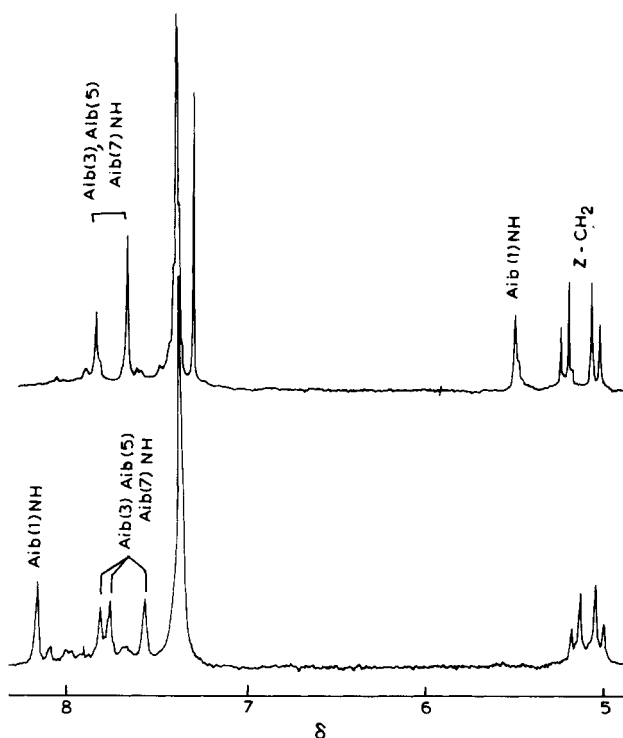

Fig. 1. The 270-MHz [1]H-nmr spectra of compound 1, low-field region: CDCl$_3$ (top) (CD$_3$)$_2$SO (bottom).   Small unidentified peaks correspond to diastereomeric peptide impurities.
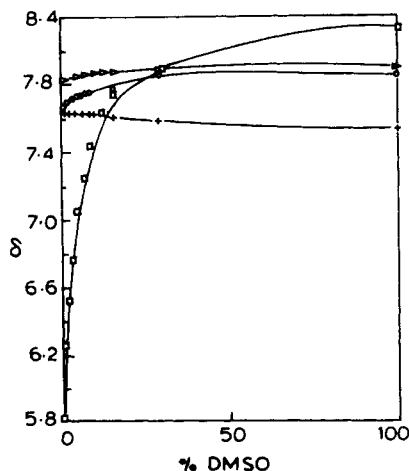
Fig. 2. Dependence of NH chemical shifts on solvent composition in $CDCl_3/(CD_3)_2SO$ mixtures.

spond to the Aib(3), (5), and (7) NH groups.   A unique assignment of these signals on the basis of the available data is not possible.   The 8.11 δ singlet in $(CD_3)_2SO$ is assigned to Aib(1) NH using spectra recorded in $(CD_3)_2SO/CDCl_3$ mixtures of varying composition.   Solvent titration,[18] variable-temperature studies of NH chemical shifts,[19] and hydrogen–deuterium (H-D) exchange experiments[20] have been carried out to evaluate the solvent accessibility of the Aib NH protons in 1.

Figure 2 shows the change in chemical shifts of the NH protons of 1 as a function of $(CD_3)_2SO$ concentration in $CDCl_3/(CD_3)_2SO$ mixtures.   While the Aib(1) NH shows a large downfield shift between 0 and 20% $(CD_3)_2SO$, the Aib(3), (5), and (7) NH groups are relatively unaffected.   This suggests that the latter are not accessible to $(CD_3)_2SO$, a strongly hydrogen bond accepting solvent, whereas the Aib(1) NH is exposed.   Table I summarizes the relevant nmr parameters for the octapeptide 1.   Studies of the tem-

TABLE I

Chemical Shifts, Temperature Coefficients, and H-D Exchange Half-Lives of NH Protons in Compound 1

| Residue[a] | δ NH | | $d\delta/dT$[c] | $t_{1/2}$[d] | |
	$CDCl_3$[b]	$(CD_3)_2SO$	(ppm/°C)	$CDCl_3$	$(CD_3)_2SO$
Aib 1	5.48 (5.19, 6.06)	8.11	5.2×10^{-3}	130 min	19 min
Aib 3	7.64 (7.61, 7.68)	7.55	1.6×10^{-3}	>3 days	>70 min
Aib 5	7.64 (7.61, 7.68)	7.75	1.0×10^{-3}	>3 days	>70 min
Aib 7	7.80 (7.79, 7.83)	7.79	1.5×10^{-3}	>3 days	>70 min

ᵃ The NH resonances of Aib(3), (5), and (7) are arbitrarily assigned.

ᵇ Chemical shifts in $CDCl_3$ at different concentrations.   Values in the column correspond to 10 mM peptide.   Values in parentheses are for 1.5 and 43 mM peptide.

ᶜ $(CD_3)_2SO$.

ᵈ $t_{1/2}$, half-life for the first-order H-D exchange process.

perature dependence of NH chemical shifts in $(CD_3)_2SO$ yield a high $d\delta/dT$ value of 5.22×10^{-3} ppm/°C for the Aib(1) NH. The other three NH groups have very low temperature coefficients of 1.49×10^{-3}, 1.03×10^{-3}, and 1.61×10^{-3} ppm/°C. Solvent-exposed peptide hydrogens generally yield $d\delta/dT$ values $>4 \times 10^{-3}$ ppm/°C.[21] Rates of H-D exchange were measured in $CDCl_3/D_2O$ and $(CD_3)_2SO/D_2O$ mixtures. In the former system, Aib(1) NH exchanged relatively fast ($t_{1/2} \sim 130$ min), while the other three NH groups exchanged slowly ($t_{1/2} > 3$ days). In the $(CD_3)_2SO/D_2O$ system, the rates of exchange were considerably greater, but again the Aib(1) NH yielded a much lower $t_{1/2}$ value (19 min) as com-pared to the Aib(3), (5) and (7) NH groups (>70 min).

The results described above strongly suggest that the Aib(3), (5), and (7) NH groups are involved in intramolecular hydrogen bonds. In order to rule out the possibility of intermolecular association, the concentration dependence of the NH chemical shifts was determined in $CDCl_3$. Over the range 1.5–43 mM the Aib(3), (5), and (7) NH groups showed very small changes in chemical shifts (Table I). The Aib(1) NH, however, moved to lower fields over this range. Thus, while peptide association may affect the urethane NH parameters, it appears that the other NH groups are unaffected. All nmr experiments described earlier were carried out in the lower concentration range of ~ 5 mM.

Figure 3 showed the 67.89-MHz ^{13}C-nmr spectrum of 1 in $CDCl_3$. The chemical shifts of the various carbons are summarized in Table II. The chemical shifts of the C^β (28.5–29.2δ) and C^γ (24.1–24.8δ) resonances of the Pro residues clearly indicate that all Aib-Pro bonds are in the *trans* con-formation. This is in agreement with earlier studies that suggest that whenever Pro is preceded by a sterically bulky group, the *trans* form is favored.[22–24] The C^β and C^γ resonances in 1 appear at higher and lower
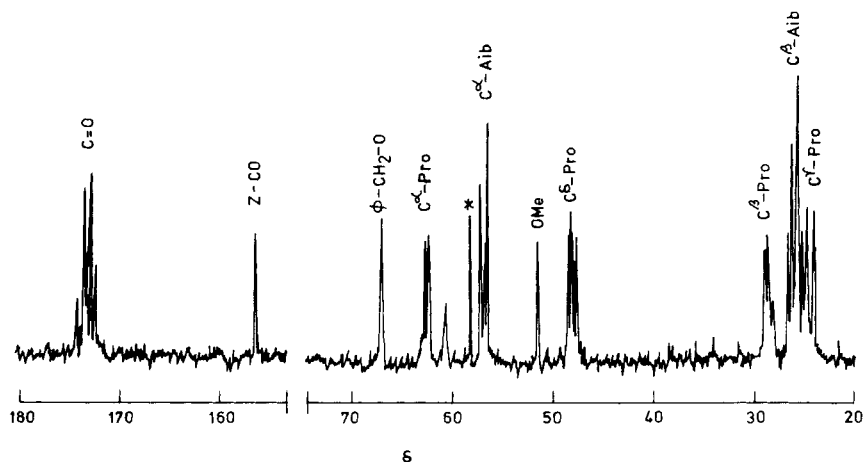

Fig. 3. The 67.89-MHz ^{13}C-nmr spectrum of 1 in $CDCl_3$. Starred peak is the CH_2 resonance of ethanol.

TABLE II

[13]Chemical Shifts (δ, ppm) in Z-(Aib-Pro)$_4$-OMe (1)

C$^\alpha$		C$^\beta$		C$^\gamma$	C$^\delta$	C=O	Others
Aib	Pro	Aib	Pro	Pro	Pro	Aib and Pro	
57.3	62.8	28.2	29.2	24.8	48.6	173.7	156.9 (urethane CO)
56.9	62.5	26.7	29.0	24.6	48.3	173.0[a]	51.7 (OCH$_3$)
56.7[a]	62.4	26.4[a]	28.7	24.2	48.2	172.8	136.6 ⎫
	60.7	26.2	28.5	24.1	47.8	172.7	128.7[a] ⎬ Phenyl
		25.9[a]				172.3[a]	128.5 ⎬ ring
		25.4				171.8	127.9[a] ⎭
							67.0 (Z-CH$_2$)

[a] Peaks correspond to two carbon resonances in intensity.

fields, respectively, than the values reported in model peptides. The C$^\beta$ and C$^\gamma$ chemical shifts compare well with the values reported in pivaloyl-Pro-NHMe (C$^\beta$, 27.2δ; C$^\gamma$, 25.7δ)[25] and pivaloyl-Pro-Pro-Ala-NHMe (C$^\beta$, 27.2, 29.6δ; C$^\gamma$, 24.6, 26.7δ).[23] This trend in C$^\beta$ and C$^\gamma$ chemical shifts is also observed in Z-(Aib-Pro)$_2$-OMe and in alamethicin fragments.[24] Three C$^\alpha$ Pro resonances in 1 occur at relatively low field (62.4, 62.5, and 62.8δ), while one appears at higher field (60.7δ). In Z-Aib-Pro-OMe, the C$^\alpha$ Pro resonance is at 60.9. The shift to lower field by $\sim 2\delta$ of the Pro C$^\alpha$ resonances in 1 tends to support a helical folded structure.[24] In Z-Aib-Pro-NHMe and Z-Aib-Pro-Aib-Ala-OMe, where type III β-turn and incipient 3_{10} helical structures have been demonstrated in both solutions[2-4] and the solid state,[2,5] the C$^\alpha$ Pro peaks occur at 62.4 and 63.8δ.[24] The low-field position of the Pro C$^\alpha$ resonance in oligopeptide fragments of alamethicin has also been noted.[24]

The NH stretching bands (ν_{NH}) in the ir spectra of octapeptide 1 and Z-(Aib-Pro)$_2$-OMe in dilute CHCl$_3$ solution are shown in Fig. 4. In the tetrapeptide two bands corresponding to ν_{NH}(free) at 3436 cm^{-1} and ν_{NH}(bonded) at 3340 cm^{-1} are observed. The presence of the band at 3340 cm^{-1} argues for the occurrence of intramolecular hydrogen-bonded conformations.[3] Z-(Aib-Pro)$_2$-OMe has been shown to adopt a type I β-turn conformation in solution and solid state, stabilized by an intramolecular $4 \rightarrow 1$ hydrogen bond.[1] It can be seen in Fig. 4 that in the octapeptide 1, two bands are observed at 3432 and 3300 cm^{-1}. The ν_{NH}(bonded) peak (3300 cm^{-1}) is significantly more intense than in Z-(Aib-Pro)$_2$-OMe, while the ν_{NH}(free) intensities are comparable in the two peptides. This suggests that both the additional NH groups in 1 participate in the formation of intramolecular hydrogen bonds.

DISCUSSION

Aib-containing peptides have been shown to be stereochemically rigid, resulting in the population of only a very limited range of conformational states in solution. This has allowed interpretation of spectral data, ob-
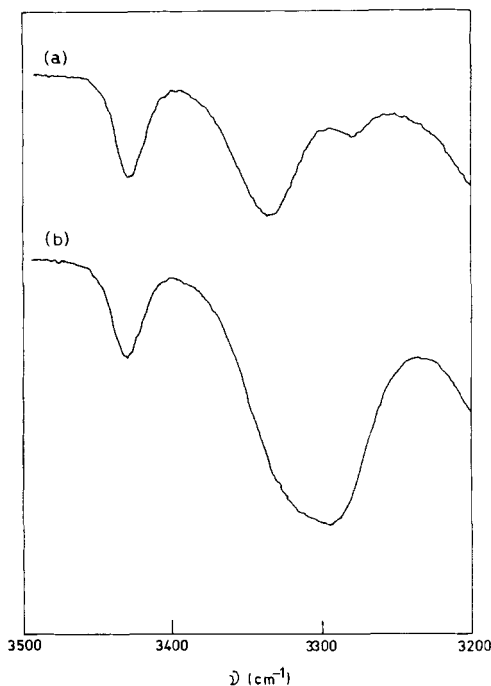
Fig. 4. ν_{NH} bands in the ir spectra of peptides in CHCl$_3$. (a) Z-(Aib-Pro)$_2$-OMe, 4.2 × 10^{-3}M. (b) Z-(Aib-Pro)$_4$-OMe (1), 3.3 × 10^{-3}M.

tained on small Aib peptides, in terms of only single, well-defined conformations. Excellent correlations have been obtained between nmr, ir, and x-ray studies. The tendency of Aib residues to occur in 3$_{10}$ helical conformations ($\phi \sim \pm 60°$, $\psi \sim \pm 30°$) has also been established.[1-7] In octapeptide 1 the alternation of Aib and L-Pro (where ϕ is restricted to $-60°$) is expected to result in a restricted range of permissible conformations. The nmr and ir results, outlined above, suggest that 1 predominantly adopts a structure in solution in which an all-trans peptide backbone is folded to yield three strong intramolecular hydrogen bonds involving the Aib(3), (5), and (7) NH groups. This is supported by the low solvent and temperature dependence of these NH chemical shifts and their slow rates of H-D exchange. A conformation compatible with the spectroscopic data is shown in Fig. 5.

The proposed structure is an approximately 3$_{10}$ helical conformation,[26] with alternate 4 → 1 hydrogen bonds interrupted by the Pro residues. In an idealized 3$_{10}$ helical model, unfavorable steric contacts exist between the CO groups of Aib(1), (3), and (5) and the C$^\delta$H$_2$ groups of Pro(4), (6), and (8), respectively. These may be relieved by slight distortions introduced by nonplanar peptide units and deviations of Aib and Pro ϕ, ψ values from those for a perfect 3$_{10}$ helix. The X_2-Pro$_3$ β-turn conformations (type III) shown in Fig. 5 have already been observed for Z-Aib-Pro-NHMe,[5] Z-Aib-Pro-Aib-Ala-OMe,[2] and Z-Aib-Pro-Aib-Pro-OMe[1] in the solid state.
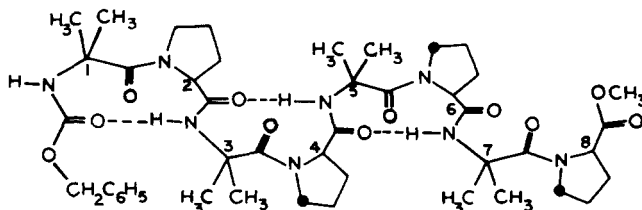
Fig. 5. Schematic representation of the intramolecularly hydrogen-bonded conformation of 1. Atoms involved in unfavorable steric contacts are shaded [CO groups of Aib(1), (3), and (5), and $C^\delta H_2$ of Pro(4), (6) and (8)].

For L-Pro to be accommodated in the approximately 3_{10} helical conformation, ψ_{Pro} should be restricted to the region $-10°$ to $-60°$. Theoretical calculations[27] suggest energy minima for L-Pro in the vicinity of $\psi \sim -50°$. A population of conformers with $\psi \sim -50°$ has also been suggested for poly(L-proline) in trifluoroethanol.[28] Further confirmation of the 3_{10} helical structure suggested for 1 must await crystal-structure determination. Attempts to obtain suitable single crystals have thus far proved fruitless.

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