X-Pro Peptides: Solution and Solid-State Conformation of Benzyloxycarbonyl-(Aib-Pro)$_2$-methyl Ester, a Type I β-Turn

Y. V. VENKATACHALAPATHI, C. M. K. NAIR, M. VIJAYAN, and P. BALARAM,* *Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India*

Synopsis

The synthesis of the tetrapeptide benzyloxycarbonyl(α-aminoisobutyryl-L-prolyl)$_2$-methyl ester (Z-(Aib-Pro)$_2$-OMe) and an analysis of its conformation in solution and the solid state are reported. Stepwise synthesis using dicyclohexylcarbodiimide leads to racemization at Pro(2). Evidence for the presence of diastereomeric tetrapeptides is obtained from 270-MHz ^{1}H-nmr and 67.89-MHz ^{13}C-nmr. The all-L tetrapeptide is obtained by fractional crystallization from ethyl acetate. The NH of Aib(3) is shown to be involved in an intramolecular hydrogen bond by variable-temperature ^{1}H-nmr and the solvent dependence of NH chemical shifts. The results are consistent with a β-turn conformation with Aib(1) and Pro(2) at the corners stabilized by a 4 $\rightarrow$ 1 hydrogen bond. The molecule crystallizes in the space group $P2_12_12_1$, with a = 8.839, b = 14.938, and c = 22.015 Å. The structure has been refined to an R value of 0.051. The peptide backbone is all-*trans*, and a 4 $\rightarrow$ 1 hydrogen bond, between the CO group of the urethane moiety and Aib(3) NH, is observed. Aib(1) and Pro(2) occupy the corner positions of a type I β-turn with $\phi = -55.4°$, $\psi = -31.3°$ for Aib(1) and $\phi = -71.6°$, $\psi = -38°$ for Pro(2). The tertiary amide unit linking Pro(2) and Aib(3) is significantly distorted from planarity ($\Delta\omega$ = 14.3°).

Peptides in which the imino acid proline occurs at every alternate position are stereochemically interesting, since conventional α-helical and β-structures are precluded because the presence of proline interrupts a regular sequence of intra- or interchain hydrogen bonds. The possibility that X-Pro tertiary amide bonds may exist in both *cis* and *trans* conformations, in solution, also needs to be considered.[1] The importance of understanding the conformations accessible to (X-Pro)$_n$ peptides is emphasized by the observation of such sequences in rabbit skeletal muscle alkali light chain (residues 13–30)[2] and in bovine β-casein A2 (residues 60–67).[3] Interestingly, the isolated heptapeptide sequence Tyr-Pro-Phe-Pro-Gly-Pro-Ile has been shown to have opioid activity.[4] A program to synthesize well-defined (X-Pro)$_n$ oligopeptides—where X = Gly, Sar, Ala, and α-aminoisobutyric acid (Aib)—and to study their solution and solid-state conformations by spectroscopic and x-ray methods has been initiated in this laboratory. The systematic variation of the bulk of the X residue should allow an evaluation of the influence of the C$^\alpha$ substituent on backbone conformation in this sequence. We describe in this report

* To whom correspondence should be addressed.

Biopolymers, Vol. 20, 1123–1136 (1981)

CCC 0006-3525/81/061123-14$01.40

the solution and solid-state conformation of the tetrapeptide benzyloxycarbonyl-(Aib-Pro)$_2$-OMe(Z-(Aib-Pro)$_2$-OMe), while the following paper summarizes the results of studies on the octapeptide Z-(Aib-Pro)$_4$-OMe. The tetrapeptide adopts a type I β-turn conformation stabilized by one strong intramolecular 4 → 1 hydrogen bond in solution and in the solid state.

EXPERIMENTAL METHODS

Synthesis of Peptides

Benzyloxycarbonyl(α-aminoisobutyryl-L-prolyl)$_2$-methyl Ester (Z-Aib-L-Pro-Aib-L-Pro-OMe, 1)

Z-Aib-Pro-Aib-OH[5] (3.3 g) was dissolved in 25 ml CH_2Cl_2 and cooled in an ice bath. L-Pro-OMe (1.0 g) was added followed by dicyclohexylcarbodiimide (DCC, 1.65 g) in 5 ml CH_2Cl_2. The mixture was stirred for 4 hr in an ice bath and overnight at room temperature. The precipitate of dicyclohexylurea (DCU) was filtered off, and the filtrate was washed successively with 1N HCl, 1N $NaHCO_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield an oil, which solidified on trituration with petroleum ether. Yield, 65%; mp 148–150°C. $[\alpha]_D^{25}$ −40.2° (c = 1.4, CH_3OH).

ANAL.: *Calcd. for* $C_{27}H_{38}O_7N_4$: C, 61.13; H, 7.16; N, 10.56. *Found:* C, 61.02; H, 6.99; N, 10.89.

The tetrapeptide prepared as above yielded a diastereomeric mixture of peptides, with racemization at Pro(2) (see Results and Discussion). Fractional crystallization from ethylacetate gave the all-L isomer. mp 184–185°C. $[\alpha]_D^{25}$ = −51° (c = 0.33, CH_3OH).

Benzyloxycarbonyl-L-prolyl-α-aminoisobutyryl-L-prolylmethyl Ester (Z-Pro-Aib-Pro-OMe)

Z-Pro-Aib-OH. Z-L-Pro (2.35 g) was dissolved in CH_2Cl_2 (20 ml) and cooled in an ice bath. Aib-OMe (1.2 g) and DCC (2.0 g) were added successively with stirring. The mixture was stirred for 4 hr in the cold and then at room temperature overnight. The precipitated DCU was filtered and the filtrate washed as described for 1. Z-Pro-Aib-OMe was obtained as an oil. Yield, 2.4 g (74%). Z-Pro-Aib-OMe (2.0 g) was allowed to stand in 10 ml methanol and 6 ml 2N NaOH for 6 hr at room temperature. After dilution with 20 ml water, the solution was washed with ethyl acetate (2 × 15 ml) to remove traces of the dipeptide ester. The aqueous layer was dried and evaporated to yield the dipeptide acid. Yield, 1.7 g (88%). mp 134–135°C. $[\alpha]_D^{25}$ = −55.7° (c = 0.1, CH_3OH).

Z-Pro-Aib-Pro-OMe. Z-Pro-Aib-OH 1.7 g was coupled to L-Pro-OMe as described above. The tripeptide ester was obtained as a chromatographically homogeneous oil. Yield, 1.6 g. $[\alpha]_D^{25}$ = −116.8° (c = 0.8, CH_3OH).

NMR Studies

^{1}H-nmr spectra were recorded at 270 MHz and ^{13}C spectra at 67.89 MHz on a Bruker WH-270 FT-nmr spectrometer at the Bangalore NMR Facility. For ^{1}H-nmr studies, a sweep width of 3012 Hz was used, with 8K data points yielding a digital resolution of 0.367 Hz/point. For ^{13}C-nmr studies, the sweep width used was 17,241 Hz, with a digital resolution of 2.104 Hz/point. All chemical shifts are expressed as δ (ppm) downfield from the corresponding ^{1}H or ^{13}C signals of internal tetramethylsilane. Concentrations of peptides used were 6 and 50 mg/ml for the ^{1}H and ^{13}C spectra, respectively.

X-Ray Diffraction

Tetrapeptide 1 crystallized by slow evaporation from ethyl acetate solution in the orthorhombic space group $P2_12_12_1$ with a = 8.839 (2), b = 14.938 (4), c = 22.015 (5) Å V 2906.8 Å^3, density (measured) = 1.203, density (calculated) = 1.210, and Z = 4. Intensity data were collected on a crystal of dimensions 0.6 × 0.4 × 0.3 mm on a CAD-4 diffractometer employing $\omega = 2\theta$ scan up to a maximum Bragg angle of 23° and using graphite monochromated MoK$_\alpha$ radiation. Of the 2123 reflections collected in this range, 1495 having $I > 2\sigma(I)$ were used for the structure determination and refinement. The intensities were corrected for Lorentz and polarization factors but not for absorption.

Structure Determination and Refinement

Attempts at structure solution employing MULTAN[6] using normal E values, as well as those normalized in each parity group and different ranges of Bragg angle, were unsuccessful. A five-atom fragment resembling a pyrrolidine ring could be identified with difficulty in the E map corresponding to the best set of phases produced by MULTAN. The E values used were calculated using group scattering factors for the benzene and pyrrolidine rings. The structure was developed from this fragment by the Karle recycling process.[7]

The structure was refined by the block diagonal SFLS method, using a modified version of the program written by R. Shiono. The heavy atoms and the hydrogen atoms located from a difference Fourier map were assigned anisotropic and isotropic thermal parameters, respectively, in the final cycles. The refinement converged at R = 0.051. The weighting function used had the form $1/(a+bF_0 + cF_0^2)$, where a = 0.533, b = 0.105, and c = −0.002. The scattering factors of the nonhydrogen and hydrogen atoms were taken from Cromer and Waber[8] and Stewart et al.,[9] respectively. The final positional parameters of the nonhydrogen atoms are given in Table I. A list of the observed and calculated structure factors is available on request.

TABLE I
Fractional Coordinates of Nonhydrogen Atoms ($\times 10^4$)[a]

Atom	x	y	z	Atom	x	y	z
C_{Z1}	3373 (15)	10968 (9)	9444 (5)	C_3^{β}	6811 (10)	7419 (6)	7843 (6)
C_{Z2}	3973 (18)	11714 (13)	9765 (6)	C_3^{α}	5325 (9)	7187 (5)	7490 (4)
C_{Z3}	3272 (17)	12519 (10)	9591 (6)	C_3	4312 (10)	6622 (5)	7884 (4)
C_{Z4}	2289 (16)	12612 (8)	9152 (6)	O_3	4742 (7)	5864 (3)	8024 (3)
C_{Z5}	1762 (13)	11879 (7)	8844 (5)	N_4	2987 (7)	6953 (4)	8074 (3)
C_{Z6}	2298 (13)	11039 (6)	8992 (4)	C_4^{α}	1791 (9)	6368 (5)	8339 (3)
C_{Z7}	1780 (14)	10224 (6)	8660 (4)	$C_4^{\beta 1}$	1216 (11)	5713 (6)	7854 (4)
C_1^{α}	2425 (7)	10256 (3)	8055 (3)	$C_4^{\beta 2}$	539 (11)	6976 (7)	8549 (4)
C_1	2250 (10)	9514 (5)	7701 (4)	C_4	2434 (10)	5861 (5)	8895 (4)
O_1	1626 (7)	8847 (3)	7893 (3)	O_4	2105 (8)	5062 (4)	8948 (3)
N_2	2836 (7)	9637 (3)	7157 (3)	N_5	3216 (8)	6270 (4)	9320 (3)
C_2^{α}	2820 (10)	8945 (5)	6687 (4)	C_5^{α}	3797 (11)	7194 (6)	9352 (4)
$C_2^{\beta 1}$	3780 (11)	9279 (6)	6154 (4)	C_5^{γ}	4323 (18)	7311 (8)	9978 (6)
$C_2^{\beta 2}$	1217 (11)	8738 (6)	6479 (4)	C_5^{β}	5010 (12)	6343 (7)	10113 (5)
C_2	3561 (9)	8051 (5)	6907 (3)	C_5^{α}	3845 (10)	5740 (6)	9815 (4)
O_2	3045 (7)	7360 (3)	6721 (3)	C_5	2670 (12)	5481 (6)	10279 (4)
N_3	4690 (7)	8067 (4)	7314 (3)	O_5	1476 (9)	5834 (5)	10363 (4)
C_3^{δ}	5707 (9)	8811 (6)	7505 (5)	O_6	3147 (9)	4765 (4)	10586 (3)
C_3^{γ}	7220 (12)	8355 (6)	7617 (6)	C_6^{α}	2208 (18)	4469 (8)	11086 (6)

[a] Standard deviations are given in parentheses.

RESULTS AND DISCUSSION

Racemization at Pro(2) During Synthesis

Z-Aib-Pro-Aib-Pro-OMe 1 was synthesized by stepwise elongation from Z-Aib, using DCC-mediated couplings. This procedure was initially adopted since the amino acids to be activated were Aib, an achiral residue, and Pro, a residue generally believed to be resistant to racemization on carboxyl activation.[10] Tetrapeptide 1 obtained by this procedure was a chromatographically homogeneous solid (TLC, 5% $CH_3OH/CHCl_3$), which yielded correct C, H, N analytical data. Figure 1(a) shows the 270-MHz ^{1}H-nmr spectrum of 1 in $CDCl_3$. Two sets of resonances are observed for the Aib(1) NH (5.62 δ, 5.65δ) and Aib(3) NH (7.47 δ, 7.25 δ) groups. The Aib(1) NH peaks are assigned on the basis of the known tendency of urethane NH groups to appear at high field in $CDCl_3$.[5,11,12] The CH_2 protons of the benzyloxycarbonyl group appear as two sets of AB quartets. Additional resonances can also be detected for the methyl resonances of the Aib residues and the ester group. These results suggest that the tetrapeptide obtained by stepwise synthesis consists of diastereomeric species. The only point at which configurational inversion can occur is Pro(2), and the species may be assigned as Z-Aib-L-Pro-Aib-L-Pro-OMe and Z-Aib-D-Pro-Aib-L-Pro-OMe. These conclusions are strengthened by studies which show that the formation of enantiomeric peptides is suppressed if 1-hydroxybenzotriazole or N-hydroxysuccinimide[13] are added in the coupling of Z-Aib-Pro-OH to Aib-OMe (R. Nagaraj and P. Balaram, unpublished).

Recrystallization of a sample of the tetrapeptide from ethyl acetate yielded single crystals, suitable for x-ray diffraction. The x-ray studies, described in a later section, established that both Pro residues have the same configuration, which must necessarily be L, since L-Pro-OMe was used in the last step of synthesis. The 270-MHz ^{1}H-nmr spectra of these crystals are shown in Figs. 1(b) and (c). In both solvents, the additional resonances noted in Fig. 1(a) are absent. The 67.89-MHz ^{13}C-nmr spectra of the diastereomeric mixture and the crystalline peptide are compared in Fig. 2. Additional resonances are seen for the CO groups and the C^α and C^δ carbons of Pro in the mixture. Furthermore, the high-field region (20–30 δ) is also complicated by extra peaks in the spectrum of the mixture as compared to the crystals. These results suggest that only one isomer has been obtained by crystallization. The subsequent conformational studies by nmr and x-ray methods have been carried out using the crystalline sample, referred to as tetrapeptide 1.

NMR Studies

The C^β resonances in 1 occur at 28.6 and 28.3δ while the C^γ resonances occur at 25.1 and 25.0δ. These positions are characteristic of *trans* X-Pro tertiary amide bonds.[14,15] There is no evidence from the ^{1}H-nmr spectra in Figs. 1(b) and (c) for the presence of minor conformations, suggesting

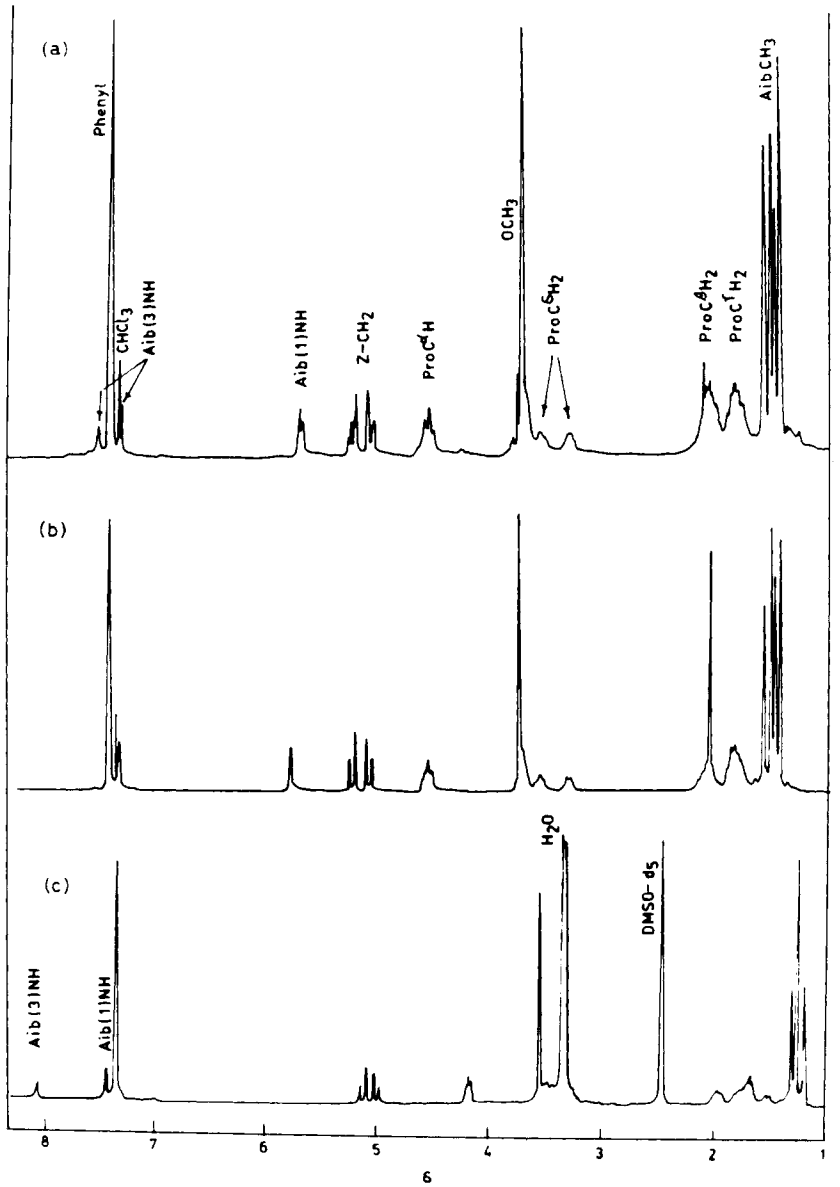

Fig. 1. The 270-MHz ^{1}H-nmr spectra of *Z*-(Aib-Pro)$_2$-OMe. (a) Diastereomeric mixture in $CDCl_3$. (b) Crystals from ethyl acetate in $CDCl_3$. (c) Crystals from ethyl acetate in $(CD_3)_2SO$.

that both Aib-Pro bonds in **1** occur only in the *trans* form in solution. Earlier studies have established the preference for *trans X*-Pro bonds in peptides where *X* is Aib[5,16] or the sterically analogous pivaloyl group.[17,18]

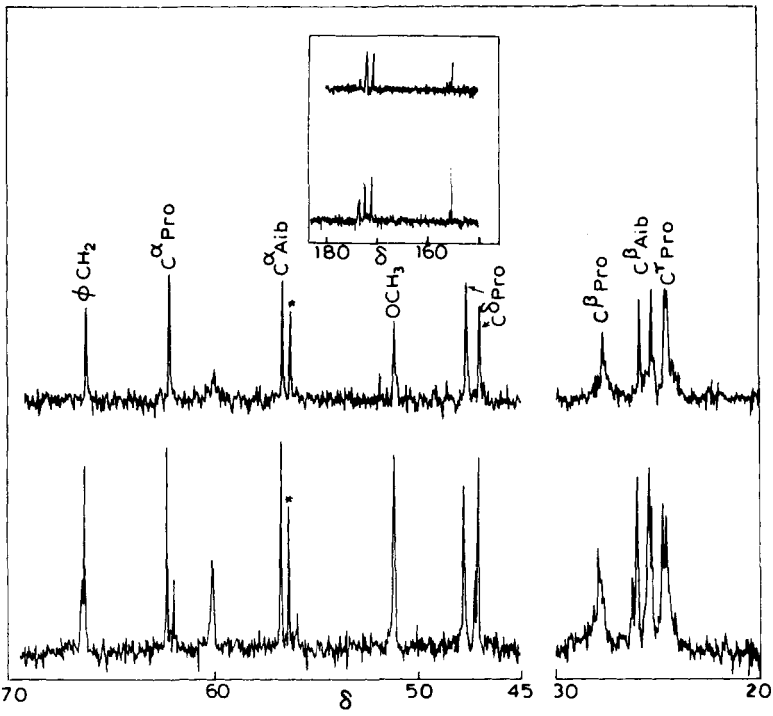

Fig. 2. The 67.89-MHz ^{13}C-nmr spectra of Z-(Aib-Pro)$_2$-OMe. Bottom: Diastereomeric mixture. Top: Crystals from ethyl acetate. Inset: Corresponding low-field region. Peaks marked with an asterisk correspond to solvent impurities or are spurious.

In the ^1H-nmr spectra, Aib(1) NH can be unambiguously assigned in $CDCl_3$, as noted earlier. The assignments in $(CD_3)_2SO$ were then made using solvent titration experiments, where spectra were obtained in $CDCl_3/(CD_3)_2SO$ mixtures. The chemical shifts of the amide NH groups are given in Table II, along with data for the related tripeptides Z-Aib-

TABLE II
NH Chemical Shifts, Temperature Coefficients ($d\delta/dT$), and Benzylic Methylene Nonequivalence Δ_{AB} in Compound 1 and Related Peptides

Peptide	Residue	NH Chemical Shifts (δ)		$d\delta/dT$, $d\delta/dT$, × 10^3 (ppm/°C)	Δ_{AB} (Hz)	
		$CDCl_3$	$(CD_3)_2SO$		$CDCl_3$	$(CD_3)_2SO$
Z-Aib-Pro-Aib-Pro-OMe **1**	Aib(1)	5.51	8.08	4.82	38.6	29.5
	Aib(3)	7.24	7.45	1.52		
Z-Pro-Aib-Pro-OMe[a]	Aib(2)	7.12	8.27	5.28		
		7.36	8.22	4.26	b	—
Z-Aib-Pro-Aib-OMe[c]	Aib(1)	5.53	8.04	5.97	29.6	31.8
	Aib(3)	7.39	7.56	2.24		

[a] Two sets of resonances are observed corresponding to *cis* and *trans* Z-Pro conformers.
[b] Broad singlet.
[c] Data taken from Refs. 5 and 16.

Pro-Aib-OMe and *Z*-Pro-Aib-Pro-OMe. In **1** the Aib(1) NH shows a downfield shift of 2.57δ on going from a poor hydrogen bond accepting solvent like $CDCl_3$, to a strong hydrogen bond acceptor like $(CD_3)_2SO$. On the contrary, the Aib(3) NH shows only a small downfield shift of 0.24δ under the same conditions. The temperature dependence of the NH chemical shifts for **1** and *Z*-Pro-Aib-Pro-OMe are shown in Fig. 3. While Aib(1) NH has a large $d\delta/dT$ value of 4.82×10^{-3} ppm/°C, the Aib(3) NH has a much lower value of 1.52×10^{-3} ppm/°C. In *Z*-Pro-Aib-Pro-OMe, two sets of resonances could be detected in the ^{1}H-nmr spectrum, corresponding to the *cis* and *trans* conformers about the urethane linkage. Both sets of NH resonances yielded relatively high $d\delta/dT$ values (5.28×10^{-3} and 4.26×10^{-3} ppm/°C).

These results suggest that **1** favors a conformation in $CDCl_3$ and $(CD_3)_2SO$ in which the Aib(3) NH group is solvent shielded or intramolecularly hydrogen bonded.[19] The solvent and temperature dependence of the NH chemical shift in an Aib residue, flanked by two Pro residues, in *Z*-Pro-Aib-Pro-OMe is in marked contrast to the behavior of Aib(3) NH in **1**. This is indicative of markedly different environments for the Aib(1) NH in the two peptides. The nmr data are consistent with a β-turn conformation[20] for **1**. (See Fig. 4 for a perspective view of this conformation in the solid state.) The structure is stabilized by an intramolecular 4 → 1 hydrogen bond between the benzyloxycarbonyl CO group and NH of Aib(3). Additional support for this structure is also obtained from the marked geminal nonequivalence observed for the benzyloxycarbonyl methylene protons. The magnitudes of the chemical-shift differences are tabulated in Table II. Previous studies of *Z*-Aib-Pro-containing peptides have shown that methylene group nonequivalence invariably occurs when the urethane CO is involved in an intramolecular hydrogen bond.[5] The ir spectrum of **1** in $CHCl_3$ ($4.2 \times 10^{-3}M$) also shows two bands at 3436 and 3340 cm^{-1}, corresponding to free (Aib(1)NH) and hydrogen bonded (Aib(3)NH) NH groups, respectively (unpublished results).

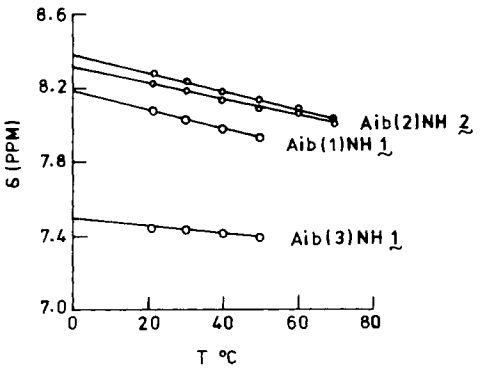

Fig. 3. Temperature dependence of NH chemical shifts.

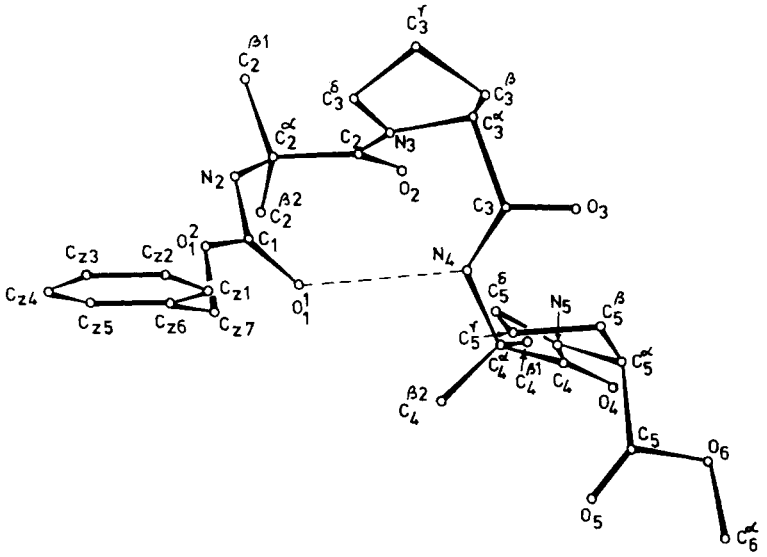

Fig. 4. Perspective view of the molecular structure of compound 1. Numbering scheme is indicated.

The tendency of Aib residues to favor formation of type III β-turns has been demonstrated in earlier nmr,[5] ir,[21,22] and x-ray studies of model peptides and alamethicin fragments.[23–26] The conformation illustrated in Fig. 4 has the Pro(2) residue occupying the $i+2$ position in the β-turn. Studies on Z-Aib-Pro-NHMe[21,25] and Z-Aib-Pro-Aib-Ala-OMe[5,21,23] have provided evidence for the formation of Aib-Pro β-turns in solution and in the solid state. The stereochemical constraints imposed by the Aib residue presumably force the Pro residue to occupy the less favorable $i+2$ position[27] in the β-turn. Since Aib peptides provide well-defined model systems for the study of solution conformations by spectroscopic methods, it is relevant to attempt studies of the solid-state structures for purposes of comparison.

Crystal and Molecular Structure of 1

The numbering scheme for the atoms in the structure of 1 is shown in Fig. 4. The bond lengths and bond angles are given in Tables III and IV, respectively. The values obtained are largely unexceptional. The $C_{Z3} - C_{Z4}$ bond in the phenyl ring and $C_5^{\delta} - C_5^{\gamma}$ bond in the pyrrolidine ring are abnormally short, and the atoms associated with these bonds have high thermal parameters. The shortening of the $C^{\beta} - C^{\gamma}$ or $C^{\gamma} - C^{\delta}$ bonds in prolyl residues is a feature observed in other structures also.[25,28] The $C^{\alpha} - C' - N$ angles ($C_2^{\alpha} - C_2 - N_3$ and $C_4^{\alpha} - C_4 - N_5$) in the Aib-Pro fragments are 120.2° and 122.0°, which are larger than the mean value of 117.8° reported by DeTar and Luthra[29] in a survey of proline-containing peptide

TABLE III
Bond Lengths in the Molecule[a]

Atoms	Bond Length (Å)	Atoms	Bond Length (Å)
$C_{Z1} - C_{Z2}$	1.423 (20)	$C_3^{\beta} - C_3^{\beta}$	1.529 (13)
$C_{Z1} - C_{Z6}$	1.380 (21)	$C_3^{\beta} - C_3^{\alpha}$	1.564 (13)
$C_{Z2} - C_{Z3}$	1.406 (24)	$C_3^{\alpha} - C_3$	1.505 (11)
$C_{Z3} - C_{Z4}$	1.307 (19)	$C_3 - O_3$	1.233 (9)
$C_{Z4} - C_{Z5}$	1.369 (17)	$C_3 - N_4$	1.338 (10)
$C_{Z5} - C_{Z6}$	1.381 (14)	$N_4 - C_4^{\alpha}$	1.491 (10)
$C_{Z6} - C_{Z7}$	1.493 (14)	$C_4^{\alpha} - C_4^{\beta 1}$	1.535 (12)
$C_{Z7} - O_1^2$	1.450 (12)	$C_4^{\alpha} - C_4^{\beta 2}$	1.505 (13)
$C_1^2 - C_1$	1.363 (9)	$C_4^{\alpha} - C_4$	1.547 (11)
$C_1 - O_1^1$	1.214 (9)	$C_4 - O_4$	1.235 (9)
$C_1 - N_2$	1.318 (11)	$C_4 - N_5$	1.314 (10)
$N_2 - C_2^{\alpha}$	1.463 (9)	$N_5 - C_5^{\alpha}$	1.476 (11)
$C_2^{\alpha} - C_2^{\beta 1}$	1.532 (12)	$N_5 - C_5$	1.456 (11)
$C_2^{\alpha} - C_2^{\beta 2}$	1.522 (13)	$C_5^{\delta} - C_5^{\gamma}$	1.464 (16)
$C_2^{\alpha} - C_2$	1.564 (11)	$C_5^{\gamma} - C_5^{\beta}$	1.598 (17)
$C_2 - O_2$	1.201 (9)	$C_5^{\beta} - C_5^{\alpha}$	1.517 (14)
$C_2 - N_3$	1.341 (10)	$C_5^{\alpha} - C_5$	1.508 (13)
$N_3 - C_3^{\delta}$	1.490 (10)	$C_5 - O_5$	1.194 (13)
$N_3 - C_3^{\alpha}$	1.481 (9)	$C_5 - O_6$	1.334 (11)
$C_3^{\delta} - C_3^{\beta}$	1.521 (13)	$O_6 - C_6^{\alpha}$	1.446 (15)

[a] The standard deviations are given in parentheses.

crystal structures. The widening of the $C^{\alpha} - C' - N$ angle has also been observed in *Z*-Aib-Pro-NHMe[25] and also in pivaloyl-D-Pro-L-Pro-L-Ala-NHMe.[30] The $C' - N - C^{\delta}$ angles ($C_2 - N_3 - C_3^{\delta}$ and $C_4 - N_5 - C_5^{\delta}$) of 130.6° and 130.7° are wider than the mean value of 124.1° reported earlier. The widening of these angles is presumably to relieve unfavorable interactions between the pyrrolidine ring and the adjacent Aib methyl groups.

A perspective view of the molecular conformation is shown in Fig. 4. Compound 1 adopts a β-turn conformation stabilized by an intramolecular hydrogen bond between the CO group of the benzyloxycarbonyl group and the Aib(3) NH. The N- - -O distance of 3.100 (8) Å and the H-N-O angle of 16(6)° are in good agreement with values for intramolecular hydrogen bonds in peptide crystal structures.[31] The backbone dihedral angles (ϕ, ψ, and ω) and the endocyclic angles that define the pyrrolidine ring conformation are summarized in Table V. The ϕ, ψ values for Aib(1) (−55.4°, −31.3°) and Pro(2) (−71.6°, −3.8°) are indicative of a type I β-turn conformation.[20] The observed ψ value for Pro(2) is much lower than those observed for similarly situated Pro residues in *Z*-Aib-Pro-NHMe (ψ_{Pro} = −25.4°)[25] and *Z*-Aib-Pro-Aib-Ala-OMe (ψ_{Pro} = −35.8).[5] In these cases the values correspond to type III β-turn structures. In 1 the presence of the Pro residue at position 4 prevents the formation of a second intramolecular hydrogen bond, as in *Z*-Aib-Pro-Aib-Ala-OMe. The ϕ, ψ values for Aib(3) are in the left-handed helical region. The urethane, ester, and

TABLE IV
Bond Angles in the Molecule[a]

Atoms	Bond Angle (deg)	Atoms	Bond Angle (deg)
$C_{Z2} - C_{Z1} - C_{Z6}$	124 (1)	$N_3 - C_3^{\alpha} - C_3^{\beta}$	104.6 (7)
$C_{Z1} - C_{Z2} - C_{Z3}$	112 (1)	$N_3 - C_3^{\alpha} - C_3$	115.1 (7)
$C_{Z2} - C_{Z3} - C_{Z4}$	126 (1)	$C_3^{\beta} - C_3^{\alpha} - C_3$	109.7 (7)
$C_{Z3} - C_{Z4} - C_{Z5}$	120 (1)	$C_3^{\alpha} - C_3 - O_3$	118.4 (7)
$C_{Z4} - C_{Z5} - C_{Z6}$	120 (1)	$C_3^{\alpha} - C_3 - N_4$	119.5 (7)
$C_{Z1} - C_{Z6} - C_{Z7}$	120 (1)	$O_3 - C_3 - N_4$	122.1 (7)
$C_{Z5} - C_{Z6} - C_{Z7}$	121 (1)	$C_3 - N_4 - C_4^{\alpha}$	121.8 (6)
$C_{Z1} - C_{Z6} - C_{Z5}$	118 (1)	$N_4 - C_4^{\alpha} - C_4^{\beta 1}$	109.6 (6)
$C_{Z6} - C_{Z7} - C_1^2$	107.7 (9)	$N_4 - C_4^{\alpha} - C_4^{\beta 2}$	106.7 (6)
$C_{Z7} - O_1^2 - C_1$	117.0 (7)	$N_4 - C_4^{\alpha} - C_4$	109.7 (6)
$O_1^2 - C_1 - O_1^1$	121.4 (7)	$C_4^{\beta 1} - C_4^{\alpha} - C_4^{\beta 2}$	110.8 (7)
$O_1^2 - C_1 - N_2$	127.5 (8)	$C_4^{\beta 1} - C_4^{\alpha} - C_4$	111.1 (7)
$C_1 - N_2 - C_2^{\alpha}$	122.7 (6)	$C_4^{\beta 2} - C_4^{\alpha} - C_4$	108.8 (7)
$N_2 - C_2^{\alpha} - C_2^{\beta 1}$	107.8 (7)	$C_4^{\alpha} - C_4 - O_4$	117.5 (7)
$N_2 - C_2^{\alpha} - C_2^{\beta 2}$	111.4 (7)	$C_4^{\alpha} - C_4 - N_5$	122.0 (7)
$N_2 - C_2^{\alpha} - C_2$	112.4 (6)	$O_4 - C_4 - N_5$	120.3 (7)
$C_2^{\beta 1} - C_2^{\alpha} - C_2^{\beta 2}$	110.6 (7)	$C_4 - N_5 - C_5^{\delta}$	130.7 (7)
$C_2^{\beta 1} - C_2^{\alpha} - C_2$	106.5 (7)	$C_4 - N_5 - C_5^{\alpha}$	118.8 (7)
$C_2^{\beta 2} - C_2^{\alpha} - C_2$	108.0 (7)	$C_5^{\delta} - N_5 - C_5^{\alpha}$	109.9 (7)
$C_2^{\alpha} - C_2 - O_2$	118.0 (7)	$N_5 - C_5^{\delta} - C_5^{\gamma}$	105.5 (8)
$C_2^{\alpha} - C_2 - N_3$	120.2 (7)	$C_5^{\delta} - C_5^{\gamma} - C_5^{\beta}$	100.8 (9)
$O_2 - C_2 - N_3$	121.7 (7)	$C_5^{\gamma} - C_5^{\beta} - C_5^{\alpha}$	101.5 (9)
$C_2 - N_3 - C_3^{\delta}$	130.6 (7)	$N_5 - C_5^{\alpha} - C_5$	112.5 (7)
$C_2 - N_3 - C_3^{\alpha}$	116.2 (6)	$N_5 - C_5^{\alpha} - C_5^{\beta}$	105.1 (7)
$C_3^{\delta} - N_3 - C_3^{\alpha}$	111.0 (6)	$C_5^{\beta} - C_5^{\alpha} - C_5$	109.1 (8)
$N_3 - C_3^{\delta} - C_3^{\gamma}$	104.0 (7)	$C_5^{\alpha} - C_5 - O_5$	126.9 (9)
$C_3^{\delta} - C_3^{\gamma} - C_3^{\beta}$	104.7 (8)	$C_5^{\alpha} - C_5 - O_6$	109.4 (8)
$C_3^{\gamma} - C_3^{\beta} - C_3^{\alpha}$	103.8 (8)	$O_5 - C_5 - O_6$	123.7 (9)
		$C_5 - O_6 - C_6^{\alpha}$	116.7 (8)

[a] The standard deviations are given in parentheses.

two peptide units in **1** are approximately planar ($\Delta\omega < 5°$). However, the tertiary amide unit linking Pro(2) and Aib(3) deviates by 14.3° from planarity. This distortion is larger than that generally observed in acyclic peptides.[32] Such nonplanar peptide units have been observed in strained cyclic peptides like dihydrochlamydocin (24°, 18°),[33] cyclo(Pro)$_3$ (12.5°),[34] and cyclo(Gly-Pro-Gly-D-Ala-Pro) (20°).[35]

The two pyrrolidine rings in **1** adopt different conformations. While Pro(2) adopts a C^{γ}-*exo* conformation, Pro(4) is in the C^{γ}-*endo* conformation.[28] The C^{γ} atoms of Pro(2) and Pro(4) are displaced from the plane containing the other four atoms by 0.517 and 0.625 Å, respectively.

A view of the molecular packing in the crystal is shown in Fig. 5. There is one intermolecular hydrogen bond between N(11) and O(23) of neighboring molecules. The N- - -O distance is 2.846 (2) Å and the H-N-O angle is 14 (4)°. A layerwise aggregation of the phenyl and pyrrolidine rings, in a direction approximately perpendicular to the *c* axis, is observed.

TABLE V
Conformational Angles for the Peptide Backbone and Pyrrolidine Ring

Angle	Atoms	Deg.	Angle	Atoms	Deg.
ω_1	$C_1^2 - C_1 - N_2 - C_2^\alpha$	179.4	θ_3	$C_3^\delta - N_3 - C_3^\alpha - C_3^\beta$	2.7
ϕ_2	$C_1 - N_2 - C_2^\alpha - C_2$	−55.4	χ_3^1	$N_3 - C_3^\alpha - C_3^\beta - C_3^\gamma$	−22.4
ψ_2	$N_2 - C_2^\alpha - C_2 - N_3$	−31.3	χ_3^2	$C_3^\alpha - C_3^\beta - C_3^\gamma - C_3^\delta$	33.7
ω_2	$C_2^\alpha - C_2 - N_3 - C_3^\alpha$	−179.6	χ_3^3	$C_3^\beta - C_3^\gamma - C_3^\delta - N_3$	−32.1
ϕ_3	$C_2 - N_3 - C_3^\alpha - C_3$	−71.6	χ_3^4	$C_3^\gamma - C_3^\delta - N_3 - C_3^\alpha$	18.2
ψ_3	$N_3 - C_3^\alpha - C_3 - N_4$	−3.8	θ_5	$C_5^\delta - N_5 - C_5^\alpha - C_5^\beta$	−7.1
ω_3	$C_3^\alpha - C_3 - N_4 - C_4^\alpha$	165.7	χ_5^1	$N_5 - C_5^\alpha - C_5^\beta - C_5^\gamma$	28.9
ϕ_4	$C_3 - N_4 - C_4^\alpha - C_4$	57.3	χ_5^2	$C_5^\alpha - C_5^\beta - C_5^\gamma - C_5^\delta$	−40.6
ψ_4	$N_4 - C_4^\alpha - C_4 - N_5$	47.1	χ_5^3	$C_5^\beta - C_5^\gamma - C_5^\delta - N_5$	36.9
ω_4	$C_4^\alpha - C_4 - N_5 - C_5^\alpha$	−177.1	χ_5^4	$C_5^\gamma - C_5^\delta - N_5 - C_5^\alpha$	−20.2
ϕ_5	$C_4 - N_5 - C_5^\alpha - C_5$	−77.0			
ψ_5	$N_5 - C_5^\alpha - C_5 - O_6$	158.1			
ω_5	$C_5^\alpha - C_5 - O_6 - C_6^\alpha$	175.1			

Solid-State and Solution Conformations

The β-turn conformation, involving the Aib(3) NH group in an intramolecular hydrogen bond, postulated from ^{1}H-nmr studies, has been shown to occur also in the crystal structure of **1**. This agreement between solution

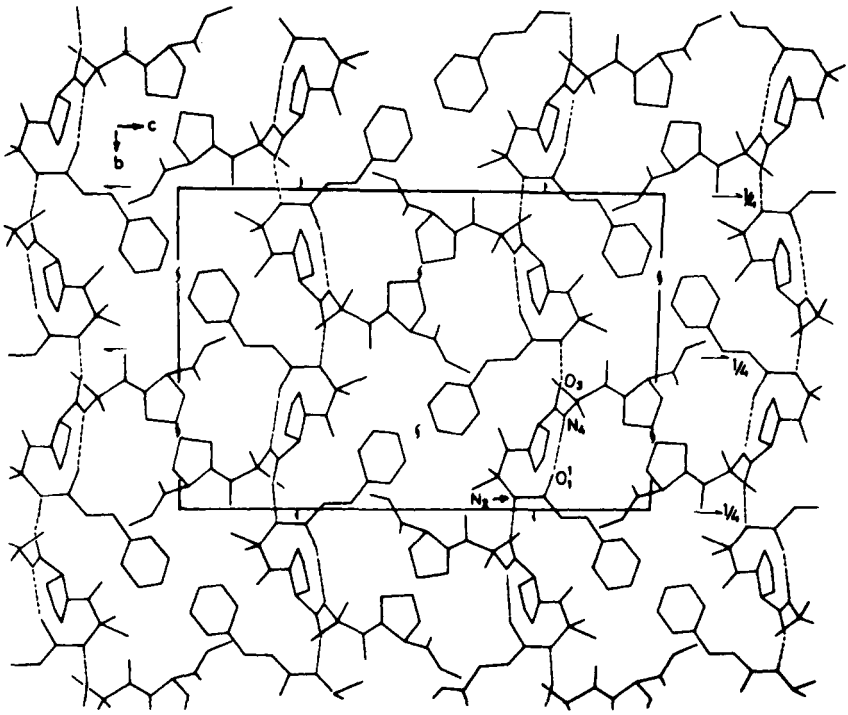

Fig. 5. The crystal structure as viewed down the *a* axis. The hydrogen bonds are indicated by broken lines. Only the atoms involved in hydrogen bonds are numbered.

and solid-state studies suggests that in Aib-containing peptides the molecular conformations are primarily influenced by intramolecular forces and not by interactions with the solvent in solution or by intermolecular packing forces in the crystal. Excellent agreement between solution and solid-state conformations has been observed in earlier studies from this laboratory of alamethicin fragments and model Aib peptides.[5,21–26] The conformational constraints introduced by Aib residues appear to limit the available range of backbone conformations in solution. This permits the unambiguous determination of solution conformations by nmr methods,[5] allows the use of ir techniques in quantitating the number of intramolecular hydrogen bonds,[21,22] and enhances the crystallizability of the peptides,[23–26] making them suitable for x-ray diffraction studies.

The research was supported by the Indian National Science Academy.

References

1. Carver, J. P. & Blout, E. R. (1976) in *Treatise on Collagen*, Vol. 1, Ramachandran, G. N., Ed., Academic Press, New York, pp. 441–526.

2. Frank, G. & Weeds, A. G. (1974) *Eur. J. Biochem.* **44,** 317–334.

3. Ribadeau Dumas, B., Brignon, G., Groselaude, F. & Mercier, J. C. (1972) *Eur. J. Biochem.* **25,** 505–514.

4. Henschen, A., Lottspeich, F., Brantl, V. & Teschemacher, H. (1979) *Hoppe Seyler's Z. Physiol. Chem.* **360,** 1217–1224.

5. Nagaraj, R., Shamala, N. & Balaram, P. (1979) *J. Am. Chem. Soc.* **101,** 16–20.

6. Germain, G., Main, P. & Woolfson, M. M. (1971) *Acta Crystallogr., Sect. A* **27,** 368–376.

7. Karle, J. (1968) *Acta Crystallogr., Sect. B* **24,** 182–186.

8. Cromer, D. T. & Waber, J. T. (1965) *Acta Crystallogr.*, **18,** 104–109.

9. Stewart, R. F., Davidson, E. R. & Simpson, W. T. (1965) *J. Chem. Phys.* **42,** 3175–3187.

10. Finn, F. M. & Hofmann, K. (1976) in *The Proteins*, Vol. 2, 3rd ed., Neurath, H. & Hill, R. L., Eds., Academic Press, New York, pp. 105–252.

11. Bystrov, V. F., Portnova, S. L., Tsetlin, V. I., Ivanov, V. T. & Ovchinnikov, Yu. A. (1965) *Tetrahedron* **25,** 493–515.

12. Pysh, E. S. & Toniolo, C. (1977) *J. Am. Chem. Soc.* **99,** 6211–6219.

13. Konig, W. & Geiger, R. (1970) *Chem. Ber.* **103,** 788–798.

14. Bovey, F. A. (1972) in *Chemistry and Biology of Peptides*, Meienhofer, J., Ed., Ann Arbor Science, Ann Arbor, Mich., pp. 3–10.

15. Gratwohl, Ch. & Wuthrich, K. (1976) *Biopolymers* **15,** 2025–2041.

16. Nagaraj, R. (1980) Ph.D. thesis, Indian Institute of Science, Bangalore.

17. Nishihara, H., Nishihara, K., Uefuji, T. & Sakota, N. (1975) *Bull. Chem. Soc. Jpn.* **48,** 553–555.

18. Venkatachalapathi, Y. V. & Balaram, P. (1979) *Nature* **281,** 83–84.

19. Wuthrich, K. (1976) *NMR in Biological Research: Peptides and Proteins*, North-Holland, Amsterdam.

20. Venkatachalam, C. M. (1968) *Biopolymers* **6,** 1425–1436.

21. Rao, Ch.P., Nagaraj, R., Rao, C. N. R. & Balaram, P. (1979) *FEBS Lett.* **100,** 244–248.

22. Rao, Ch.P., Nagaraj, R., Rao, C. N. R. & Balaram, P. (1980) *Biochemistry* **19,** 425–431.

23. Shamala, N., Nagaraj, R. & Balaram, P. (1977) *Biochem. Biophys. Res. Commun.* **79,** 291–298.

24. Shamala, N., Nagaraj, R. & Balaram, P. (1978) *J. Chem. Soc. Chem. Commun.*, 996–997.

25. Prasad, B. V. V., Shamala, N., Nagaraj, R., Chandrasekharan, R. & Balaram, P. (1979) *Biopolymers* **18,** 1635–1646.

26. Prasad, B. V. V., Shamala, N., Nagaraj, R. & Balaram, P. (1980) *Acta Crystallogr., Sect. B* **36,** 107–110.

27. Zimmerman, S. S. & Scheraga, H. A. (1977) *Biopolymers* **16,** 811–843.

28. Ashida, T. & Kakudo, M. (1974) *Bull. Chem. Soc. Jpn.* **47,** 1129–1133.

29. De Tar, D. F. & Luthra, N. (1977) *J. Am. Chem. Soc.* **99,** 1232–1244.

30. Nair, C. M. K., Vijayan, M., Venkatachalapathi, Y. V. & Balaram, P. (1979) *J. Chem. Soc. Chem. Commun.*, 1183–1184.

31. Karle, I. L. (1975) *Peptides: Chemistry, Structure and Biology*, Walter, R. & Meienhofer, J., Eds., Ann Arbor, Science, Ann Arbor, Mich., pp. 61–84.

32. Benedetti, E. (1977) *Peptides: Proceedings of the Fifth American Peptide Symposium*, Goodman, M. & Meienhofer, J., Eds., pp. 257–276.

33. Flippen, J. L. & Karle, I. L. (1976) *Biopolymers* **15,** 1081–1092.

34. Druyan, M. E., Coulter, C. L., Walter, R., Kartha, G. & Ambady, G. K. (1976) *J. Am. Chem. Soc.* **98,** 5498–5502.

35. Karle, I. L. (1978) *J. Am. Chem. Soc.* **100,** 1286–1289.

Received August 19, 1980
Accepted November 19, 1980