Crystal and Molecular Structure of Benzyloxycarbonyl-a-Aminoisobutyryl-L-Prolyl Methylamide: The Observation of an X_2-Pro$_3$ Type III β-Turn*

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Synopsis

The crystal and molecular structure of N-benzyloxycarbonyl-α-aminoisobutyryl-L-prolyl methylamide, the amino terminal dipeptide fragment of alamethicin, has been determined using direct methods. The compound crystallizes in the orthorhombic system with the space group $P2_12_12_1$. Cell dimensions are $a = 7.705$ Å, $b = 11.365$ Å, and $c = 21.904$ Å. The structure has been refined using conventional procedures to a final R factor of 0.054. The molecular structure possesses a 4 ▬ 1 intramolecular N-H---O hydrogen bond formed between the CO group of the urethane moiety and the NH group of the methylamide function. The peptide backbone adopts the type III β-turn conformation, with $\phi_2 = -51.0°$, $\psi_2 = -39.7°$, $\phi_3 = -65.0°$, $\psi_3 = -25.4'$. An unusual feature is the occurrence of the proline residue at position 3 of the β-turn. The observed structure supports the view that Aib residues initiate the formation of type III β-turn conformations. The pyrrolidine ring is puckered in C^γ-*exo* fashion.

INTRODUCTION

α-Aminoisobutyric acid (Aib) is a major constituent of the membrane-modifying antibiotic alamethicin[1–3] and related microbial peptides.[4–7] An understanding of the conformational role of Aib residues would aid in establishing correlations between the structure and biological function in Aib-containing polypeptide ionophores. Theoretical studies[8–10] have shown that the replacement of the hydrogen atom at C^α by a methyl group results in a considerable restriction of the conformational freedom of the peptide backbone. Experimental studies of the conformation of peptides containing Aib residues have been limited but have revealed interesting structural features. We have previously demonstrated the occurrence of an incipient 3_{10} helical structure in the crystalline state for the tetrapeptide Z-Aib-Pro-Aib-Ala-OMe[11] and provided [1]H-nmr evidence for the existence

of this conformation in solution.[12] A folded structure involving two in-tramolecular hydrogen bonds has been established in the solid state for Boc-Pro-Aib-Ala-Aib-OBz.[13] An Aib residue has been shown to be in-volved in the formation of a y-turn in the cyclic tetrapeptide dihydro-chlamydocin.[14] In this paper we describe the crystal and molecular structure of Z-Aib-Pro-NHCH$_3$ (Fig. 1), which forms a type III β-turn, with the Pro residue at the unusual $i + 2$ position.

EXPERIMENTAL

Synthesis of Benzyloxycarbonyl-a-Aminoisobutyryl-Prolyl-N-methylamide (Z-Aib-Pro-NHCH$_3$)

Pro-NHCH$_3$

Z-Pro[15] (2.3 g, 9 mmol) in tetrahydrofuran (25 ml) was cooled to 0°C and N-methylmorpholine (1.01 ml, 9 mmol) and isobutylchloroformate (1.2 ml, 9 mmol) added with stirring. After 12 min a saturated solution of methylamine in dry tetrahydrofuran (20 ml) was added and the mixture stirred overnight at room temperature. N-Methylmorpholinium hydro-chloride was filtered off and the solvent evaporated. The residue was taken up in ethyl acetate (50 ml) and washed with water and $1N$ NaHCO$_3$. The organic layer was dried over anhydrous Na$_2$SO$_4$ and evaporated to give an oil. Yield of Z-Pro-NHCH$_3$, 2.2 g (88%). [1]H-nmr (60 MHz): 7.46, s, 5H phenyl; 6.66, broad multiplet, 1H, NH CH$_3$; 5.26, s, 2H, benzyl CH$_2$; 4.46, m, 1H, C$^\alpha$H Pro; 3.66, m, 2H, C$^\delta$H$_2$ Pro; 2.86, d, 3H, NHCH$_3$; 2.66, m, 4H, C$^\beta$H$_2$ and C$^\gamma$H$_2$ Pro: Z-Pro-NHCH$_3$ (0.75 g, 3 mmol) was dissolved in ethanol (15 ml). Cyclohexene (4 ml) and 10%Pd/C catalyst (0.40 g) were added and the mixture was refluxed for 1 hr.[16] The catalyst was filtered and the solvent evaporated to yield Pro-NHCH$_3$ as an oil, which was used without further purification.

Z-Aib-Pro-NHCH$_3$

Z-Aib[17] (0.585 g, 2.5 mmol) was dissolved in dichloromethane (10 ml) and cooled to 0°C. Pro-NHCH$_3$ (0.320 g, 2.5 mmol) was added to the stirred solution, followed by dicyclohexylcarbodiimide (0.515 g, 2.5 mmol). The mixture was stirred at room temperature for 24 hr and the dicyclo-hexylurea filtered off. The organic layer was washed with $1N$ HCl, water,
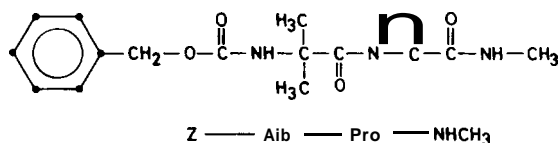
Fig. 1. Structure of Z-Aib-Pro-NHCH$_3$.

and $1N$ $NaHCO_3$. The organic layer was dried and evaporated to yield an oil, which solidified on triturating with petroleum ether. Yield 0.60 g (70%) mp 168°C, $[\alpha]_D = 20.4°$ (MeOH). ^{1}H-nmr (270 MHz): 7.346, m, 5H phenyl; 7.236, m, 1H NH CH_3; 5.636, s, 1H, Aib NH; 5.226, d, 1H, 4.986, d, 1H, benzyl CH_2; 4.616, **q,** 1H, Pro C^α-H; 3.596, m, 1H, 3.116, m, 1H, Pro C^δ-H_2; 2.756, d, 3H, $NHCH_3$; 1.986, m, 2H, 1.686, m, 2H, $C^\beta H_2$, $C^\gamma H_2$, Pro; 1.576, s, 3H, 1.416, s, 3H, Aib CH_3.

X-Ray Crystallography

Crystals of Z-Aib-L-Pro-methylamide were grown from methanol−ether solution as colorless needles elongated along the a axis. Rotation and Weissenberg photographs indicated an orthorhombic lattice with the space group $P2_12_12_1$. Unit-cell dimensions were obtained from 2θ measurements both on photographs and on the diffractometer. The density was measured by a flotation method using a KCl water mixture. Crystal data are summarized in Table **I**. Intensities of 1760 reflections were measured on a CAD-4 diffractometer with MoK_α radiation ($\lambda = 0.7106$ Å) by w-28 scan. About 640 reflections were considered to be unobserved since their intensities were less than 3σ. Three standard reflections were measured after every 50 reflections, and there was no significant change in the intensities during the period of data collection. The intensities were corrected for Lorentz and polarization factors but not for absorption, since the size of the crystal was very small (0.8 X 0.3 X 0.08 mm).

Structure Determination and Refinement

The normalized structure factors[18] E were obtained using the overall temperature factor ($B = 4.7$ Å^2) and scale factor determined from a Wilson plot.[19] The structure was solved by direct methods[20] using the Multan program.[21] Several sets of phases were generated with 250 input reflections having $E \geqslant 1.4$. An E map[22] computed with the phases obtained from the set of highest combined figure of merit revealed the structure in part; 20

TABLE I
Crystal Data for Z-Aib-Pro-$NHCH_3$

Molecular formula: $C_{18}N_3O_4H_{25}$
Molecular weight: 347 amu
Crystal system: orthorhombic
Space group: $P2_12_12_1$
$a = 7.705(1)$ **8,**
$b = 11.365(1)$ **8,**
$c = 21.904(2)$ **8,**
Volume: 1918.07 Å^3
Z: **4**
Density (measured) = 1.19 g cm^{-3}
Density (calculated) = **1.20**g cm^{-3}

nonhydrogen atoms were located. Calculated structure factors for the trial coordinates of the partial structure gave an R value of 0.27. The remaining 5 atoms were located from a difference Fourier map. The refinement of positional and isotropic thermal parameters using a block diagonal least-squares program (R. Shiono, personal communication) lowered the R value to 0.144. Refinement of all nonhydrogen atoms with anisotropic temperature factor and an overall scale factor yielded an R value of 0.094. The difference Fourier map computed at this stage revealed the positions of 19 out of 25 hydrogen atoms. The remaining hydrogen atoms were fixed using stereochemical considerations. The hydrogen atoms attached to carbons were fixed assuming a C-H distance of 1.1 Å. Bond angles of 109.5" or 120.0" were used for tetrahedral and trigonal atoms, respectively. For hydrogens bonded to nitrogen atoms, an N-H distance of 1.0 Å and a bond angle of 120.0" were used. The hydrogens were used only in structure factor calculations and were assigned the temperature factor of the heavy atom to which they are bonded. Final refinement with anisotropic thermal parameters for nonhydrogen atoms and isotropic thermal factors for hydrogen atoms lowered the R value to 0.058. With σ-weighting scheme and further cycles of refinement, the R factor converged to a final value of 0.054 using 1120 observed reflections. The shifts in the parameters at the end of last cycles were less than 0.1σ. The final difference Fourier map was featureless. The scattering factors were those of Cromer and Waber[23] for nonhydrogen atoms and of Stewart et al.[24] for hydrogen atoms. The atomic and thermal parameters with their standard deviations are recorded in Tables II and III. A listing of the observed and calculated structure factors is available on request.

RESULTS AND DISCUSSION

A perspective diagram of the molecular structure of Z-Aib-Pro-NHCH$_3$, viewed down the a axis, is shown in Fig. 2. An intramolecular $\mathbf{4} \rightarrow 1$ hydrogen bond, with an N- - -O distance of 3.12 Å and an H-N-O angle of 28.6°, is formed between the O_1^1 of the benzyloxycarbonyl protecting group and the N_4 of the methylamide group. The observed bond angles and bond lengths are largely unexceptional and are listed in Table IV. The standard deviations are 0.008 Å for lengths and 0.5" for angles. The peptide and urethane groups in the molecule do not show significant deviations from planarity. The peptide backbone adopts a type III β-turn conformation ($\phi = -60°, \psi = -30°$).[25] The relevant conformational angles (ϕ, ψ, and ω) are listed in Table V.

The β-turn conformation stabilized by a $\mathbf{4} \rightarrow 1$ intramolecular hydrogen bond has been observed in the crystal structures of a number of acyclic proline-containing peptides and also in cyclic peptides.[26] The β-turns reported in these oligopeptide structures have been either of type I ($\phi_{i+1} = -60°, \psi_{i+1} = -30°, \phi_{i+2} = -90°, \psi_{i+2} = 0°$), type I' ($\phi_{i+1} = 60°, \psi_{i+1} = 30°, \phi_{i+2} = 90°, \psi_{i+2} = 0°$), type II ($\phi_{i+1} = -60°, \psi_{i+1} = 120°, \phi_{i+2} = 80°$,

TABLE II

Final Atomic Coordinates and Thermal Parameters for the Nonhydrogen Atoms[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C_{Z1}	0.4471(15)	0.3265(10)	0.4967(4)	0.0699(41)	0.0339(18)	0.0034(2)	-0.0011(26)	-0.0006(9)	0.0011(6)
C_{Z2}	0.5138(13)	0.4287(8)	0.4708(4)	0.0493(33)	0.0208(12)	0.0060(3)	0.0038(17)	-0.0038(8)	0.0035(5)
C_{Z3}	0.5302(11)	0.4304(6)	0.4079(4)	0.0338(20)	0.0113(7)	0.0046(2)	0.0002(10)	-0.0040(6)	-0.0012(4)
C_{Z4}	0.4862(8)	0.3361(5)	0.3709(3)	0.0183(13)	0.0084(5)	0.0038(2)	-0.0001(7)	-0.0013(4)	0.0003(3)
C_{Z5}	0.4233(13)	0.2360(7)	0.4002(4)	0.0412(23)	0.0146(8)	0.0047(2)	-0.0063(12)	-0.0035(7)	0.0026(4)
C_{Z6}	0.3942(18)	0.2323(10)	0.4622(5)	0.0649(36)	0.0256(15)	0.0071(4)	-0.0127(23)	-0.0021(11)	0.0059(7)
C_{Z7}	0.4982(8)	0.3408(5)	0.3040(3)	0.0179(13)	0.0085(5)	0.0039(2)	0.0024(8)	-0.0010(4)	-0.0006(3)
C_1	0.2849(7)	0.4804(4)	0.2726(3)	0.0168(11)	0.0050(4)	0.0030(2)	-0.0007(6)	-0.00004(4)	-0.0006(2)
C_2'	0.0348(8)	0.6026(4)	0.2431(3)	0.0190(12)	0.0048(4)	0.0032(2)	0.0008(6)	-0.0018(4)	0.0001(2)
$C_2^{\beta 1}$	-0.1628(8)	0.5829(5)	0.2434(3)	0.0186(13)	0.0062(5)	0.0043(2)	0.0001(7)	-0.0018(4)	0.0001(2)
$C_2^{\beta 2}$	0.0961(10)	0.6453(5)	0.1815(3)	0.0350(19)	0.0099(6)	0.0029(2)	-0.0029(10)	-0.0024(5)	0.0003(3)
C_2	0.0728(7)	0.6951(4)	0.2925(3)	0.0165(12)	0.0049(4)	0.0026(1)	0.0002(6)	-0.0018(4)	0.0000(2)
C_3'	0.0949(9)	0.7652(5)	0.3969(3)	0.0333(14)	0.0086(6)	0.0036(2)	-0.0002(8)	-0.0016(5)	-0.0018(3)
$C_3^{\beta 1}$	0.0733(12)	0.7052(8)	0.4576(3)	0.0389(24)	0.0227(11)	0.0036(2)	-0.0166(16)	0.0042(6)	-0.0020(4)
$C_3^{\beta 2}$	-0.0108(14)	0.5920(8)	0.4458(4)	0.0743(39)	0.0174(10)	0.0038(2)	-0.0036(19)	0.0043(8)	-0.0000(4)
C_3^{δ}	0.0399(9)	0.5541(5)	0.3833(3)	0.0316(18)	0.0087(6)	0.0033(2)	-0.0014(9)	0.0026(4)	0.0010(3)
C_3	0.2668(10)	0.8270(5)	0.3939(3)	0.0355(19)	0.0082(6)	0.0028(2)	-0.0022(9)	-0.0018(5)	-0.0011(3)
C_4'	0.5672(10)	0.8272(8)	0.3684(3)	0.0279(25)	0.0240(11)	0.0035(2)	-0.0092(13)	-0.0003(5)	-0.0007(4)
O_1'	0.3288(5)	0.3655(3)	0.2786(2)	0.0209(8)	0.0061(3)	0.0033(1)	0.0017(4)	-0.0013(3)	-0.0003(2)
O_1''	0.3854(5)	0.5616(3)	0.2800(2)	0.0188(8)	0.0072(3)	0.0036(1)	-0.0013(5)	-0.0003(3)	-0.0003(2)
O_2	0.0979(5)	0.7983(3)	0.2774(2)	0.0268(9)	0.0041(3)	0.0036(1)	-0.0016(5)	-0.0003(3)	0.0004(2)
O_3	0.2752(8)	0.9289(4)	0.4152(3)	0.0439(15)	0.0120(5)	0.0051(2)	-0.0049(8)	0.0003(4)	-0.0031(3)
N_2	0.1177(6)	0.4897(3)	0.2580(2)	0.0161(9)	0.0044(3)	0.0034(1)	-0.0000(5)	-0.0000(3)	-0.0002(2)
N_3	0.0744(6)	0.6689(4)	0.3519(2)	0.0212(10)	0.0072(4)	0.0021(1)	-0.0000(6)	0.0000(3)	-0.0006(2)
N_4	0.3995(7)	0.7708(4)	0.3723(2)	0.0196(10)	0.0099(5)	0.0034(1)	-0.0007(7)	-0.0005(4)	-0.0007(2)

[a] $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{23}kl + 2\beta_{13}hl)]$.

TABLE III
Positional Coordinates for Hydrogen Atoms

Atom	x	y	z
H_{Z1}	0.436	0.328	0.542
H_{Z2}	0.576	0.501	0.496
H_{Z3}	0.581	0.510	0.388
H_{Z5}	0.405	0.172	0.370
H_{Z6}	0.323	0.154	0.486
H_{Z7}^{1}	0.584	0.407	0.286
H_{Z7}^{2}	0.525	0.259	0.281
H_{2}	0.060	0.423	0.245
$H_{2}^{\beta 11}$	−0.192	0.551	0.289
$H_{2}^{\beta 12}$	−0.225	0.656	0.233
$H_{2}^{\beta 13}$	−0.193	0.515	0.212
$H_{2}^{\beta 21}$	0.058	0.589	0.152
$H_{2}^{\beta 22}$	0.049	0.718	0.167
$H_{2}^{\beta 23}$	0.208	0.651	0.178
H_{3}^{α}	0.007	0.822	0.384
$H_{3}^{\beta 1}$	0.176	0.683	0.472
$H_{3}^{\beta 2}$	0.045	0.746	0.484
$H_{3}^{\gamma 1}$	0.027	0.531	0.473
$H_{3}^{\gamma 2}$	−0.145	0.615	0.439
$H_{3}^{\delta 1}$	−0.056	0.497	0.355
$H_{3}^{\delta 2}$	0.173	0.503	0.385
H_{4}	0.392	0.709	0.366
$H_{4}^{\alpha 1}$	0.612	0.850	0.413
$H_{4}^{\alpha 2}$	0.671	0.783	0.352
$H_{4}^{\alpha 3}$	0.573	0.905	0.345

$\psi_{i+2} = 0°$) or type II$'$($\phi_{i+1} = 60°$, $\psi_{i+1} = −120°$, $\phi_{i+2} = −80°$, $\psi_{i+2} = 0°$) categories.[26] The experimentally determined dihedral angles vary by about $10°–15°$ from the theoretical values.[26] In Z-Aib-Pro-NHCH$_3$ the conformational angles for the residues at the corners of the β-turn (Fig. 3) are ϕ_{i+1}(Aib) = $−51°$, ψ_{i+1}(Aib) = $−39.7°$, ϕ_{i+2}(Pro) = $−65°$, and ψ_{i+2}(Pro) = $−25.4°$. These values correspond to a type III β-turn conformation (ϕ_{i+1} = $−60°$, ψ_{i+1} = $−30°$, ϕ_{i+2} = $−60°$, ψ_{i+2} = $−30'$). It may be noted that type III β-turns have also been reported recently in the crystal structures of Z-Aib-Pro-Aib-Ala-OMe[11] and Boc-Pro-Aib-Ala-Aib-OBz.[13] Theoretical calculations have yielded energy minima for acetyl-α-aminoisobutyryl methylamide, (Ac-Aib-NHCH$_3$) in the right- and left-handed 3_{10} or α-helical regions of the conformational map.[8,9] An x-ray study of Ac-Aib-NHCH$_3$ has yielded values of $\phi = −55.5''$ and $\psi = −39.3'$ for the Aib residue.[27] A fiber diffraction study of poly(α-aminoisobutyric acid) has suggested that a 3_{10} helical conformation may occur in the polymer.[28] Single-crystal x-ray diffraction studies of the pentapeptide, tosyl-(Aib)$_5$-OMe, have yielded a structure consisting of three consecutive type III β-bends stabilized by three intramolecular $4 \rightarrow 1$ hydrogen bonds.[29] These studies, together with results reported in this paper, suggest that Aib residues have a strong tendency to initiate the formation of β-turns and
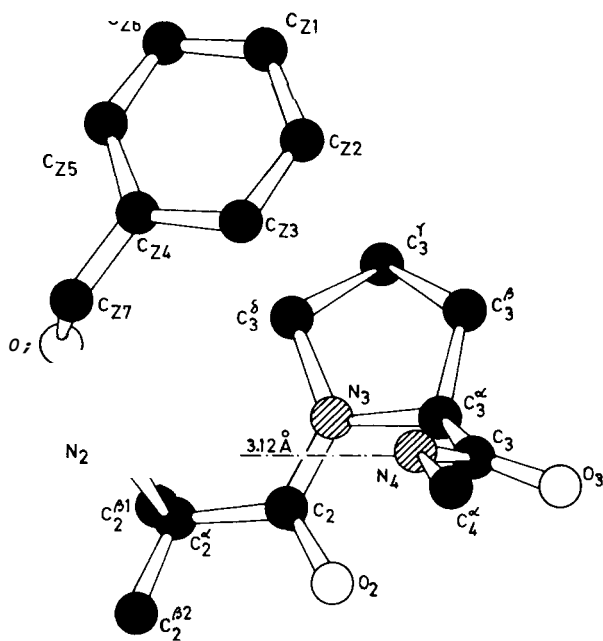
Fig. 2. Perspective view of the molecular structure of Z-Aib-Pro-NHCH$_3$.

that Aib residues can stabilize the 3_{10} helical conformation.[30] The only exception so far is the Aib residue in the cyclic tetrapeptide dihydrochlamydocin,[14] which adopts a C_7 conformation. It is noteworthy that this structure is highly distorted by the constraints of accommodating four *trans* peptide bonds in a 12-membered ring and shows considerable deviations from planarity for all four peptide units.

An unusual conformational feature observed in the structure of Z-Aib-Pro-NHCH$_3$ is the 8-turn accommodating the Pro residue at the $i+2$ position. Following the notation in Ref. 31, the benzyloxycarbonyl and methylamide groups are defined as residues i and i+3 (Fig. 3). Proline has been found to occur frequently at the i+1 position in small peptides and proteins.[32-34] The only case where a Pro residue has been detected at the i+2 position, in a small peptide, is in the tetrapeptide Z-Aib-Pro-Aib-Ala-OMe.[12] An analysis of **459** β-turn structures, from the crystallographic data available for 29 proteins, has shown the occurrence of 58 turns with Pro at the i+1 position and 12 with Pro at the i+2 position.[34] Furthermore, 8 of the bends with Pro at the i+2 position have cis X-Pro bonds (type VI β-turn). Theoretical studies also suggest that bends are not favored in X-Pro sequences because of unfavorable interactions between the X residue and the pyrrolidine ring, which restricts the X residue to conformations not generally found in β-turns.[31,35] However, a change in the configuration of the X residue from **L** to **D** may facilitate the formation of the type **ll'** bend for D-X-L-Pro sequences.[29] Such a 8-turn has indeed been postulated for gramicidin S[36-38] and has been observed in a recent crystal

Atoms	Length (Å)	Atoms	Angle (deg)
$C_{Z1}-C_{Z2}$	1.391(4)	$C_{Z1}-C_{Z2}-C_{Z3}$	116.8(9)
$C_{Z2}-C_{Z3}$	1.384(12)	$C_{Z2}-C_{Z3}-C_{Z4}$	123.3(7)
$C_{Z3}-C_{Z4}$	1.386(9)	$C_{Z3}-C_{Z4}-C_{Z5}$	116.6(6)
$C_{Z4}-C_{Z5}$	1.393(10)	$C_{Z4}-C_{Z5}-C_{Z6}$	122.3(8)
$C_{Z5}-C_{Z6}$	1.378(13)	$C_{Z5}-C_{Z6}-C_{Z1}$	118.1(10)
$C_{Z6}-C_{Z1}$	1.372(15)	$C_{Z6}-C_{Z1}-C_{Z2}$	122.5(10)
$C_{Z4}-C_{Z7}$	1.470(9)	$C_{Z3}-C_{Z4}-C_{Z7}$	122.6(6)
$C_{Z7}-O_1^2$	1.446(7)	$C_{Z5}-C_{Z4}-C_{Z7}$	120.7(6)
$O_1^2-C_1$	1.356(6)	$C_{Z4}-C_{Z7}-O_1^2$	109.4(5)
$C_1-O_1^1$	1.215(6)	$C_{Z7}-O_1^2-C_1$	116.7(4)
C_1-N_2	1.332(7)	$O_1^2-C_1-O_1^1$	124.0(4)
$N_2-C_2^\alpha$	1.470(6)	$O_1^2-C_1-N_2$	109.9(5)
$C_2^\alpha-C_2^{\beta1}$	1.538(8)	$O_1^1-C_1-N_2$	126.0(5)
$C_2^\alpha-C_2^{\beta2}$	1.510(8)	$C_1-N_2-C_2^\alpha$	122.9(4)
$C_2^\alpha-C_2$	1.536(7)	$N_2-C_2^\alpha-C_2^{\beta1}$	107.6(4)
C_2-O_2	1.234(5)	$N_2-C_2^\alpha-C_2^{\beta2}$	110.1(5)
C_2-N_3	1.335(5)	$N_2-C_2^\alpha-C_2$	111.0(4)
$N_3-C_3^\alpha$	1.482(7)	$C_2^{\beta1}-C_2^\alpha-C_2$	106.6(4)
$C_3^\alpha-C_3^\beta$	1.505(10)	$C_2^{\beta1}-C_2^\alpha-C_2^{\beta2}$	111.1(5)
$C_3^\beta-C_3^\gamma$	1.463(13)	$C_2^{\beta2}-C_2^\alpha-C_2$	110.4(5)
$C_3^\gamma-C_3^\delta$	1.489(7)	$C_2^\alpha-C_2-N_3$	122.4(5)
$C_3^\delta-N_3$	1.499(7)	$C_2-N_3-C_3^\alpha$	119.0(4)
$C_3^\alpha-C_3$	1.501(10)	$O_2-C_2-N_3$	118.1(5)
C_3-O_3	1.250(7)	$C_2-N_3-C_3^\delta$	129.7(4)
C_3-N_4	1.295(8)	$N_3-C_3^\alpha-C_3^\beta$	104.0(5)
$N_4-C_4^\alpha$	1.445(9)	$C_3^\alpha-C_3^\beta-C_3^\gamma$	107.0(7)
		$C_3^\beta-C_3^\gamma-C_3^\delta$	107.5(7)
		$C_3^\gamma-C_3^\delta-N_3$	102.5(5)
		$C_3^\delta-N_3-C_3^\alpha$	110.9(4)
		$N_3-C_3^\alpha-C_3$	114.3(5)
		$C_3^\alpha-C_3-N_4$	118.8(6)
		$C_3^\alpha-C_3-O_3$	117.6(6)
		$O_3-C_3^\alpha-N_4$	123.6(6)
		$C_3-C_3^\alpha-C_3$	110.4(6)
		$C_3-N_4-C_4^\alpha$	120.5(5)

structure determination of a hydrated gramicidin–urea complex.[39] However, in the case of X-Aib, the type 1l' structure is energetically unfavorable due to the presence of a second C^β atom. Type VI bends involving a *cis* Aib-Pro bond are also unfavorable, since the bulky Aib group leads to an almost exclusive preference for the *trans* X-Pro structure.[40] The sterically analogous pivaloyl group has been shown to restrict the pivaloyl-Pro bond to the *trans* configuration.[41] In compounds containing -Aib-Pro- sequences, we have found no evidence for the *cis* form in solution by ^{13}C- and ^{1}H-nmr (R. Nagaraj and P. Balaram, unpublished results). Therefore, the stereochemical constraints imposed by the Aib residue compel the pyrrolidine ring to occupy the $i+2$ position in the type III β-turn structure obtained for Z-Aib-Pro-NHCH$_3$.

Conformation	Angle (deg)
$\omega_1(O_1^2\text{-}C_1\text{-}N_2\text{-}C_2^\alpha)$	$-174.5(4)$
$\phi_2(C_1\text{-}N_2\text{-}C_2^\alpha\text{-}C_2)$	$-51.0(7)$
$\psi_2(N_2\text{-}C_2^\alpha\text{-}C_2\text{-}N_3)$	$-39.7(7)$
$\omega_2(C_2^\alpha\text{-}C_2\text{-}N_3\text{-}C_3^\alpha)$	$-174.0(5)$
$\phi_3(C_2\text{-}N_3\text{-}C_3^\alpha\text{-}C_3)$	$-65.0(7)$
$\psi_3(N_3\text{-}C_3^\alpha\text{-}C_3\text{-}N_4)$	$-25.4(8)$
$\omega_3(C_3^\alpha\text{-}C_3\text{-}N_4\text{-}C_4^\alpha)$	$179.7(6)$
$\theta(C_3^\delta\text{-}N_3\text{-}C_3^\alpha\text{-}C_3^\beta)$	$0.5(6)$
$\chi_3^1(N_3\text{-}C_3^\alpha\text{-}C_3^\beta\text{-}C_3^\gamma)$	$-17.6(8)$
$\chi_3^2(C_3^\alpha\text{-}C_3^\beta\text{-}C_3^\gamma\text{-}C_3^\delta)$	$28.8(9)$
$\chi_3^3(C_3^\beta\text{-}C_3^\gamma\text{-}C_3^\delta\text{-}N_3)$	$-27.2(8)$
$\chi_3^4(C_3^\gamma\text{-}C_3^\delta\text{-}N_3\text{-}C_3^\alpha)$	$16.2(7)$

The conformational angles describing the geometry of the pyrrolidine ring in Z-Aib-Pro-NHCH$_3$ are listed in Table V. The ring puckering may be classified as C^γ-exo.[42] The C^β and C^γ atoms are displaced on either side of the $NC^\alpha C^\delta$ plane by 0.013 and 0.405 Å, respectively. C_3 is displaced from the $NC^\alpha C^\delta$ plane on the side opposite to C^γ by 1.174Å. The C_2^α-C_2-N_3 angle of 122.4' is slightly wider than the mean value of 117.8' reported by De Tar and Luthra[43] from a survey of crystal structures of proline-containing peptides. The C_2-N_3-C_3^δ angle of 129.7' is also larger than the mean value of 124.1' reported in Ref. **43**. The widening of these angles possibly relieves unfavorable contacts between the proline residue and the methyl group of the neighboring Aib residue. The C_3^β-C_3^γ distance of 1.46Å is shorter than the reported average value of 1.51Å. This shortening probably arises from the large temperature factor ($B \simeq 11.2$Å^2) associated with the C^γ atom. Such effects have been noted in earlier analyses of proline-containing
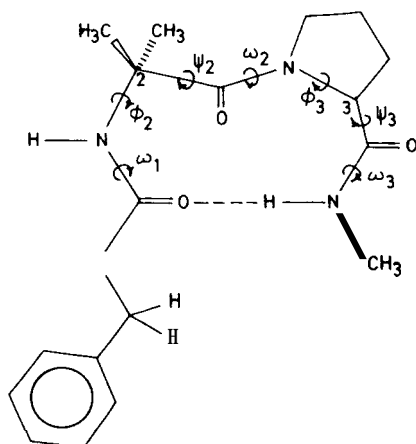

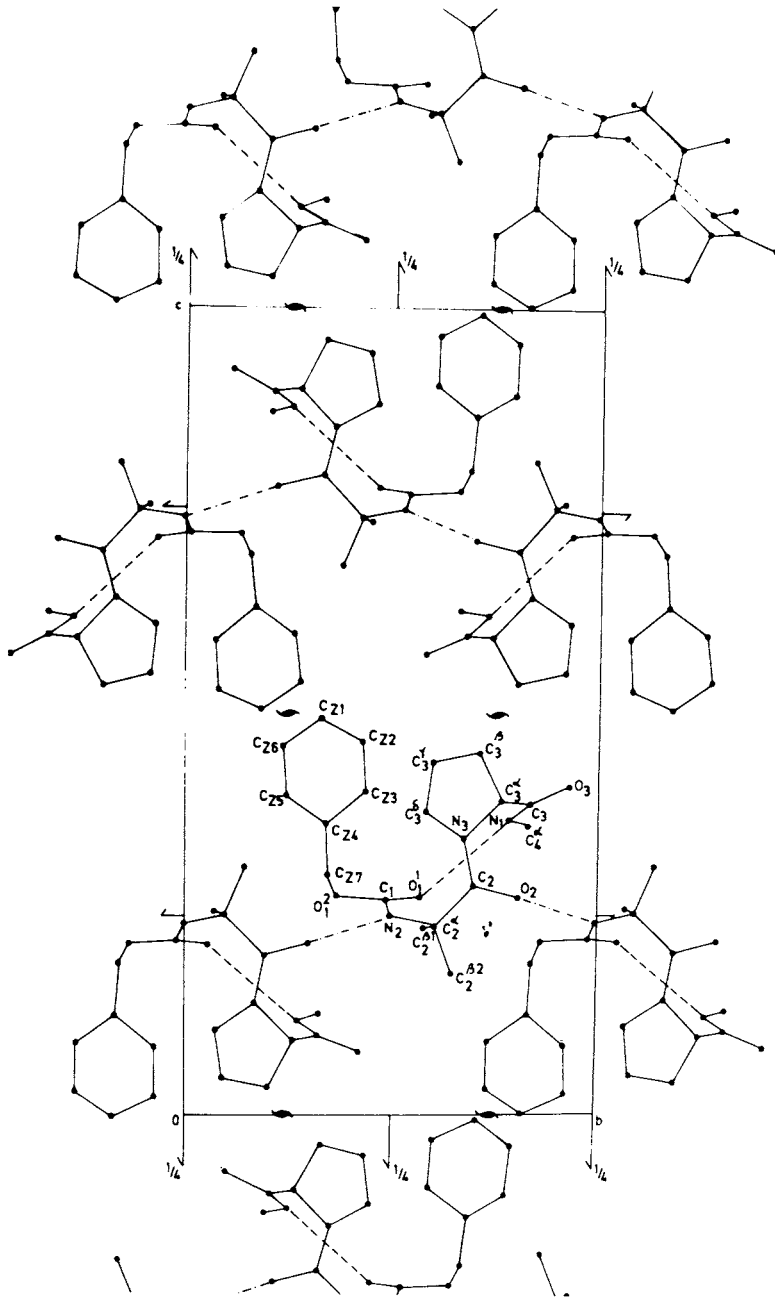Fig. 3. Schematic representation **of** the β-turn in Z-Aib-Pro-NHCH$_3$.

Fig. 4. Packing of Z-Aib-Pro-NHCH$_3$ molecules as viewed along a axis.

peptide structures,[42] indicating that the pyrrolidine ring assumes somewhat different conformations from cell to cell and that the conformation reported is an "average."

Molecular Packing

A view of the molecular packing in the crystal is shown in Fig. **4.** In this projection the benzene and pyrrolidine rings are aligned almost parallel to the longest axis. All the NH and CO groups are involved in intra- and intermolecular hydrogen bonds, with the exception of the Pro CO group. In addition to van der Waals interactions, the crystal is stabilized by a network of intermolecular hydrogen bonds involving the CO and NH groups of Aib residues on neighboring molecules. The observed N- - -O distance is 2.84 Å and the H-N- - -O angle is 7.28'.

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