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Conformations of peptides containing 1-aminocyclohexanecarboxylic acid (Acc^6)

Crystal structures of two model peptides

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The crystal structures of two peptides containing 1-aminocyclohexanecarboxylic acid (Acc^6) are described. Boc-Aib-Acc^6-NHMe·H_2O adopts a β-turn conformation in the solid state, stabilized by an intramolecular 4 → 1 hydrogen bond between the Boc CO and methylamide NH groups. The backbone conformational angles ($\phi_{Aib} = -50.3°$, $\psi_{Aib} = -45.8°$; $\phi_{Acc^6} = -68.4°$, $\psi_{Acc^6} = -15°$) lie in between the values expected for ideal Type I or III β-turns. In Boc-Aib-Acc^6-OMe, the Aib residue adopts a partially extended conformation ($\phi_{Aib} = -62.2°$, $\psi_{Aib} = 143°$) while the Acc^6 residue maintains a helical conformation ($\phi_{Acc^6} = 48°$, $\psi_{Acc^6} = 42.6°$). ^1H n.m.r. studies in $CDCl_3$ and $(CD_3)_2SO$ suggest that Boc-Aib-Acc^6-NHMe maintains the β-turn conformation in solution.

Key words: α-aminoisobutyryl peptides; 1-aminocyclohexanecarboxylic acid peptides; β-turns; n.m.r.; peptide conformation; X-ray diffraction

The presence of α,α-dialkylated α-aminoacids in peptides significantly restricts the available range of backbone conformations. Recent interest in the stereochemistry of peptides containing α,α-dialkylated residues has stemmed from studies on the structural aspects of the trans-membrane channel formed by alamethicin (1–8) and related antibiotics (8–11), which contain a high proportion of α-aminoisobutyric acid (Aib). Studies in this area have also been stimulated by the possibility that α,α-dialkylated residues can be used as local structure determinants in designing conformationally restricted analogs of biologically active peptides (12–17). These in turn may help in delineating the nature of receptor bound conformations and in the rational design of analogs of high biological potency.

The structures of some α,α-dialkylated α-aminoacids, with acyclic and cyclic side-chains are illustrated in Fig. 1. Over the past several years the preferred conformations of Aib peptides have been examined by conformational energy calculations (13, 18–22), X-ray diffraction (7, 8, 21) and spectroscopic methods (23–25). These studies have established an overwhelming preference of the Aib residue for right or left handed 3_{10}/α-helical conformations ($\phi \sim \pm 60° \pm 20°$, $\psi \sim \pm 30° \pm 20°$). More recently, similar studies have also been reported for other α,α-dialkylated residues like isovaline (Iva)(26–28), α,α-diethylglycine (Deg)(27, 19) and α,α-di-*n*-propylglycine (Dpg) (26, 27, 29). It has been observed that Deg and Dpg peptides show a marked tendency to form extended structures. This is presumably

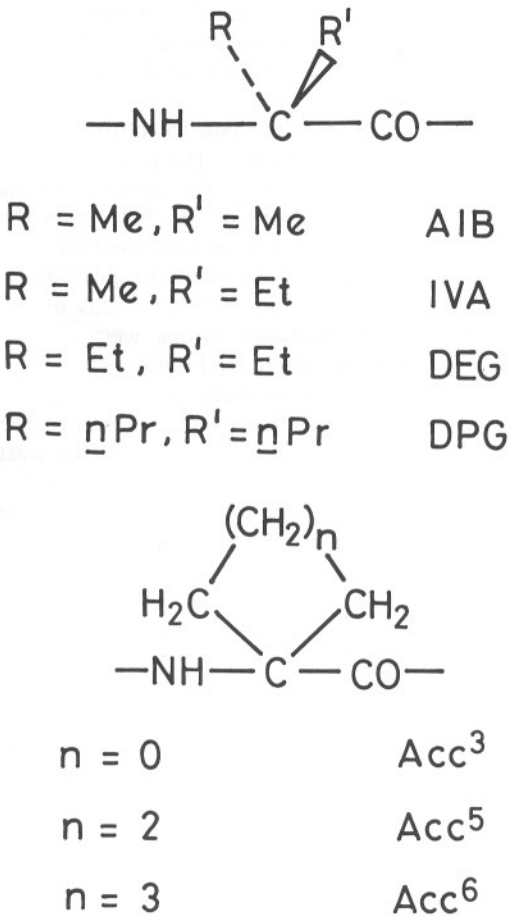

FIGURE 1

Structures of some α,α-dialkylated amino acid residues with cyclic and acyclic sidechains.

because unfavourable contacts between the substituents on C^{α} and the flanking peptide units destabilize the folded helical conformation, for an all *trans* alkyl side chain orientation. An examination of molecular models suggests that these unfavourable interactions are absent in the cycloalkane aminoacids, 1-aminocyclopentanecarboxylic acid (Acc^5) and 1-aminocyclohexanecarboxylic acid (Acc^6).* A very recent theoretical analysis suggests that various helical conformations are favoured for the Acc^3 residue; with selective substitution at C^{β} forcing folding into specific backbone conformations (30). The crystal structure of a fully protected derivative of the 2-chloro substituted Acc^3 residue (E isomer) established ϕ, ψ values close to those of a 3_{10} helix (30).

As part of our investigations on the conformations of peptides containing α,α-dialkylated aminoacid residues, we describe in this report the crystal structures of the peptides Boc-Aib-Acc^6-OMe (**I**) and Boc-Aib-Acc^6-NHMe · H_2O (**II**). Apart from the free aminoacid itself (H-Acc^6-OH · HCl)(31), these are the first crystal structures of Acc^6 derivatives or peptides. The synthesis and characterization of Acc^6 containing peptides have been described (32–34).

EXPERIMENTAL PROCEDURES

Synthesis of peptides

1-aminocyclohexanecarboxylic acid (32–34) was synthesized from cyclohexanone, adapting the procedure described for the synthesis of Aib from acetone (35). H-Acc^6-OMe · HCl was prepared by the thionyl chloride–methanol procedure (36). Neutralization of the methyl ester hydrochloride with Na_2CO_3 solution, followed by extraction with $CHCl_3$ and evaporation of the organic solvent yielded H-Acc^6-OMe, which was used immediately.

Boc-Aib-Acc^{6}OMe (**I**)

200 mg (1 mmol) Boc-Aib-OH was dissolved in 2 ml CH_2Cl_2 and cooled in an icebath. H-Acc^6-OMe extracted from 240 mg of H-Acc^6-OMe · HCl was added, followed by 200 mg dicyclohexylcarbodiimide (DCC). The reaction mixture was stirred at room temperature for 12 h and the precipitated dicyclohexylurea was filtered. The filtrate was diluted with 50 ml EtOAc and washed with 1 N $NaHCO_3$ (3 × 15 ml), 1 N HCl (3 × 15 ml) and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield **I** as a solid, which was recyrstallized from EtOAc-petroleum ether. Yield 130 mg (39%). M.p. = 111–112°. T.l.c. R_f = 0.4 (silica gel; 92:8 $CHCl_3$-CH_3OH). Satisfactory 80 MHz ^{1}H n.m.r. spectra were obtained.

*We suggest the general abbreviation Acc^n for the family of 1-aminocycloalkanecarboxylic acids, where *n* stands for the number of carbon atoms in the cycloalkane ring.

Boc-Aib-Acc^6*-NHMe* **(II)**

100 mg **I** was dissolved in 5 ml dry methanol and saturated with dry CH_3NH_2 gas. The solution was kept tightly stoppered for 4 days at room temperature. T.l.c. established conversion to **II**, although traces of **I** were detectable. Evaporation of methanol yielded a white solid, which was chromatographed on a silica gel column (eluant 98:2 $CHCl_3$-methanol) to yield **II** as a crystalline solid. Yield 80 mg (80%). M.p. = 179–180°. 80 MHz ^{1}H n.m.r. spectra in full agreement with the structure were obtained.

Spectroscopic studies

^{1}H n.m.r. spectra were recorded on a Varian FT-80A spectrometer. Sweep widths of 1000 Hz were employed with a digital resolution of 0.244 Hz/point. All chemical shifts are expressed as δ (p.p.m.) downfield from internal tetramethylsilane. I.r. spectra were recorded in dry $CHCl_3$ solutions on a Perkin Elmer model 297 spectrometer using a pathlength of 3.5 mm.

X-ray diffraction

Single crystals of Boc-Aib-Acc^6-OMe **(I)** and Boc-Aib-Acc^6-NHMe **(II)** were grown by slow evaporation from ethyl acetate-petroleum ether mixtures. X-ray diffraction data were collected on a Philips PW 1100 four-circle diffractometer, using MoK_α radiation monochromatized by a graphite crystal ($\lambda = 0.71069$ Å). Intensities were corrected for Lorentz and polarization effects and put on an absolute scale by Wilson's method. In the case of **I** absorption corrections were applied (37).

The crystallographic data for both peptides are summarized in Table 1. The structures were solved by application of the direct methods program MULTAN 80 (38). In both cases the E-maps of the set of phases with the best combined figure of merit revealed the position of 20 non-hydrogen atoms. The positions of the remaining non-hydrogen atoms were derived from subsequent difference Fourier maps. In **II**, the difference Fourier maps also revealed one water molecule in the asymmetric unit. The structures were refined by full matrix least squares procedures. The function $\Sigma W[|F_o| - |F_c|]^2$ was minimized and unit weights were applied. Scattering factors were taken from the International Tables for X-ray Crystallography (39). The refinement was carried out with anisotropic temperature factors for non-hydrogen atoms. Hydrogen atoms were calculated in idealized positions and not varied. In **II**, the two hydrogen atoms of the water molecule were located by a difference Fourier map. The structures of **I** and **II** were refined to final R values of 0.060 and 0.064, respectively. Calculations were carried out using the SHELX-76 program (40). The final positional parameter of the non-hydrogen atoms, along with equivalent isotropic thermal factors are listed in Tables 2

TABLE 1

Crystal data for peptides

	Boc-Aib-Acc^6-OMe (I)	Boc-Aib-Acc^6-NHMe · H_2O (II)
Molecular formula	$C_{17}H_{30}N_2O_5$	$C_{17}H_{31}N_3O_4 \cdot H_2O$
M.w. (a.m.u.)	342.4	359.5
Density (calc.)	1.222	1.177
Density (exp.)	1.23	1.17
Space group	$P2_1/n$	$P2_1/c$
Z	4	4
a(Å)	15.923(3)	8.660(3)
b(Å)	10.829(3)	12.477(3)
c(Å)	10.856(3)	18.830(3)
β(°)	95.87 (10)	94.2 (1)
Reflections ($I \geqslant 3\sigma(I)$)	2470	2345
R value	0.060	0.064

TABLE 2

Fractional coordinates (× 10^4) and equivalent isotropic temperature factors (Å^2 × 10^3) for Boc-Aib-Acc^6-OMe (I). ESD's are given in parentheses

Atom	x	y	z	U_{eq}
O(1)	−1119(1)	2917(2)	5996(2)	42(1)
O(2)	128(2)	2100(3)	6804(2)	56(1)
O(3)	1085(1)	4877(2)	6237(2)	39(1)
O(4)	2188(2)	5659(2)	8763(2)	54(1)
O(5)	1641(2)	3765(2)	8627(2)	48(1)
N(1)	− 6(2)	3183(3)	5003(2)	33(1)
N(2)	2136(2)	3488(2)	6356(2)	34(1)
C(1)	−1279(3)	2915(5)	8194(4)	74(2)
C(2)	−2476(2)	2960(4)	6531(4)	59(1)
C(3)	−1627(3)	1038(4)	6885(5)	66(2)
C(4)	−1616(2)	2426(3)	6936(3)	43(1)
C(5)	− 287(2)	2674(3)	6011(3)	38(1)
C(6)	890(2)	3169(3)	4833(3)	33(1)
C(7)	1190(2)	1850(3)	4680(3)	42(1)
C(8)	993(2)	3906(3)	3668(3)	40(1)
C(9)	1370(2)	3905(3)	5897(3)	31(1)
C(10)	2661(2)	4238(3)	7273(3)	34(1)
C(11)	3377(2)	3427(3)	7868(3)	42(1)
C(12)	4021(2)	3103(4)	6975(4)	51(1)
C(13)	4367(2)	4225(4)	6410(4)	62(2)
C(14)	3658(2)	5004(4)	5768(4)	54(1)
C(15)	3023(2)	5361(3)	6649(3)	44(1)
C(16)	2134(2)	4662(3)	8277(3)	38(1)
C(17)	1037(3)	4123(5)	9460(4)	70(2)

and 3. Anisotropic temperature factors, hydrogen positional parameters and structure factor tables are available on request from Dr. Bardi, Padua.

RESULTS AND DISCUSSION

The molecular structures of the two peptides Boc-Aib-Acc^6-OMe (**I**) and Boc-Aib-Acc^6-NHMe·H_2O (**II**), with the numbering schemes for the atoms are shown in Figs. 2 and 3. Bond lengths and bond angles together with their estimated standard deviations are given in Tables 4 and 5. The backbone and cyclohexyl ring torsional angles (41) are listed in Tables 6 and 7. Both peptides are achiral and crystallize in centrosymmetric space groups, containing molecules of both handedness. The torsion angles listed correspond to molecules of one handedness only.

Structural parameters

The bond lengths and bond angles observed for **I** and **II** are in broad agreement with previously described results for the geometry of the Boc urethane group (42), secondary peptide unit (43), methyl ester group (44), Aib residue (20, 21) and cyclohexane ring (45–47).

Both cyclohexyl rings of the two Acc^6 residues adopt an approximate chair conformation, with the following Cremer-Pople puckering parameters (48): $q_2 = 0.025$ Å, $q_3 = 0.551$ Å, $\phi_2 = -161°$ for **I** and $q_2 = 0.031$ Å, $q_3 = -0.567$ Å, $\phi_2 = 49°$ for **II**. The overall mean endocyclic torsion angle of 54.4° for **I** and 55.5° for **II** should be compared with the expected torsion angle of 54.4° for free cyclohexane having a C–C–C bond angle of 111.5° (46).

TABLE 3

Fractional coordinates (× 10^4) and equivalent isotropic temperature factors (Å^2 × 10^3) for Boc-Aib-Acc^{6}NHMe · H_2O (II). ESD's are given in parentheses

Atom	x	y	z	U_{eq}
O(1)	6286(3)	6392(2)	8861(2)	48(1)
O(2)	6183(3)	4579(2)	8734(1)	43(1)
O(3)	4904(3)	3051(2)	7355(1)	40(1)
O(4)	9475(4)	2261(3)	7281(2)	67(1)
O_w	7543(4)	6634(3)	7170(2)	68(1)
N(1)	4905(4)	5706(3)	7962(2)	35(1)
N(2)	6589(3)	4353(2)	7100(2)	30(1)
N(3)	8381(4)	3252(3)	8094(2)	45(1)
C(1)	8979(6)	5938(5)	9191(3)	65(2)
C(2)	7705(8)	7578(5)	9590(4)	84(3)
C(3)	7000(8)	5786(6)	10078(3)	82(2)
C(4)	7523(5)	6389(4)	9446(2)	49(2)
C(5)	5826(4)	5481(3)	8540(2)	36(1)
C(6)	4113(4)	4879(3)	7523(2)	34(1)
C(7)	3476(6)	5409(4)	6838(2)	48(2)
C(8)	2807(5)	4399(4)	7913(3)	49(2)
C(9)	5252(4)	4003(3)	7325(2)	31(1)
C(10)	7813(4)	3670(3)	6839(2)	36(1)
C(11)	9069(5)	4408(4)	6578(2)	46(1)
C(12)	8551(6)	5026(4)	5905(2)	59(2)
C(13)	7957(7)	4288(5)	5315(3)	70(2)
C(14)	6647(6)	3591(4)	5563(2)	58(2)
C(15)	7191(5)	2947(3)	6216(2)	43(1)
C(16)	8602(5)	2991(3)	7427(2)	41(1)
C(17)	9173(6)	2729(4)	8701(3)	56(2)

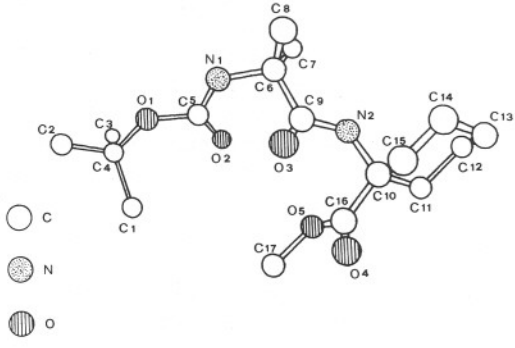

FIGURE 2
Molecular structure of Boc-Aib-Acc^6-OMe (**I**).

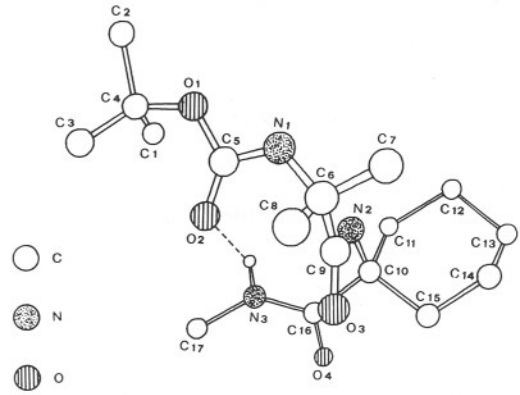

FIGURE 3
Molecular structure of Boc-Aib-Acc^6-NHMe · H_2O (**II**).

TABLE 4

Bond lengths (Å) *and angles* (°) *in Boc-Aib-Acc^{6}OMe* (*I*). *ESD's are given in parentheses*

Bond lengths		Bond angles	
C(4) –C(1)	1.511(5)	C(1) –C(4) –C(2)	110.9(3)
C(4) –C(2)	1.510(5)	C(1) –C(4) –C(3)	112.6(3)
		C(1) –C(4) –O(1)	109.7(3)
C(4) –C(3)	1.504(5)	C(2) –C(4) –C(3)	111.4(4)
		C(2) –C(4) –O(1)	101.4(3)
C(4) –O(1)	1.454(4)	C(3) –C(4) –O(1)	110.2(3)
O(1) –C(5)	1.349(4)	C(4) –O(1) –C(5)	121.6(3)
C(5) –O(2)	1.203(4)	O(1) –C(5) –O(2)	125.2(3)
		O(1) –C(5) –N(1)	108.6(3)
C(5) –N(1)	1.342(4)	O(2) –C(5) –N(1)	126.2(4)
N(1) –C(6)	1.457(5)	C(5) –N(1) –C(6)	120.7(3)
		N(1) –C(6) –C(7)	110.2(3)
C(6) –C(7)	1.521(5)	N(1) –C(6) –C(8)	106.9(3)
		N(1) –C(6) –C(9)	108.5(3)
C(6) –C(8)	1.518(5)	C(7) –C(6) –C(9)	115.5(3)
C(6) –C(9)	1.540(4)	C(8) –C(6) –C(9)	105.4(3)
		C(7) –C(6) –C(8)	109.9(3)
C(9) –O(3)	1.218(4)	C(6) –C(9) –O(3)	120.2(3)
C(9) –N(2)	1.347(4)	C(6) –C(9) –N(2)	117.7(3)
		O(3) –C(9) –N(2)	121.9(3)
N(2) –C(10)	1.476(4)	C(9) –N(2) –C(10)	119.6(3)
C(10)–C(11)	1.529(4)	N(2) –C(10)–C(11)	108.4(3)
		N(2) –C(10)–C(15)	110.5(3)
C(11)–C(12)	1.522(5)	N(2) –C(10)–C(16)	109.6(3)
C(12)–C(13)	1.492(6)	C(10)–C(16)–O(4)	124.7(3)
		C(10)–C(15)–O(5)	111.8(3)
C(13)–C(14)	1.520(5)	O(4) –C(16)–O(5)	123.3(3)
C(14)–C(15)	1.511(5)	C(16)–O(5) –C(17)	115.8(3)
		C(10)–C(11)–C(12)	112.6(3)
C(15)–C(10)	1.533(5)	C(11)–C(12)–C(13)	112.0(3)
C(10)–C(16)	1.513(5)	C(12)–C(13)–C(14)	110.6(3)
		C(13)–C(14)–C(15)	111.4(3)
C(16)–O(4)	1.201(4)	C(14)–C(15)–C(10)	112.5(3)
C(16)–O(5)	1.329(4)	C(15)–C(10)–C(11)	110.1(3)
		C(15)–C(10)–C(16)	109.8(3)
O(5) –C(17)	1.439(6)	C(11)–C(10)–C(16)	108.3(3)

Peptide backbone conformation

Peptide **II** adopts a folded, β-turn conformation stabilized by a 4 → 1 intramolecular hydrogen bond between the methylamide NH and Boc CO groups. The N(3)---O(2) distance of 2.855 Å is in good agreement with values reported for NH---CO hydrogen bonds in peptide crystal structures (49). The ϕ, ψ values for the Aib ($\phi = -50.3°$, $\psi = -45.8°$) and Acc^6 ($\phi = -68.4°$, $\psi = -15°$) residues lie in between the values expected for ideal Type I or III β-turn conformations (50). It may be noted that these two β-turn classes differ only slightly in the dihedral angles of the right hand corner (i + 2) residue (50).

Peptide **I**, which lacks the methylamide group, adopts a significantly different conformation. The signs of the ϕ, ψ values for the Aib and Acc^6 residues are opposite. While the Acc^6 residue again adopts a conformation in the

TABLE 5

Bond lengths (Å) *and angles* (°) *in Boc-Aib-Acc⁶-NHMe · H_2O (II). ESD's are given in parentheses*

Bond lengths		Bond angles	
C(4) –C(1)	1.492(7)	C(1) –C(4) –C(2)	110.3(5)
		C(1) –C(4) –C(3)	112.1(5)
C(4) –C(2)	1.514(8)	C(1) –C(4) –O(1)	110.1(4)
C(4) –C(3)	1.505(8)	C(2) –C(4) –C(3)	112.4(5)
		C(2) –C(4) –O(1)	101.0(4)
C(4) –O(1)	1.479(5)	C(3) –C(4) –O(1)	110.3(5)
O(1) –C(5)	1.334(5)	C(4) –O(1) –C(5)	120.7(4)
		O(1) –C(5) –O(2)	126.2(4)
C(5) –O(2)	1.216(4)	O(1) –C(5) –N(1)	109.4(3)
C(5) –N(1)	1.331(5)	O(2) –C(5) –N(1)	124.4(4)
		C(5) –N(1) –C(6)	122.8(4)
N(1) –C(6)	1.461(5)	N(1) –C(6) –C(7)	107.4(3)
C(6) –C(7)	1.517(6)	N(1) –C(6) –C(8)	109.9(4)
C(6) –C(8)	1.516(6)	N(1) –C(6) –C(9)	110.9(4)
		C(7) –C(6) –C(9)	107.7(3)
C(6) –C(9)	1.536(5)	C(8) –C(6) –C(9)	110.6(3)
		C(7) –C(6) –C(8)	110.3(4)
C(9) –O(3)	1.228(4)	C(6) –C(9) –O(3)	120.9(4)
C(9) –N(2)	1.335(5)	C(6) –C(9) –N(2)	115.6(3)
N(2) –C(10)	1.472(5)	O(3) –C(9) –N(2)	123.5(4)
		C(9) –N(2) –C(10)	125.4(3)
C(10)–C(11)	1.533(7)	N(2) –C(10)–C(11)	107.7(3)
C(11)–C(12)	1.523(7)	N(2) –C(10)–C(15)	111.8(4)
		N(2) –C(10)–C(16)	112.1(3)
C(12)–C(13)	1.504(7)	C(10)–C(16)–O(4)	120.2(4)
C(13)–C(14)	1.529(7)	C(10)–C(16)–N(3)	117.8(4)
		O(4) –C(16)–N(3)	121.9(4)
C(14)–C(15)	1.515(7)	C(16)–N(3) –C(17)	123.1(5)
C(15)–C(10)	1.545(7)	C(10)–C(11)–C(12)	113.5(5)
		C(11)–C(12)–C(13)	111.6(5)
C(10)–C(16)	1.516(5)	C(12)–C(13)–C(14)	110.1(5)
C(16)–O(4)	1.228(5)	C(13)–C(14)–C(15)	110.8(5)
		C(14)–C(15)–C(10)	112.1(5)
C(16)–N(3)	1.325(5)	C(15)–C(10)–C(11)	108.9(3)
N(3) –C(17)	1.445(6)	C(15)–C(10)–C(16)	110.0(3)
		C(11)–C(10)–C(16)	106.1(4)
O_W –H_{W_1}	0.965(3)	H_{W_1} –O_W –H_{W_2}	98.0(9)
O_W –H_{W_2}	0.912(4)		

$3_{10}/\alpha$-helical region ($\phi = 48°$, $\psi = 42.6°$; where ψ is defined as the angle N(2)–C(10)–C(16)–O(5)), the Aib residue takes up a partially extended conformation ($\phi = -62.2°$, $\psi = 143°$). This conformation is rarely observed in Aib peptides (21, 51, 52).

The urethane moiety, the peptide units, the methylamido and methyl ester groups of **I** and **II** are approximately *trans* planar (Table 6). The C(4)–O(1) bond of the urethane moiety of both compounds is in the usual *trans* arrangement relative to the C(5)–N(1) bond, the C(4)–O(1)–C(5)–N(1) torsion angle being 176.5° for **I** and −169.6° for **II**. This structural

TABLE 6

Backbone[a] and sidechain torsion angles (°) in Boc-Aib-Acc^6-OMe (I) ESD's are given in parentheses

O(1) –C(5) –N(1) –C(6)	ω	174.2(3)
C(5) –N(1) –C(6) –C(9)	ϕ_{Aib}	– 62.2(4)
N(1) –C(6) –C(9) –N(2)	ψ_{Aib}	143.0(3)
C(6) –C(9) –N(2) –C(10)	ω	172.9(3)
C(9) –N(2) –C(10)–C(16)	ϕ_{Acc^6}	48.0(4)
N(2) –C(10)–C(16)–O(5)	ψ_{Acc^6}	42.6(4)
C(10)–C(11)–C(12)–C(13)		54.6(4)
C(11)–C(12)–C(13)–C(14)		– 56.0(4)
C(12)–C(13)–C(14)–C(15)		56.6(4)
C(13)–C(14)–C(15)–C(10)		– 55.5(4)
C(14)–C(15)–C(10)–C(11)		52.4(4)

[a]Backbone dihedral angles defined following the convention in ref. 41.

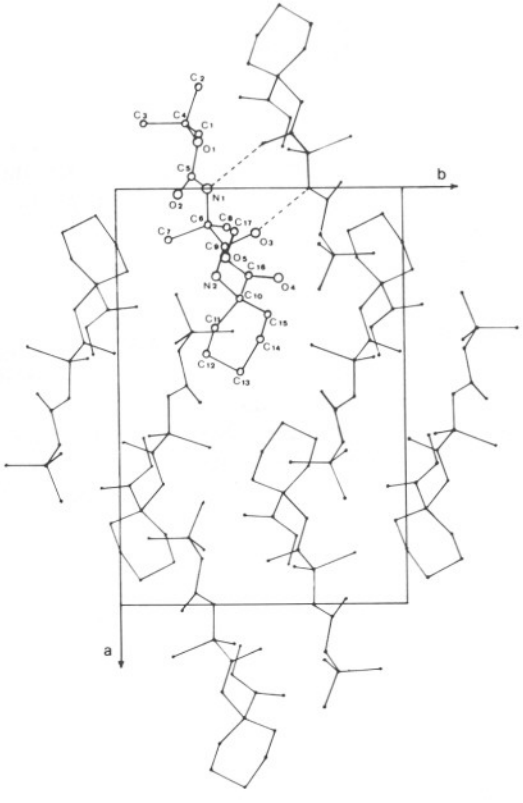

FIGURE 4

Molecular packing in the crystals of Boc-Aib-Acc^6-OMe (I) viewed down the *c* axis. Hydrogen bonds are shown as dashed lines.

property, accompanied by the *trans* conformation of the CO–NH moiety, allows us to classify the urethane group of both compounds as type *b* (42, 53).

Crystal packing

The crystal structure of **I** (Fig. 4) is characterized by a single intermolecular hydrogen bond between Aib NH and Aib CO groups of symmetry related ($\bar{x}$, $1-y$, $1-z$) molecules. The N···O distance of 2.950 (4) Å agrees well with the average value determined from a large number of peptide structures (54). The H···O distance is 2.055 Å and H–N–O angle is 147.9°. The Acc^6 NH, urethane CO and ester CO groups are not involved in intermolecular hydrogen bonding; a somewhat unusual observation, since most NH and CO groups are generally hydrogen bonded in crystal structures of amides and carboxylic acid derivatives (55, 56). There is, however, a rather short N···O contact of 3.25 Å between the Acc^6 NH and the ester CO of a symmetry related molecule ($0.5-x$, $-0.5+y$, $1.5-z$). This may be indicative of a hydrogen bond, weakened by steric constraints.

A view of the molecular packing in crystals of **II** along the *a* axis is illustrated in Fig. 5.

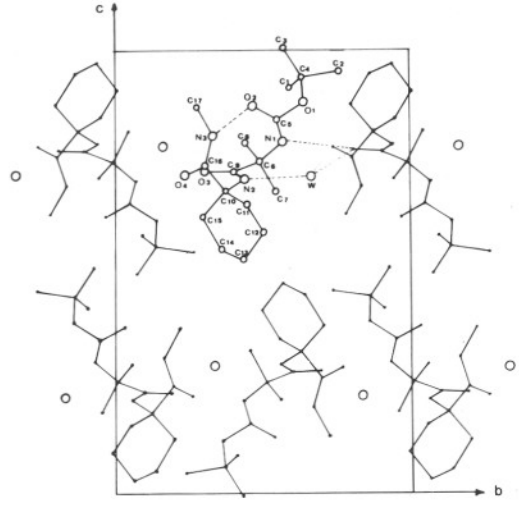

FIGURE 5

Molecular packing in the crystals of Boc-Aib-Acc^6-NHMe·H_2O (**II**) viewed down the *a* axis. Hydrogen bonds are shown as dashed lines. For the symmetry codes of the hydrogen bonds, see Table 8.

TABLE 7

Backbone[a] *and sidechain torsion angles (°) in Boc-Aib-Acc⁶-NHMe·H_2O (II) ESD's are given in parentheses*

O(1) –C(5) –N(1) –C(6)	ω	−175.2(4)
C(5) –N(1) –C(6) –C(9)	ϕ_{Aib}	− 50.3(6)
N(1) –C(6) –C(9) –N(2)	ψ_{Aib}	− 45.8(5)
C(6) –C(9) –N(2) –C(10)	ω	−175.9(4)
C(9) –N(2) –C(10)–C(16)	ϕ_{Acc^6}	− 68.4(5)
N(2) –C(10)–C(16)–N(3)	ψ_{Acc^6}	− 15.0(6)
C(10)–C(16)–N(3) –C(17)	ω	−174.4(4)
C(10)–C(11)–C(12)–C(13)		− 54.9(6)
C(11)–C(12)–C(13)–C(14)		55.9(6)
C(12)–C(13)–C(14)–C(15)		− 57.9(6)
C(13)–C(14)–C(15)–C(10)		57.9(5)
C(14)–C(15)–C(10)–C(11)		− 54.0(5)

[a]Backbone dihedral angles defined following the convention in ref. 41.

Once again, there is an intermolecular hydrogen bond between the Aib NH and Aib CO groups of symmetry related molecules. In addition, a complex network of hydrogen bonds involving the water molecule stabilizes the crystal structure. The water molecule interacts with the Acc^6 NH of one peptide molecule and the Acc^6 CO and Aib CO groups of a symmetry related peptide. The details of the hydrogen bond parameters in **II** are summarized in Table 8. The observed N---O (54) and O---O (57, 58) distances agree well with values reported in related crystal structures.

Solution conformation of **II**

In order to determine whether the β-turn conformation, observed in the solid state for **II**, was maintained in solution, ^{1}H n.m.r. studies were carried out in $CDCl_3$ and $(CD_3)_2SO$. The involvement of NH groups in intramolecular hydrogen bonds was probed using temperature and solvent induced chemical shifts and paramagnetic radical induced broadening of NH resonances as diagnostic parameters (24). Fig. 6 summarizes the results of these experiments. Addition of the hydrogen bonding solvent $(CD_3)_2SO$ to $CDCl_3$ solutions of **II** results in a large downfield shift of the Aib NH group and a smaller shift of the Acc^6 NH, while the methylamide NH group is almost unaffected (Fig. 6a). Addition of the paramagnetic probe TEMPO to $CDCl_3$ solutions of II results in a dramatic broadening of the Aib NH resonance (Fig. 6b). Once again the Acc^6 NH is less affected, and the terminal methylamide NH is largely unaffected. In $(CD_3)_2SO$ solution the observed temperature coefficients ($d\delta/dT$) follow the order Aib NH > Acc^6 NH > NHMe (Fig. 6c). The very low value of 0.001 p.p.m./°K observed for the methylamide NH is characteristic of a strongly solvent shielded (hydrogen bonded) proton. The n.m.r. parameters also suggest that the Acc^6 NH is partially solvent shielded, presumably due to the steric bulk of the cyclohexyl residue. Further, support for the existence of intramolecularly hydrogen bonded solution conformations in **II** is obtained from i.r. studies in $CHCl_3$ (0.0053 M). In **II** NH stretching bands (ν_{NH}) are observed at 3440 cm^{-1} and 3395 cm^{-1} corresponding to free and intramolecularly hydrogen bonded NH groups (59). In contrast, the i.r. spectra of the ester **I** are characterized by a single ν_{NH} based at

TABLE 8

Geometry of the hydrogen bonds in the crystal of Boc-Aib-Acc⁶NHMe·H_2O (II)

Donor D–H	Acceptor A	Symmetry equivalence of A	Distances (Å) D---A	H---A	Angle D–H---A (°)
N(3)–H	O(2)	x, y, z	2.85	1.92	154.0
N(2)–H	O_W	x, y, z	2.96	1.97	170.1
N(1)–H	O(3)	$\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z$	2.99	2.02	162.9
O_W–H_{W_1}	O(4)	$2-x, \frac{1}{2}+y, \frac{1}{2}-z$	2.82	1.86	174.2
O_W–H_{W_2}	O(3)	$\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z$	2.95	2.07	161.6

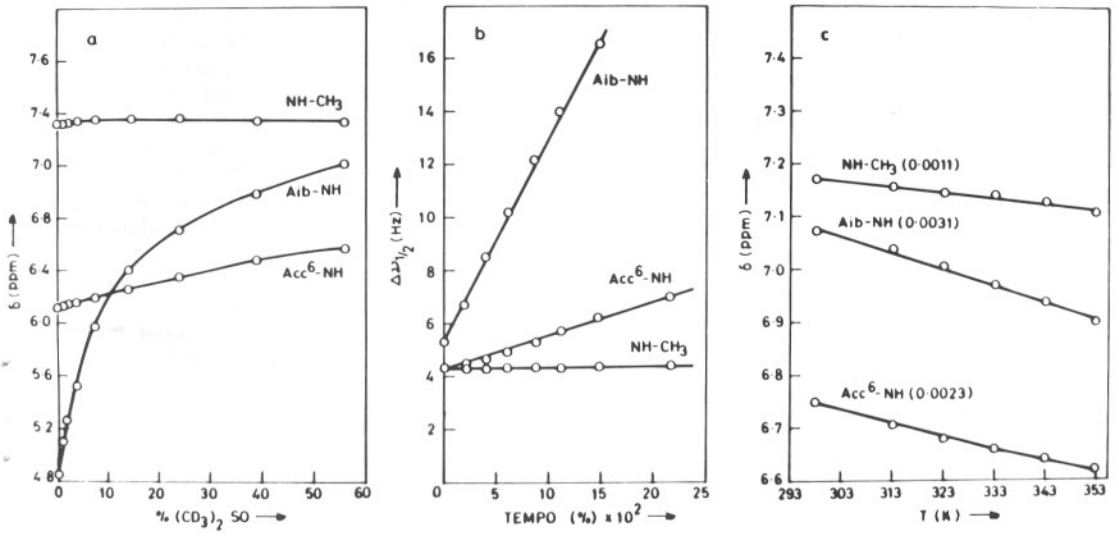

FIGURE 6

^{1}H n.m.r. data for NH groups in Boc-Aib-Acc^6-NHMe. a) Dependence of NH chemical shifts on $(CD_3)_2SO$ concentration in mixtures of $CDCl_3$ and $(CD_3)_2SO$. b) Dependence of NH linewidths on TEMPO concentration in $CDCl_3$ solution. c) Temperature dependence of NH chemical shifts in $(CD_3)_2SO$. Temperature coefficient values ($d\delta/dT$, p.p.m./°K) are shown in parentheses. Peptide concentrations of 10 mg/ml were used in all experiments.

3450 cm^{-1} corresponding to free NH groups. The spectral data thus suggest that the β-turn conformation of **II** is maintained in solution.

CONCLUSION

The crystal structures of Boc-Aib-Acc^6-NHMe and Boc-Aib-Acc^6-OMe establish that the Acc^6 residue preferentially adopts conformations in the right or left handed $3_{10}/\alpha$-helical region of ϕ, ψ space. The β-turn (Type I(I′) or III(III′)) conformation observed in **II** appears to be stabilized by the intramolecular 4 → 1 hydrogen bond, since replacement of the NH group by O in peptide **I** results in the observation of a distinctly different conformation. The β-turn conformation of **II** is maintained in solution. It thus appears that the Acc^6 residue can be used to stabilize folded, helical conformations in oligopeptides.

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