Stereochemistry of Peptides Containing 1-Aminocyclopentanecarboxylic Acid (Acc^5): Solution and Solid-state Conformations of Boc-Acc^5-Acc^5-NHMe

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Synopsis

The conformational analysis of a protected homodipeptide of 1-aminocyclopentanecarboxylic acid (Acc^5) has been carried out. ^{1}H-nmr studies establish a β-turn conformation for Boc-Acc^5-Acc^5-NHMe in chloroform and dimethylsulfoxide solutions involving the methylamide NH in an intramolecular hydrogen bond. Supportive evidence for the formation of an intramolecular hydrogen bond is obtained from ir studies. X-ray diffraction studies reveal a type III β-turn conformation in the solid state stabilized by a $4 \rightarrow 1$ hydrogen bond between the Boc CO and methylamide NH groups. The ϕ, ψ values for both Acc^5 residues are close to those expected for an ideal 3_{10}-helical conformation ($\phi \simeq \pm 60°$, $\psi = \pm 30°$).

INTRODUCTION

The ability of α,α-dialkylated amino acid residues to impart conformational rigidity to peptides is well recognized.[1-4] The utility of such residues in developing conformationally constrained analogs of biologically active peptides has been established.[5] a-Aminoisobutyric acid (Aib) is the best-studied member of this class of amino acids.[6,7] The 1-aminocycloalkanecarboxylic acids (abbreviated as Acc^n, where n is the number of carbon atoms in the cycloalkane ring)[8] have been less-extensively investigated. These residues may be expected to impose backbone conformational constraints similar to those observed for Aib. We describe in this report the solution and solid-state conformation of the model peptide Boc-Acc^5-Acc^5-NHMe (**1**) (Boc, *t*-butyloxycarbonyl; NHMe, methylamido. Note that the abbreviation "Cle" (cycloleucine) has also been used in the literature for the residue 1-aminocyclopentanecarboxylic acid).

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MATERIALS AND METHODS

Synthesis of Peptides

H-Acc^5-OMe . HCl was prepared by the thionyl chloride-methanol procedure[9] (yield 95%). Neutralization of the methyl ester hydrochloride with Na_2CO_3 solution, followed by extraction with CHCl, and evaporation of the organic solvent, yielded H-Acc^5-OMe, which was used immediately. Boc-Acc^5-OH was prepared by a standard procedure.[10]

Boc-Acc^5-Acc^5-OMe

500 mg (2.1 mmol) Boc-Acc^5-OH was dissolved in 3 mL CH_2Cl_2 and cooled in an ice bath. H-Acc^5-OMe, extracted from 540 mg (3 mmol) of H-Acc^5-OMe . HCl was added, followed by 500 mg of dicyclohexylcarbodiimide (DCC). The reaction mixture was stirred at room temperature for 12 h and the precipitated dicyclohexylurea was filtered. The filtrate was diluted with 100 mL ethylacetate and washed with 1N $NaHCO_3$ (3 × 20 mL), 1*N* HCl (3 × 20 mL), and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield Boc-Acc^5-Acc^5-OMe as a solid of high purity, as judged by thin-layer chromatography (TLC). Yield: 595 mg (77%).mp: 140–141°C. Nmr ($CDCl_3$): δ 1.36 [s, 9H, C—(CH,),]; δ 1.64, 2.00, 2.20 [m, 16H, $C^\beta H_2$, $C^\gamma H_2$ Acc^5(1) and Acc^5(2)]; δ 3.6 (s, 3H, COO—CH,); δ 6.16 [s, 1H, NH-Acc^5(1)]; δ 6.60 [s, 1H, NH-Acc^5(2)].

Boc-Acc^5-Acc^5-NHMe

250 mg of Boc-Acc^5-Acc^5-OMe was dissolved in 10 mL of dry methanol and saturated with dry CH_3NH_2 gas. The solution was kept tightly stoppered for 3 days at room temperature. Conversion to the methylamide was established by TLC. Evaporation of methanol yielded a white solid, which showed the presence of traces of the starting material, by TLC. The crude product was chromatographed on a silica gel column (eluent 98 :2 $CHCl_3$-MeOH) to yield Boc-Acc^5-Acc^5-NHMe as a white crystalline solid. Yield: 200 mg (84%).mp: 167–168°C. Nmr ($CDCl_3$) (Fig. 1): δ 1.4 [s, 9H, C—(CH,),]; δ 1.75, 1.95, 2.25 [m, 16H, $C^\beta H_2$, $C^\gamma H_2$ Acc^5(1) and Acc^5(2)]; δ 2.75 (d, 3H, NH—CH_3); δ 5.00 [s, 1H, NH Acc^5(1), δ 6.3 (s, 1H, NH Acc^5(2)]; δ 7.25 (broad multiplet, 1H, NH—CH_3).

Spectroscopic Studies

'H-nmr 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 (ppm) downfield from internal tetramethylsilane. The nitroxide, 2,2,6,6-tetramethylpiperidine-1-oxyl was obtained from Sigma Chemical Company. Ir absorption spectra were recorded in dry $CHCl_3$ solutions on a Perkin-Elmer model 297 spectrophotometer, using cells of pathlength 3.5 mm.

X-Ray Diffraction

Single crystals of **1** ($C_{18}H_{31}N_3O_4$) in the space group, $P2_1/a$ [$Z = 4$, $a = 12.100(3)$, $b = 17.247(3)$ $c = 11.143(3)$ Å, $\beta = 117.5(1)°$], were grown by slow evaporation from **a** methanol–water mixture. X-ray diffraction data were collected on **a** Philips PW 1100 four-circle diffractometer using M_0K_α radiation ($\lambda = 0.71069$ Å). 2019 reflections with $I \geq 3\sigma(I)$ were used in the solution of the structure by application of the direct-methods program MULTAN 80.[11] The E-maps of the set of phases with the best-combined figure of merit revealed the position of 20 nonhydrogen atoms. The positions of the remaining nonhydrogen atoms were derived from subsequent difference Fourier maps. The structure was refined by full-matrix least-squares procedures, with isotropic temperature parameters for all atoms to an R factor of 0.161. At this stage a difference Fourier map revealed disorder of one cyclopentane ring (hereafter called ring II)—i.e., C(12), C(13), C(14), C(15), and C(16). Population parameters were applied to C(15) and C(150) carbon atoms of ring 11. Refinement with population parameters of 0.650 and 0.350 for C(15) and C(150), respectively, resulted in a change of the thermal parameters to values in accord with those of other carbon atoms. The structure was refined by **a**

TABLE I
Fractional Coordinates ($\times 10^4$), Population Parameters, and Equivalent Isotropic Temperature Factors (Å^2 $\times$ 10^3) for Boc-Acc^5-Acc^5-NHMe[a]

Atom	Population parameter	x	y	z	U_{eq}/U[b]
O(1)	1.0	– 258 (3)	9362 (2)	8295 (3)	45 (2)
O(2)	1.0	1695(3)	9070 (2)	8610 (3)	50 (2)
O(3)	1.0	3104 (3)	7305 (2)	8801 (3)	47 (2)
O(4)	1.0	3353 (3)	7815 (3)	5707 (4)	79 (3)
N(1)	1.0	440 (3)	8158 (2)	8825 (4)	37 (2)
N(2)	1.0	1198(3)	7495 (2)	7041 (4)	40 (2)
N(3)	1.0	2539 (4)	8660 (3)	6626 (5)	62 (3)
C(1)	1.0	749 (5)	10641 (3)	8768 (6)	69 (4)
C(2)	1.0	– 1555(5)	10444 (3)	7522 (6)	72 (4)
C(3)	1.0	– 250 (6)	10066 (3)	6413 (6)	74 (4)
C(4)	1.0	– 288 (5)	10146 (3)	7752 (5)	48 (3)
C(5)	1.0	713 (4)	8881 (3)	8587 (5)	39 (3)
C(6)	1.0	1414 (4)	7559 (2)	9332 (5)	34 (3)
C(7)	1.0	2386 (4)	7718 (3)	10784 (5)	46 (3)
C(8)	1.0	1808 (6)	7404 (4)	11646 (6)	75 (4)
C(9)	1.0	999 (6)	6752 (4)	10892 (6)	73 (4)
C(10)	1.0	816 (4)	6784 (3)	9447 (5)	44 (3)
C(11)	1.0	1987 (4)	7450 (2)	8359 (5)	35 (3)
C(12)	1.0	1605 (4)	7372 (3)	6000 (5)	46 (3)
C(13)	1.0	465 (5)	7462 (4)	4574 (5)	58 (3)
C(14)	1.0	25 (6)	6652 (4)	4061 (6)	82 (4)
C(15)	0.65	826 (9)	6065 (5)	5179 (10)	68 (3)*
C(150)	0.35	1233(9)	6190 (5)	4602 (10)	69 (5)*
C(16)	1.0	2037 (5)	6530 (3)	6000 (6)	61 (3)
C(17)	1.0	2608 (4)	7969 (3)	6146 (5)	52 (3)
C(18)	1.0	3333 (5)	9310 (4)	6697 (7)	81 (4)

[a] Estimated standard deviations are given in parentheses.
[b] Temperature factors for atoms having partial occupancy factors.

block-matrix least-squares procedure. The scattering factors were taken from the International Tables for X-ray Crystallography." The refinement was carried out, allowing all nonhydrogen atoms [except C(15) and C(150)] to vibrate anisotropically, while hydrogen atoms [except those as C(15) and C(150)] were put in calculated idealized positions (C—H, N—H = 1.0 Å) and were included in the last cycle. Calculations were carried out using the SHELX-76 program.[13] The final conventional R value for 2019 observed reflections [$I \geq 3\sigma(I)$] was 0.0815 ($R_w = 0.0745$). The final positional parameters of the nonhydrogen atoms, along with equivalent isotropic thermal factors, are listed in Table I. Anisotropic temperature factors, hydrogen positional parameters, and structure factor tables are available from Dr. R. Bardi.

RESULTS AND DISCUSSION

NMR Studies

Figure 1 shows the 80 MHz ^{1}H-nmr spectrum of **1** in $CDCl_3$. The assignment of the three NH groups is straightforward. The singlet at 4.98 δ corresponds to the Acc^5(1) NH (urethane) by virtue of its high-field position. The singlet at 6.35 δ is due to the Acc^5(2) NH, while the broad quartet at 7.29

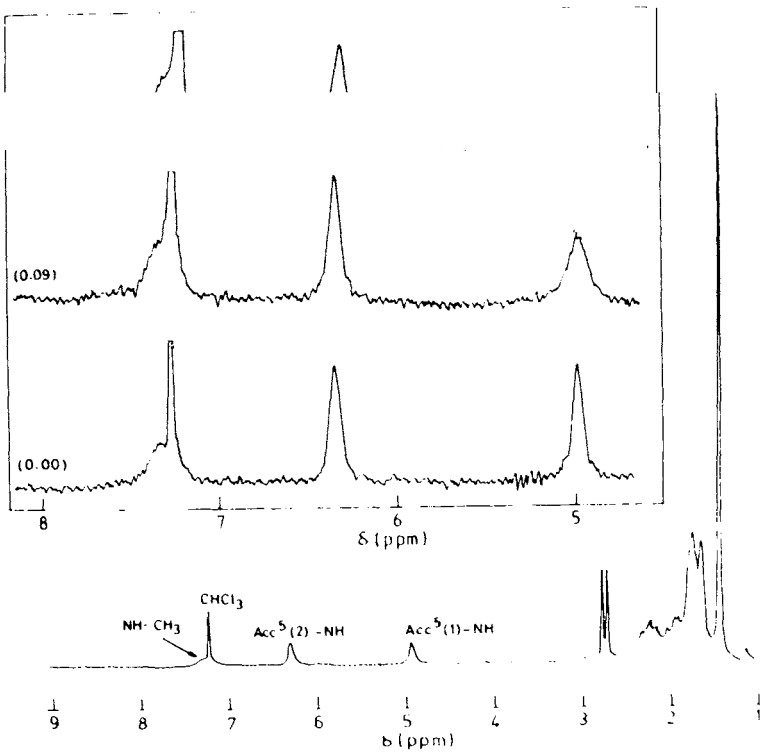

Fig. 1. 80-MHz 'H-nmr spectrum of Boc-Acc^5-Acc^5-NHMe in CDCl,,. Inset: effect of addition of the free radical TEMPO on peptide NH resonances. TEMPO concentrations (%) are indicated against the traces.

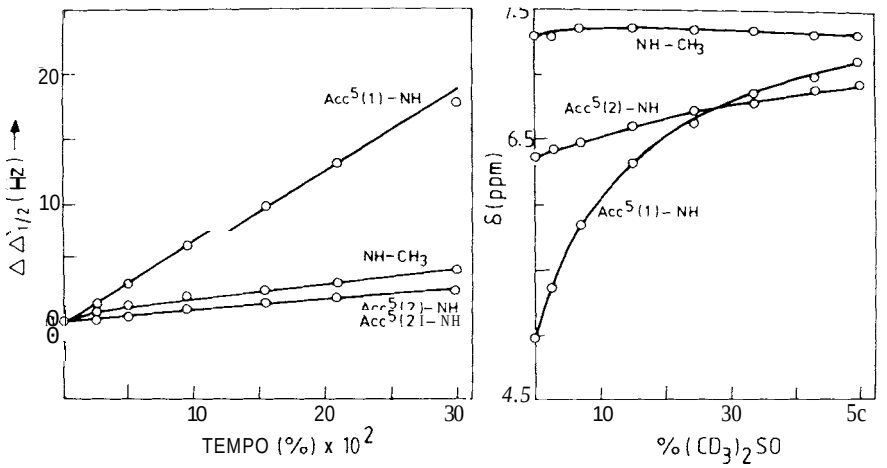

Fig. 2. Left: effect of increasing concentrations of TEMPO on NH resonance linewidths. $\Delta\Delta\nu_{1/2}$ is the line-broadening induced. Right: solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures.

6 is assigned to the methylamide NH group. The involvement of NH groups in intramolecular hydrogen bonding was probed using the following methods'*: (1) paramagnetic radical-induced line-broadening in $CDCl_3$, *(2)* solvent dependence of NH chemical shifts in $CDCl_3$—$(CD_3)_2SO$ mixtures, and (3) temperature dependence of NH chemical shifts in $(CD_3)_2SO$. Figure **1** (inset) shows the effect of the addition of the free radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) on the linewidth of the NH resonances in **1.** The results of these experiments and the solvent dependences of NH chemical shifts are summarized in Fig. *2.*

The urethane NH, $Acc^5(1)NH$, is clearly solvent exposed since it shows large changes in chemical shifts and linewidths in the solvent and free radical perturbation experiments. The behavior of the $Acc^5(2)$ and methylamide NH groups in these experiments is characteristic of solvent-shielded NH protons.[14] In $(CD_3)_2SO$ the following temperature coefficients $(d\delta/dT)$ were obtained: $Acc^5(1)$ NH 0.0035, $Acc^5(2)$ NH 0.003, and NHMe 0.0015 ppm/K. The results suggest that the methylamide NH group is inaccessible to the solvent. The relatively low $d\delta/dT$ values for the other two NH groups may be a consequence of steric shielding by the bulky, cyclopentyl groups. The presence of intramolecularly hydrogen-bonded conformations is evident from ir absorption studies in $CHCl_3$ solution.[15,16] A ν_{NH} (hydrogen-bonded) band at 3390–3400 cm^{-1} and a ν_{NH} (free) band at 3440–3450 cm^{-1} are observed over the concentration range $5 \times 10^{-3}M$–$0.7 \times 10^{-4}M$. The relative intensities of these bands is almost constant over this range of peptide concentration. It is noteworthy that the hydrogen-bonded NH-stretching frequency is significantly higher in **1** than that observed in several β-turn peptides.[15,16] **A** structure compatible with the nmr data would involve the NHMe group in a $4 \rightarrow 1$ hydrogen bond with the Boc CO group, stabilizing a β-turn conformation. Clear support for such a possibility is obtained by x-ray diffraction studies.

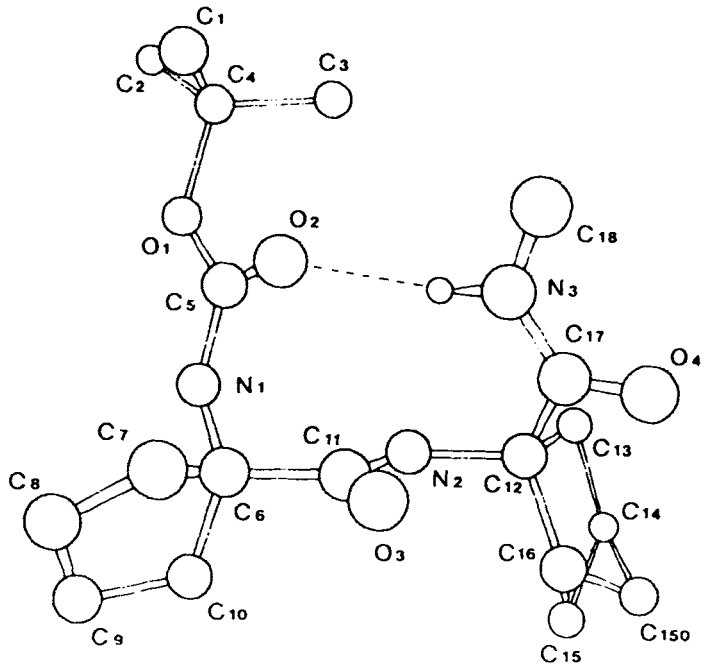

Fig. 3. Perspective view of the molecular conformation of Boc-Acc^5-Acc^5-NHMe in the solid state.

Crystal Structure

The molecular structure of Boc-Acc^5-Acc^5-NHMe (1) in the solid state, with the atomic numbering scheme, is shown in Fig. **3.** Bond lengths and bond angles are given in Table **11.** The backbone and side-chain torsion angles[17] are listed in Table III. The geometry of the inter- and intramolecular hydrogen bonds are given in Table IV and the molecular packing in the crystal is illustrated in Fig. 4.

Structural Parameters

The observed bond lengths and angles for the Boc and amide groups are largely unexceptional. For cyclopentane ring 1 the C—C bond lengths range from 1.473 (9) to 1.553 (7) Å, with a mean value of 1.519 (8) Å. The short C(8)–C(9) distance of 1.473 (9) A is probably a result of the large thermal parameters for these atoms. Cyclopentane ring II is disordered and the C—C bond lengths range from 1.510 (9) to 1.558 **(6)** Å. The mean value are 1.541 (8) and 1.531 **(9)** A, for the two conformations. Mean bond angles of 105.7 (5)°, 105.1 (5)°, and 105.1 (7)" are obtained for ring I and the two conformations of ring II, respectively. All the cyclopentane parameters are in good agreement with **expected values.**[18,19] Ring I adopts a half-chair (C_s) conformation [puckering coordinates" $q_2 = 0.334$ (6) Å and @ $= -157.1°$] while both forms of ring II adopt envelope (C_s) conformations [$q_2 = 0.371$ (12) A and $\phi_2 = -40$ (1)"; $q_2 = 0.371$ (12) Å and $\phi = 77.1°$][21].

TABLE II
Bond Lengths (Å) and Bond Angles (°) in Boc-Acc^5-Acc^5-NHMe[a]

Bond Lengths		Bond Angles	
O(1)–C(4)	1.475 (6)	C(4)-O(1)-C(5)	121.1 (4)
O(1)–C(5)	1.350 (6)	C(5)-N(1)-C(6)	120.0 (4)
O(2)–C(5)	1.221 (6)	C(11)-N(2)-C(12)	122.0 (4)
O(3)–C(11)	1.232 (6)	C(17)-N(3)-C(18)	123.5 (5)
O(4)–C(17)	1.237 (7)	C(2)-C(4)-C(3)	110.5 (5)
N(1)–C(5)	1.347 (6)	C(1)-C(4)-C(3)	113.3 (5)
N(1)–C(6)	1.470 (5)	C(1)-C(4)-C(2)	111.1 (5)
N(2)–C(11)	1.334 (6)	O(1)-C(4)-C(3)	108.3 (4)
N(2)–C(12)	1.470 (8)	O(1)-C(4)-C(2)	102.2 (4)
N(3)–C(17)	1.325 (7)	O(1)-C(4)-C(1)	110.9 (4)
N(3)–C(18)	1.455 (8)	O(2)-C(5)-N(1)	124.6 (5)
C(1)–C(4)	1.508 (7)	O(1)-C(5)-N(1)	110.5 (5)
C(2)–C(4)	1.522 (8)	O(1)-C(5)-O(2)	124.8 (5)
C(3)–C(4)	1.520 (9)	N(1)-C(6)-C(11)	110.8 (3)
C(6)–C(7)	1.523 (6)	N(1)-C(6)-C(10)	108.4 (4)
C(6)–C(10)	1.553 (7)	N(1)-C(6)-C(7)	111.7 (3)
C(6)–C(11)	1.545 (9)	C(10)-C(6)-C(11)	109.3 (3)
C(7)–C(8)	1.525 (9)	C(7)-C(6)-C(11)	113.0 (4)
C(8)–C(9)	1.473 (9)	C(7)-C(6)-C(10)	103.2 (4)
C(9)–C(10)	1.522 (9)	C(6)-C(7)-C(8)	104.6 (5)
C(12)–C(13)	1.558 (6)	C(7)-C(8)-C(9)	106.8 (5)
C(12)–C(16)	1.543 (7)	C(8)-C(9)-C(10)	108.0 (5)
C(12)–C(17)	1.542 (7)	C(6)-C(10)-C(9)	105.8 (4)
C(13)–C(14)	1.510 (9)	N(2)-C(11)-C(6)	116.1 (5)
C(14)–C(15)	1.549 (9)	O(3)-C(11)-C(6)	120.6 (5)
C(14)–C(150)	1.523 (9)	O(3)-C(11)-N(2)	123.2 (5)
C(15)–C(16)	1.546 (9)	N(2)-C(12)-C(17)	110.8 (4)
C(150)–C(16)	1.522 (9)	N(2)-C(12)-C(16)	111.9 (4)
		N(2)-C(12)-C(13)	109.2 (5)
		C(16)-C(12)-C(17)	112.2 (5)
		C(13)-C(12)-C(17)	108.9 (4)
		C(13)-C(12)-C(16)	103.5 (4)
		C(12)-C(13)-C(14)	106.6 (4)
		C(13)-C(14)-C(15)	108.6 (6)
		C(13)-C(14)-C(150)	103.2 (9)
		C(14)-C(15)-C(16)	101.9 (6)
		C(14)-C(150)-C(16)	104.3 (9)
		C(12)-C(16)-C(15)	105.0 (6)
		C(12)-C(16)-C(150)	107.8 (8)
		N(3)-C(17)-C(12)	116.7 (5)
		O(4)-C(17)-C(12)	119.8 (5)
		O(4)-C(17)-N(3)	123.1 (6)

[a] ESD's *are* given in parentheses.

Backbone Conformation

The peptide adopts a β-turn conformation stabilized by a 4 → 1 intramolecular hydrogen bond between the methylamide NH and Boc CO groups (Fig. 3). The N(3)–O(2) distance of 2.918 (8) Å agrees well with the average value determined from a large number of peptide structures.[22] The ϕ, ψ values for

TABLE III
Conformational Angles (°) in 1

Peptide Backbone		
ω_1	O(1)-C(5)-N(1)-C(6)	−172.2 (4)
ϕ_2	C(5)-N(1)-C(6)-C(11)	−57.6 (6)
ψ_2	N(1)-C(6)-C(11)-N(2)	−38.1 (6)
ω_2	C(6)-C(11)-N(2)-C(12)	−177.5 (4)
ϕ_3	C(11)-N(2)-C(12)-C(17)	−60.2 (6)
ψ_3	N(2)-C(12)-C(17)-N(3)	−31.1 (7)
ω_3	C(12)-C(17)-N(3)-C(18)	−173.6 (5)
Cyclopentane Ring		
Ring I		
	C(6)-C(7)-C(8)-C(9)	30.3 (6)
	C(7)-C(8)-C(9)-C(10)	−13.8 (7)
	C(8)-C(9)-C(10)-C(6)	−7.6 (7)
	C(9)-C(10)-C(6)-C(7)	25.8 (5)
	C(10)-C(6)-C(7)-C(8)	−34.0 (5)
Ring II		
	C(12)-C(13)-C(14)-C(15)	3.2 (7)
	C(13)-C(14)-C(15)-C(16)	−24.7 (8)
	C(14)-C(15)-C(16)-C(12)	37.0 (7)
	C(15)-C(16)-C(12)-C(13)	−35.6 (7)
	C(16)-C(12)-C(13)-C(14)	19.8 (6)
	C(12)-C(13)-C(14)-C(150)	−35.8 (9)
	C(13)-C(14)-C(150)-C(16)	37.7 (12)
	C(14)-C(150)-C(16)-C(12)	−25.8 (12)
	C(150)-C(16)-C(12)-C(13)	3.9 (9)

TABLE IV
Geometry of the Hydrogen Bonds in the Crystal of Boc-Acc^5-Acc^5-NHMe

Donor	Acceptor	Symmetry equivalence of	Distances (Å)		Angle(°)
D-H	A	A	D–A	H–A	D-H–A
N(3)-H	O(2)	x, y, z	2.918 (8)	2.05 (6)	154 (4)
N(2)-H	O(4)	$x + 1/2, -y + 1/2, z$	3.102 (5)	2.30 (4)	159 (4)
N(1)-H	O(3)	$x + 1/2, -y + 1/2, z$	2.925 (6)	2.04 (5)	167 (4)

the two Acc^5 residues [$\phi_2 = -57.6$ (6)°, $\psi_2 = -38.1$ (6)"; $\phi_3 = -60.2$ (6)°, $\psi_3 = -31.1$ (7)°] are in good agreement with the values expected for a type III β-turn conformation.[23] The achiral peptide crystallizes in a centrosymmetric space group and the enantiomeric type III and III' conformations are present in the crystal.

Crystal Packing

The crystal packing, as viewed down the c axis in Fig. 2, is characterized by a network of N—H ... O hydrogen bonds involving symmetry-related (x, y, z and $x + 1/2, 1/2 - y, z$) molecules. The N ... O distances of 2.925 (6) and

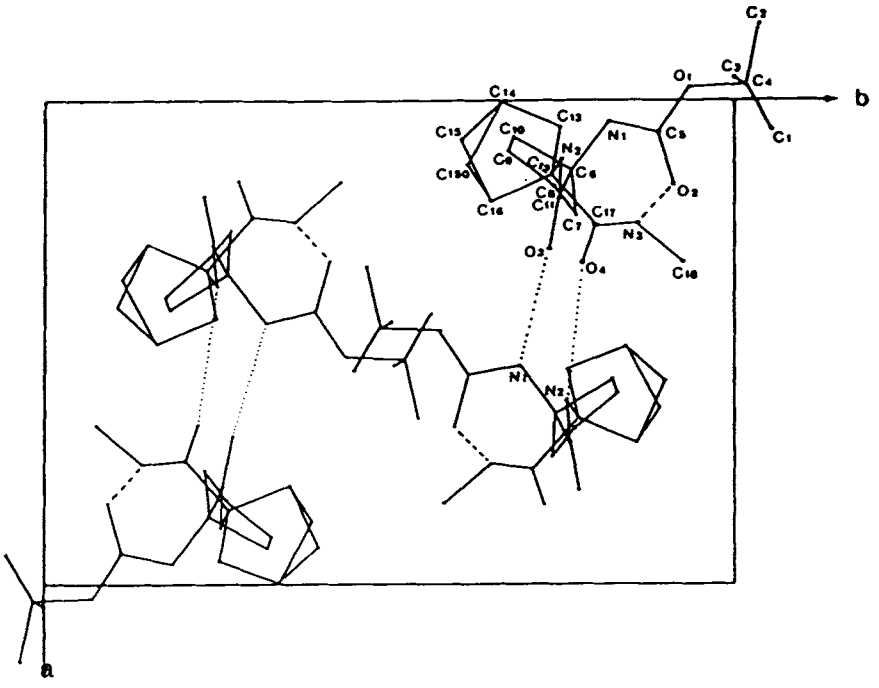

Fig. 4. Molecular packing in the crystal structure of Boc-Acc^5-Acc^5-NHMe viewed down the c axis.

3.102 (5) Å are close to the average range observed for a large number of intermolecular hydrogen bonds in peptide structures (between 2.9 and **3.0** Å).[22] The details of the hydrogen-bond parameters are summarized in Table IV.

CONCLUSIONS

The results of the present study suggest that Acc^5 residues favor conformations in the helical/type III β-turn region of the conformational map. The ability of Acc^5 residues to impart stereochemical rigidity to peptide backbones is indicated by the similarity between the solution and solid-state conformations **of** Boc-Acc^5-Acc^5-NHMe. It may be expected that the introduction of Acc^5 residues into biologically active peptides can result in the stabilization of β-turn conformations. Indeed, evidence in support of this expectation has been obtained in recent spectroscopic studies on analogs of enkephalins[24] and chemotactic peptides.[25,26]

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