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# A combined extended and helical backbone for Boc-(Ala-Leu-Ac<sub>7</sub>c-)<sub>2</sub>-OMe<sup>\*</sup>

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**Key words:** crystal structure analysis; helix with extended backbone; lack of hydrogen-bond formation; stacking of cycloheptyl rings

**Abstract:** The structure of the peptide Boc-Ala-Leu-Ac<sub>7</sub>c-Ala-Leu-Ac<sub>7</sub>c-OMe (Ac<sub>7</sub>c, 1-aminocycloheptane-1-carboxylic acid) is described in crystals. The presence of two Ac<sub>7</sub>c residues was expected to stabilize a 3<sub>10</sub>-helical fold. Contrary to expectation the structural analysis revealed an unfolded amino terminus, with Ala(1) adopting an extended  $\beta$ -conformation ( $\phi = -93^\circ, \psi = 112^\circ$ ). Residues 2–5 form a 3<sub>10</sub>-helix, stabilized by three successive intramolecular hydrogen bonds. Notably, two NH groups Ala(1) and Ac<sub>7</sub>c(3) do not form any hydrogen bonds in the crystal. Peptide assembly appears to be dominated by packing of the cycloheptane rings that stack against one another within the molecule and also throughout the crystal in columns.

**Abbreviations:** Aib,  $\alpha$ -amino isobutyric acid, dimethylglycine; Ac<sub>7</sub>c, 1-amino-1-cycloheptane-1-carboxylic acid.

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<sup>\*</sup>Dedicated to the memory of a thoroughly nice person and a scholar with great imagination.

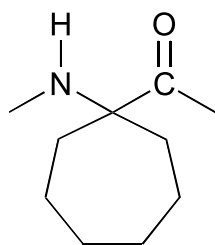
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Numerous crystal structure analyses of peptides containing  $\alpha$ ,  $\alpha$ -dialkyl amino acids, of which  $\alpha$ -aminoisobutyric acid (dimethylglycine, Aib) is the prototype, have demonstrated the strong helix promoting tendencies of these residues (1–5). The crystallinity of hydrophobic helical peptides provides an opportunity to examine interactions in crystals involving a variety of side chains (6). 1-aminocycloalkane-1-carboxylic acids (Ac<sub>*n*</sub>c, where *n* represents the number of carbon atoms in the cycloalkane ring) have also been shown to be effective promoters of helix formation (7,8). Recent structure determinations of Ac<sub>8</sub>c peptides have provided a view of helical scaffolds with pendant cyclo-octane rings

(9). Relatively few studies have been reported on peptides containing the cycloheptane analogue Ac<sub>7</sub>c.



1-aminocycloheptane-1-carboxylic acid residue (Ac<sub>7</sub>c)

Previously reported structures of short peptides containing Ac<sub>*n*</sub>c residues have revealed β-turn and <sub>3<sub>10</sub></sub>-helix formation analogous to that obtained for Aib residues (10–12). This report describes the structure of the hexapeptide Boc-Ala-Leu-Ac<sub>7</sub>c-Ala-Leu-Ac<sub>7</sub>c-OMe **1** which reveals some unanticipated conformational features. Notably, the amino-terminus is unfolded with Ala(1) adopting φ,ψ values, which lie close to the extended strand region of the Ramachandran map. Interestingly, two NH groups do not appear to participate in any significant hydrogen bonding interaction. The cycloheptane side chains stack over each other both in the individual molecule and also throughout the crystal.

## Experimental Procedures

### Crystal structure analysis of **1**

Crystals of **1**, grown by slow evaporation of a MeOH solution, were in the form of fine needles (0.58 × 0.10 × 0.09 mm) with good optical extinction along the long direction, but weak X-ray scattering. At –50 °C and

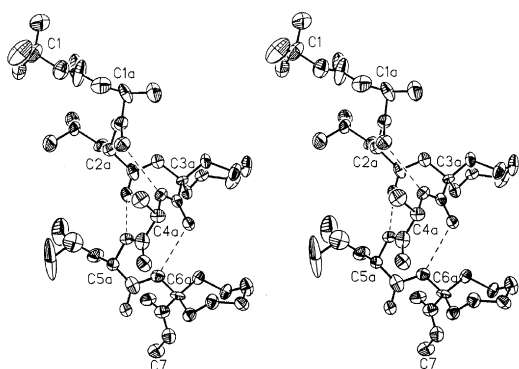


Figure 1. Stereodiagram of Boc-(Ala-Leu-Ac<sub>7</sub>c)<sub>2</sub>OMe. The C<sub>1</sub> and C<sub>7</sub> atoms at the two termini and the C<sup>α</sup> atoms are labelled. The three intrahelical <sub>3<sub>10</sub></sub>-hydrogen bonds, N<sub>4</sub>···O<sub>1</sub>, N<sub>5</sub>···O<sub>2</sub> and N<sub>6</sub>···O<sub>3</sub>, are indicated by dashed lines.

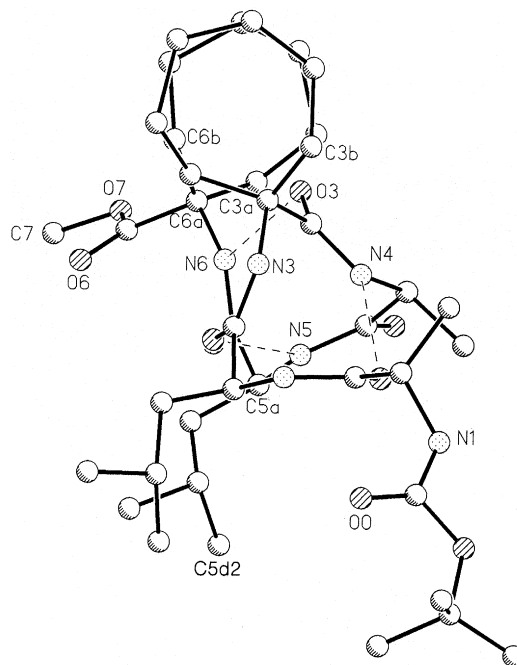


Figure 2. The molecule Boc-(Ala-Leu-Ac<sub>7</sub>c)<sub>2</sub>-OMe viewed down the *a* direction. The backbone forms 1–2/3 turns of a <sub>3<sub>10</sub></sub>-helix extending from C<sub>2a</sub> to C<sub>6a</sub>. The backbone at the N-terminus (C<sub>1</sub>–C<sub>1a</sub>) is rotated away from the helix, as is the backbone at the C-terminus (C<sub>6a</sub>–C<sub>7</sub>). Note the surroundings of atoms O<sub>o</sub>, N<sub>1</sub>, N<sub>3</sub> and O<sub>4</sub> that do not participate in any hydrogen bonding.

copper radiation, the mean I/σ value of the X-ray data dropped below 2 at a resolution of 1.2 Å, a resolution considered not amenable to direct phase determination for a structure composed of only C, H, N and O atoms. However, a partial structure of 35 atoms in two separate fragments was eventually found in a run of 20 000 trials of the TREF programme (13). Partial structure expansion with the tangent formula led to the complete molecule in several cycles (14). Hydrogen atoms were added in idealized positions. Anisotropic refinement on F<sup>2</sup> values resulted in R<sub>1</sub> = 15.1% for 497 parameters and 1477 independent data with |F<sub>o</sub>| > 2σ (nearly two quadrants of data related by symmetry were measured). Although the ratio of observed data to number of parameters is only 2.97 : 1, and the R<sub>1</sub> factor is relatively high because of the large number of weak data, the values of the bond distances and bond angles<sup>1</sup> are entirely acceptable and the thermal parameters are reasonable, as shown in the stereodiagram in Fig. 1.

The crystal data for C<sub>40</sub>H<sub>70</sub>N<sub>6</sub>O<sub>9</sub> are: space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, a = 11.363(10) Å, b = 19.035(13) Å, c = 20.64(2) Å, Z = 4, V = 4464(6) Å<sup>3</sup>, d<sub>X-ray</sub> = 1.159 g/cm<sup>3</sup>.

<sup>1</sup>Coordinates, bond lengths, bond angles, anisotropic thermal parameters and idealized hydrogen atom coordinates have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, Ref. no. 218494.

**Table 1. Hydrogen bonds<sup>a</sup>**

Type	Donor	Acceptor	D···A (Å)	H···A (Å)	Angle (°) D···O = C
Head-to-tail	N1	None <sup>b</sup>			
	N2	O5 <sup>c</sup>	2.797	1.91	135
	N3	None <sup>d</sup>			
4 → 1	N4	O1	3.059	2.18	131
4 → 1	N5	O2	3.040	2.07	127
4 → 1	N6	O3	3.091	2.26	123

a. Hydrogen atoms were placed in idealized positions with N-H = 0.90 Å.

b. Nearest approach to a possible acceptor is N1···O4<sup>c</sup> = 3.83 Å.

c. At symmetry equivalent 1 + x, y, z.

d. Nearest approach to a possible acceptor is N3···O7<sup>c</sup> = 4.03 Å.

## Results and Discussion

The folding of the backbone in **1** is shown in the stereodiagram in Fig. 1 and in a view down the helix axis (parallel to the *a* direction in the crystal) in Fig. 2. Three well-formed consecutive  $3_{10}$ -type hydrogen bonds (Table 1) are formed in the central part of the molecule where the backbone assumes a  $3_{10}$ -helix containing 1–2/3 turns from C2a to C6a.

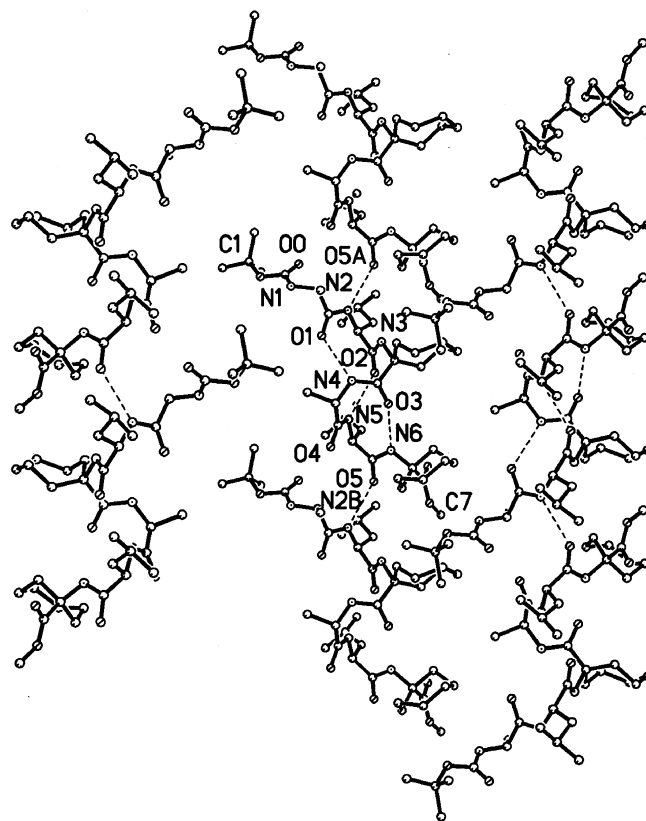
**Table 2. Torsional angles**

Residue	Angle	Value (°)	$\chi_{n1}$	$\chi_{n2}$	$\chi_{n3}$	$\chi_{n4}$	$\chi_{n5}$	$\chi_{n6}$	$\chi_{n7}$
Boc 0	$\psi_0$	174							
	$\omega_0$	179							
Ala 1	$\phi_1$	-93							
	$\psi_1$	112							
	$\omega_1$	-173							
Leu 2	$\phi_2$	-66	-64	-74					
	$\psi_2$	-24		164					
	$\omega_2$	175							
Ac <sub>7</sub> C3	$\phi_3$	-51	-52	+90	-67	+53	-76	+85	-27
	$\psi_3$	-30							
	$\omega_3$	-175							
Ala 4	$\phi_4$	-67							
	$\psi_4$	-18							
	$\omega_4$	176							
Leu 5	$\phi_5$	-67	-74	-68					
	$\psi_5$	-25		172					
	$\omega_5$	-178							
Ac <sub>7</sub> C 6 <sup>a</sup>	$\phi_6$	+50	+40	-92	+75	-54	+69	-85	+39
	$\psi_6$	-141							
	$\omega_6$	-177							

a. Terminal torsional angles  $\psi_6 \equiv \text{N6 C6A C6'O7}$  and  $\omega_6 \equiv \text{C6A C6'O7 C7}$ .

The N-terminus of the backbone extends away from the helix thereby precluding a N3···Oo hydrogen bond that would ordinarily be expected in a  $3_{10}$ -helix with a Boc or Z end group (11). The C-terminus also turns away from the helix, thus preventing a head-to-tail N1···O6 hydrogen bond when the helices are stacked. In both locations, the extended backbone is reflected by the positive sign of a torsional angle, i.e.  $\psi_1 = +112$  and  $\phi_6 = +50$  (Table 2).

In the crystal, the helical portions of the molecules are stacked and connected by one head-to-tail hydrogen bond, N2···O5a, so that the helices appear as continuous, parallel to the *a* cell direction, Fig. 3. The only four hydrogen bonds in the crystal are listed in Table 1. The extended backbone at the Boc end (C1) interdigitates with a symmetry equivalent to form a herring bone pattern. The N1H and carbonyl Oo moieties are completely surrounded by hydrocarbon groups and do not participate in hydrogen bonding. Figure 4 (a detail from Fig. 3) shows that the nearest intermolecular approaches to atom Oo are hydrogen atoms from C<sub>5</sub>AA (Oo···H = 2.56 Å) and C<sub>5</sub>DB



**Figure 3.** View perpendicular to the helical columns of Boc-(Ala-Leu-Ac<sub>7</sub>c)<sub>2</sub>-OMe. The columns are formed by head-to-tail hydrogen bonds N2b···O5, N2···O5a, etc. The pair of adjacent helices on the left are related by a vertical twofold screw axis. The pair of adjacent helices on the right are related by a horizontal twofold screw axis. A single molecule extends from C1 to C7 (centre of diagram).

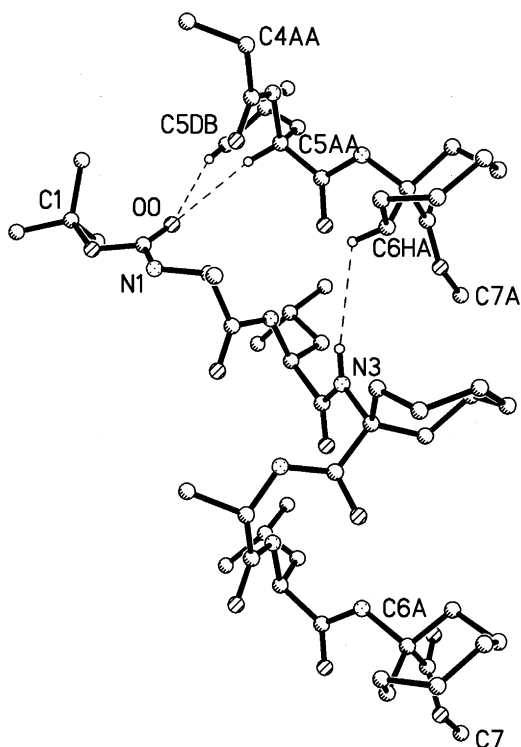


Figure 4. A detail from Figure 3 showing Oo and N<sub>3</sub>H, potential hydrogen bond acceptor and donor, respectively, surrounded by CH moieties, but not close enough for any consideration of CH···O hydrogen bond formation.

(Oo···H = 2.69 Å) where both C atoms are part of a leucyl residue. The nearest approach to N<sub>1</sub>H is the H on C<sub>6</sub>E (heptyl ring) at 2.55 Å (not shown). Similarly, the nearest approach to N<sub>3</sub>H is the H on C<sub>6</sub>HA at 2.69 Å.

Peptide molecules tend to assume conformations so that all possible NH groups are satisfied with hydrogen bond formation with carbonyl O's or with solvent molecules where each NH···O bond contributes 4–5 Kcal/mol to the total energy (15,16). As two of the six NH groups do not contribute to the total energy, it may be assumed that these energetic penalties may be offset by other structural features. An examination of the environment of the 'free' C=O moieties shows that only Oo could possibly participate in CH···O hydrogen bonds, that could contribute 1–2 Kcal/mol. However, the H···O distances of 2.56 Å and 2.69 Å between C<sub>5</sub>AAH···Oo and C<sub>5</sub>DBH···Oo, respectively, in adjacent molecules, appear to be too large to provide significant stabilization since observed CH···O bonds occur at *c.* 2.1–2.4 Å (17).

Another possible source for stabilizing the conformation may be the stacking of the cycloheptyl rings throughout the crystal, Figs 2 and 5. The positioning of the ring that contains the C<sub>6</sub>a atom under the ring that contains the C<sub>3</sub>a atom requires a positive value of +50° for the φ<sub>6</sub> torsional angle about the N<sub>6</sub>-C<sub>6</sub>a bond. A positive φ-value for the last

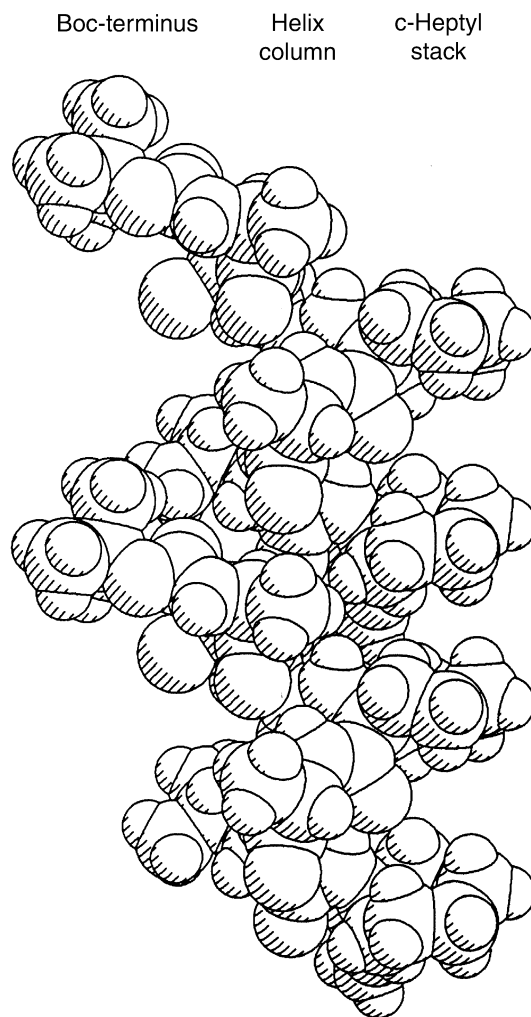


Figure 5. Space-filling drawing of two molecules of an infinite 3<sub>10</sub>-helix column formed by head-to-tail hydrogen bonding, see Figures 2 and 3. The Boc-termini are extended to the left, the c-heptyl groups containing C<sup>3</sup> and C<sup>6</sup> form an infinite stack on the right and the C-termini are extended behind the helix (not visible). The view is in the c-direction.

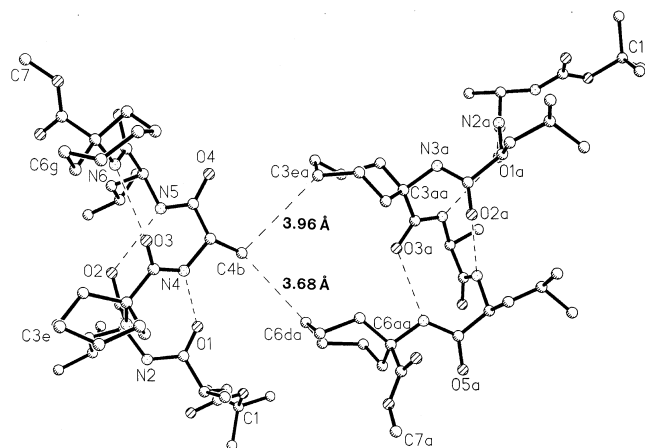


Figure 6. The closest C···C approach to the cyclic-heptyl side chains. The two molecules shown are related by a horizontal twofold screw.

residue in a helix has been observed often. Nevertheless, the distances between C<sub>3a</sub> and C<sub>6a</sub> (indicative of average distances between the rings) are 5.70 Å for the intramolecular separation and 5.96 Å for the intermolecular separation in the stack. There are very few close van der Waals' approaches between C···C atoms in adjacent rings. The closest approaches are C<sub>3b</sub>···C<sub>6h</sub> = 4.12 Å between molecules stacked over each other in a column and C<sub>3b</sub>···C<sub>6h</sub> = 4.32 Å within the same molecule. All other C···C approaches in the stacked rings are greater than 4.5 Å. Except for the two values of 4.1 and 4.3 Å, there does not seem to be the kind of attraction that has been found for saturated hydrocarbon chains in other crystals, such as the many contacts with values of c. 4.23 Å between parallel hydrocarbon chains in crystals of Boc-β-Ala-pentadecylamine (18) and C···C distances of 4.2–4.3 Å across the cavities of macrocycles related to cyclic Nylons (19).

The closest van der Waals' approaches to the cycloheptyl rings involve the C<sub>4b</sub> atom from a neighbouring column, Fig. 6. The C<sub>4b</sub> atom is inserted into the space between C<sub>3e</sub> and C<sub>6d</sub> with C<sub>4b</sub>···C<sub>3e</sub> = 3.96 Å and C<sub>4b</sub>···C<sub>6d</sub> = 3.68 Å.

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## Conclusions

The middle portion of the sequence Boc-(Ala-Leu-Ac<sub>7c</sub>-)<sub>2</sub>-OMe folds into a partial <sub>3<sub>10</sub></sub>-helix with the backbone at the two termini extended away from the helix. The helical portions, each containing three <sub>3<sub>10</sub></sub>-type hydrogen bonds, stack over each other to form a classical continuous column connected by one NH···O=C hydrogen bond at each juncture. The cycloheptyl rings are placed over each other in each molecule and also in the continuous columns of molecules. Two of the six NH moieties are devoid of any hydrogen bonds. There are no distinct reasons for the extension of the backbone and the absence of hydrogen bonds at N<sub>1</sub>H and N<sub>3</sub>H near the N-terminus.

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