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ACKNOWLEDGEMENTS. G.S. acknowledges CSIR, New Delhi for awarding a Senior Research Fellowship. This study was supported by the Department of Biotechnology, Government of India.

Received 20 November 2002; revised accepted 4 March 2003

Water-mediated supramolecular β -sheet from a short synthetic peptide containing non-coded amino acids

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The crystal structure of a synthetic, terminally blocked tetrapeptide *t*-Boc- β -Ala-L-Leu-Aib-L-Val-OMe reveals that the peptide adopts an overall extended backbone conformation. It self-assembles in the solid state to form an intermolecularly hydrogen-bonded β -sheet-like structure mediated by water molecules.

β -ALANINE (β -Ala) is the first member of ω -amino acids, which has been recently implicated in *de novo* peptide and protein design. The presence of additional polymethylene spacers in these amino acids, between N and C $^{\alpha}$ atoms, provides increased flexibility to the peptide backbone and allows us to design and characterize several diverse structures^{1–3}. Many crystal and solution structures of β -Ala and γ -aminobutyric acid (γ -Abu) in both linear and cyclic peptides, reveal a variety of supramolecular helices and β -sheet structures^{4,5}. The supramolecular architecture has numerous applications in material and biological sciences⁶, e.g. the design and synthesis of model peptides, which form supramolecular β -sheets in crystals and amyloid-like fibrils in the solid state, is one of the convenient approaches to elucidate and understand the fibrillogenesis process at the atomic

level. Previously, it has been demonstrated that synthetic peptides containing alkyl spacers (β -alanyl residues) form supramolecular β -sheets and they further self-assemble into amyloid-like fibrils^{4,5}. As a continuation of our research in designing and constructing a unique supramolecular peptide architecture, we report the investigations on conformational analysis of a tetrapeptide, *t*-Boc- β -Ala-L-Leu-Aib-L-Val-OMe (Figure 1).

The title peptide has been synthesized by conventional solution phase methodology⁷. Single crystals, obtained from methanol and water mixtures (in 1:1 ratio), were monoclinic. Intensity data were recorded with CuK $_{\alpha}$ radiation on an Enraf–Nonius CAD-4 diffractometer⁸ using variable scan speed ($\Delta\omega = 0.80^{\circ} + 0.14^{\circ} \tan\Theta$), with $\omega/2\Theta$ scan in bisecting geometry mode. Crystals were stable during data collection. Data intensity and background counts were taken in the ratio 2:1. A total of 3823 intensities were measured and corrected only for Lorentz and polarization effects. Reduced data had merged *R*-factors: $R_{\text{int}} = 0.021$ and $R_{\sigma} = 0.015$. Applying direct-phase determination technique in SHELX 97 (ref. 9) 80 phase-sets were generated from 352 largest *E*-values above 1.2. There were two solutions having the least value of combined figure-of-merit. *E*-maps were computed for both the sets, and the one which gave interpretable results had a molecular fragment containing 24 non-hydrogen atoms. Using this as a model, difference Fourier maps were generated by least-square refinement carried on F^2 . The remaining atoms, including one water molecule, were located from successive Fourier maps. Polar axis restraints in the space group C2 were applied using the method of Flack and Schwarzenbach¹⁰. Hydrogen atoms, attached to the backbone and side chain atoms, were geometrically idealized and were assigned isotropic displacement parameters, 20% more than the atoms to which they are bonded (25% in case of methyl groups). Hydrogen atom positions of water molecule were not

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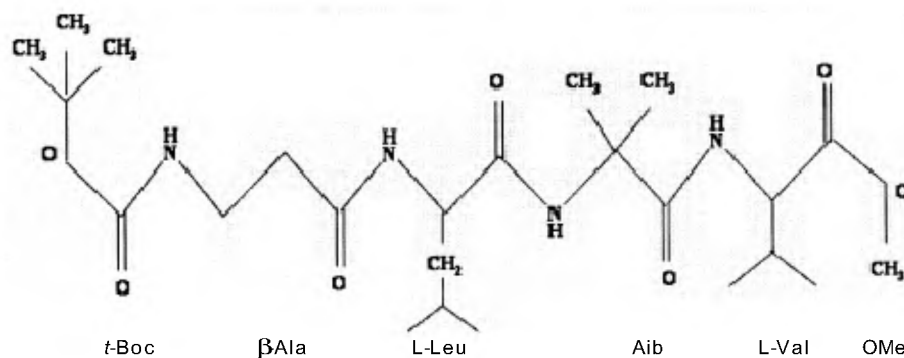


Figure 1. Chemical diagram.

Table 1. Crystal and diffraction parameters for peptide, *t*-Boc- β -Ala-L-Leu-Aib-L-Val-OMe

Empirical formula	C ₂₄ H ₄₄ N ₄ O ₇ .H ₂ O
Crystal habit	Plate, colourless
Crystal size (mm)	0.7 × 0.5 × 0.1
Crystallizing solvent	Methanol/water
Space group	C2
Cell parameters	
<i>a</i> (Å)	20.987 (1)
<i>b</i> (Å)	9.619 (2)
<i>c</i> (Å)	17.225 (2)
β (°)	113.20 (1)
Volume (Å ³)	3196.1 (8)
<i>Z</i>	4
Molecule/asymmetric unit	1
Cocrystallized solvent	One water molecule
Molecular weight	518.65
Density ((g/cm ³)(cal)	1.078
<i>F</i> (000)	1128
Radiation	CuK α (λ = 1.5418 Å)
Temperature (K)	293 (2)
Θ -range (°)	2.8–73.0
Absorption coefficient, μ (mm ⁻¹)	0.665
Independent reflections	3143
Observed reflections	2468
[<i>F</i>] > 4 σ (<i>F</i>)	
Index ranges	–22 ≤ <i>h</i> ≤ 25 –10 ≤ <i>k</i> ≤ 11 –21 ≤ <i>l</i> ≤ 19
Final <i>R</i> (%)	0.0745
Final <i>wR</i> ₂ (%)	0.1930
Goodness-of-fit (<i>S</i>)	1.056
(Δ/σ) _{max}	0.043
$\Delta\rho_{max}$ (eÅ ⁻³)	0.316
$\Delta\rho_{min}$ (eÅ ⁻³)	–0.288
Data/parameters ratio	3143/325
Absolute structure parameter	–0.7 (4)
Weight	$w = 1/[\sigma^2(F_o^2) + (0.1748P)^2 + 0.02P]$, where $P = (\max(F_o^2, 0) + 2F_c^2)/3$

determined. Crystal data are reported in Table 1. Crystallographic data have been deposited at the Cambridge Crystallographic Data Center (reference CCDC 209239).

The displacement ellipsoid diagram of the molecule is shown in Figure 2. Bond lengths and bond angles agree

well with the average values reported for peptides¹¹. The urethane moiety (atoms, C1B, N1, C05, O01 and O02) is essentially planar. The maximum deviation of the atoms defining the plane is –0.045(6) Å. Peptide and ester units are *trans* planar only with maximum deviation of 11° from the ideal *trans* value. Ester unit, as commonly found¹², is in plane with C4A atom of valine residue with maximum atomic deviation about the mean plane being 0.09(2) Å.

Conformational parameters of the peptide are given in Table 2. Boc-NH urethane group is in the most populous (*trans*, *trans*) conformation: $\omega_0 = 174.2(5)^\circ$, $\theta^1 = 179.2(5)^\circ$. The three methyl groups have the usual g^+ , t , g^- conformation (values are 60.2(9)°, –179.0(7)°, –61.3(8)°, respectively), i.e. they are staggered with respect to the O01–C05 bond¹³. Important conformational features of the molecule are: Aib residue is left-handed helical, while β -alanyl (ϕ , ψ alone) and leucyl residues have conformations in the β -sheet region. The (ϕ , ψ) values of β -Ala, in previously reported crystal structures of peptides containing this residue, have largely been observed in the β -region of the Ramachandran map. The C–C torsion angles along the polymethylene chains are defined¹ as θ_n , with numbering starting from the N-terminus. For β -Ala, this angle is referred as either θ or θ_1 . The torsion angle, θ (N–C1B–C1A–C), in the present peptide, is 172° and *trans* is the most favoured conformation in linear peptides¹. Valine adopts an extended conformation with the ester unit. Side-chain conformations of valine and leucine are (g^+g^-) and $g^-(g^-t)$, respectively, which according to the study on side-chain analysis¹⁴, are among the most favoured conformations. The Aib residue, in the typical helical conformation, produces a kink in the peptide backbone. However, (ϕ , ψ) values for other amino acids fall within the extended (β -sheet) region of the Ramachandran map, which leads to the formation of an overall extended backbone conformation of the molecule.

Packing diagram of the peptide molecule is shown in Figure 3. The peptides are interlinked by: (i) intermolecular hydrogen bonds and (ii) intermolecular bridges formed by water. These networks of hydrogen-bonds

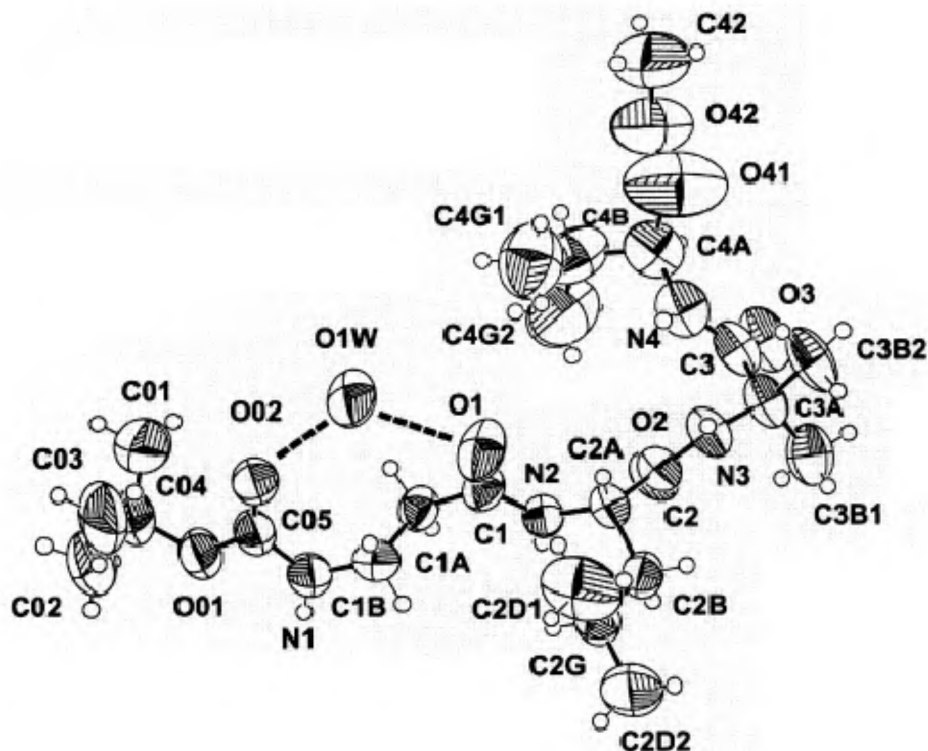


Figure 2. Displacement ellipsoid plot of the molecule, including water with IUPAC-IUB¹⁵ and α -amino acids¹ atom-labelling scheme drawn at 50% probability level. Hydrogen atoms are shown with small circles of arbitrary radii and hydrogen bonds with dotted lines.

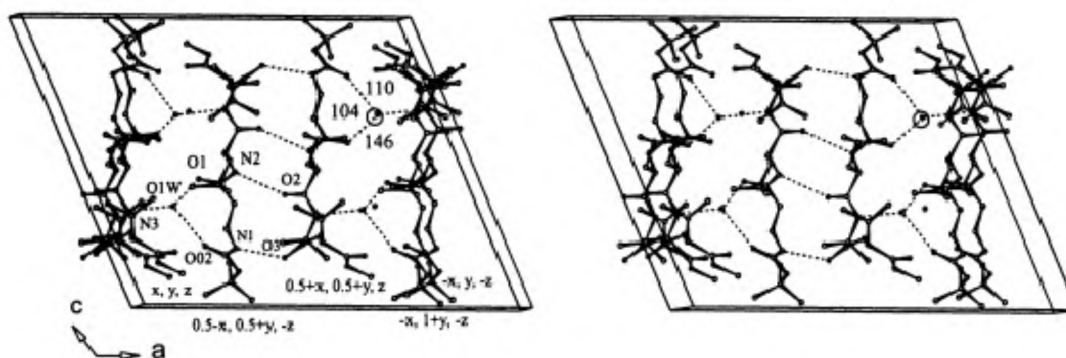


Figure 3. Stereo-view of crystal packing, showing intermolecular interactions. Values indicate angles subtended at the water molecule, describing planarity of configuration around water.

Table 2. Torsion angles for peptide *t*-Boc- β -Ala-L-Leu-Aib-L-Val-OMe

Residue	ϕ (deg)	θ (deg)	ψ (deg)	ω (deg)	χ^1 (deg)	χ^2 (deg)
β -Ala	-78.7 (6) ¹	172.1 (4)	103.3 (5)	-176.2 (4)		
Leu	-117.4 (4)		153.7 (4)	171.9 (4)	-57.1 (5)	-60.9 (9), 171.8 (7)
Aib	62.5 (6)		38.6 (5)	169.0 (6)		
Val	-115.2 (9)		168.2 (10) ²	171.6 (13) ³	62.9 (12), -63.2 (12)	

¹C05-N1-C1B-C1A; ²N4-C4A-C4-O42; ³C4A-C4-O42-C42.

Table 3. Hydrogen-bond parameters for peptide *t*-Boc- β -Ala-L-Leu-Aib-L-Val-OMe

Type	Donor	Acceptor	N...O (Å)	H...O (Å)	\angle O...HN (deg)
Water-peptide interactions	O1W	O02	2.811 (8)	–	–
	O1W	O1	2.774 (7)	–	–
	N3	O1W ¹	2.802 (6)	1.947	172.2(3)
Peptide-peptide interactions	N1	O3 ²	2.889 (7)	2.103	151.7(4)
	N2	O2 ²	2.969 (5)	2.132	164.4(2)

Symmetry related by: ¹1/2 - x, y - 1/2, 1 - z; ²1 - x, y, 1 - z.

form an unusual type of anti-parallel β -sheet structure. As shown in Figure 3, in the *ac*-plane the extended peptides are inter-connected by two distinct types of hydrogen-bond networks. On one side, each molecule is connected by lateral N-H...O type of standard hydrogen bonds observed in anti-parallel β -sheets. On the other side, it is joined with two molecules through water-mediated hydrogen bonds. Each water molecule makes three hydrogen bonds and forms a triangular intermolecular bridge between the two peptides. Water acts as donor in (Boc) C05 = O02...O1W and (β -Ala) C1 = O1...O1W bonds and as an acceptor in N3-H...O1W (Table 3). Further, indirect support of the presence of hydrogen-bond with water is provided by the observation that pertinent carbonyl groups, O02 and O1 are not involved in any other intermolecular hydrogen-bonding scheme. The sum of angles, \angle N3...O1W...O02, \angle N3...O1W...O1 and \angle O1...O1W...O02 around water is 359.8°, indicating that such configuration is of planar-type.

We have observed an uncommon β -sheet structure with distinct hydrogen-bonding patterns. Correlating our study on β -Ala containing peptide with previous work suggests a new type of β -sheet assemblage, which has implications in protein *de novo* design.

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ACKNOWLEDGEMENTS. We thank Prof. P. Balaram and Prof. N. Shamala for generously providing computational and experimental facilities. R.S.R. thanks to University Grants Commission, for fellowship.

Received 30 April 2003; revised accepted 30 July 2003