20. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
21. Hoffmann, P. G., Lego, M. C. and Galetto, W. G., *J. Agric. Food Chem.*, 1983, **31**, 1326–1330.
22. Iwai, K., Lee, K-R., Kobashi, M. and Suzuki, T., *Agric. Biol. Chem.*, 1977, **41**, 1873–1876.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265–275.
24. Flores, H. E. and Galston, A. W., *Plant Physiol.*, 1982, **69**, 701–706.
25. Redmond, J. W. and Tseng, A., *J. Chromatogr.*, 1979, **170**, 479–481.
26. Bais, H. P., Sudha, G. and Ravishankar, G. A., *J. Plant Growth Regul.*, 1999, **18**, 159–165.
27. Chi, G-L., Pus, E. G. and Goh, C-J., *Plant Physiol.*, 1991, **96**, 178–183.
28. Bais, H. P., Sudha, G. and Ravishankar, G. A., *Plant Cell Rep.*, 2001, **20**, 547–555.
29. Freund, J. E. and Perles, B. M., *Statistics. A First Course*, Prentice Hall, 1999, pp. 261–288.
30. Leslie, C. A. and Romani, R. J., *Plant Physiol.*, 1988, **88**, 833–837.
31. Saniewski, M. and Wegrzynowicz-Lesiak, E., *J. Fruit Ornam. Plant Res.*, 1994, **2**, 79–90.
32. Saniewski, M., Miszczak, A., Kawa-Miszczak, L., Wegrzynowicz-Lesiak, E., Miyamoto, K. and Ueda, J., *J. Plant Growth Regul.*, 1998, **17**, 33–37.
33. Vidal, S., Leon, I., Denecke, J. and Palva, E. T., *Plant J.*, 1997, **11**, 115–123.
34. Schweizer, P., Buchala, A., Silverman, P., Seskar, M., Raskin, I. and Metraux, J-P., *Plant Physiol.*, 1997, **114**, 79–88.
35. Kauss, H., *Biochem. Soc. Symp.*, 1994, **60**, 95–1 00.
36. Fang, Y., Smith, M. A. L. and Pepin, M-E., *In Vitro Cell Dev. Biol.-Plant*, 1999, **35**, 106–113.
37. Weiler, E. W., *Naturwissenchaften,* 1997, **84**, 340–349.

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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^{α} 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 sciences[6], 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 methodology[7]. Single crystals, obtained from methanol and water mixtures (in 1 : 1 ratio), were monoclinic. Intensity data were recorded with CuK_{α} radiation on an Enraf–Nonius CAD-4 diffractometer[8] using variable scan speed ($\Delta\omega = 0.80° + 0.14° \tan\theta$), 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: $R_{int} = 0.021$ 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 Schwarzenbach[10]. 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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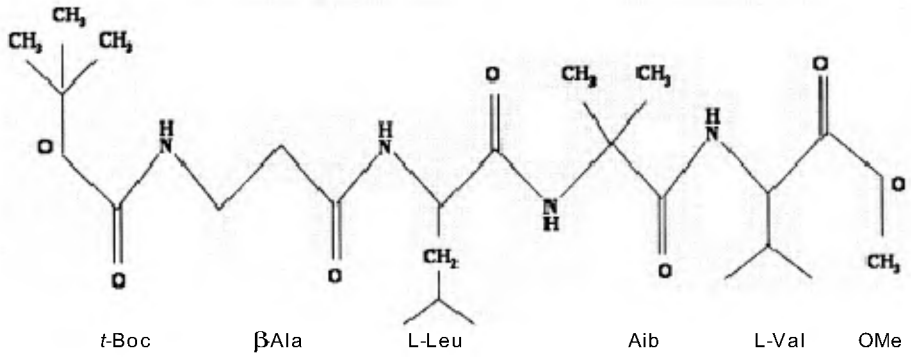

Figure 1. Chemical diagram.

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

Empirical formula	$C_{24}H_{44}N_4O_7.H_2O$
Crystal habit	Plate, colourless
Crystal size (mm)	0.7 × 0.5 × 0.1
Crystallizing solvent	Methanol/water
Space group	C2
Cell parameters	
a (Å)	20.987 (1)
b (Å)	9.619 (2)
c (Å)	17.225 (2)
β(°)	113.20 (1)
Volume (Å^3)	3196.1 (8)
Z	4
Molecule/asymmetric unit	1
Cocrystallized solvent	One water molecule
Molecular weight	518.65
Density ((g/cm^3)(cal)	1.078
F (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 [$\|F\| > 4\sigma(F)$]	2468
Index ranges	$-22 \le h \le 25$ $-10 \le k \le 11$ $-21 \le l \le 19$
Final *R* (%)	0.0745
Final wR_2 (%)	0.1930
Goodness-of-fit (*S*)	1.056
$(\Delta/\sigma)_{max}$	0.043
$\Delta\rho_{max}$ (eA^{-3})	0.316
$\Delta\rho_{min}$ (eA^{-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 peptides[11]. 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 found[12], 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: $\omega_0 = 174.2(5)°$, $\theta^1 = 179.2(5)°$. 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 bond[13]. 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 defined[1] 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 peptides[1]. 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 analysis[14], 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

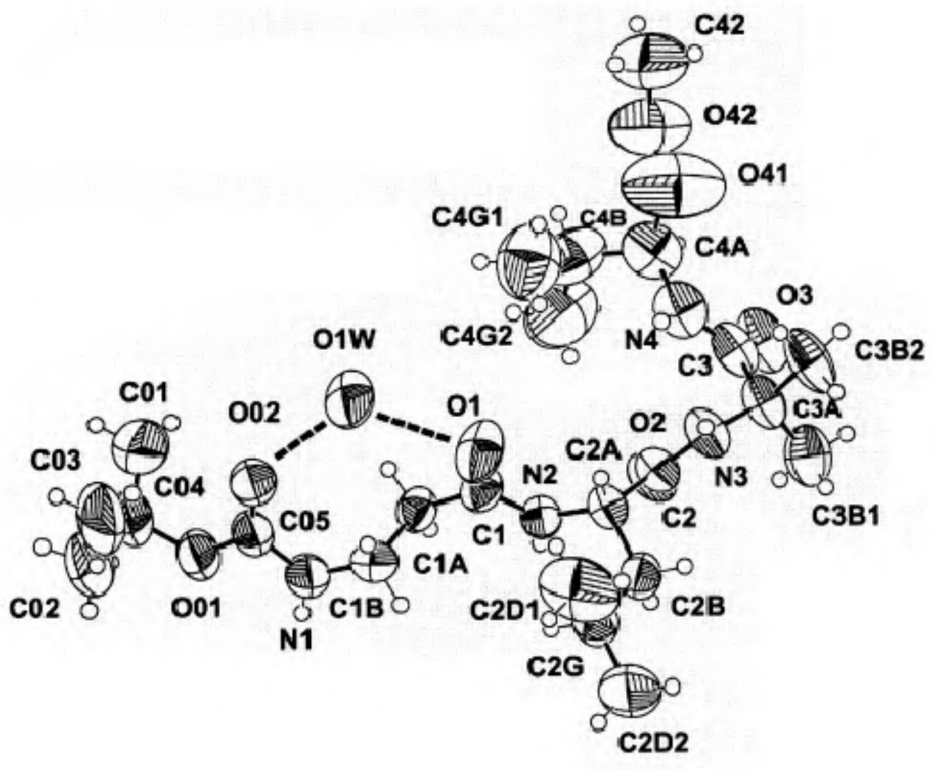

Figure 2. Displacement ellipsoid plot of the molecule, including water with IUPAC-IUB[15] and ω-amino acids[1] atom-labelling scheme drawn at 50% probability level. Hydrogen atoms are shown with small circles of arbitrary radii and hydrogen bonds with dotted lines.

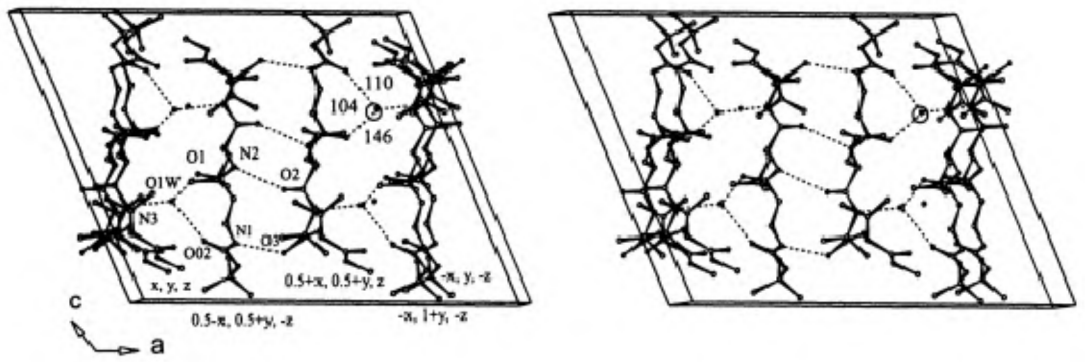

Figure 3. Stereo-view of crystal packing, showing intermolecular interactions. Values indicate angles subtended at the water molecule, describing planarity of configuration around water.

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)	

[1]C05-N1-C1B-C1A; [2]N4-C4A-C4-O42; [3]C4A-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)	∠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: [1] $1/2 - x, y - 1/2, 1 - z$; [2] $1 - x, y, 1 - z$.

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, ∠N3···O1W···O02, ∠N3···O1W···O1 and ∠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.

1. Banerjee, A. and Balaram, P., Stereochemistry of peptides and polypeptides containing omega amino acids. *Curr. Sci.*, 1997, **73**, 1067–1077.
2. Gellman, S. H., Foldamers: A manifesto. *Acc. Chem. Res.*, 1998, **31**, 173–180; Appella, D. H., Christianson, L. A., Klein, D. A., Powell, D. R., Huang, X., Barchi, J. J. and Gellman, S. H., Residue-based control of helix shape in beta-peptide oligomers. *Nature*, 1997, **387**, 381–384.
3. Pagani Zecchini, G., Morera E., Nalli, M., Paglialunga Paradisi, M., Lucente, G. and Spisani, S., Synthesis and activity on human neutrophil functions of fMLF-OMe analogs containing alkyl spacers at the central position. *Farmaco*, 2001, **56**, 851–858.
4. Banerjee, A., Maji, S. K., Drew, M. G. B., Haldar, D. and Banerjee, A., Amyloid-like fibril-forming supramolecular β-sheets from a β-turn forming tripeptide containing non-coded amino acids: the crystallographic signature. *Tetrahedron Lett.*, 2003, **44**, 335–339.
5. Maji, S. K., Drew, M. G. B. and Banerjee, A., First crystallographic signature of amyloid-like fibril forming beta-sheet assemblage from a tripeptide with non-coded amino acids. *Chem. Commun.*, 2001, **19**, 1946–1947.
6. Vauthey, S., Santoso, S., Gong, H., Watson, N. and Zhang, S., Molecular self-assembly of surfactant-like peptides to form nanotubes and nanovesicles. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 5355–5360; Wang, W. and Hecht, M. H., Rationally designed mutations convert *de novo* amyloid-like fibrils into monomeric beta-sheet proteins. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 2760–2765.
7. Bodanszky, M. and Bodanszky, A., in *The Practice of Peptide Synthesis*, Springer, New York, 1984, pp. 1–282.
8. Enraf–Nonius, CAD-4 PC Software, Enraf-Nonius, Delft, The Netherlands, 1995.
9. Sheldrick, G. M., SHELX 97: Program for the solution and refinement of crystal structures, University of Göttingen, Germany, 1997.
10. Flack, H. D. and Schwarzenbach, D., On the use of least-squares restraints for origin fixing in polar space groups. *Acta Crystallogr., Sect. A*, 1988, **44**, 499–506.
11. Engh, R. A. and Huber, R., Accurate bond and angle parameters for X-ray protein structure refinement. *Acta Crystallogr., Sect. A*, 1991, **47**, 392–400.
12. Schweizer, W. B. and Dunitz, J. D., Structural characteristics of the carboxylic ester group. *Helv. Chim. Acta*, 1982, **65**, 1547–1554.
13. Benedetti, E., Pedone, C., Toniolo, C., Némethy, G., Pottle, M. S., and Scheraga, H. A., Preferred conformation of the tert-butoxycarbonyl-amino group in peptides. *Int. J. Pept. Protein Res.*, 1980, **16**, 156–172.
14. Benedetti, E., Morelli, G., Némethy, G. and Scheraga, H. A., Statistical and energetic analysis of side-chain conformations in oligopeptides. *Int. J. Pept. Protein Res.*, 1983, **22**, 1–15; Lovell, S. C., Word, J. M., Richardson, J. S. and Richardson, D. C., The penultimate rotamer library. *Proteins*, 2000, **40**, 389–408.
15. IUPAC Commission on Biochemical Nomenclature, Abbreviations and symbols for the description of the conformation of polypeptide chains. *Biochem. J.*, 1971, **121**, 577–585.

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