Aggregation modes in sheets formed by protected β-amino acids and β-peptides

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Received 14th November 2005, Accepted 13th January 2006
First published as an Advance Article on the web 30th January 2006
DOI: 10.1039/b516088j

The crystal structures of four protected β-amino acid residues, Boc-(*S*)-β^3-HAla-NHMe (**1**); Boc-(*R*)-β^3-HVal-NHMe (**2**); Boc-(*S*)-β^3-HPhe-NHMe (**3**); Boc-(*S*)-β^3-HPro-OH (**6**) and two β-dipeptides, Boc-(*R*)-β^3-HVal-(*R*)-β^3-HVal-OMe (**4**); Boc-(*R*)-β^3-HVal-(*S*)-β^3-HVal-OMe (**5**) have been determined. *Gauche* conformations about the C$^\beta$–C$^\alpha$ bonds ($\theta \sim \pm 60°$) are observed for the β^3-HPhe residues in **3** and all four β^3-HVal residues in the dipeptides **4** and **5**. *Trans* conformations ($\theta \sim 180°$) are observed for β^3-HAla residues in both independent molecules in **1** and for the β^3-HVal and β^3-HPro residues in **2** and **6**, respectively. In the cases of compounds **1–5**, molecules associate in the crystals *via* intermolecular backbone hydrogen bonds leading to the formation of sheets. The polar strands formed by β^3-residues aggregate in both parallel (**1**, **3**, **5**) and antiparallel (**2**, **4**) fashion. Sheet formation accommodates both the *trans* and *gauche* conformations about the C$^\beta$–C$^\alpha$ bonds.

Introduction

Extended polypeptide chains can spontaneously self-assemble to form sheet like structures, held together by inter-chain hydrogen bonds between acceptor carbonyl and donor NH groups.[1] The β-sheet, first proposed by Pauling and Corey has been widely found in both globular and fibrous proteins.[2] In polypeptides generated from α-amino acids, both antiparallel and parallel β-sheet structures are frequently observed.[1] Sheet like structures have been suggested for insoluble polypeptide deposits, termed amyloids, formed in a variety of pathological conditions.[3] The ability of polypeptides formed from β-amino acids to adopt well defined structures has stimulated a considerable body of recent work on the conformation of β-polypeptides[4–8] and hybrid sequences containing α, β and higher ω-amino acids.[9–16] The homologation of the backbone in β-residues results in extended strands in which the NH groups of successive residues lie on one face, while successive CO groups lie on the other. Sheets formed by association of extended strands from β-peptides are therefore "polar", in contrast to the corresponding structures derived from α-amino acids, which have a regular alternation of orientation of amide groups. Fig. 1 illustrates possible modes of association of two proximal β-peptide strands. In β-residues, the additional degree of torsional freedom about the C$^\beta$–C$^\alpha$ bond (θ) results in a larger number of conformational possibilities as compared to peptides of α-residues. For fully extended strands, θ is approximately 180°. Both antiparallel and parallel sheets may be formed as illustrated in Fig. 1, which also provides examples of experimentally determined crystal structures.[6,9,17] The torsion angle θ can also adopt *gauche* conformations ($\approx \pm 60°$), which support the formation of folded helical structures.[5,18] A proposal by Gellman and coworkers[19] suggests that antiparallel sheet formation by β-polypeptide strands may also be possible for β-peptide backbones adopting *gauche* conformations (Fig. 1e). While no example of such a structure has been reported so far, the crystal structure of a hybrid β-hairpin peptide incorporating two facing β-residues (Fig. 1f) establishes the possibility of accommodating a *gauche* conformation within the framework of antiparallel orientation of two proximal strands.[9] Sheets formed by β-peptides have also been the subject of recent computational studies, which suggest a considerable degree of cooperativity in hydrogen bond formation, driven by electrostatic effects in a 'polar' strand arrangement.[20] In order to probe possible conformational variability in intermolecular sheet structures formed by β-amino acid residues (Fig. 3), we present the crystal structures of a series of protected β-amino acid derivatives and dipeptides. This report describes the crystal structure of six model compounds containing β-residues:

Boc-(*S*)-β^3-HAla-NHMe (**1**); Boc-(*R*)-β^3-HVal-NHMe (**2**); Boc-(*S*)-β^3-HPhe-NHMe (**3**); Boc-(*R*)-β^3-HVal-(*R*)-β^3-HVal-OMe (**4**); Boc-(*R*)-β^3-HVal-(*S*)-β^3-HVal-OMe (**5**); Boc-(*S*)-β^3-HPro-OH (**6**).

The observed aggregation patterns in crystals exemplify the modes of sheet formation involving β residues.

Results and discussion

Molecular conformation

The solid state conformations of compounds **1–6** are shown in Fig. 3. The relevant backbone and side chain torsion angles are given in Table 1. Both independent molecules of derivative **1** and **2** adopt *trans* conformations about the C$^\beta$–C$^\alpha$ bond ($\theta \approx 180°$). In

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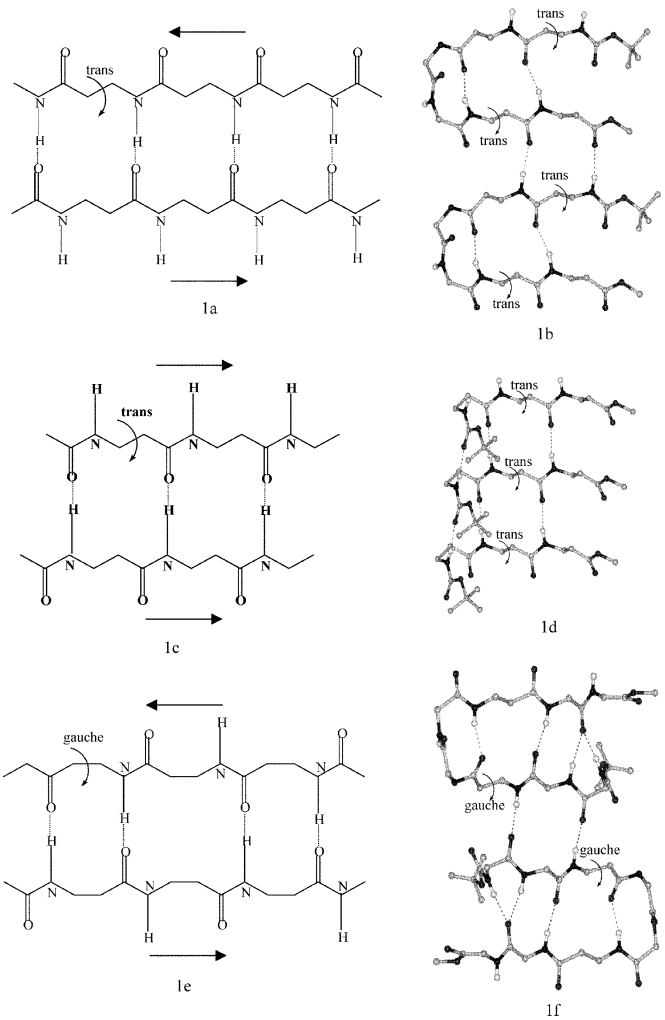

Fig. 1 Schematic representations of two proximal strands of β-peptides: 1a and 1b[17] represent the arrangements of antiparallel β-sheet when the β-residue adopts the *trans* conformation ($\theta \sim 180°$). 1c and 1d[6] show the parallel association of β-strands formed by β-residues with θ values ~180°. 1e and 1f[9] represent the antiparallel β-sheet, when θ values correspond to the *gauche* form. In structures 1b, 1d and 1f side chains have been removed for clarity.

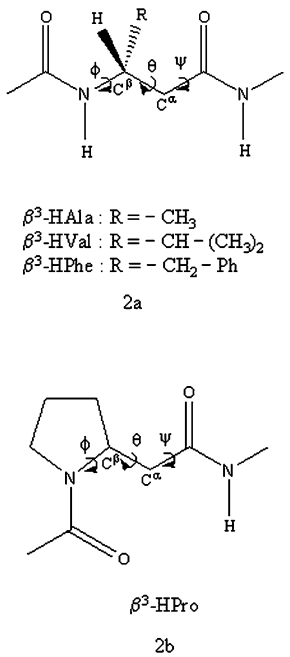

Fig. 2 Chemical structures of β-amino acid residues.

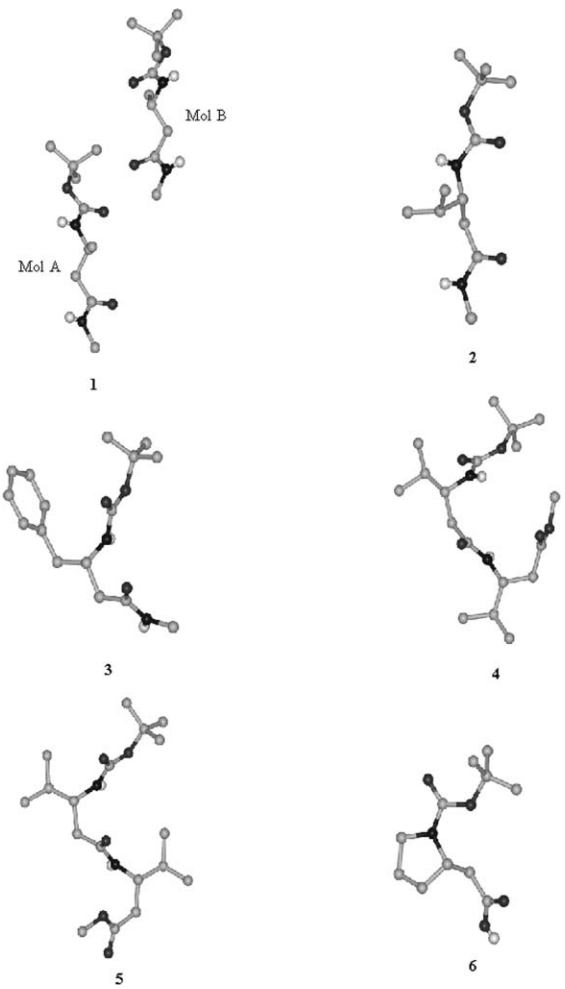

Fig. 3 Molecular conformations of compounds **1–6** in the solid state. In **1**, the two independent molecules are represented as A and B.

contrast, in Boc-(*S*)-β^3-HPhe-NHMe (**3**), the dipeptide Boc-(*R*)-β^3-HVal-(*R*)-β^3-HVal-OMe (**4**) and the diastereomeric dipeptide Boc-(*R*)-β^3-HVal-(*S*)-β^3-HVal-OMe (**5**), all observed θ values take *gauche* ($\theta \approx 60°$) conformations (for βVal the configuration derived after homologation is opposite to that of the precursor, Boc-(*S*)-Val). In peptide **5**, the alternating configurations of two residues results in g^+ and g^- conformations about the C^β–C^α bond. In derivative **6** (Boc-(*S*)-β^3-HPro-OH), the observed θ values correspond to a *trans* orientation about the C^β–C^α bond. Intramolecular hydrogen bonds are absent in all cases. Inspection of the molecular conformation in Fig. 3 reveals that the peptide backbone is folded in the case of Boc-(*R*)-β^3-HVal-(*R*)-β^3-HVal-OMe (**4**), in which successive *gauche* forms are observed. In the case of alternating configurations in peptide **5**, the backbone adopts an 'S' shaped structure. In the cases of **1** and **2**, extended conformations are observed, while in Boc-(*S*)-β^3-HPhe-NHMe (**3**) the backbone is bent. The local conformation of the (*S*)-β^3-HPhe

Table 1 Torsion angles (deg) for compounds **1–6**[a]

Compound	Residue	ϕ	θ	ψ	ω	χ^1	χ^2
1	β³-HAla (Mol A)	−110.6(4)	168.5(3)	122.4(3)	178.9(4)		
	β³-HAla (Mol B)	−110.3(4)	168.0(3)	123.6(3)	178.3(3)		
2	(*R*)-β³-HVal	−115.9(3)	167.3(2)	126.4(3)	−179.5(3)	61.9(4), −67.0(3)	
3	β³-HPhe	−135.1(6)	65.8(8)	−141.0(6)	−176.7(7)	−61.8(8)	−86.7(7), 90.8(9)
4	(*R*)-β³-HVal(1)	−130.9(3)	66.3(3)	−141.3(3)	−174.0(3)	−60.5(4), 176.5(3)	
	(*R*)-β³-HVal(2)	−130.5(3)	57.7(4)	−116.7(4)	173.7(4)	−53.2(4), −177.7(3)	
5	(*R*)-β³-HVal(1)	−132.5(6)	59.4(6)	−135.8(5)	−177.6(5)	−52.7(8), −174.2(7)	
	β³-HVal(2)	127.6(6)	−64.0(7)	−13.5(9)	179.9(6)	55.0(8), 176.8(8)	
6	β³-HPro[b]	−81.8(4)	173.0(4)	117.6(5)	—		

[a] Esd values in parentheses. See Fig. 2 for torsion angle definitions. The torsion angles for rotation about bonds of the amino acid side chains (χ^1 χ^2) as suggested by the IUPAC-IUB commission on Biochemical Nomenclature.[28] Where configurations are not indicated the residues are (*S*). [b] Ring geometry for β³-HPro is: χ^1(N–C$^\alpha$–C$^\beta$–C$^\gamma$) = 26.5(5); χ^2(C$^\alpha$–C$^\beta$–C$^\gamma$–C$^\delta$) = −35.6(6); χ^3(C$^\beta$–C$^\gamma$–C$^\delta$–N) = 29.7(5); χ^4(C$^\gamma$–C$^\delta$–N–C$^\alpha$) = −13.5(5) and θ(C$^\delta$–N–C$^\alpha$–C$^\beta$) = −7.7(4).

residue (ϕ = − 135.1°, ψ = − 141.0°) corresponds to the value observed in the C_{14}-helical structure of oligo β-peptides.[5]

Molecular association in crystals

Fig. 4 shows a view of the intermolecular hydrogen bonding patterns observed in crystals **1–5**. The intermolecular hydrogen bond parameters are summarized in Table 2. A notable feature of Fig. 4 is the fact that in all cases the observed hydrogen bonding pattern corresponds to the formation of β-peptide sheets, all of which are polar. In Boc-(*S*)-β³-HAla-NHMe (**1**), Boc-(*S*)-β³-HPhe-NHMe (**3**), Boc-(*R*)-β³-HVal-(*S*)-β³-HVal-OMe (**5**), the hydrogen bond arrangement corresponds to parallel sheets. In Boc-(*R*)-β³-HVal-NHMe (**2**) and Boc-(*R*)-β³-HVal-(*R*)-β³-HVal-OMe (**4**), the arrangement is antiparallel. Fig. 5 illustrates the packing observed in crystals of **1–6**. In the case of Boc-(*S*)-β³-HAla-NHMe (**1**), the two independent molecules in the asymmetric unit give rise to the formation of two separate layers of parallel sheets, which are oriented at an angle of ∼51°. In all cases of derivative and peptides **1–5**, the basic repeating unit possesses two intermolecular hydrogen bonds. Boc-(*S*)-β³-HPro-OH (**6**) lacks an NH group and the molecules in the crystal are held together by a single OH···O=C hydrogen bond involving the Boc CO group and OH group of the terminal carboxylic acid.

Conclusions

The structures of Boc-(*S*)-β³-HPhe-NHMe (**3**), Boc-(*R*)-β³-HVal-(*R*)-β³-HVal-OMe (**4**) and Boc-(*R*)-β³-HVal-(*S*)-β³-HVal-OMe (**5**) illustrate the fact that inter-strand hydrogen bond leading to sheet formations can result even when the backbone adopts a *gauche* conformation about the C$^\beta$–C$^\alpha$ bond of β-residues. For θ *trans*, ϕ and ψ values for the β-residues adopt almost precisely those found for α-residues in β-sheet conformations *i.e.* $\phi \approx -120°$, $\psi \approx 120°$. In these cases, insertion of an additional atom into the backbone merely extends the length of the residue and switches the orientation of the neighboring peptide bond as compared to the α-analogs. When θ is *gauche*, a significant readjustment of ψ is necessary ($\psi \approx -140°$) in order to facilitate intermolecular hydrogen bond to stabilize the sheet formation. The structures

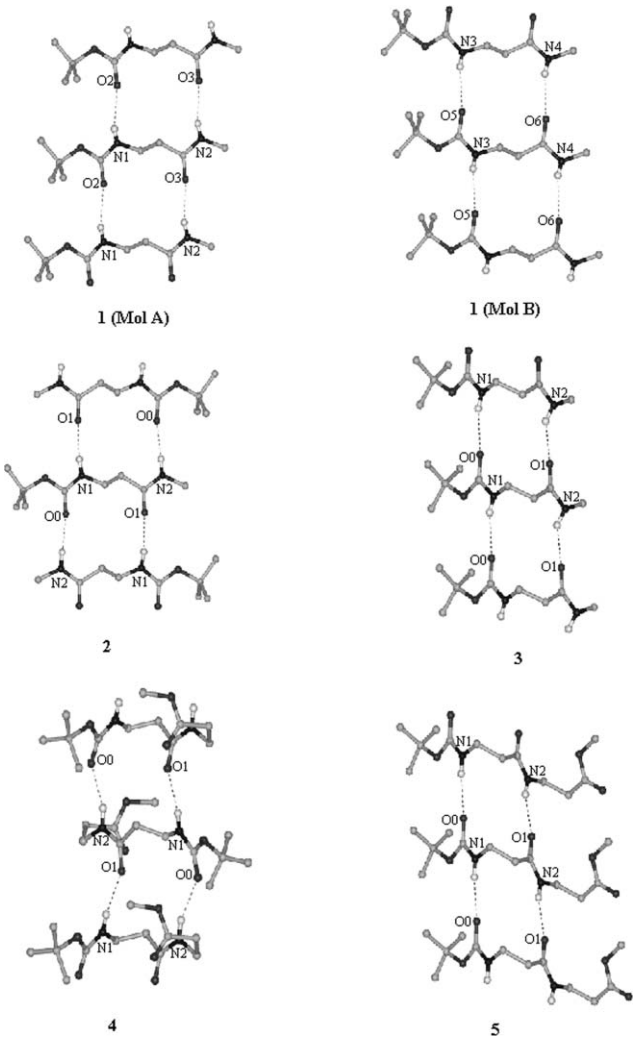

Fig. 4 Intermolecular hydrogen bonding patterns stabilizing sheets in crystals of **1–5**. For the geometrical details of hydrogen bond and symmetry relationship between molecules see Table 2.

of the model amino acid derivatives and peptides **1–5**, establish that sheet formation, both parallel and antiparallel in β peptides can accommodate the various rotameric states about the central

Table 2 Hydrogen bond parameters in compounds **1–6**[a]

Compound	Type	Donor (D)	Acceptor (A)	D···A (Å)	H···A (Å)	C=O···H (deg)	C=O···D (deg)	D–H···A (deg)
1	Intermolecular	N(1)	O(2)[b]	2.947(3)	2.12	166.7	171.5(2)	162.0
		N(2)	O(3)[b]	2.977 (4)	2.14	167.3	171.4(2)	165.2
		N(3)	O(5)[c]	2.960 (3)	2.14	165.3	170.9(2)	160.0
		N(4)	O(6)[c]	2.981 (4)	2.14	168.8	172.6(2)	166.1
2	Intermolecular	N(1)	O(1)[d]	2.921 (2)	2.08	169.6	171.2(1)	167.3
		N(2)	O(0)[d]	2.886 (3)	2.03	169.7	170.1(1)	177.8
3	Intermolecular	N(1)	O(0)[e]	2.995 (6)	2.25	160.5	169.9(3)	145.2
		N(2)	O(1)[e]	3.173 (15)	2.32	167.0	168.6(8)	172.8
4	Intermolecular	N(1)	O(1)[f]	2.963 (3)	2.12	152.3	153.4(2)	166.6
		N(2)	O(0)[f]	2.893 (3)	2.06	142.6	147.7(2)	161.9
5	Intermolecular	N(1)	O(0)[g]	3.013 (6)	2.18	170.0	174.8(3)	162.8
		N(2)	O(1)[g]	2.993 (6)	2.18	163.5	169.8(3)	157.5
6	Intermolecular	O(2)	O(0)[g]	2.673(6)	1.86	170.1	169.0(3)	168.5

[a] Esd values in parentheses. [b] Symmetry related by ($x + 1$, y, z). [c] Symmetry related by ($x - 1$, y, z). [d] Symmetry related by ($-x$, $y - 1/2$, $-z + 1/2$. [e] Symmetry related by (x, $y - 1$, z). [f] Symmetry related by ($x - 1/2$, $-y + 1/2$, $-z$). [g] Symmetry related by ($x - 1$, y, z). [h] Symmetry related by ($x + 1$, $y + 1$, z).

C^{β}–C^{α} bond. While helices invariably require a *gauche* conformation about the C^{β}–C^{α} bond, both *trans* and *gauche* forms can be accommodated in extended sheets of β-residues.[5,18,21–25]

Experimental

Synthesis of homologated amino acid derivatives

The synthesis of the *N-tert*-butyloxycarbonyl (Boc) protected derivatives of β^3-amino acids were done by Arndt–Eistert homologation of the corresponding Boc-α-amino acids using published procedures.[26] Boc-(*S*)-β^3-HPhe, Boc-(*R*)-β^3-HVal, Boc-(*S*)-β^3-HAla and Boc-(*S*)-β^3-HPro were thus prepared.

N-tert-Butyloxycarbonyl-(*S*)-β^3-homophenylalanyl-*N*-methylamide (Boc-(*S*)-β^3-HPhe-NHMe (3))

Boc-(*S*)-β^3-HPhe-OH (~0.80 g, 2.8 mmol) was dissolved in dry tetrahydrofuran (THF) and stirred in an ice bath for about 20 minutes. Triethylamine (Et_3N) (0.3 ml, 1 equiv.), $ClCOOC_2H_5$ (0.3 ml, 1 equiv.) were added to this mixture and stirred for about 30 minutes. Dry THF saturated with methylamine gas was added and stirred for about 2–3 hours. THF was removed under reduced pressure and the resulting mixture was extracted with ethyl acetate and sodium carbonate (Na_2CO_3). The organic layer was washed with HCl (20 ml) to ensure removal of excess Et_3N. Ethyl acetate was removed under reduced pressure, yielding Boc-(*S*)-β^3-HPhe-NHMe (**3**) as a pale brown solid. Single crystals of **3** were obtained from an ethyl acetate–toluene mixture by slow evaporation. Mp 155–156 °C; δ_H (400 MHz, $CDCl_3$) 1.41 [9 H, s, -$C(CH_3)_3$], 2.27 (1H, dd, -$CH_2CONHCH_3$), 2.41 (1 H, dd, -$CH_2CONHCH_3$), 2.76 (3 H, d, -$NHCH_3$), 2.97 (2 H, dd, -CH_2Ph), 4.05 (1 H, m, -$CHCH_2CONHCH_3$), 5.41 [1 H, d, -NH-Boc], 5.81 (1 H, q, -$NHCH_3$), 7.17–7.32 (5 H, m, ArH); *m/z* (ESI) 293.2 ($[M + H]^+$), 607.3 ($[2M + Na]^+$).

N-tert-Butyloxycarbonyl-(*R*)-β^3-homovalyl-*N*-methylamide (Boc-(*R*)-β^3-HVal-NHMe (2))

Boc-(*R*)-β^3-HVal-OH (~0.60 g, 2.5 mmol) was converted to Boc-(*R*)-β^3-HVal-NHMe (**2**) following the above procedure. The product **2** was obtained as a white powder. Single crystals of **2** suitable for X-ray diffraction were obtained from methanol–water mixture by slow evaporation. Mp 149–150 °C; δ_H (400 MHz, $CDCl_3$) 0.85 [6 H, d, -$CH(CH_3)_2$], 1.35 [9 H, s, -$C(CH_3)_3$], 1.75 [1 H, m, -$CH(CH_3)_2$], 2.31 (1 H, dd, -$CH_2CONHCH_3$), 2.40 (1 H, dd, -$CH_2CONHCH_3$), 2.71 (3 H, d, -$NHCH_3$), 3.57 (1 H, m, -$CHCH_2CONHCH_3$), 4.92 (1 H, d, -NH-Boc), 6.08 (1 H, q, -$NHCH_3$); *m/z* (ESI) 245.2 ($[M + H]^+$), 267.3 ($[M + Na]^+$), 511.4 ($[2M + Na]^+$).

N-tert-Butyloxycarbonyl-(*S*)-β^3-homoalanyl-*N*-methylamide (Boc-(*S*)-β^3-HAla-NHMe (1))

Boc-(*S*)-β^3-HAla-OH (~0.53 g, 2.6 mmol) was converted to Boc-(*S*)-β^3-HAla-NHMe following the procedure described for **3**. The product was obtained as a greyish white powder and single crystals suitable for X-ray diffraction were obtained from a tetrahydrofuran–water mixture by slow evaporation. Mp 113–114 °C; δ_H (400 MHz, $CDCl_3$) 1.16 (3 H, d, -CH_3), 1.35 [9 H, s, -$C(CH_3)_3$], 2.32 (2 H, dd, -$CH_2CONHCH_3$), 2.72 (3 H, d, -$NHCH_3$), 3.85 (1 H, m, -$CHCH_2CONHCH_3$), 5.10 (1 H, d, -NH-Boc), 5.90 (1 H, q, -$NHCH_3$); *m/z* (ESI) 217.2 ($[M + H]^+$), 455.3 ($[2M + Na]^+$).

N-tert-Butyloxycarbonyl-(*S*)-β^3-homoprolyl-*N*-methylamide (Boc-(*S*)-β^3-HPro-NHMe)

Boc-(*S*)-β^3-HPro-OH (~0.56 g, 2.4 mmol) was converted to Boc-(*S*)-β^3-HPro-NHMe following the procedure employed for **3**. The product was obtained as a yellow, viscous substance. Several attempts to crystallize Boc-(*S*)-β^3-HPro-NHMe did not yield any crystals, always resulting in a gummy substance. δ_H (400 MHz, $CDCl_3$) 1.47 [9 H, s, -$C(CH_3)_3$], 1.79–1.98 [4 H, br., -CH_2-CH_2-(ring)], 2.62 (1 H, dd, -$CH_2CONHCH_3$), 2.66 (1 H, dd, -$CH_2CONHCH_3$), 2.77 (3 H, d, -$NHCH_3$), 3.32 [2 H, br., -CH_2N (ring)], 4.01 [1 H, br., -CHN (ring)], 6.40 (1 H, br., -$NHCH_3$); *m/z* (ESI) 243.1 ($[M + H]^+$), 265.1 ($[M + Na]^+$), 507.4 ($[2M + Na]^+$). Diffraction quality crystals were however obtained from the starting material

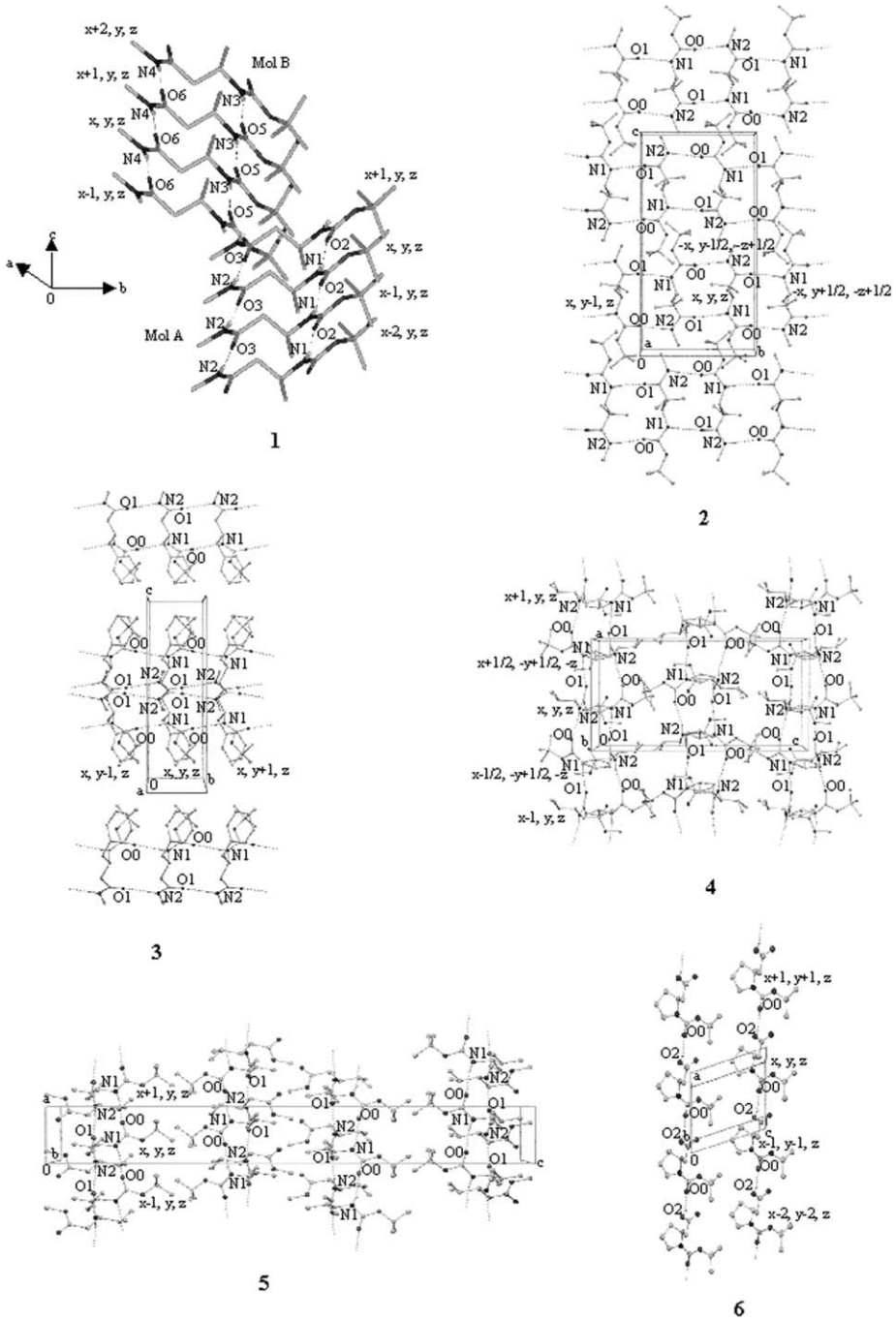

Fig. 5 Assembly of molecules **1–6** are stabilized by intermolecular hydrogen bonds in the crystal unit cell.

Table 3 Crystal and diffraction parameters for compounds **1–6**

	Compound **1**	Compound **2**	Compound **3**	Compound **4**	Compound **5**	Compound **6**
Empirical formula	$C_{10}H_{20}N_2O_3$	$C_{12}H_{24}N_2O_3$	$C_{16}H_{24}N_2O_3$	$C_{18}H_{34}N_2O_5$	$C_{18}H_{34}N_2O_5$	$C_{11}H_{19}N_1O_4$
Crystal habit	Clear plate	Clear rod	Clear plate	Clear rod	Clear plate	Clear rod
Crystal size (mm)	0.58 × 0.4 × 0.1	0.5 × 0.15 × 0.1	0.5 × 0.2 × 0.02	0.6 × 0.15 × 0.05	0.28 × 0.06 × 0.02	0.6 × 0.28 × 0.1
Crystallizing solvent	THF–water	methanol–water	ethyl acetate–toluene	methanol–water	DMF–CCl_4–methanol	methanol–water
Space group	$P1$	$P2_12_12_1$	$C2$	$P2_12_12_1$	$P2_12_12_1$	$P1$
Cell parameters						
a/Å	5.1040(15)	8.730(2)	20.536(12)	9.3852(17)	5.170(4)	5.989(2)
b/Å	9.469(3)	9.741(3)	5.165(3)	11.899(2)	10.860(8)	6.651(2)
c/Å	13.780(4)	17.509(5)	16.872(10)	19.199(4)	37.30(3)	8.661(3)
α/deg	80.141(5)	90	90	90	90	70.749(5)
β/deg	86.042(5)	90	109.824(9)	90	90	77.415(5)
γ/deg	89.932(5)	90	90	90	90	86.976(5)
Volume/Å^3	654.5(3)	1489.1(7)	1683.4(17)	2144.1(7)	2094(3)	317.78(19)
Z	2	4	4	4	4	1
Molecules/asym.unit	2	1	1	1	1	1
Cocrystallized solvent	None	None	None	None	None	None
Molecular weight	216.28	244.33	292.37	358.47	358.47	229.27
Density (g cm^{-3})	1.097	1.090	1.154	1.110	1.137	1.198
F(000)/Radiation	236/MoK$_\alpha$	536/MoK$_\alpha$	632/MoK$_\alpha$	784/MoK$_\alpha$	784/MoK$_\alpha$	124/MoK$_\alpha$
Temperature/°C	20	20	20	20	20	20
2θ max (°)/R_{int}	54.30/0.0334	50.02/0.0345	51.06/0.0335	53.48/0.0200	46.50/0.0752	54.46/0.0629
Measured reflections	7006	10641	3565	16116	13067	3471
Independent reflections	5070	2622	2547	3988	3012	2499
Unique reflections	2657	1529	1561	2362	1807	1312
Observed reflections [$\lvert F_o \rvert > 4\sigma(\lvert F_o \rvert)$]	2377	1433	1182	2271	1393	1221
Final R (%)/wR_2 (%)	4.94/13.59	4.83/13.26	9.09/19.12	5.85/15.59	7.87/15.88	5.66/16.41
Goodness-of-fit	1.098	1.086	1.184	1.307	1.273	1.122
$\Delta\rho_{max}$ (e Å^{-3})/$\Delta\rho_{min}$/e Å^{-3}	0.173/−0.138	0.176/−0.202	0.391/−0.171	0.263/−0.197	0.284/−0.261	0.369/0.309
Number of parameters	271	154	190	226	226	146
Data-to-parameter ratio	8.7 : 1	9.3 : 1	6.2 : 1	10.0 : 1	6.2 : 1	8.4 : 1

Boc-(*S*)-β³-HPro-OH (**6**) by slow evaporation from methanol–water mixtures. Mp 88 °C.

N-tert-Butyloxycarbonyl-(*R*)-β³-homovalyl-methyl ester (Boc-(*R*)-β³-HVal-OMe)

Boc-(*R*)-β³-HVal-OH (2.31 g, 10 mmol) was dissolved in methanol (5 ml) and diluted with diethyl ether (100 ml). Diazomethane gas was passed into the solution until it turned yellow. The ether was evaporated under reduced pressure yielding compound Boc-(*R*)-β³-HVal-OMe.

N-tert-Butyloxycarbonyl-(*R*)-β³-homovalyl-(*R*)-β³-homovalyl-methyl ester (Boc-(*R*)-β³-HVal-(*R*)-β³-HVal-OMe (4))

3.5 g (14.3 mmol) of Boc-(*R*)-β³-HVal-OMe was deprotected with 98% formic acid (75 ml) and the deprotection was monitored by TLC. After 2 hours, the formic acid was evaporated and the residue was taken in distilled water (15 ml). The pH of the aqueous layer was adjusted to ~8 by addition of Na_2CO_3 and extracted with ethyl acetate (3 × 30 ml). The combined organic layer was washed with brine solution (30 ml) and was concentrated *in vacuo* to ~6 ml. The resulting solution was then added to pre-cooled solution of the Boc-(*R*)-β³-HVal-OH (3.0 g, 13 mmol) in dry THF (10 ml), followed by addition of dicyclohexylcarbodiimide (DCC); 2.88 g, 14.0 mmol. The reaction mixture was allowed to attain room temperature and was stirred for one day. THF was evaporated, residue taken in ethyl acetate and the precipitated dicyclohexylurea (DCU) was filtered off. The organic layer was washed successively with brine (3 × 30 ml), HCl (2 M, 3 × 30 ml), brine (1 × 30 ml), Na_2CO_3 (3 × 30 ml) and brine (1 × 30 ml). This layer was dried over anhydrous sodium sulfate and evaporated *in vacuo*, thereby yielding **4** as a white solid. The crude peptide was purified by medium-pressure liquid chromatography on a reverse-phase C_{18} (40–63 μ) column using methanol–water mobile phase. δ_H (500 MHz, $CDCl_3$) 0.91 [12 H, m, (*R*)-β³-HVal1 $C^{\delta}H_3$, (*R*)-β³-HVal2 $C^{\delta}H_3$], 1.45 [9 H, s, -$C(CH_3)_3$], 1.82 [2 H, m, (*R*)-β³-HVal1 $C^{\gamma}H$, (*R*)-β³-HVal2 $C^{\gamma}H$], 1.92, 1.95, 2.37, 2.43 [4 H, m, (*R*)-β³-HVal1 $C^{\alpha}H_2$, (*R*)-β³-HVal2 $C^{\alpha}H_2$], 3.66 [1 H, m, (*R*)-β³-HVal $C^{\beta}H$], 3.67 [3 H, s, -OCH_3], 4.07 [1 H, m, (*R*)-β³-HVal $C^{\beta}H$], 5.12 [1 H, d, (*R*)-β³-HVal1 NH), 6.28 [1 H, d, (*R*)-β³-HVal2 NH]; *m/z* (ESI) 359.9 ([M + H]⁺), 397.2 ([M + K]⁺), 739.5 ([2M + Na]⁺), 755.5 ([2M + K]⁺). Single crystals suitable for X-ray diffraction were obtained from a methanol–water mixture by slow evaporation.

N-tert-Butyloxycarbonyl-(*R*)-β³-homovalyl-(*S*)-β³-homovalyl-methyl ester (Boc-(*R*)-β³-HVal-(*S*)-β³-HVal-OMe (5))

Boc-(*R*)-β³-HVal-(*S*)-β³-HVal-OMe was synthesized and purified following the procedure described above for **3**. Boc-(*R*)-β³-HVal-OH was coupled to (*S*)-β³-HVal-OMe. δ_H (500 MHz, $CDCl_3$) 0.95 [12 H, m, (*R*)-β³-HVal1 $C^{\delta}H_3$, (*S*)-β³-HVal2 $C^{\delta}H_3$], 1.45 [9 H, s, -$C(CH_3)_3$], 1.85 [2 H, m, (*R*)-β³-HVal1 $C^{\gamma}H$, (*S*)-β³-HVal2 $C^{\gamma}H$], 2.42 [3 H, m, (*R*)-β³-HVal1 $C^{\alpha}H_2$, (*S*)-β³-HVal2 $C^{\alpha}H$], 2.55 [1 H, d, (*S*)-β³-HVal2 $C^{\alpha}H$], 3.65 [1 H, m, (*R*)-β³-HVal1 $C^{\beta}H$], 3.69 [3 H, s, -OCH_3], 4.11 [1 H, m, (*S*)-β³-HVal2 $C^{\beta}H$], 5.21 [1 H, d, (*R*)-β³-HVal1 NH], 6.32 [1 H, d, (*S*)-β³-HVal2 NH]; *m/z* (ESI) 397.2 ([M + K]⁺), 739.5 ([2M + Na]⁺), 755.5 ([2M + K]⁺). Single crystals suitable for X-ray diffraction were obtained from a dimethyformamide–carbon tetrachloride–methanol mixture by slow evaporation.

X-Ray diffraction

X-Ray intensity data for crystals **1–6**, were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer using MoKα ($\lambda = 0.71073$ Å) radiation. ω scan type was used. The structures of **1–6**, were determined by direct phase determination using the program SHELXS-97.[27a] Refinements of all six structures were carried out against F^2, with a full matrix anisotropic least-squares method using the program SHELXL-97.[27b] In compound **1**, the asymmetric unit contained two molecules (Mol A and Mol B). All the hydrogen atoms of compounds **1–6** were fixed geometrically in the idealized positions and refined in the final cycle of refinement as riding over the atoms to which they were bonded. In these all-light-atom structures with no significant anomalous scatterers the Friedel pairs were merged before the final refinement cycles. The relevant crystallographic data collection parameters and the structure refinement details are summarized in Table 3. CCDC reference numbers 289146 (**1**), C289147 (**2**), 289148 (**3**), 289149 (**4**), 289150 (**5**) and 289151 (**6**). For crystallographic data in CIF or other electronic format see DOI: 10.1039/b516088j.

Acknowledgements

This research was supported by a grant from the Council of Scientific and Industrial Research, India and a program grant from the Department of Biotechnology, India in the area of Molecular Diversity and Design. VS thank CSIR for a Senior Research Fellowship. X-Ray diffraction data were collected on the CCD facility funded under the IRHPA program of the Department of Science and Technology, India.

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