Molecular Conformation and Packing of Peptide β Hairpins in the Solid State: Structures of Two Synthetic Octapeptides Containing 1-Aminocycloalkane-1-Carboxylic Acid Residues at the *i*+2 Position of the β Turn

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Abstract: Peptide β-hairpin formation is facilitated by centrally positioned D-Pro-Xxx segments. The synthetic peptides Boc-Leu-Phe-Val-D-Pro-Ac_6c-Leu-Phe-Val-OMe (**1**) and Boc-Leu-Phe-Val-D-Pro-Ac_8c-Leu-Phe-Val-OMe (**2**) were synthesized in order to explore the role of bulky 1-aminocycloalkane-1-carboxylic acid residues (Ac_nc, where *n* is the number of carbon atoms in the ring), at the *i*+2 position of the nucleating β turn in peptide β hairpins. Peptides **1** and **2** crystallize in the monoclinic space group $P2_1$ with two molecules in the asymmetric unit. The crystal structures of **1** and **2** provide conformational parameters for four peptide hairpin molecules. In all cases, the central segments adopts a type II′ β-turn conformation, and three of the four possible cross-strand hydrogen bonds are observed. Fraying of the hairpins at the termini is accompanied by the observation of NH···π interaction between the Leu(1)NH group and Phe(7) aromatic group. Cross strand stabilizing interactions between the facing residues Phe(2) and Phe(7) are suggested by the observed orientation of aromatic rings. Anomalous far-UV CD spectra observed in solution suggest that close proximity of the Phe rings is maintained even in isolated molecules. In both peptides **1** and **2**, the asymmetric unit consists of approximately orthogonal hairpins, precluding the formation of a planar β-sheet arrangement in the solid state. Solvent molecules, one dioxane and one water in **1**, three water molecules in **2**, mediate peptide association. A comparison of molecular conformation and packing motifs in available β-hairpin structures permits delineation of common features. The crystal structures of β-hairpin peptides provide a means of visualizing different modes of β-sheet packing, which may be relevant in developing models for aggregates of polypeptides implicated in disease situations.

Keywords: aggregation · conformation analysis · peptides · X-ray diffraction

Introduction

β Hairpins are one of the smallest units of autonomously folded polypeptide structures.[1] The hairpin formation is dictated by the propensity of dipeptide segments to form tight turns of appropriate stereochemistry. Analysis of protein structures reveals a preponderance of type I′ and II′ β turns in hairpin segments.[2] This observation has provided the basis for the de novo design of synthetic β hairpins, by inserting central dipeptide segments with a high probability of formation of type I′ and II′ β turns. Both D-Pro-Gly[3] and Asn-Gly[4] segments have been extensively investigated in designed synthetic peptides, successfully permitting the construction of well-defined hairpin structures. D-Pro-Gly segments have proved to be more effective in stabilizing β-hairpin conformations in synthetic peptides, than Asn-Gly sequences.[5] Aib-D-Ala segments which form a type I′ β-turn conformation also promote hairpin formation, as determined in the crystal structures of a synthetic octapeptide Boc-Leu-Phe-Val-Aib-D-Ala-Leu-Phe-Val-OMe.[6] The availability of well-defined peptide β-hairpins has facilitated attempts to understand the packing of β-sheet peptides in the

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solid state, providing some insights into their modes of aggregation. These studies are particularly important in view of intense current interest in delineating the nature of aggregates formed in amyloid fibrils, which are predominately composed of peptide chains in extended conformations.[7]

We have undertaken a systematic program to examine the effect of residues at the turn and strand positions of β hairpins on the local conformation of peptides, the mode of aggregation in the solid state and the existence of the cross strand interactions in hairpins.[6,8,9] The limited tendency of β-hairpin peptides to yield single crystals has restricted the rate of accumulation of structural information. We describe in this report the crystal structures of the crystalline octapeptide hairpins Boc-Leu-Phe-Val-D-Pro-Ac_6c-Leu-Phe-Val-OMe (**1**) and Boc-Leu-Phe-Val-D-Pro-Ac_8c-Leu-Phe-Val-OMe (**2**), in which the residue at *i*+2 position of the β turn is a bulky 1-aminocycloalkane-1-carboxylic acid (Ac_nc, where *n* is the number of carbon atom in the ring) constrained to adopt backbone conformational angles in the α_R or α_L region of conformational space. Ac_nc residues are cyclic analogues of the well-studied conformationally constrained residue Aib.[10] A notable feature of the central β-turn in hairpin structures in peptides and proteins is the hydration/solvation of the central peptide unit, which lies in an orientation approximately perpendicular to the plane of the β sheet.[11] The incorporation of a bulky hydrophobic residue at the *i*+2 position of the β turn was intended to probe the effects of side chains on this solvation process. The crystal structures of the two designed β hairpins revealed an unexpected orthogonal arrangement of adjacent peptide molecules in the solid state, a feature thus far been observed only in the crystal structure of the peptide Boc-Leu-Phe-Val-D-Pro-Ala-Leu-Phe-Val-OMe.[9] The analysis of the crystal structures of ten β-hairpin peptides determined so far,[3b,6,9,12] permits a classification of the major packing modes observed thus far.

Results

Molecular conformation and hydrogen bonds: Both peptides **1** and **2** crystallized in the monoclinic space group $P2_1$ with two molecules in the asymmetric unit. Figures 1 and 2 illustrate the molecular conformation observed in crystals for peptides **1** and **2**. Crystal and diffraction data and refinement details are summarized in Table 1. The backbone and side chain torsion angles for the two peptides are summarized in Table 2, while the intra- and intermolecular hydrogen bond parameters are listed in Table 3. All four independent peptide hairpins are stabilized by three intramolecular hydrogen bonds (Val(3) NH···OC Leu(6), Leu(6) NH···OC Val(3), Val(8) NH···OC Leu(1)). The fourth intramolecular hydrogen bond which may be anticipated in an ideal β hairpin, Leu(1) NH···OC Val(8) is not observed, because of the conformational distortions at the termini of the two antiparallel strands. In all four molecules the central D-Pro-Ac_6c/Ac_8c turn adopts a type II′ β-turn conformation. Residues 1 to 3 adopt ϕ,ψ values characteristic of an extended β-sheet conformation. In all four molecules, the ϕ value at residues 6,7 is $\approx -80°$, a deviation of nearly 40° from the values ideally expected in β sheets. The conformational deviations are more significant at Val(8). In peptide **1**, two solvent molecules water(O1w) and dioxane interact with the peptide. While O1w bridges adjacent hairpins, the oxygen atom of dioxane hydrogen bonds to the Ac_6c NH of the central peptide unit of the β turn. The asymmetric unit in crystals of peptide **2** contains three independent water molecules. O1w and O3w link adjacent molecules via the strand segment, while O2w forms a hydrogen bond to Ac_8c NH. Figure 3 shows the edge on view of the four independent β-hairpin molecules observed in the structure of peptides **1** and **2**, which illustrates the disposition of the side chains with respect to the hairpin plane.

Conformation of cycloalkane rings: The cyclohexane ring in both the molecules **1A** and **1B** adopts almost ideal chair conformations, with the amino group occupying an axial orientation.[10b] The cyclooctane rings in both molecules **2A**

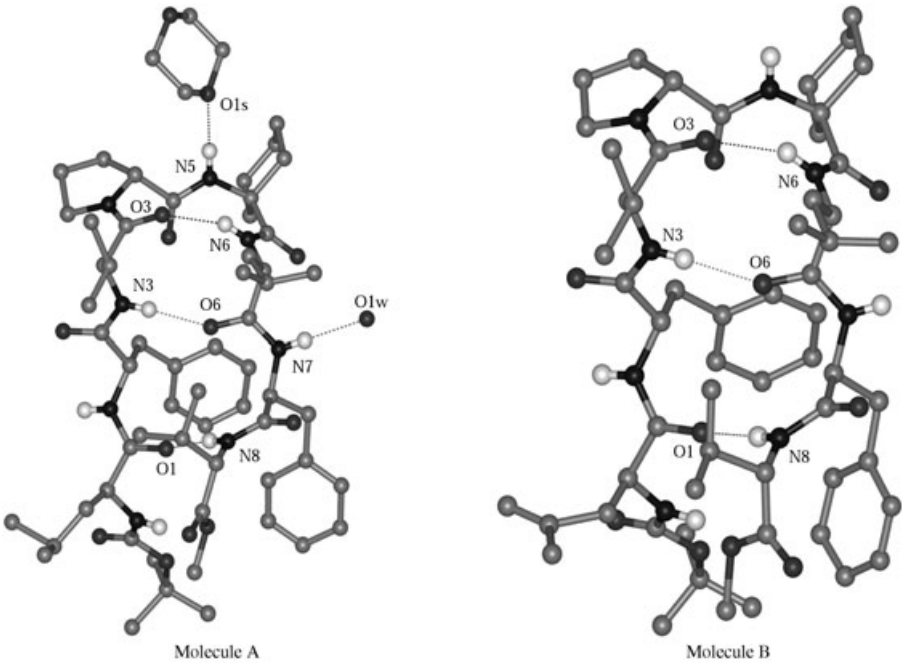

Figure 1. Molecular conformation of Boc-Leu-Phe-Val-D-Pro-Ac_6c-Leu-Phe-Val-OMe (**1**) in the solid state. Hydrogen bonds are shown as dotted lines.

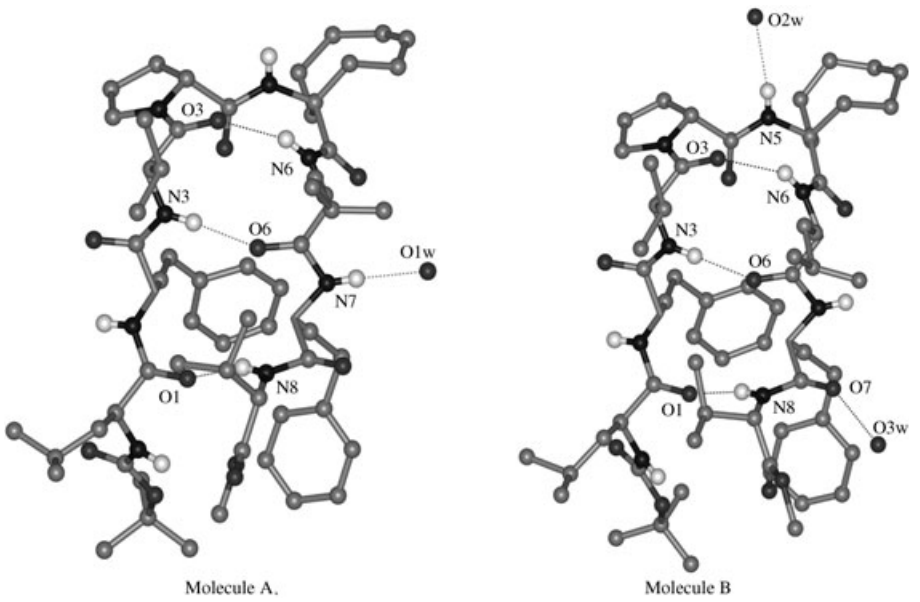

Figure 2. Molecular conformation of Boc-Leu-Phe-Val-D-Pro-Ac_8c-Leu-Phe-Val-OMe (**2**) in the solid state. Hydrogen bonds are shown as dotted lines.

Table 1. Crystal and diffraction parameter for peptides **1** and **2**.

	Peptide **1**	Peptide **2**
empirical formula	$C_{58}H_{88}N_8O_{11}+0.5\,H_2O+0.5\,C_4H_8O_2$	$C_{60}H_{92}N_8O_{11}+1.5\,H_2O$
crystal habit	clear	clear
crystal size [mm]	$0.4\times0.1\times0.04$	$0.37\times0.11\times0.05$
recryst. solvent	methanol/isopropanol/1,4-dioxane	methanol/water
space group	$P2_1$	$P2_1$
cell parameters		
a [Å]	18.143(12)	15.039(3)
b [Å]	24.858(16)	25.900(5)
c [Å]	18.449(12)	19.083(4)
α [°]	90	90
β [°]	117.02(13)	108.498(3)
γ [°]	90	90
V [Å^3]	7412(8)	7049(3)
Z	4	4
molecules/asym. unit	2	2
cocrystallized	$1\times H_2O$	$3\times H_2O$
solvent	+1,4-dioxane	
M_w	1072+9+44 = 1125	1100+27 = 1127
ρ_{calcd} [g cm^{-3}]	1.008	1.060
F(000)	2436	2444
radiation	$Mo_{K\alpha}$	$Mo_{K\alpha}$
λ [Å]	0.71073	0.71073
T [°C]	21	21
$2\theta_{max}$ [°]	50.0	46.5
scan type	ω	ω
measured reflns	70405	44024
independent reflns	25632	17837
unique reflns	13277	10387
obsd reflns [$\|F\|>4\sigma(F)$]	8697	8345
final R [%]	10.57	9.67
final $wR2$ [%]	24.80	18.61
GoF (S)	1.166	1.009
$\Delta\rho_{max}$ [e Å^{-3}]	0.46	0.52
$\Delta\rho_{min}$ [e Å^{-3}]	−0.39	−0.20
no. restraints/parameters	4/1450	14/1402
data-to-parameter ratio	6:1	5.9:1

and **2B** adopt a boat-chair conformation,[13] with the amino group occupying a quasi-axial orientation. The observed geometries closely resemble those established in model Ac_6c and Ac_8c peptides, from previous crystallographic studies.[10a,b,l]

Side chain interactions: Peptides **1** and **2** contain aromatic residues at residues 2 and 7, which occupy facing, non-hydrogen bonded positions in the antiparallel β-hairpin. Figure 4 illustrates the observed orientations and distance between the aromatic pair in each of the four crystallographically observed molecules. In all four examples, the distance between the centroids of the aromatic rings lie between 4.98 and 5.41 Å, and the interplanar angle lies between 20 and 60°. In the case of **1A**, **2A** and **2B** relatively small interplanar angles (20° to 45°) are observed. These parameters fall well within the limits considered as diagnostic for favorable aromatic–aromatic interactions.[14] Recent experimental and theoretical studies suggest that the interaction potential between a pair of phenyl rings has a relatively broad minimum and stringent orientation dependences are not suggested.[15] In both crystals, there are no potential intermolecular aromatic contacts.

A notable feature of all four hairpin molecules is the absence of the N1···O8 hydrogen bond (Leu(1) NH···OC Val(8)). Interestingly, in all the cases the aromatic ring of Phe(7) folds back over the body of the hairpin; this suggests a potential NH···π interactions. The range of observed NH··· Phe (centroid) distances (4.924–5.332 Å) and angle subtended by the NH bond and the perpendicular to the aromatic plane (112.8–160.5°) lie within the ranges

Table 2. Torsion angles [°] for peptides **1** and **2**.[a]

Residue	Peptide **1** (Xx*x* = Ac_6c)						Peptide **2** (Xxx = Ac_8c)					
	Molecule A			Molecule B			Molecule A			Molecule B		
	ϕ	ψ	ω	ϕ	ψ	ω	ϕ	ψ	ω	ϕ	ψ	ω
Leu(1)	−123.7[b]	138.3	−175.1	−126.0[e]	129.6	−172.8	−116.2[b]	129.9	−179.0	−103.5[e]	138.5	178.1
Phe(2)	−132.1	144.4	168.3	−132.7	144.7	165.3	−124.4	149.6	162.8	−122.6	143.0	165.5
Val(3)	−113.5	96.0	−164.8	−111.6	99.5	−165.3	−117.4	91.0	−167.4	−110.8	88.3	−166.3
D-Pro(4)	**55.0**	**−138.2**	178.7	**53.8**	**−134.1**	−179.9	**55.0**	**−130.1**	−174.9	**52.1**	**−133.0**	−176.2
Xxx(5)	**−71.7**	**−9.1**	−175.7	**−74.2**	**−12.5**	−174.9	**−67.1**	**−21.2**	−171.2	**−56.8**	**−29.8**	−171.4
Leu(6)	−82.4	122.3	179.4	−81.8	118.5	175.8	−86.2	122.6	176.7	−77.6	121.4	170.2
Phe(7)	−88.3	119.0	−174.9	−83.5	130.1	−178.8	−90.8	117.1	−177.2	−84.4	117.2	−172.6
Val(8)	−124.5	6.3[c]	179.6[d]	−111.9	−64.9[f]	−179.0[g]	−114.5	0.0[c]	−179.5[d]	−76.2	150.1[f]	174.1[g]
Side chain	χ^1	χ^2		χ^1	χ^2		χ^1	χ^2		χ^1	χ^2	
Leu(1)	−72.4	−74.6, 177.2		−97.1	−45.6, −163.4		−79.4	−62.7, 169.9		−64.1	−73.0, 166.8	
Phe(2)	−72.5	−96.7, 80.8		−75.4	−93.5, 86.2		−74.8	−94.9, 86.7		−70.8	78.2, −103.1	
Val(3)	−61.6, 176.1			−60.4, 175.9			−58.6, −178.9			−57.8, 179.3		
Leu(6)	−173.7	70.6, −163.4		−179.1	65.3, −170.2		-174.7	67.9, −173.9		176.5	64.0, −173.4	
Phe(7)	−174.0	33.3, −150.0		180	76.4, −99.4		−175.1	53.5, −126.6		−177.8	44.9, −140.4	
Val(8)	−61.5, 63.1			−56.8, 178.3			63.7, −62.3			−63.7, 173.0		

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ and ω) and about bonds of the amino acid side chains (χ^1,χ^2) as suggested by the IUPAC-IUB Commission on Biochemical Nomenclature.[22] [b] C′(0)-N1-C$^\alpha$1-C′(1). [c] N8-C$^\alpha$8-C′8-O1(OMe). [d] C$^\alpha$8-C′8-O1(OMe)-C1(OMe). [e] C′(10)-N11-C$^\alpha$11-C′11. [f] N18-C$^\alpha$(18)-C′(18)-O2(OMe). [g] C$^\alpha$(18)-C′(18)-O2(OMe)-C2(OMe).

suggested for such interactions from analysis of protein crystal structures.[16]

Circular dichroism analysis: The observation of close interstrand aromatic contacts between residues Phe(2) and Phe(7) in peptides **1** and **2** in the solid state prompted us to examine the CD spectra of both peptides. A feature of aromatic interactions is the possibility of observing exciton effects leading to anomalous near UV spectrum. In peptides lacking aromatic residues the CD bands in the region 190 to 240 nm can be assigned unambiguously to the backbone amide chromophore permitting delineation of secondary structural features from observed band positions. The presence of interacting aromatic groups can however result in anomalous spectral features as illustrated in Figure 5, for peptides **1** and **2**. In both cases, negative bands centred at 230 and 210 nm are observed. The long wavelength 230 nm band presumably arises from an exciton interaction,[6] and may be the long wavelength component of an exciton split doublet, in which the short wavelength component is masked by overlap with backbone amide CD bands. In the cases of hairpins with proximal aromatic groups conformational assignments in solution by CD may be fraught with uncertainty. In such cases NMR and the use of vibrational CD may be more reliable.[17]

Crystal packing: Figures 6 and 7 illustrate the interactions between the two independent molecules in the asymmetric unit of peptides **1** and **2**. Adjacent β hairpins are arranged in an approximately orthogonal fashion (the angle between the mean plane of the β hairpins is 60.9° in peptide **1** and 67.0° in peptide **2**). A consequence of this twisted arrangement is the limited hydrogen bond interaction between the adjacent molecules, which form the crystallographic asymmetric unit, with only the N2···O2 hydrogen bond being observed. In the absence of extensive intermolecular hydrogen bonding, crystal packing is facilitated by solvent bridges.

Figure 8 shows the molecular packing of peptide **1** in crystals. The asymmetric units are linked along the 101 direction (inclined to the crystallographic *c* axis) by a direct inter-peptide hydrogen bond (N7B···O7A) and a water bridge (N7A···O1w···O5B). The dioxane molecule bridges N5A and N5B of two different dimeric asymmetric unit which are translationally related 100 direction.

Figure 9 illustrates two views of the molecular packing in crystals of peptide **2**. The orthogonal arrangement of two independent hairpins in the asymmetric unit necessitates the representation along two orthogonal directions in order to visualize role of bridging solvent molecules. Along the *b* axis the A molecules are linked by the N5···O8 hydrogen bond, which is formed between the Ac_8c NH and Val(8) CO groups linking the central peptide unit of one molecule with the C terminus of the symmetry related molecule. The central peptide NH (Ac_8c NH) of molecule B hydrogen bonds to water molecule O2w, which in turn interacts with O3w, which is anchored by two hydrogen bonds to O7 and O8 of a translated unit. The dimeric asymmetric units are arranged along 001 direction by a direct N7B···O7A hydrogen bond and a water mediated N7A···O1w···O5B interaction.

Discussion

Crystal structures of peptide **1** and **2** suggest that in designed hairpins, position *i*+2 of the β turn readily accommodates sterically bulky side chains. Crystallization of the octapeptide hairpin with Ac_6c and Ac_8c at position 5 establish that packing into crystalline arrays is feasible for a range of

Table 3. Hydrogen bonds in peptides **1** and **2**.

Donor	Acceptor	N···O [Å])	H···O [Å]	C=O···H [°]	C=O···N [°]	O···H-N [°]
			peptide **1** (Xxx = Ac_6c)			
intermolecular						
N2A	O2B	2.893	2.127	143.4	151.2	148.2
N5A	O1S	3.049	2.229			159.3
N7A	O1w	2.947	2.088			176.9
N2B	O2A	2.946	2.198	142.3	150.4	145.3
N5B	O4S[a]	2.930	2.114			158.3
N7B	O7A[b]	2.939	2.090	168.0	166.5	169.2
O1w	O5B[c]	2.686				
intramolecular						
molecule A						
N3	O6	2.875	2.031	152.1	153.8	167.5
N6	O3	2.958	2.158	134.5	132.5	154.6
N8	O1	2.918	2.064	155.0	155.8	172.1
molecule B						
N3	O6	2.893	2.059	152.1	153.2	163.3
N6	O3	2.987	2.193	135.5	133.0	153.4
N8	O1	2.895	2.038	160.4	158.8	174.5
			peptide **2** (Xxx = Ac_8c)			
intermolecular						
N2A	O2B	2.893	2.061	151.6	156.3	162.7
N5A	O8A[d]	2.985	2.135	165.4	162.4	169.4
N7A	O1w	2.942	2.089			171.8
N2B	O2A	2.879	2.068	152.1	157.2	156.8
N5B	O2w	3.043	2.241			155.2
N7B	O7A[e]	2.986	2.135	163.5	164.4	170.4
O1w	O5B[f]	2.779				
O2w	O3w[a]	2.766				
O3w	O8A[e]	2.932				
O3w	O7B	2.663				
intramolecular						
molecule A						
N3	O6	2.851	2.004	154.6	156.4	167.8
N6	O3	3.028	2.219	133.7	130.7	156.5
N8	O1	2.955	2.131	153.6	158.0	160.4
molecule B						
N3	O6	2.854	2.026	150.5	151.7	161.2
N6	O3	3.028	2.256	136.9	130.5	149.3
N8	O1	2.895	2.043	159.4	160.5	171.2

[a] Symmetry related by $x+1, y, z$; [b] symmetry related by $x+1, y, z+1$; [c] symmetry related by $x-1, y, z-1$; O1S and O4S are oxygen atom of 1,4-dioxane; [d] symmetry related by $-x+1, y-{}^1/_2, -z$; [e] symmetry related by $x, y, z+1$; [f] symmetry related by $x, y, z-1$.

amino acid residues in both the strand and turn positions. β-hairpin packing in crystals has often featured molecular association by two dimensional sheet formation, both parallel and antiparallel, in which strand segments of the adjacent molecules are properly registered. Non-polar interactions and solvent bridges involving the central peptide unit of the β turn have provided stabilization along the third dimension. Thus far the crystal structures of nine β-hairpin peptides (including **1** and **2**) containing only α-amino acid residues have been determined. These provide a view of 17 crystallographic independent β-hairpin molecules. The turn segment in eight peptide sequences of the type D-Pro-Xxx, while in one case Aib-D-Ala segment is employed. In eight out of nine hairpins molecules the central β-turn adopts the type II′ β-turn conformation. The sole exception is the Aib-D-Ala segment, which adopts a type I′ β-turn conformation. It should be noted that entries 10–12 in Table 4, are β-hairpin peptides, which contain both α- and β-amino acids in the strands. In these the introduction of an additional backbone atom results in the creation of polar strands, with different modes of association.[12b–d] In these hybrid peptides, type I′ turns have been observed for the D-Pro-Gly segments.[12b,d] The main difference between hairpin nucleating type I′ and type II′ β-turns is that in the former the antiparallel strands are appreciably twisted while the type II′ turn promotes the formation of a flatter hairpin.[6]

A notable feature of the octapeptide hairpin structures is the fraying of the hydrogen bond involving N and C-terminal residues, (N1···O8). Of the examples reported so far, summarized in Table 4, there is only one molecule in which this cross strand hydrogen bond is observed in crystals.[3b] In the remaining cases, the backbone torsion angles of residues

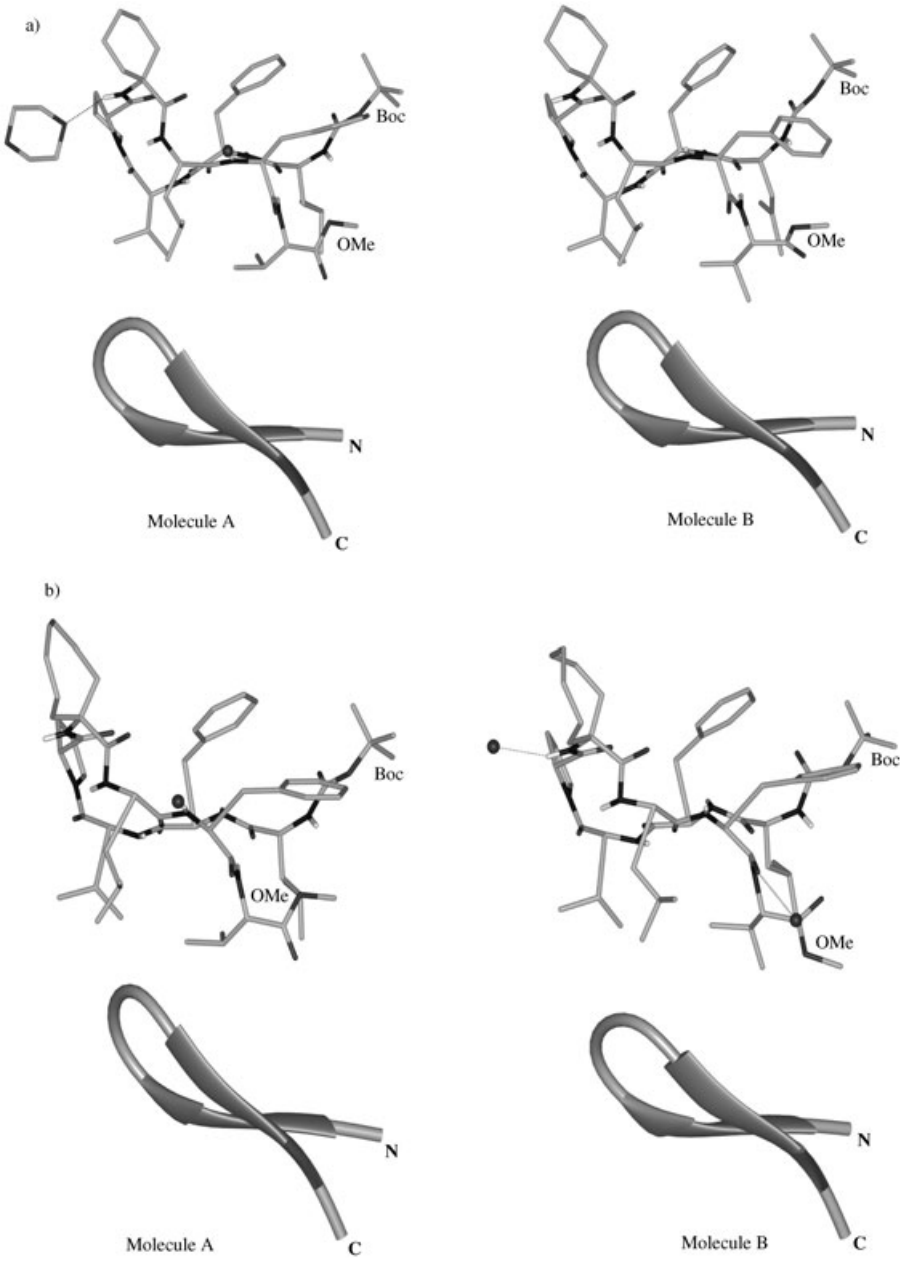

Figure 3. Edge on view of the independent molecules observed in peptides a) **1** and b) **2**. Schematic ribbon representation is also shown.

1 and 8 deviate sufficiently from β-sheet values permitting the Leu(1) NH and Val(8) CO groups to participate in intermolecular interaction. The structures of peptides **1** and **2** reveal that in all four molecules Leu(1) NH and the Phe(7) aromatic ring are oriented in a manner indicative of a stabilizing NH···π interaction. The survey of crystal structures listed in Table 4 reveals that in four cases where residue 7 is an aliphatic amino acid, NH(1) is involved in an intermolecular hydrogen bond with a neighboring molecule, while in one case the intramolecular NH(1)···OC(8) interaction is observed. In seven cases, when an appropriately positioned Phe residue is available, the NH(1)···π interaction is observed. The sole exception is the structure of Boc-Leu-Val-Val-D-Pro-Gly-Leu-Phe-Val-OMe,[9] where NH(1) participates in an intermolecular interaction and no NH···π contact is observed.

The packing arrangements determined in the peptide hairpins **1** and **2** reveal an orthogonal arrangement, which is relatively rare as a packing motif in this class of peptides. Peptides **1**, **2** and Boc-Leu-Phe-Val-D-Pro-Ala-Leu-Phe-Val-OMe,[9] determined earlier are the only three examples in which this twisted arrangement is observed. Normally peptide hairpins pack into a two dimensional sheets in which adjacent hairpins in a row are arranged in either parallel or

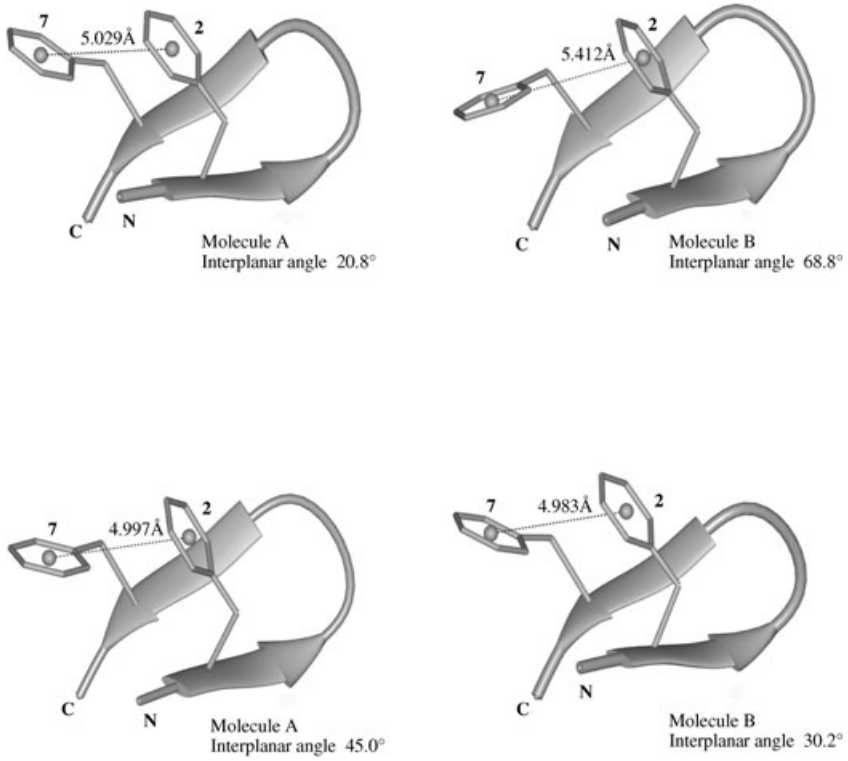

Figure 4. Aromatic–aromatic interaction between the Phe residues observed in peptides **1** (top) and **2** (bottom). The distance between centroid of aromatic rings and the interplanar angles are indicated.

antiparallel fashion. The packing of β-sheets in crystals is of special interest in view of the importance of the association of β-sheet polypeptide structures in forming insoluble precipitates, most notably in the case of amyloid deposits associating with pathology of neurodegenerative diseases.[7a,b,f]

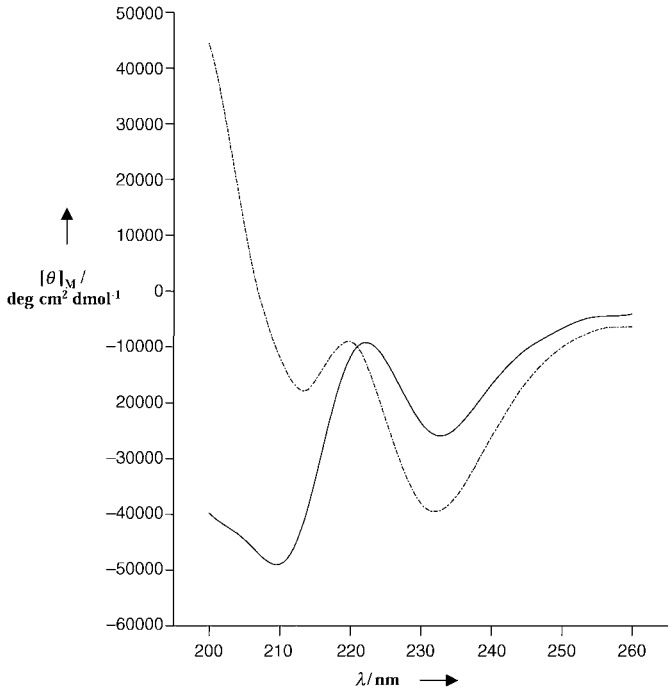

Figure 5. Electronic CD spectra of peptides **1** (——) and **2** (–•–•) methanol.

Conclusion

The determination of the crystal structures of peptide hairpins **1** and **2** establish that the D-Pro-Xxx unit is a robust β-hairpin nucleating segment capable of accommodating a range of residues at the $i+2$ position of the β turn. In **1** and **2** bulky 1-aminocycloalkane-1-carboxylic acid residues, Ac_6c and Ac_8c, occupy the $i+2$ position of the type II′ β turn. The packing of hairpins **1** and **2** in crystals reveals a rare, orthogonal packing arrangement in which the angle between mean plane of the two hairpin in the asymmetric unit is between 60–70°. The structures of **1** and **2** add to a limited number of available β-hairpin structures in crystals, which provide views of the packing of β-sheets that may be useful in understanding the structures of aggregates formed in protein folding diseases, where extended polypeptide strands self associate into fibrillar structures.

Experimental Section

Peptide synthesis: Peptides **1** and **2** were synthesized by conventional solution phase methods by using a fragment condensation strategy. The

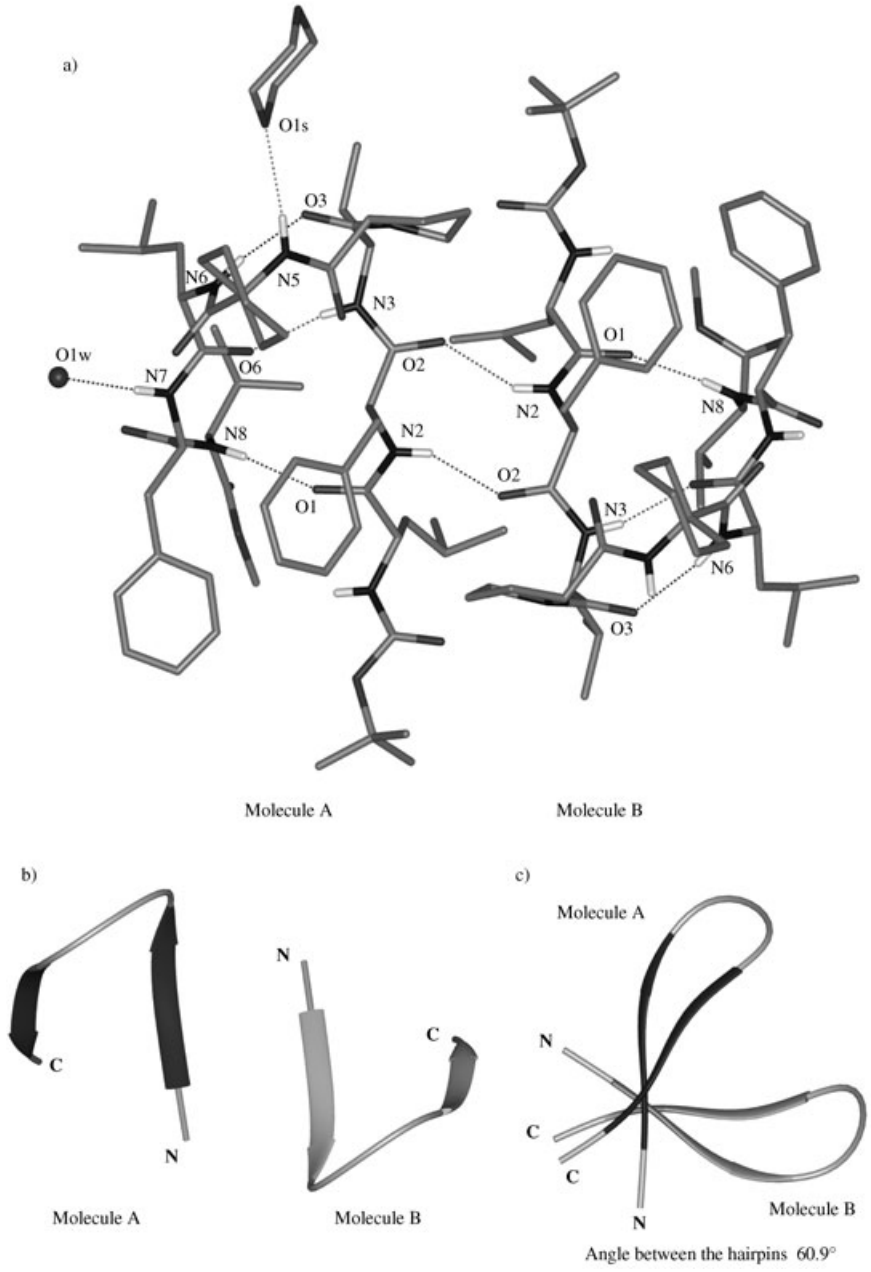

Figure 6. a) Asymmetric unit of peptide **1**. All the intra- and intermolecular hydrogen bonds in the asymmetric unit are shown as dotted lines. b) Relative orientation of the two molecules in the asymmetric unit, viewed as in a). c) Viewed after a 90° rotation.

tert-butyloxycarbonyl (Boc) group was used to protect the N terminus; the C terminus was protected as a methyl ester. Deprotections were performed by using 98% formic acid and saponification for the N- and C-terminal protection groups, respectively. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBT). All the intermediates were characterized by ^{1}H NMR (80 MHz) and TLC (silica gel, chloroform/methanol 9:1), and were used without further purification. The final peptides were purified by medium pressure liquid chromatography (MPLC) on a C_{18} column (40–60 μ) followed by HPLC (C_{18}, 5–10 μ), employing methanol-water gradients. The homogeneity of the purified peptides was ascertained by analytical HPLC. The purified peptides were characterized by electrospray and MALDI mass spectrometry, and by complete assignment of the 500 MHz NMR spectra. (M_{calcd} 1072; M_{obs} 1095.7 [M+Na$^+$] for peptide **1**, M_{calcd} 1100; M_{obs} 1123.5 [M+Na$^+$] for peptide **2**).

Circular dichroism: CD spectra were recorded on a JASCO J-715 spectropolarimeter. The instrument was calibrated with d-10 camphorsulfonic acid. The path length used was 1 mm. The data were acquired in the wavelength scan mode, using a 1 nm bandwidth, with a step size of 0.5 nm. Typically, four scans were acquired from 260 to 200 nm by using a scan speed of 20 nm min^{-1}. The resulting data were baseline corrected and smoothened. Peptide concentrations of 0.1 mg mL^{-1} were used.

X-ray diffraction: Single crystals of peptides **1**, and **2** were grown from methanol/isopropanol/1,4-dioxane and methanol/water solutions, respectively. The crystallographic data and other details for the compounds **1**

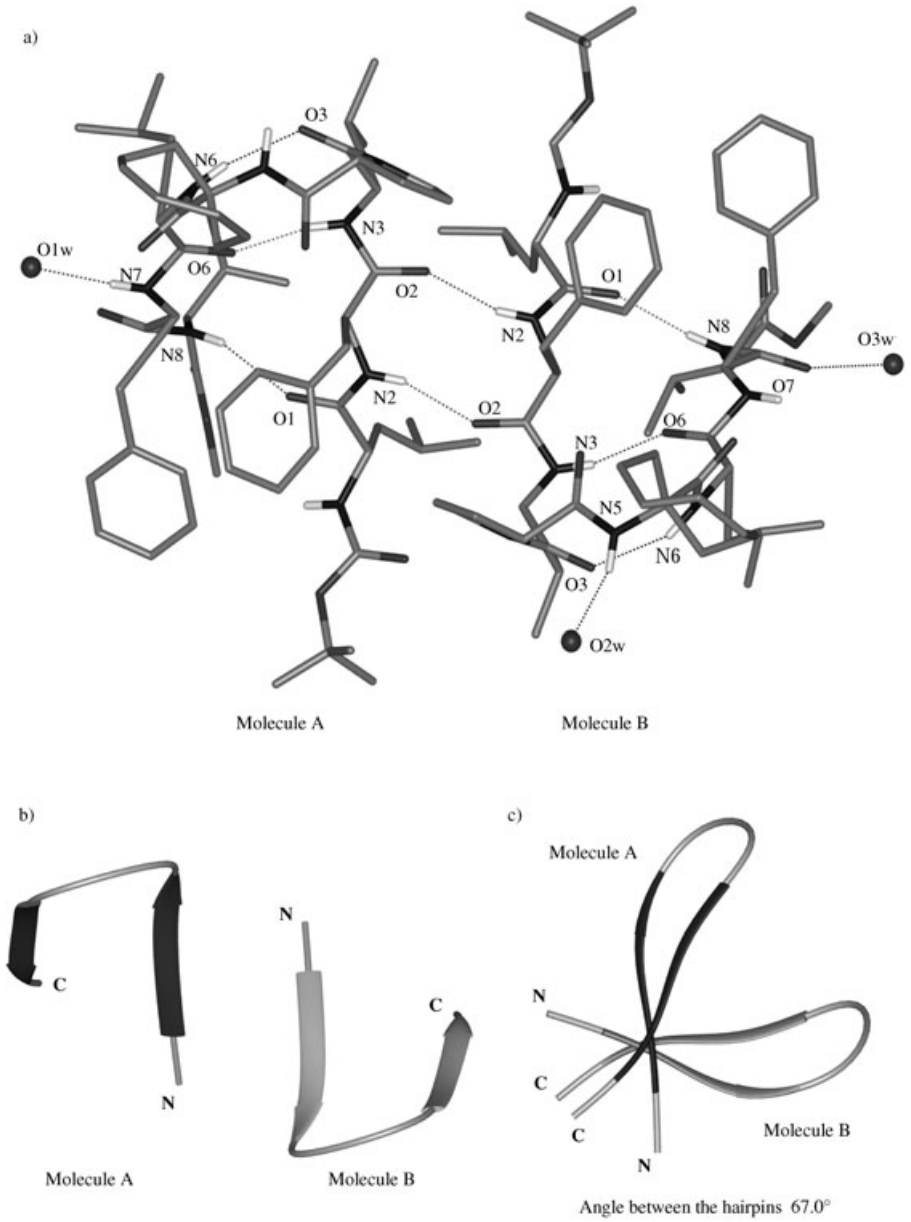

Figure 7. a) Asymmetric unit of peptide **2**. All intra- and intermolecular hydrogen bonds in the asymmetric unit are shown as dotted lines. b) Relative orientation of the two molecules in the asymmetric unit, viewed as in a). c) Viewed after a 90° rotation.

and **2** are summarized in Table 1, X-ray diffraction data were collected on a Bruker AXS SMART APEX CCD diffractometer by using $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). For peptide **1**, the data collection covered a hemisphere of reciprocal space, by a combination of four sets with different ϕ angles (0, 60, 120 and 180°), with detector kept at $2\theta = -23°$. Each set consists of 606 frames and exposure time for each frame was 30 s for 0.3° ω scan. For peptide **2** the data collection covered a hemisphere of reciprocal space, by a combination of three sets with different ϕ angles (0, 90 and 180°), with detector kept at $2\theta = -23°$. Each set consists of 606 frames and exposure time for each frame was 25 s for 0.3° ω scan. There is a higher redundancy in the data set for peptide **1**. The structures were solved by direct methods by using SHELXD[18] for peptide **1** and Shake-and-Bake method (SnB)[19] for peptide **2**. For peptide **1** two fragments were obtained containing 68 and 62 atoms. For peptide **2**, two fragments containing 60 and 45 atoms were obtained. The data upto 0.9 Å were used for structure solution and refinement for peptide **2**. The remaining atoms and solvent molecules were located from difference Fourier-maps. Full matrix least squares refinement was carried out by using SHELXL-97.[20] The hydrogen atoms were fixed geometrically in the idealized positions and refined in the final cycle of refinement as riding over the atoms to which they are bonded. In the refinement restraints were applied on bond length in the cyclooctane rings in peptide **2**. The final R factor was 10.57% for peptide **1** and 9.67% for peptide **2**. The standard deviations in bond lengths, bond angles and dihedral angles are approximately 0.01 Å, 1.0 and 1.5°, respectively for peptides **1** and **2**.

CCDC-252393 (**1**), -252394 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Figure 1 to 4 and 6 to 9 were generated by using the program MOLMOL.[21]

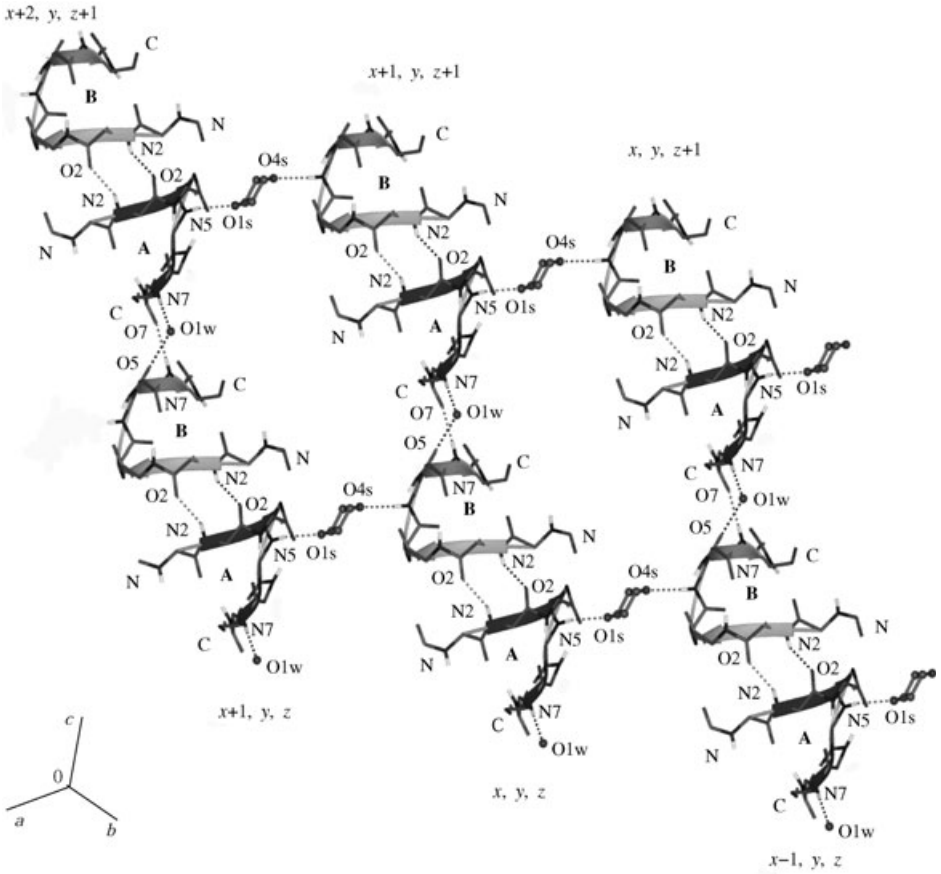

Figure 8. Schematic representation of molecular packing of peptide **1** in crystals.

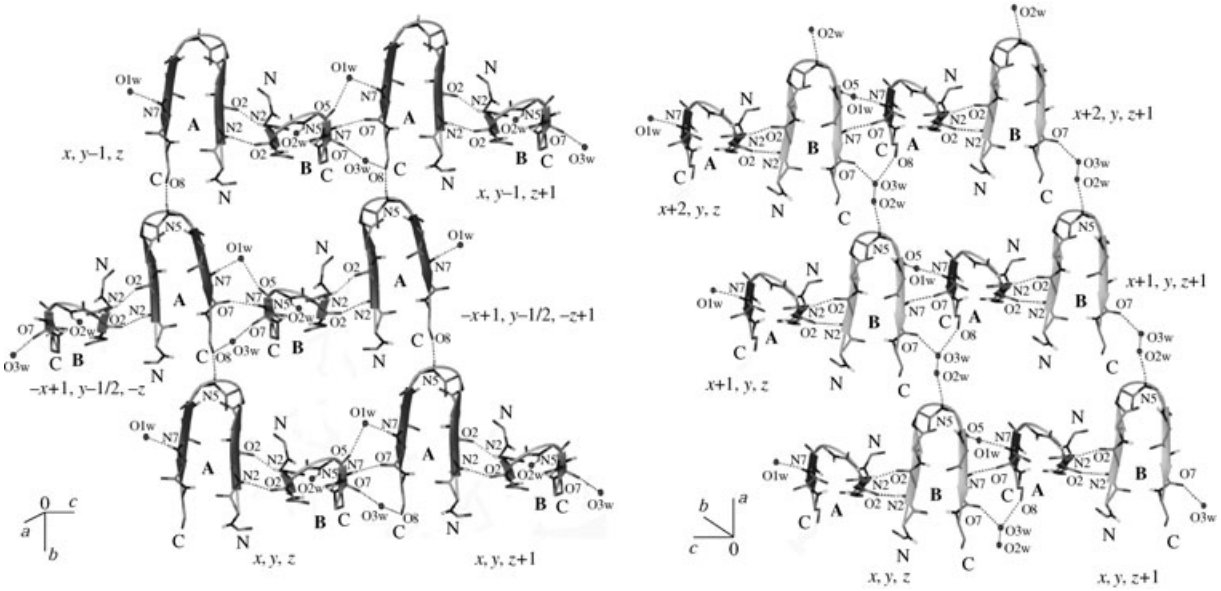

Figure 9. Schematic representation of molecular packing of peptide **2** in the solid state (left) along *b* axis (right) along axis.

Table 4. Comparison of β hairpins.

No	Sequence	Space group	No. of molecules in the asymmetric unit	Type of β-turn	Cocrystallized solvents in the asymmetric unit	Packing	Ref.
1	Boc-L-V-V-D-**P-G**-L-V-V-OMe	$P\bar{1}$	2	type II′	two water molecules	parallel	[3b]
2	Boc-L-V-V-D-**P-G**-L-F-V-OMe	$P2_12_12_1$	1	type II′	one water molecule	parallel	[9]
3	Boc-L-V-V-D-**P-A**-L-V-V-OMe	$P1$	2	type II′	one water molecule	parallel	[12a]
4	Boc-L-F-V-D-**P-A**-L-F-V-OMe	$P2_12_12_1$	2	type II′	three water molecules + isopropanol	orthogonal	[9]
5	Boc-M-L-F-V-D-**P-A**-L-V-V-F-OMe	$P1$	4	type II′	four water molecules	antiparallel	[9]
6	Boc-L-V-V-D-**P-U**-L-V-V-OMe	$P\bar{1}$	1	type II′	one water + DMF	parallel	[9]
7	Boc-L-F-V-**U**-D-**A**-L-F-V-OMe	$P2_12_12_1$	1	type I′	four water molecules	parallel	[6]
8	Boc-L-F-V-D-**P-Ac$_6$c**-L-F-V-OMe (**1**)	$P2_1$	2	type II′	one water + 1,4-dioxane	orthogonal	present study
9	Boc-L-F-V-D-**P-Ac$_8$c**-L-F-V-OMe (**2**)	$P2_1$	2	type II′	three water molecules	orthogonal	present study
10	Boc-L-V-β-Phe-V-D-**P-G**-L-β-Phe-V-V-OMe	$P2_12_12_1$	1	type I′	three water molecules	antiparallel	[12b]
11	Boc-β-Phe-β-Phe-D-**P-G**-β-Phe-β-Phe-OMe	$P2_1$	1	type II′	methanol	parallel	[12c]
12	Boc-L-V-β-Val-D-**P-G**-β-Leu-V-V-OMe	C_2	1	type I′	one water	antiparallel	[12d]

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