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Peptide Hairpins with Strand Segments Containing α - and β -Amino Acid Residues : Cross – Strand Aromatic Interactions of Facing Phe Residues.

Rituparna S. Roy¹, Hosahudya N. Gopi¹, Srinivasarao Raghothama², Richard D. Gilardi³, Isabella L Karle³, and Padmanabhan Balam¹

¹Molecular Biophysics Unit, Indian Institute of Science, Bangalore - 560012, India.

²Sophisticated Instruments Facility, Indian Institute of Science, Bangalore - 560012, India.

³Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C 20375-5341

Abstract

The incorporation of β -amino acid residues into the strand segments of designed β -hairpin leads to the formation of *polar sheets*, since in the case of β -peptide strands, all adjacent carbonyl groups point in one direction and the amide groups orient in the opposite direction. The conformational analysis of two designed peptide hairpins composed of α/β -hybrid segments are described: Boc-Leu- β Phe-Val-^DPro-Gly-Leu- β PheVal-OMe (**1**) and Boc- β Leu-Phe- β Val-^DPro-Gly- β Leu-Phe- β Val-OMe (**2**). 500 MHz ¹HNMR analysis in methanol supports a significant population of hairpin conformations in both the peptides. Diagnostic nuclear Overhauser effects are observed in both cases. X-ray diffraction studies on single crystals of peptide **1** reveal a β -hairpin conformation in both the molecules, that constitute the crystallographic asymmetric unit. Three cross-strand hydrogen bonds and a nucleating Type II' β -turn at the ^DPro-Gly segment are observed in the two independent molecules. In peptide **1**, the β Phe residues at positions 2 and 7, occur at the *non-hydrogen bonding* position with the benzyl sidechains pointing on opposite faces of the β -sheet. The observed aromatic centroid –to – centroid distances are 8.92 Å (molecule A) and 8.94 Å (molecule B). In peptide **2**, the aromatic rings must occupy facing positions in anti-parallel strands, in the NMR derived structure.

Peptide **1** yields a normal “hairpin-like” CD spectrum in methanol with a minimum at 224 nm. The CD spectrum of peptide **2** reveals a negative band at 234 nm and a positive band at 221 nm, suggestive of an exciton split doublet. Modelling of the facing Phe sidechains at the hydrogen bonding position of a canonical β -hairpin suggests that inter-ring separation is ~ 4.78 Å for the *gauche*⁺*gauche*[−] (*g*⁺*g*[−]) rotamer. A previously reported peptide β -hairpin composed of only α -amino acids, Boc-Leu-Phe-Val-^DPro-Gly-Leu-Phe-Val-OMe also exhibited an anomalous far UV CD spectrum, which was interpreted in terms of interactions between facing aromatic chromophores, Phe 2 and Phe 7

Keywords

peptide hairpins; hybrid peptides; β -peptides; anomalous circular dichroism; cross-strand aromatic interactions; exciton split doublet; peptide crystal structure

INTRODUCTION

The ability of a centrally positioned ^DPro-Xxx segment to stabilize β -hairpin conformations in short synthetic peptides is well established by NMR studies in solution¹⁻⁸ and X-ray diffraction studies in crystals.⁹⁻¹⁴ Robust synthetic hairpin scaffolds provide an opportunity to examine the effect of turn stereochemistry on the orientation of the anti-parallel strands¹⁴ and to probe cross-strand interactions between sidechains placed at facing positions.^{14,15} Hybrid peptides incorporating β , γ and δ - amino acid residues in the strand facilitate changes of local polarity of the sheets.¹⁶⁻¹⁸ In the case of ^DPro-Gly segments, the nucleating turn can adopt either Type I' ($\phi_{i+1} = 60^\circ$, $\psi_{i+1} = 30^\circ$, $\phi_{i+2} = 90^\circ$, $\psi_{i+2} = 0^\circ$) or Type II' ($\phi_{i+1} = 60^\circ$, $\psi_{i+1} = -120^\circ$, $\phi_{i+2} = -80^\circ$, $\psi_{i+2} = 0^\circ$) conformations, since the achiral Gly residue can readily be accommodated at the $i+2$ positions of both turn types. Sequence variations in the strand segment permit analysis of cross-strand interactions and intra-strand interactions between proximal sidechains. The nature of sidechain interactions in ideal β -hairpins is schematically illustrated in Figure 1. In an earlier study, we have characterized a β -hairpin conformation for the peptide Boc-Leu-Phe-Val-^DPro-Gly-Leu-Phe-Val-OMe, in solution by NMR spectroscopy.¹⁵ Interestingly, an anomalous circular dichroism spectrum for this octapeptide was observed in the region 210 - 240 nm suggesting of cross-strand aromatic interactions.¹⁵ This segment has also been shown to exist as a β -hairpin, when incorporated into a larger 17-residue sequence that adopts a mixed helix-hairpin structure.¹⁰ Similar sidechain interactions were also established in the peptide, Boc-Leu-Phe-Val-Aib-^DAla-Leu-Phe-Val-OMe, in which an Aib-^DAla (Type I' β - turn) nucleated a β -hairpin conformation in crystals.¹⁴ In order to investigate the effects of cross-strand aromatic interactions in β -hairpins, we have designed and subjected to conformational analysis the following octapeptides:

1(Boc-Leu- β Phe-Val-^DPro-Gly- Leu- β Phe-Val-OMe)

(β Phe = (S)- β^3 - homophenylalanine)

2(Boc- β Leu- Phe- β Val-^DPro-Gly- β Leu-Phe- β Val-OMe).

(β Leu = (S)- β^3 - homoleucine) and (β Val = (R)- β^3 - homovaline)

These sequences were based on the parent peptide Boc-Leu-Phe-Val-^DPro-Gly-Leu-Phe-Val-OMe (peptide **3**). Replacement of Phe 2 and Phe 7 by β Phe and retention of the β -hairpin conformation should place the two Phe rings on opposite faces of an approximately planar β -sheet structure, eliminating cross-strand interactions. In peptide **2**, replacement of Val 3 and Leu 6 by their higher homologs places the phenyl rings, Phe 2 and Phe 7, at facing *hydrogen-bonded* positions in a β -sheet, in contrast to the parent peptide **3**, where the Phe rings are at facing *non-hydrogen bonded* positions. Peptide **2** also contains β -residues at positions 1 and 8, β Leu and β Val, respectively. This substitution does not affect the aromatic sidechain position but merely alters the sheet polarity at the two termini. The results described in this paper establish β -hairpin conformations in solution for both peptides **1** and **2**. In addition, X-ray diffraction studies establish a β -hairpin structure for peptide **1** in crystals. Peptide **1** yields a CD spectrum similar to that normally observed in peptide β -hairpins, while peptide **2** yields an anomalous CD spectrum.

MATERIALS AND METHODS

Peptide Synthesis and Crystallization

The two octapeptides peptide **1** and peptide **2** were synthesized by conventional solution-phase procedures, using a fragment condensation strategy. t-Butyloxycarbonyl (Boc) and methyl groups are used for N- and C-terminal protection. Boc-(S)- β Phe-OH, Boc-(S)- β Leu-OH and Boc-(R)- β Val-OH were synthesized by Arndt-Eistert homologation of Boc-(S)-Phe-OH, Boc-(S)-Leu-OH and Boc-(S)-Val-OH (note the formal change of configuration assignment upon

homologation), respectively.^{19,20} Peptide couplings were mediated by N,N' -dicyclohexylcarbodiimide and 1-hydroxy benzotriazole.¹⁸ Crude peptide **1** was purified by medium-pressure liquid chromatography on a reverse-phase C_{18} (40–63 μ) column and crude peptide **2** was purified by HPLC on a C_{18} (5–10 μ) column using methanol–water gradients.

Peptides were characterized by ESI-MS: Peptide **1**, $M+H^+ = 1033.7$ Da and $M+Na^+ = 1055.6$ Da (M calc = 1032 Da) ; Peptide **2** , $M+Na^+ = 1083.6$ Da (M calc = 1060 Da) and complete analysis of the 500 MHz 1H -NMR spectrum. Single crystals suitable for X-ray diffraction for peptide **1** were obtained by slow evaporation from ethanol–toluene–xylene solvent mixtures. The synthesis and characterization of peptide **3** has been previously described.¹⁵

X-ray Diffraction

Crystals in the form of thin plates were obtained by slow evaporation from ethanol containing some toluene and xylene. A crystal, $1.00 \times 0.56 \times 0.06$ mm in size, was used to collect X-ray diffraction data on a Bruker Smart CCD 6K diffractometer. A high intensity rotating anode (Cu radiation) X-ray source was used to collect data to a preset limit of $\theta=67^\circ$ (0.84 Å resolution) at $-75^\circ C$. Even at the limit of the setting of the apparatus, there were reflections with usable intensities. However, the structure solution was not automatic, possibly because the $hk\ell$ reflections with $h=2n$ were much stronger than those with $h \neq 2n$. A vector search procedure based on a hairpin model taken from the decapeptide Boc-Leu-Val- β Phe-Val- D Pro-Gly-Leu- β Phe-Val-Val-OMe molecule was not successful, in retrospect, possibly because the β -turns are of different types in the two crystals. However, 15,000 trials with the SHELX program²¹ led to a successfully placed fragment that was used with the tangent formula expansion²² to obtain the entire structure consisting of two independent peptide molecules and two ethanol solvent molecules in an asymmetric unit. Hydrogen atoms were placed in idealized positions and allowed to ride on the C or N atom to which they are bonded. Anisotropic least-squares refinement produced an R-factor of 7.5% for 14439 data with $|F_{obs}| > 4\sigma(F_o)$ and 8.7% for all 17056 data. For the crystal with a formula content of 2 ($C_{55}H_{84}N_8O_{11} \cdot 2(C_2H_5OH)$), the crystallographic parameters are: space group $P2_1$, $a = 19.555$ (1) Å, $b = 11.352$ (1) Å, $c = 28.912$ (1) Å, $\beta = 101.909$ (2) $^\circ$, $V = 6259.8$ (4) Å³, $Z = 4$, $d_{calc} = 1.145$ gm/cm³.

Details of data collection, coordinates, bond lengths and angles, anisotropic thermal parameters and hydrogen coordinates are deposited in the Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, UK, ref. CCDC #225008.

RESULTS AND DISCUSSION

Conformational Analysis of Peptide 1

Molecular conformation in crystals—The conformations of the two crystallographically independent molecules A and B of peptide **1**, molecule A shown in stereo in Figure 2, are very similar, but not identical. There are four areas of significant differences, that is, more than 30° in the torsional angles that are listed in Table I. In the backbone, three differences occur at ψ_3 , 37° for the $N_3C_3AC_3'N_4$ torsion just preceding the D Pro residue; at ψ_5 , 33° for the $N_5C_5AC_5'N_6$ torsion; and at ϕ_6 , 33° for the $C_5'N_6C_6AC_6'$ torsion. In the side chains there is one difference at Val 3 where the rotation about C_3A-C_3B is *gauche*⁺ in one conformer and *gauche*[−] in the other. All these differences occur in the vicinity of the hairpin turn. Despite the differences in several of the torsional angles, the hydrogen bonds in both conformers A and B are essentially the same, both cross-strand, Figure 2, and intermolecular, Table II.

Figure 3 compares the structures determined previously for the decapeptide, Boc-Leu-Val- β Phe-Val- D Pro-Gly-Leu- β Phe-Val-Val-OMe, which differs from peptide **1** in having an additional Val residue at both the N and C-termini. The decapeptide hairpin has four

intramolecular hydrogen bonds, whereas peptide **1** has three intramolecular hydrogen bonds. The nature of the nucleating turn differs in the two cases. In peptide **1**, a Type II' turn has formed, a feature found commonly in hairpins, whereas in the decapeptide a Type I' turn is seen. The superposition of structures shown in Figure 3 clearly illustrates the differences in orientation of the turns in two cases. In each case, the NH and C=O moieties at the top of the hairpin participate in hydrogen bonding with mediating solvent molecules. In the direction lateral to the β -hairpins, hydrogen bonds between the strands of separate molecules link them into infinite β -sheets in both peptide **1** and the decapeptide crystals. The individual hairpins in peptide **1** have their head groups pointed in the same direction (Figure 4).

A view of the crystal structure of **1** edge-on to the β -sheets is shown in Figure 5. There is a continuous vertical connection between alternating right and left hairpin molecules by an EtOH molecule that forms an $\text{OH}\cdots\text{O}=\text{C}$ hydrogen bond with the peptide "head" of the molecule below [$\text{O1EA}\cdots\text{O4}$] and an $\text{O}\cdots\text{HN}$ with the peptide "head" of the molecule above [$\text{O1EA}\cdots\text{N5}$]. At the "tail-to-tail" region there is a slight interdigitation of the nonpolar tails (not shown).

Conformational Analysis of Peptide 1—Peptide **1** yields sharp 500 MHz ^1H -NMR spectrum in methanol- d_3 (CD_3OH). In chloroform and benzene, a broad spectrum, characteristic of aggregated species are observed. Addition of a small amount of (5.66 % v/v) methanol to CDCl_3 , results in sharpening of NH resonances. All subsequent studies were done in neat CD_3OH at a peptide concentration of (~ 6.8 mM) at which aggregation effects are insignificant. Sequence specific assignments of the amide resonances were readily achieved using TOCSY and ROESY experiments. The relevant NMR parameters are summarized in Table III. The large $^3\text{J}_{\text{NH}-\text{C}^\alpha\text{H}}$ values (for α - residues) are consistent with extended strand conformations for residues 1-3 and 6-8. The observation of the Val 3 (NH) $\leftrightarrow$ Leu 6 (NH) NOE (d_{NN} NOE) supports the antiparallel registry of the two strands (Figure 6). The strong Gly 5 (NH) $\leftrightarrow$ Leu 6 (NH) NOE (d_{NN} NOE) is characteristic of the Gly residue occupying the $i+2$ position of a β -turn. The strong NOE between $^{\text{D}}\text{Pro}$ 4 (C^αH) and Gly 5 (NH) ($d_{\alpha\text{N}}$ NOE) is supportive of a ψ value $\sim +120^\circ$ at $^{\text{D}}\text{Pro}$ 4, providing support for a $^{\text{D}}\text{Pro}$ – Gly Type II' β - turn. A weak NOE between Leu1 (NH) and Val 8 (C^αH) (Figure 6) is notable. This NOE may arise when Leu1 (NH) points inward into the hairpin. The strong Leu 1(NH) $\leftrightarrow$ βPhe2 (NH) NOE suggests that local helical conformations may be also populated. It should be noted that the orientations of the Leu 1 and Val 8 residues are essentially unconstrained by hairpin formation. Interestingly, in the crystal structure of peptide **1**, both Leu1 and Val 8 adopt extended conformations:

Leu 1 $\phi = -94.4^\circ$, $\psi = 125.6^\circ$ (molecule A), $\phi = -113.9^\circ$, $\psi = 125.5^\circ$ (molecule B) ;

Val 8 $\phi = -156.8^\circ$, $\psi = 143.8^\circ$ (molecule A), $\phi = -154.8^\circ$, $\psi = 122.2^\circ$ (molecule B)

(Note Val 8 ψ is determined using the co-ordinate of the oxygen atom of the terminal OMe group as the fourth atom.)

This is undoubtedly a consequence of the formation of intermolecular β - sheet hydrogen bonds involving the backbone NH and CO groups of Leu 1 and Val 8 residues. Figure 7 shows a schematic view of the hairpin in peptide **1**, illustrating the major NOEs which are diagnostic of a β - hairpin structure.

Conformational Analysis of Peptide 2—Peptide **2** yielded broad backbone resonances in CDCl_3 solution, indicative of extensive aggregation. Sharp well- resolved 500 MHz ^1H -NMR spectra were obtained in deuterated methanol (CD_3OH) solution. Resonance assignments were readily achieved using a combination of TOCSY and ROESY experiments.

The relevant NMR parameters are summarized in Table IV. Figure 8 shows a partial ROESY spectrum illustrating key NOEs. The observation of the Phe 2 (NH) $\leftrightarrow$ Phe 7 (NH) (d_{NN} NOE) is supportive of a β -hairpin structure. The NOE β Val 3 (C^β H) $\leftrightarrow$ β Leu 6(NH) ($d_{\beta N}$ NOE) and β Leu 1(NH) $\leftrightarrow$ β Val 8 (C^β H) ($d_{N\beta}$ NOE) also supports the anti-parallel hairpin structure. The Gly 5(NH) and β Leu 6(NH) (d_{NN} NOE) is indicative of the anticipated chain reversal at the D Pro-Gly segment. The NOEs supportive of a β -hairpin in peptide **2** are schematically illustrated in Figure 7. Interestingly, a strong D Pro4 (C^α H) $\leftrightarrow$ Gly 5 (NH) ($d_{\alpha N}$ NOE) and a weak D Pro4 (C^δ H) $\leftrightarrow$ Gly 5 (NH) ($d_{\delta N}$ NOE) are both observed. The simultaneous observation of these NOEs suggests that both Type I' and Type II' β -turn conformations are populated at the D Pro-Gly turn segment. While the majority of D Pro-Gly turn segments characterized crystallographically in peptide β -hairpins adopt Type II' conformations, Type I' turns have also been observed.^{16,18} The weak d_{NN} NOEs β Leu 1(NH) $\leftrightarrow$ Phe 2 (NH), Phe 2 (NH) $\leftrightarrow$ β Val 3 (NH) and Phe 7 (NH) $\leftrightarrow$ β Val 8 (NH) are not compatible with a completely rigid hairpin and suggest fraying of the structure at the N- and C- terminus ends. Such fraying is a relatively common feature in CD₃OH solution, since solvent competition for backbone hydrogen bonding sites tends to destabilize the hairpins.

Circular Dichroism—Figure 9 illustrates the CD spectra for peptides **1** to **3** in methanol solution. Peptide **1** has a broad negative CD band with a minimum at 224 nm. The CD spectra of model peptide β -hairpins which do not contain aromatic residues have been shown to yield a broad negative band at 214 - 220 nm.^{8, 14-15, 23-24} The origins of the red shift in the case of **1**, is not clear. It is conceivable that this is a consequence of the fact that the strand residues are made up of both α - and β - amino acids, resulting in differences in the orientation of peptide chromophores as compared to all α - peptide cases. Notably, the CD spectrum of peptide **2** is anomalous, yielding a negative band at 234 nm and a positive band at 221 nm. This observation may be rationalized by invoking an exciton interaction between the phenyl chromophores of Phe 2 and Phe 7. A comparison of the CD spectrum of peptide **2**, with that of parent peptide **3** is also instructive. In peptide **3**, negative bands are observed at 235 nm and 210 nm, Figure 9.¹⁵ A positive band at 224 nm overlapped with a lower wavelength negative CD band may be the reason for non-observation of an exciton split doublet, which is seen for peptide **2** and also for octapeptide Boc-Leu-Phe-Val-Aib- D Ala-Leu-Phe-Val-OMe.¹⁴ Figure 9 also illustrates the orientation of the facing aromatic residues observed in crystals of peptide **1** and the β -hairpin segment corresponding to the segment of peptide **3** in a 17-residue helix-hairpin peptide. In peptide **1**, the centroid-to-centroid distance between the aromatic rings are 8.92 Å (for molecule A) and 8.94 Å (for molecule B). The corresponding distance between facing Phe residues in the *non-hydrogen bonded* position determined for the Boc-Leu-Phe-Val- D Pro-Gly-Leu-Phe-ValOMe hairpin segment in a 17- residue peptide is 5.03 Å. In the crystal structure of BocLeu-Phe-Val-Aib- D Ala-Leu-Phe-Val-OMe, the Phe-Phe centroid-to-centroid distance is 5.52 Å.¹⁴ Crystals of peptide **2** suitable for X-ray diffraction were not obtained, despite several attempts. In order to estimate the inter- aromatic distance, Phe rings were modeled into the *hydrogen bonding position* of a β -hairpin. Analysis of protein structures reveals that for most aromatic pairs at *hydrogen bonding* sites in β -sheets, the favoured combination of sidechain conformations are g^-t and g^+g^- .^{25, 26} For this combination of sidechain torsion angles, aromatic rings are proximal (centroid – to – centroid distance: 4.78 Å (g^+g^-)) (Figure 9). However, in these orientations, the closest contact involves the C^β atom of one residue and the aromatic ring of the other.

Differences in the orientations of the two facing aromatic rings in peptide **1** to **3** are also evident from a comparison of the NMR chemical shifts of the aromatic proton resonances. From the data presented in Figure 10, it is clear that the aromatic protons in peptides **1** and **3** are shifted distinctly upfield, as compared to the corresponding resonances in peptide **2**. Indeed, in peptide **3**, there is an upfield shift of ~ 0.4 ppm of the Phe 7 [H2, H6] resonances. The ring current shifts observed for peptide **2** are much less pronounced. Interestingly, a small downfield shift

is observed for the Phe ring proton resonances of peptide **2** as compared to peptide **1**. This feature is consistent with the inter-ring orientation illustrated in Figure 9, which would place the aromatic protons of Phe **2** in the deshielding region of the proximal Phe **7** group. It is pertinent to note that the crystallographically determined Phe orientations in peptide **3** (Figure 9) places the C^βH of Phe **2** in the shielding region of the aromatic ring current of Phe **7**. Interestingly, for peptide **3**, the observed C^βH₂ proton chemical shifts reveal pronounced nonequivalence of the two Phe2 C^βH₂ protons (2.79 ,3.12 ppm) as compared to Phe **7** C^βH₂ protons (2.85 ppm).

Aromatic interactions may have been frequently invoked as contributors to the stability of folded structures in proteins and peptides.²⁷⁻³² Analysis of inter-ring orientation has revealed that there may be strong preference for either stacked (interplanar angle = 0°) or perpendicular (interplanar angle = 90°) ring orientation. A survey of Phe ring orientations in several peptide crystal structures is consistent with a broad energy minimum for an interacting aromatic pair and the absence of pronounced angular dependence. These observations are also consistent with the result of theoretical calculations for interacting benzene rings.³³ Aromatic interactions are generally considered to be stabilizing for inter-ring distances ~ 4.5 - 7 Å.^{34, 35} It is likely that the close approach of aromatic rings may result in anomalous circular dichroism. In addition to proximity, restriction of sidechain mobility and adoption of a fixed rotamer about the C^α – C^β may also be contributors to the observed circular dichroism.

CONCLUSIONS

Defined peptide hairpins provide an opportunity to explore sidechain interactions in β-sheet structures. The result presented here establish that aromatic interactions may be important contributors to the observed circular dichroism, when residues involved occupy facing positions across a pair of antiparallel strands. Anomalous CD spectra are observed when the interacting pair occupies either *hydrogen bonding* or *non-hydrogen bonding* positions in an anti-parallel sheet. Our results emphasize the utility of DPro-Gly segments in nucleating hairpins in hybrid peptide containing α- and β- amino acids. The utility of mixed α/β-sequences in altering sidechain dispositions in designed peptides is also exemplified in these structures described here.

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Figure 1. Schematic of a peptide β -hairpin indicating the nature of sidechain interactions. (a) $i/i+2$ intra-strand interactions (b) cross-strand interaction between facing residues. Two distinct sites are defined, hydrogen bonding and non-hydrogen bonding sites. (c) cross-strand diagonal interactions.

FIGURE 2

Figure 2.
Stereodiagram of conformer A of Boc-Leu- β Phe-Val-^DPro-Gly-Leu- β Phe-Val OMe (Peptide 1).

FIGURE 3 **Figure 3.**

Superposition of peptide **1** (solid line) and Boc-Leu-Val-βPhe-Val-^DPro-Gly-Leu-βPhe-Val-Val-OMe (dashed line) by least squares fit of corresponding C^α atoms in the strands. Note the differences between type I' β-turn for Boc-Leu-Val-βPhe-Val-^DPro-Gly-Leu-βPhe-Val-Val-OMe and Type II' β-turn for peptide **1**.

FIGURE 4

Figure 4. Assembly of peptide **1** conformers A and B into an extended β -sheet by intermolecular $\text{NH}\cdots\text{OC}$ hydrogen bonds. All the hairpin molecules have their “head” groups directed to the top of the diagram.

FIGURE 5

Figure 5. Packing viewed edge-on to the β -sheets in peptide **1**. The hydrogen bonds, mediated by EtOH molecules, in the polar “head-to-head” region are indicated.

FIGURE 6 **Figure 6.**

Partial 500 MHz ROESY spectrum of Boc-Leu-βPhe-Val-^DPro-Gly-Leu-βPhe Val-OMe (**1**) in CD₃OH at 300K. (Top) C^αH↔NH NOEs (α-residues) and C^βH↔NH NOEs (β-residues). (Bottom) NH↔NH NOEs. Key NOEs are marked.

FIGURE 7

Figure 7. Schematic representation of hairpin structures for peptides **3**, **1** and **2** illustrating key NOEs, which are consistent with the hairpin conformation. For other NOEs, see the text.

Figure 8. Partial 500MHz ROESY spectrum of Boc-βLeu-Phe-βVal-^DPro-Gly-βLeu-Phe βVal-OMe (2) in CD₃OH at 300K. (Top) $C^{\alpha}H \leftrightarrow NH$ NOEs (α-residues) and $C^{\beta}H \leftrightarrow NH$ NOEs (β-residues). (Bottom) $NH \leftrightarrow NH$ NOEs. Key NOEs are marked.

Figure 9.

Far UV CD spectra of peptides **3**, **1** and **2** in methanol at 300K. “d” refers to the centroid-to-centroid distance.

FIGURE 10

Figure 10. Partial 500 MHz ^1H -NMR spectra of peptides **3**, **1** and **2** in CD_3OH at 300K. Aromatic proton resonances are shown.

Table ITorsion Angles For Peptide 1^a

Residue Name	Torsion Angles (°)					
	ϕ	θ	ψ	χ^1	χ^2	χ^3
Leu1	-94.4 (-113.9)		125.6 (125.5)	176 (173.4)	63.7, -174.1 (62.7, -175.5)	
β Phe2	-141.1 (-145.4)	150.2 (160.5)	158.8 (151.1)	56.3 (54.6)	-76.7, 99 (-78.2, 100.7)	
Val 3	-153.7 (-139.3)		122.6 (86.3)	50, 179.1 (-45.4, -169.7)		
^D Pro 4 ^b	55.7 (64.5)		-130.5 (-122.2)	21.6 (11.2)	-28.9 (-29.1)	24.2 (35.2)
Gly 5	-83.3 (-73.1)		20.4 (-11.5)			
Leu 6	-117.6 (-85.8)		113.1 (109.2)	170.5 (174.2)	58.6, -176.2 (-175.9, 57.3)	
β Phe7	-101 (-91.9)	166.7 (166.3)	118.5 (115.5)	-50.1 (-58.9)	150.8, -26.5 (-15.3, 163.6)	
Val 8	-156.8 (-154.8)		143.8 (122.2 ^c)	57.8, -174.1 (179, 54.4)		

^aThe torsion angle values given without bracket are for molecule A and the torsion angle values given within bracket are for molecule B.

^b χ^4 : -9.7 (-29.3); χ^5 (C δ -N-C α -C β): -7.7 (11.4) .

^cTorsion around N8-C8-C8'-O9.

Table II

Hydrogen bonds in Peptide 1

H-Bond type	Donor ^a	Acceptor ^a	d(D...A) [Å]	d(H...A) [Å]	D...O=C angle [°]
Intramol.	N2 (Mol A)	O7 (Mol A)	2.92	2.02	175.61
Intramol.	N3 (Mol A)	O6 (Mol A)	2.91	2.04	161.45
Intramol.	N6 (Mol A)	O3 (Mol A)	2.99	2.06	160.40
Intramol.	N12 (Mol B)	O17 (Mol B)	2.95	2.06	171.88
Intramol.	N13 (Mol B)	O16 (Mol B)	2.82	1.93	172.90
Intramol.	N16 (Mol B)	O13 (Mol B)	2.99	2.15	155.83
Intermol.	N1 (Mol A)	O18 (Mol B)	2.96	2.13	152.84
Intermol.	N7 (Mol A)	O12 ^b (Mol B)	2.95	2.05	175.24
Intermol.	N8 (Mol A)	O11 ^c (Mol B)	2.94	2.06	161.37
Intermol.	N11 (Mol B)	O8 ^d (Mol A)	3.02	2.13	167.96
Intermol.	N17 (Mol B)	O2 (Mol A)	2.95	2.06	169.36
Intermol.	N18 (Mol B)	O1 (Mol A)	2.94	2.05	171.60
EtOH-pept.	O2EA	O14	2.76	1.91	179.46
EtOH-pept.	N5	O1EA ^e	2.83	1.93	173.81
EtOH-pept.	N15	O2EA ^f	2.85	1.96	170.66

^aFor assignments see Figure 2 and Figure 4.

^bAt symmetry equivalent $x-1, +y, +z$

^cAt symmetry equivalent $x-1, +y, +z$

^dAt symmetry equivalent $x+1, +y, +z$

^eAt symmetry equivalent $-x+1, +y+1/2, -z+1$

^fAt symmetry equivalent $-x+2, +y+1/2, -z+1$

Table III¹H-NMR parameters for Peptide 1 in CD₃OH at 300K

Residue Name	δ (ppm)						$^3J_{\text{NH-C}^{\alpha}\text{H}}$ (Hz) ^a
	NH	C ^{α} H	C ^{β} H	C ^{γ} H	C ^{δ} H	Others	
Leu 1	6.51	4.08	C ^{β} H ₂ / C ^{γ} H (1.57/1.34)		0.88		8.7
β Phe 2	7.98	2.50	4.50	2.78,2.87		aro: ~7.25	8.8
Val 3	8.36	4.53	2.10	1.00			7.5
^D Pro 4	—	4.36	C ^{β} H ₂ / C ^{γ} H ₂ (2.03,2.16/2.26)		3.74,3.89		—
Gly 5	8.41	3.80,3.88	C ^{β} H ₂ / C ^{γ} H (1.51,1.74)		0.92		—
Leu 6	8.05	4.38	C ^{β} H ₂ / C ^{γ} H (1.51,1.74)		0.92		8.4
β Phe 7	7.95	2.50	4.44	2.75,2.85		aro: ~7.25	8.6
Val 8	8.28	4.43	2.15	0.95			8.3

^aFor β -residues, the vicinal coupling constant ($^3J_{\text{NH-C}^{\beta}\text{H}}$) is given.

Table IV¹H - NMR parameters for Peptide 2 in CD₃OH at 300K

Residue	$\delta(\text{ppm})$							$^3J_{\text{NH-C}^\alpha\text{H}}^a$ (Hz)
	NH	C $^\alpha$ H	C $^\beta$ H	C $^\gamma$ H	C $^\delta$ H	C $^\epsilon$ H	Others	
β Leu 1	6.24	2.36,2.45	3.94	C $^\gamma$ H ₂ $^\delta$ H (1.09,1.30,1.61)		0.87		9.3
Phe 2	8.33	4.68	2.98,3.12				Aro: ~7.25	8.1
β Val 3	7.84	2.34,2.60	4.32	1.82	0.90			9.2
β Pro 4	—	4.28	C $^\beta$ H ₂ /C $^\gamma$ H ₂ (1.98/2.06/2.25)		3.63			—
Gly 5	8.51	3.80						—
β Leu 6	7.67	2.38,2.66	4.39	C $^\gamma$ H ₂ $^\delta$ H (1.10,1.53,1.63)		0.86		9.4
Phe 7	8.61	4.72	3.03,3.10				Aro: ~7.25	7.8
β Val 8	7.94	2.32,2.45	4.07	1.79	0.88			9.3

^aFor β -residues, the vicinal coupling constant ($^3J_{\text{NH-C}^\beta\text{H}}$) is given.