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Hybrid Peptides: Expanding the β Turn in Peptide Hairpins by the Insertion of β-, γ-, and δ-Residues

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Abstract: The β turn segment in designed peptide hairpins has been expanded by the insertion of β-, γ- and δ-amino acids at the *i*+2 position. The model octapeptides Boc-Leu-Phe-Val-^{D}Pro-Ac_6c-Leu-Phe-Val-OMe (**1**), Boc-Leu-Phe-Val-^{D}Pro-β^3-Ac_6c-Leu-Phe-Val-OMe (**2**), and Boc-Leu-Phe-Val-^{D}Pro-Gpn-Leu-Phe-Val-OMe (**3**) have been shown to adopt β hairpin conformations in methanol by the observation of key diagnostic nuclear Overhauser effects. Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe (**4**) adopts a β hairpin conformation in crystals; this is stabilized by three cross-strand hydrogen bonds as demonstrated by X-ray diffraction. The canonical C_{10} turn in an α–α segment is expanded to C_{11}, C_{12}, and C_{13} turns in α–β, α–γ, and α–δ segments, respectively. The crystal structures of Piv-^{L}Pro-β^3-Ac_6c-NHMe (**5**) and Boc-Ac_6c-Gpn-Ac_6c-OMe (**6**) reveal intramolecularly hydrogen-bonded C_{11} and C_{12} conformations, respectively. Computer modeling of octapeptide sequences that contain centrally positioned hybrid-turn segments, by using turn parameters derived from the structures of peptides **5** and **6**, establishes the stereochemical acceptability of the β hairpins in the cases of peptides **2** and **3**. Accommodation of ω-amino acids into the turn segments is achieved by the adoption of *gauche* conformations around the backbone C–C bonds.

Keywords: amino acids · beta hairpins · peptides · structure elucidation

Introduction

De novo polypeptide design is based on an understanding of the factors that govern the local folding of peptide chains and their subsequent assembly into compact globular structures that are stabilized by tertiary interactions.[1] Modular approaches to the construction of synthetic protein mimics that are being developed in this laboratory have focused primarily on the rational design of the stable elements of secondary structures.[2] β Hairpins, in which two antiparallel strands are connected by a short loop segment, have proved to be an attractive target for "first principles" design.[3] In this structural feature, which is commonly found in proteins, the key elements that determine the chain folding are the centrally positioned turn segments and cross-strand hydrogen-bond registry. In proteins, tight two-residue β turns are most often found at the site of polypeptide chain reversal.[4] Three- and four-residue connecting loops are also found; these are essentially "turning hinges" that are determined by short-range interactions.[4c] The principles underlying the design of peptide β hairpins are understood; there are many reported examples of well-characterized, isolated β hairpins[5,6] and multistranded β sheets.[7] The use of obligatory "prime" β turn segments formed by ^{D}Pro-Xxx sequences facilitates the design of conformationally stable β hairpins.[1b,2b,c]

The generation of β hairpins that contain centrally positioned two- and three-residue turns has been a focus of at-

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tention (Figure 1). Two-residue turns are stabilized by a 10-atom (C_{10}) 4→1 hydrogen bond between the CO_i and NH_{i+3} groups. β Turns were first recognized as a key element in protein structures almost four decades ago and have been

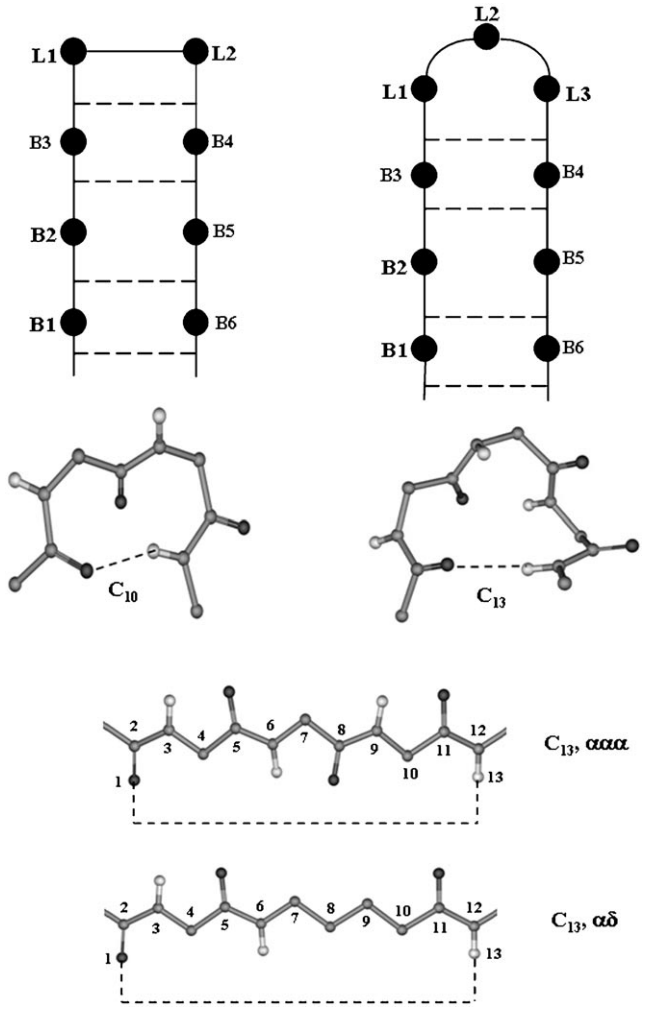

Figure 1. Top: Schematic representation of β hairpins generated with central two-residue (left) and three-residue (right) loops. B*n* are the strand residues and L*n* are the turn residues. Middle: C_{10}, 4→1 (left) and C_{13}, 5→1 (right) hydrogen-bonded turns. Bottom: Schematic view of 13-atom (C_{13}) hydrogen-bonded rings formed in α–α–α and α–δ sequences.

the subject of extensive study for the last several years.[8] Tight two-residue turns, which result in a 180° chain reversal of the polypeptide backbone, can be described by considering the positions of four backbone C^α atoms of residues that are designated as $i \rightarrow i+3$. In tight turns, the $C^\alpha_i \rightarrow C^\alpha_{i+3}$ distance is ≤ 7.0 Å.[8d] The conformational characteristics of β turns are conveniently described by the ϕ and ψ torsion angles at the $i+1$ and $i+2$ residues, which form the corners of the turn: type I, ($\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$; $\phi_{i+2} = -90°$, $\psi_{i+2} = 0°$), type II ($\phi_{i+1} = -60°$, $\psi_{i+1} = +120°$, $\phi_{i+2} = +80°$, $\psi_{i+2} = 0°$) and type III ($\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$; $\phi_{i+2} = -60°$, $\psi_{i+2} = -30°$). For, the enantiomeric type I′, II′, and III′ β turns, the signs of all torsion angles are inverted. Types I/I′ and III/III′ are extremely closely related and can be considered together. Types I (I′) and II (II′) are related by a 180° flip of the central peptide unit; this involves a simultaneous change in the torsion angles ϕ_{i+1} and ψ_{i+2}.[9] Type I′ and II′ β turns favor the formation of β hairpins. In a recent study, the successful construction of a β hairpin that incorporates a three-residue turn has been demonstrated for the nonapeptide Boc-Leu-Phe-Val-^{D}Pro-^{L}Pro-^{D}Ala-Leu-Phe-Val-OMe.[10] The three-residue loop is stabilized by a 13-atom (C_{13}) 5→1 hydrogen bond between the CO_i and NH_{i+4} groups (Figure 1). Earlier studies in this laboratory have demonstrated the feasibility of inserting β- and γ-residues into host α-peptide sequences without the disruption of the helical folding patterns.[11] Stimulated by the recent explosion of work on the structural properties of peptides that contain β-, γ-, and higher ω-amino acids,[12] we have undertaken a program to systematically explore the construction of folded peptide structures by using hybrid sequences of α- and ω-amino acids.[13] In this report, we examine the possibility of expanding the β turn segment in peptide hairpins by the insertion of α-, β-, γ- and δ-amino acid residues. The conventional C_{10} β turn that is formed by an α–α segment may be expanded to C_{11}, C_{12}, and C_{13} turns in α–β, α–γ, and α–δ sequences, respectively. A α–δ sequence may be viewed as a surrogate for a three-residue α–α–α segment, because both contain the same number of backbone atoms[11b] (Figure 1). We describe herein the definitive characterization of peptide β hairpins that contain centrally positioned α–β, α–γ, and α–δ segments. The β-residue, 1-aminocyclohexaneacetic acid, (β^3-Ac_6c) and the γ-residue, 1-(aminomethyl)cyclohexaneacetic acid, (gabapentin, Gpn)[14] have been chosen because the presence of dialkyl substituents at the C^α- and C^β-positions imposes constraints on the flanking backbone torsion angles. The Thorpe–Ingold effect dictates the conformational restrictions around C–C single bonds that are attached to tetrasubstituted carbon atoms.[15] In the case of amino acid residues, the most extensively studied system that illustrates the effect of *gem*-dialkyl substituents on restricting allowed conformational space is the Aib residue.[16]

NMR spectroscopic studies of three octapeptides, Boc-Leu-Phe-Val-^{D}Pro-Ac_6c-Leu-Phe-Val-OMe (**1**; Ac_6c, 1-aminocyclohexane-1-carboxylic acid), Boc-Leu-Phe-Val-^{D}Pro-β^3-Ac_6c-Leu-Phe-Val-OMe (**2**), and Boc-Leu-Phe-Val-^{D}Pro-Gpn-Leu-Phe-Val-OMe (**3**) have evidenced hairpin conformations in solution. Peptide **1** has previously been shown to adopt a β hairpin conformation in the solid state.[6i] The β hairpin conformation for the octapeptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe (**4**; δ-Ava, δ-aminovaleric acid) is demonstrated in the solid state by X-ray diffraction, which supports an earlier conformational assignment based on NMR analysis in solution.[17] The crystal structures of the model peptides Piv-^{L}Pro-β^3-Ac_6c-NHMe (**5**) and Boc-Ac_6c-Gpn-Ac_6c-OMe (**6**), which permit the definition of expanded C_{11} and C_{12} β turns, are also described. Characterization of the conformation of the expanded turns permits the construction of stereochemically acceptable models for peptide hairpins that have centrally positioned α–ω hybrid segments.

Results and Discussion

Solution conformation of peptides 1–3: Peptides **1**–**3** yielded sharp, well-resolved NMR spectra in methanol (CD_3OH). Sequence-specific assignments of the backbone $C^{\alpha}H$ and NH resonances were readily achieved by using a combination of TOCSY and ROESY experiments. The relevant NMR parameters for peptides **1**–**3** are summarized in Table 1. Inspection of $^3J(HN,C^{\alpha}H)$ values in all three peptides reveals that residues 1–3, 6, and 8 show very high values, ≥ 8.5 Hz; this strongly supports a ϕ value of $\approx 120°$, which is fully consistent with extended β strand conformations. The NH resonance of Phe7 is the only resonance that exhibits a somewhat smaller $^3J(HN,C^{\alpha}H)$ value of 7.2–7.4 Hz. The temperature dependence of the NH chemical shifts establish that in peptide **1**, the NH groups of the Leu1, Val3, Leu6, and Val8 residues have significantly lower $d\delta/dT$ values than the other three NH groups. In peptide **2**, the NH groups of the Leu1, Val3, Leu6, and Val8 residues have the lowest $d\delta/dT$ values, while in peptide **3**, the NH resonances of Leu1, Leu6, and Val8 have the lowest values. Low $d\delta/dT$ values are consistent with the involvement of NH groups in cross-strand hydrogen bonds, which renders them less accessible to solvation by methanol compared to free NH groups. In an ideal β hairpin structure with ^{D}Pro-Xxx turn segments, the NH groups of the Leu1, Val3, Leu6, and Val8 residues are expected to be intramolecularly hydrogen bonded. In solution, strand fraying can result from solvent competition for hydrogen-bond donor and acceptor groups. Hairpin conformations may be most reliably characterized in solution by the observation of diagnostic NOEs.[6j] Figures 2 and 3 display the partial ROESY spectra of peptides **1**–**3** in CD_3OH; these illustrate the NH↔NH and $C^{\alpha}H$↔$C^{\alpha}H$ NOEs, respectively. The key NOEs that are diagnostic of a hairpin conformation are observed; these are d_{NN} Val3↔Leu6, Leu1↔Val8 and $d_{\alpha\alpha}$ Phe2↔Phe7. In peptide **1**, overlap of the NH resonances of Phe2 and Val8 precludes a clear separation of d_{NN} Leu1↔Val8 and d_{NN} Leu1↔Phe2 NOEs. In peptide hairpins, the observation of the d_{NN} Leu1↔Phe2 NOE suggests fraying of the structure at the termini, a feature that is also observed in crystal structures.[6h,i] The observed backbone NOEs for peptides **1**–**3** are summarized in Figure 4. For peptide **1**, a β hairpin conformation, which is stabilized by three cross-strand hydrogen

Table 1. NMR parameters of peptides **1**, **2**, and **3**.[a]

Residue	Peptide 1				Peptide 2				Peptide 3			
	Chemical shift [ppm]		$^3J(NH,C^{\alpha}H)$ [Hz]	$d\delta/dT$ [ppb K^{-1}]	Chemical shift [ppm]		$^3J(NH,C^{\alpha}H)$ [Hz]	$d\delta/dT$ [ppb K^{-1}]	Chemical shift [ppm]		$^3J(NH,C^{\alpha}H)$ [Hz]	$d\delta/dT$ [ppb K^{-1}]
	NH	$C^{\alpha}H$			NH	$C^{\alpha}H$			NH	$C^{\alpha}H$		
Leu (1)	6.23	4.17	8.6	2.43	6.43	4.16	8.6	2.93	6.55	4.18	8.7	1.20
Phe (2)	8.19	5.30	9.8	8.70	8.02	5.35	9.4	6.47	8.16	5.25	9.0	6.46
Val (3)	8.95	4.49	9.4	5.49	8.78	4.62	8.9	5.89	8.83	4.64	9.2	6.55
^{D}Pro(4)	–	4.47	–	–	–	4.34	–	–	–	4.41	–	–
Xxx(5)	8.14	–	–	9.50	7.56	3.24	–	9.45	7.94	2.34	–	7.18
Leu(6)	8.12	4.45	8.5	4.24	8.41	4.66	8.8	8.18	8.27	4.69	8.8	5.47
Phe(7)	8.47	4.48	7.4	10.38	8.47	4.64	7.3	9.50	8.70	4.67	7.2	9.85
Val(8)	8.16	4.07	9.6	4.45	8.22	4.19	8.9	4.03	8.23	4.22	8.8	5.26

[a] Xxx = Ac_6c (**1**), β^3-Ac_6c (**2**) and Gpn (**3**); solvent CD_3OH.

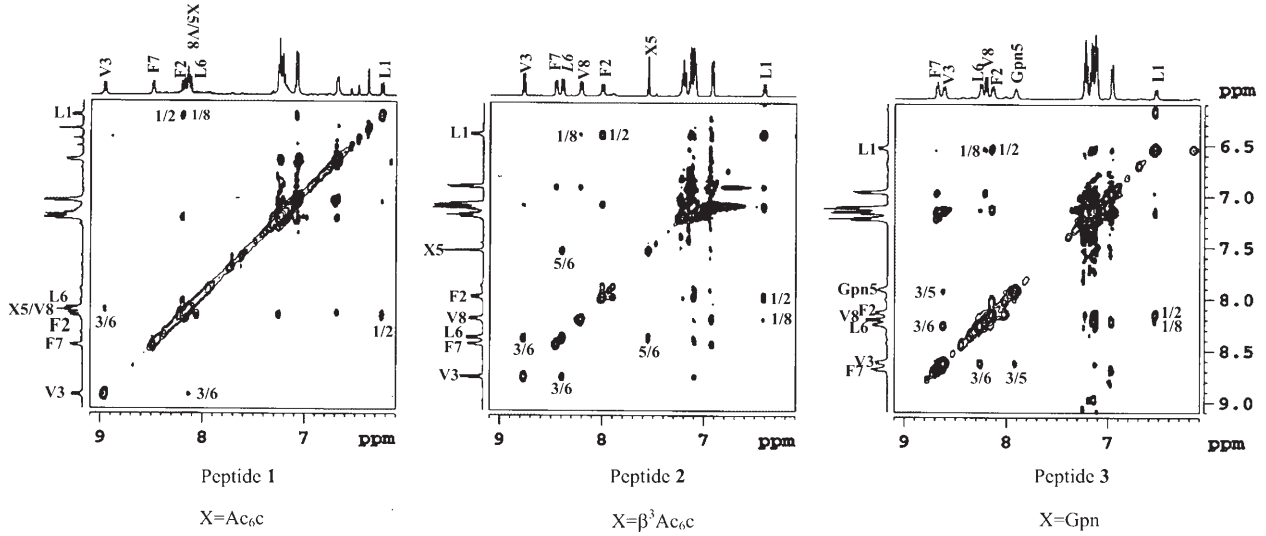

Figure 2. Partial 500 MHz ROESY spectra of peptides Boc-Leu-Phe-Val-^{D}Pro-Ac_6c-Leu-Phe-Val-OMe (**1**), Boc-Leu-Phe-Val-^{D}Pro-β^3-Ac_6c-Leu-Phe-Val-OMe (**2**) and Boc-Leu-Phe-Val-^{D}Pro-Gpn-Leu-Phe-Val-OMe (**3**) in CD_3OH illustrating NH↔NH NOEs.

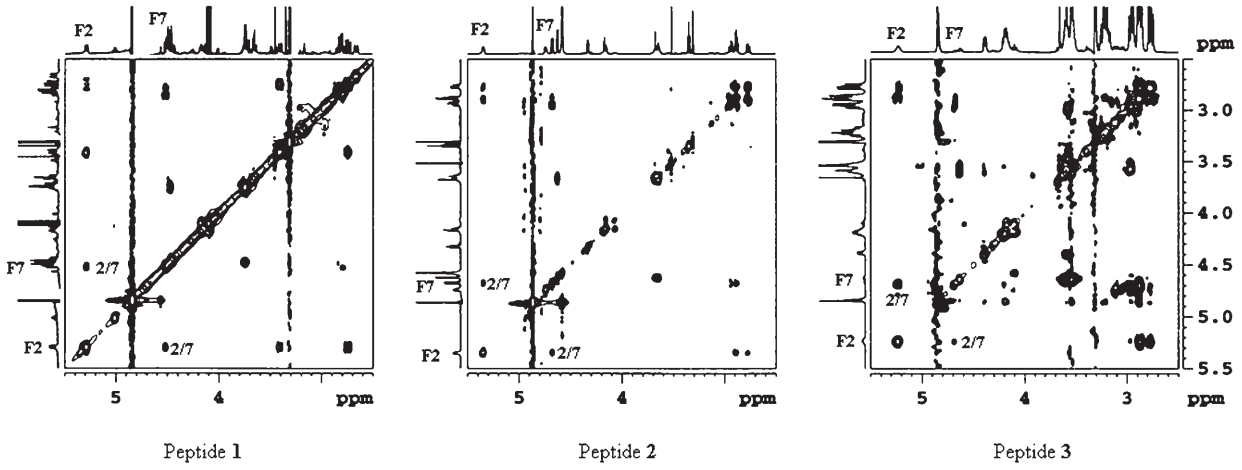

Figure 3. Partial 500 MHz ROESY spectra of peptides **1–3** in CD_3OH illustrating $C^{\alpha}H \leftrightarrow C^{\alpha}H$ NOEs.

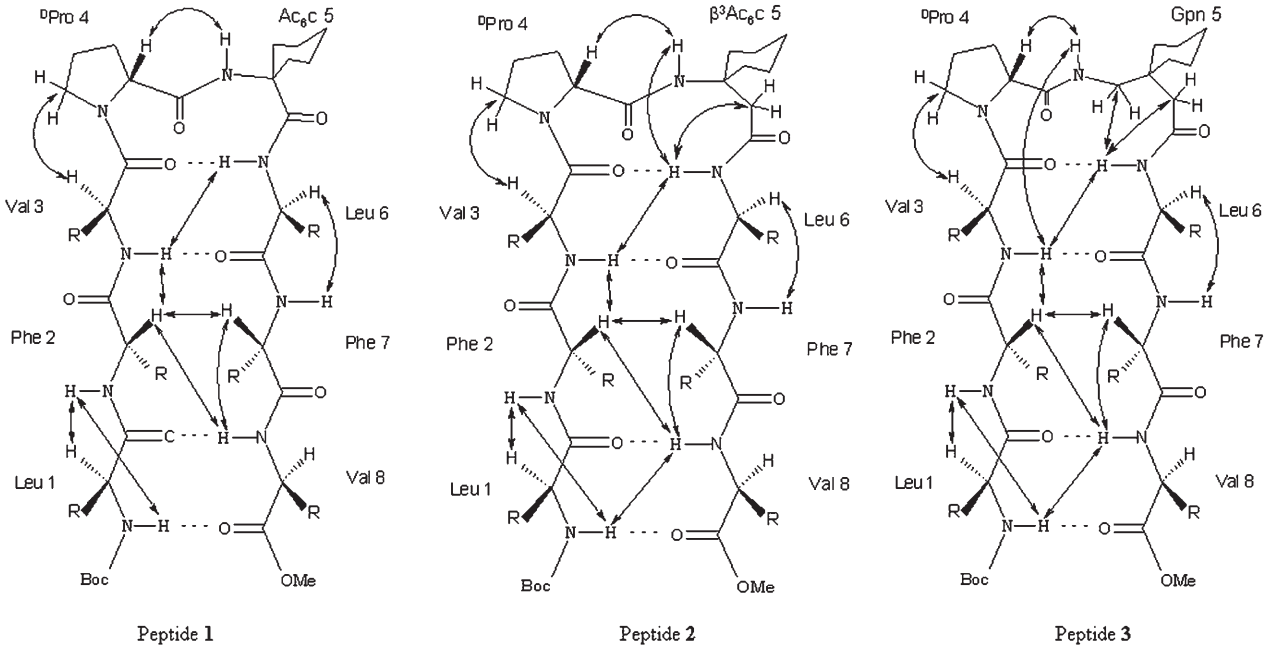

Figure 4. Summary of observed NOEs for peptides **1–3**. Note that with the exception of the Leu1 NH↔Phe2 NH NOE, all other observed NOEs are consistent with the hairpin conformation shown.

bonds, has been established in crystals.[6i] The NMR spectra strongly suggest that the overall backbone conformations of peptides **1–3** are similar.

Crystal structure of peptide 4: In an earlier study, expansion of the turn segment in β hairpins has been reported for the peptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe.[17] NMR spectroscopic studies were consistent with a registered antiparallel hairpin that contains a central ^{D}Pro-δ-Ava (α,δ-hybrid turn), stabilized by a C_{13} hydrogen bond between the Val3 CO and Leu7 NH groups. Single crystals of peptide **4** were obtained several years after the initial solution-phase study, and this permitted the solid-state structural characterization. Figure 5 shows two views of the molecule. The peptide folds into a β hairpin conformation that is stabilized by three interstrand hydrogen bonds. The backbone and side-chain torsion angles are listed in Table 2 (See Table S2, Supporting Information, for hydrogen-bond parameters). A superposition of the β hairpin conformations in peptide **4** and the parent octapeptide Boc-Leu-Val-Val-^{D}Pro-Gly-Leu-Val-Val-OMe[6b] by using a least-squares fit between the backbone atoms of residues 1–3 and 6–8 yields an RMSD of 0.22 Å (See Figure S3, Supporting Information). The arrangement of the antiparallel strands, the end-groups, the side-chains and terminal N–H···O=C hydrogen bonds are very close in the two peptides. The insertion of the -CH_2-CH_2-CH_2- segment into the backbone by the substitution of δ-Ava in peptide **4** for Gly in the parent peptide in the turn

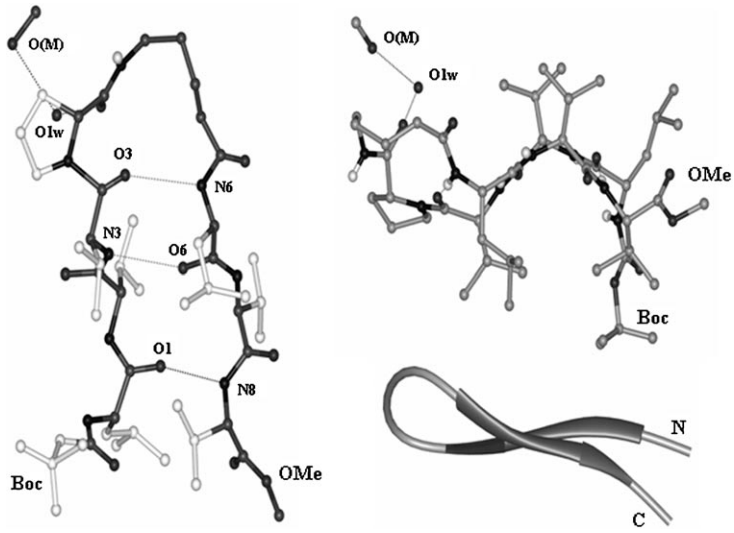

Figure 5. Left: Molecular conformation of the peptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe (**4**) in crystals. Intramolecular hydrogen bonds are shown as dotted lines (N3···O6 = 2.96 Å, ∢N3–H···O0 = 160.5°; N6···O3 = 3.03 Å, ∢N6–H···O3 = 163.2°, N8..O1 = 3.01 Å, ∢N8–H···O1 = 173.2°). Right: Side view of the molecule showing the pleated sheet formation (top) and ribbon representation (bottom).

region has created an extended loop in the backbone and has spread the backbone strands in the region of the pyrrolidine ring by ≈0.9 Å. The edge-on view of the peptide **4** in Figure 5 shows the pleated sheet that is formed by the strands and the extension in the turn region that contains the C5B-C5G-C5D chain from the δ-Ava residue. The assembly of peptide molecules into extended β sheets are facilitated by intermolecular NH···O=C hydrogen bonds between laterally displaced hairpins and are identical to that found in the crystals of β hairpins (see Supporting Information). The β sheet motif is quite similar to that displayed in the crystals of a number of other β hairpin peptides.[6e,h,i]

While the overall β hairpin fold that was determined for peptide **4** in crystals closely resembles the β hairpin fold that was proposed from NMR spectroscopy studies in solution,[17] there are significant differences in the torsion angles that were reported for the δ-Ava residue. It should be noted that the NMR-derived structure used only NOE restraints between the two-strand segments. The absence of key restraints in the turn region renders the precise determination of the conformational parameters of the δ-Ava residue uncertain. It is likely that in expanded turn segments with several inserted methylene groups, multiple loop conformations can be generated which are all compatible with β hairpin conformations.

There are several possible reasons for the different folding of the -CH_2-CH_2-CH_2- moiety. First, aliphatic chains are very flexible. Their flexibility has been demonstrated in a number of crystal structures. For instance, the conformers of a β hairpin peptide with four different γ-residues in the strands gave a distinct conformation for the inserted aliphatic chain for each.[6g] Another example is the polymethylene-bridged cystine-based cyclobisureas; in this case the inserted aliphatic chains of variable length are always disordered.[18] Another reason is that the environment of the peptide molecules is different. In solution, the molecules are surrounded by $CDCl_3$. In the solid state, the turn regions are involved in strong hydrogen bonds with the co-crystallized water and methanol molecules (Figure 5). The water molecule has an array of tetrahedrally disposed, strong hydrogen bonds, with three different peptide molecules, and a methanol molecule (primarily a space-filler; see Figure S4, Supporting Information). The presence and placement of the solvent molecules may influence the conformation of the trimethylene chain in the turn. The final reason is that the torsional angles in a polymethylene chain fragment are usually observed to have values near ±60° and 180°.[19]

It is difficult to evaluate whether the conformation in the turn region is significantly different in the solid state compared to the solution phase. The constant factor is that the strand region of a β hairpin peptide remains unaffected, independent of the physical state and/or insertions of polymethylene segments into the turn region. Furthermore, the bulge that is created in the turn region by the extra -CH_2-CH_2-CH_2- segment provides a scaffold for the desired designed modifications.

Table 2. Backbone and side-chain torsion angles in peptide **4**.[a]

Residue	ϕ	ψ	ω	θ_1	θ_2	θ_3	χ^1	χ^2
Leu (1)	−110.4	−56.7	173.9				−179.6	−172.3, 62.9
Val (2)	−121.0	122.6	−169.3				−54.9, −178.4	
Val (3)	−128.9	119.4	177.4				−52.6, −178.8	
^{D}Pro(4)	64.1	−156.9	−177.7					
δAva (5)	−126.8	76.9	174.5	53.2	63.7	166.5		
Leu (6)	−78.8	115.4	172.1				175.5	-167.7, 64.9
Val (7)	−100.4	113.1	−173.7				−51.4, −175.4	
Val (8)	−90.2	140.1	174.3				−57.3, 175.8	

[a] For nomenclature of amino acid backbone torsion angles (see reference [12f]).

Crystal structures of model α,β and α,γ-hybrid turns: The studies described above established that the loop region in the canonical all-α-residue octapeptide β hairpins can be expanded by the insertion of β-, γ- and δ-amino acids at the *i*+2 position of the central two-residue β turn. While crystallographic evidence is available for the hairpin that incorporates the ^{D}Pro-δ-Ava (α–δ) turn, attempts to obtain crystals for the peptides Boc-Leu-Phe-Val-^{D}Pro-β^{3}-Ac$_6$c-Leu-Phe-Val-OMe (**2**) and Boc-Leu-Phe-Val-^{D}Pro-Gpn-Leu-Phe-Val-OMe (**3**) have been unsuccessful thus far. Structural determination of isolated hybrid turns proved feasible, and permitted further modeling of the expanded loop segment. Single crystals of the model peptides Piv-Pro-β^{3}-Ac$_6$c-NHMe (**5**) and Boc-Ac$_6$c-Gpn-Ac$_6$c-OMe (**6**) were readily obtained. Figure 6 shows the molecular conformation that was determined in the solid state for these two sequences. In peptide **5**, the molecule forms an expanded β turn structure that is stabilized by a C_{11} hydrogen bond between the Piv CO and the NHMe groups. The torsion angles for the Pro residue are similar to those found at the *i*+1 position of type II β turns in α–α sequences. The β^{3}-Ac$_6$c residue adopts a compact structure, with a *gauche* conformation observed about the C^{β}–C^{α} bond. Two water molecules serve as both intra- and intermolecular bridges. In peptide **6**, the Boc-Ac$_6$c-Gpn segment forms an expanded α,γ turn, that is stabilized by a C_{12} hydrogen bond between Boc CO and Ac$_6$c NH groups. In this case, the Ac$_6$c residue at the *i*+1 position adopts a helical (α_R or α_L) conformation, which is similar to that observed in type III/III′ β turns. *Gauche* conformations are observed for the Gpn residue C^{γ}–C^{β} (θ_1) and C^{β}–C^{α} (θ_2) bonds, a feature commonly observed in Gpn peptides.[13b,20] Restrictions on θ_1 and θ_2 are a consequence of the *gem*-dialkyl substitution at the central C^{β} atom of the Gpn residue.

The backbone conformation of the C_{12} α,γ turn is illustrated in Figure 6 (bottom left) and the relevant torsion angles are indicated. Previously, C_{11} α,β turns have been observed in the following structures: Boc-Aib-Aib-βAla-NHMe (Aib: $\phi=-65°$, $\psi=-14°$; β-Ala: $\phi=-88°$, $\theta=71°$, $\psi=-101°$)[21] and CF_3CO-Aib-*cis*-8-aminocyclooct-4-ene carboxylic acid Aib-OMe (Aib: $\phi=-47°$, $\psi=-130°$; β-residue: $\phi=-84.5°$, $\theta=69°$, $\psi=-94°$).[22] It can be noted that the turns fall into two distinct categories, which can be classified in a way analogous to the conventional β turns that are formed by α–α segments.[4a,b,8a] These are type I (α,β),$\phi_\alpha=-65°$, $\psi_\alpha=-14°$ and $\phi_\beta=-88°$, $\theta_\beta=71°$, $\psi_\beta=-101°$ and type II (α,β), $\phi_\alpha=-54.8°$, $\psi_\alpha=135.75°$ and $\phi_\beta=74$, $\theta_\beta=72$, $\psi_\beta=-84.6$. The type I (α,β) turn can be incorporated into helical structures in hybrid sequences that contain both α- and β-amino acid residues.[13a,23] This structural feature may be considered to be a formal expansion of the 3_{10}-helical turn that is formed in all-α-residue sequences. The type II α,β turn can result in an isolated feature that causes polypeptide chain reversal. Figure 6 (bottom right) shows a superposition of the two distinct α,β turn types; these differ in the orientation of the central peptide unit following an approximately 150° flip around the torsion angles $\psi(i+1)$ and $\phi(i+2)$, with concomitant changes in the $\psi(i+2)$ of the β-residue. In both turn types the β-residue adopts a *gauche* conformation around the C^{α}–C^{β} bond.

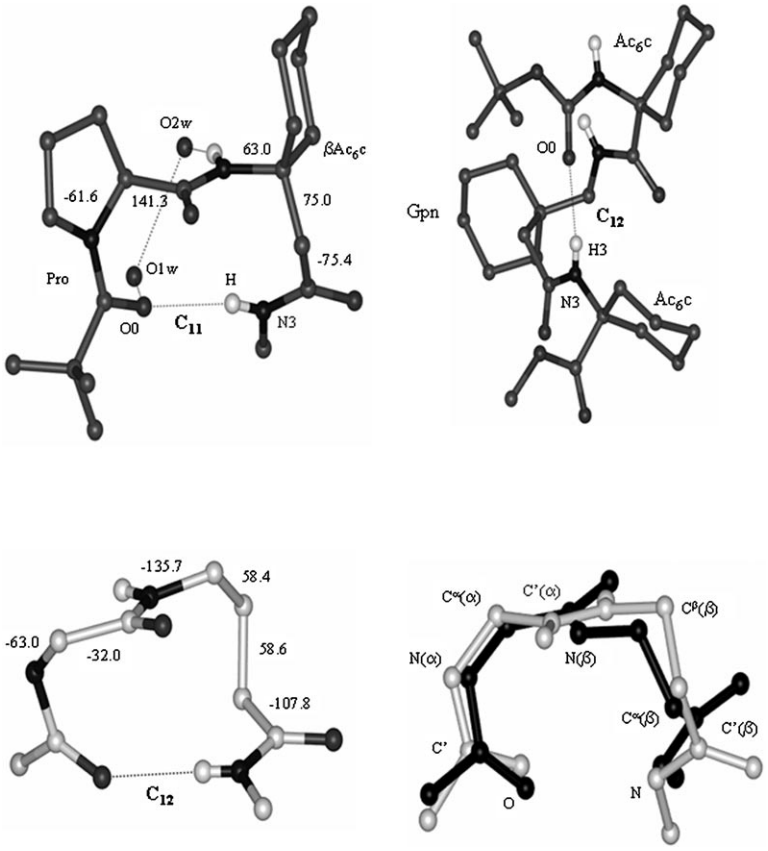

Figure 6. Top left: Molecular conformation in crystals of Piv-Pro-β^{3}-Ac$_6$c-NHMe **5** (C_{11} hydrogen bond, N3···O0 = 2.99 Å; H3···O0 = 2.26 Å; ∢N3–H···O0 = 153.0°. O0···O1w = 2.85 Å, O2w···N2 = 2.99 Å). Top right: Molecular conformation in crystals of Boc-Ac$_6$c-Gpn-Ac$_6$c-OMe, **6** (the figure represents one enantiomeric form of this achiral peptide which crystallized in a centrosymmetric space group; C_{12} Hydrogen bond, N3···O0 = 3.02 Å; H3···O0 = 2.21 Å; ∢N3-H···O0 = 173.5°). Bottom left: 12-Atom turn in Boc-Ac$_6$c-Gpn-Ac$_6$c-OMe. The backbone torsion angles are indicated. Bottom right: Superposition of an 11-atom turn in peptide **5** (gray) and Boc-Aib-Aib-β-Ala-NHMe (black)[22] RMSD = 0.45 Å. (Note the original designation of β-alanine (β-Ala), refers to 3-aminopropanoic acid, which is currently referred to as β-Gly.)[12c]

Modeling of hairpins with expanded turn types: Our inabili-

ty to produce crystals of peptides **2** and **3** prompted us to undertake an evaluation of the stereochemical acceptability of β hairpins with α,β and α,γ turn segments. Starting models were generated by using the α,β (C_{11}) and α,γ (C_{12}) turns that were determined by X-ray crystallography in the structures of peptides **5** and **6**. Three residues were attached to the N and C termini in ideal antiparallel β sheet conformations. Energy minimization with hydrogen-bonding constraints that were determined from NMR experiments resulted in models with completely satisfactory stereochemical parameters. Figure 7 illustrates the structures of the hairpins with expanded β turn segments ^{D}Pro-β^{3}-Ac$_6$c, ^{D}Pro-γ-Abu (γ-Abu, γ-aminobutyric acid) and ^{D}Pro-Gpn. Hydrogen-bond lengths and interstrand C^{α}–C^{α} distances compare well with those determined by crystallography for the ^{D}Pro-Ac$_6$c (α,α)-[6i] and ^{D}Pro-δ-Ava (α,δ) hairpins. [17] The overall similarity of strand registry in all these cases is evident; this suggests that expansion of the central β turn segment is stereochemically feasible, without appreciably distorting the gross fold of peptide's β hairpins. The extent of twist between the strands, which was estimated by using the virtual torsion angle $C3^{\alpha}$-$C4^{\alpha}$-$C5^{\alpha}$-$C6^{\alpha}$, is relatively small in all cases (<30°). Figure 8 compares the turn conformations in the hairpins that contain central α–β and α–γ segments with those that were crystallographically determined in the α,α and α,δ turns. It is clear that α,β, α,γ, and α,δ turns are formal expansions of the canonical type II′ β turn. In all cases, the ^{D}Pro residue retains the conformation necessary for occupying the *i*+1 position in a type II′ β turn. We have demonstrated earlier that type I/III helical turns can be expanded by the insertion of β- and γ-residues.[13a] Notably, the α,δ turn may be viewed as a mimic of the three-residue hairpin-supporting turn, in which a central peptide unit has been replaced by -CH_2-CH_2-CH_2- segment.

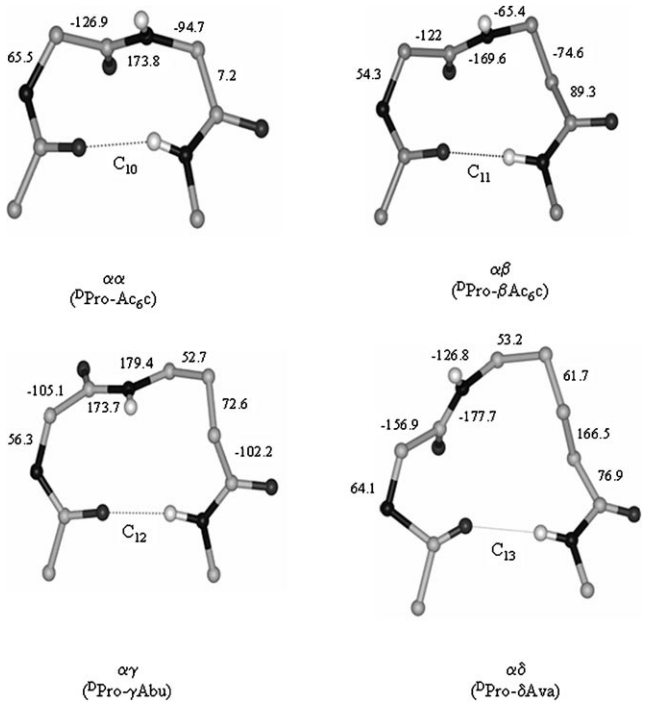

Figure 8. Backbone torsion angles and hydrogen bond parameters for the turn segments in hairpins. Top left: α,α Turn (^{D}Pro-Ac$_6$c), N···O = 2.89 Å; H···O = 2.40 Å; ∢N–H···O = 158° (crystal structure). Top right: α,β Turn (^{D}Pro-β^{3}-Ac$_6$c), N···O = 3.05 Å; H···O = 2.19 Å; ∢N–H···O = 141° (Model). Bottom left: α,γ Turn (^{D}Pro-γ-Abu), N···O = 3.04 Å; H···O = 2.05 Å; ∢N–H···O = 167° (Model). Bottom right: α,δ Turn (^{D}Pro-δ-Ava), N···O = 3.03 Å; H···O = 2.16 Å; ∢N–H···O = 163° (crystal structure).

Conclusion

Hybrid sequences are acquiring increasing importance in the design of biologically active peptides, because of the en-

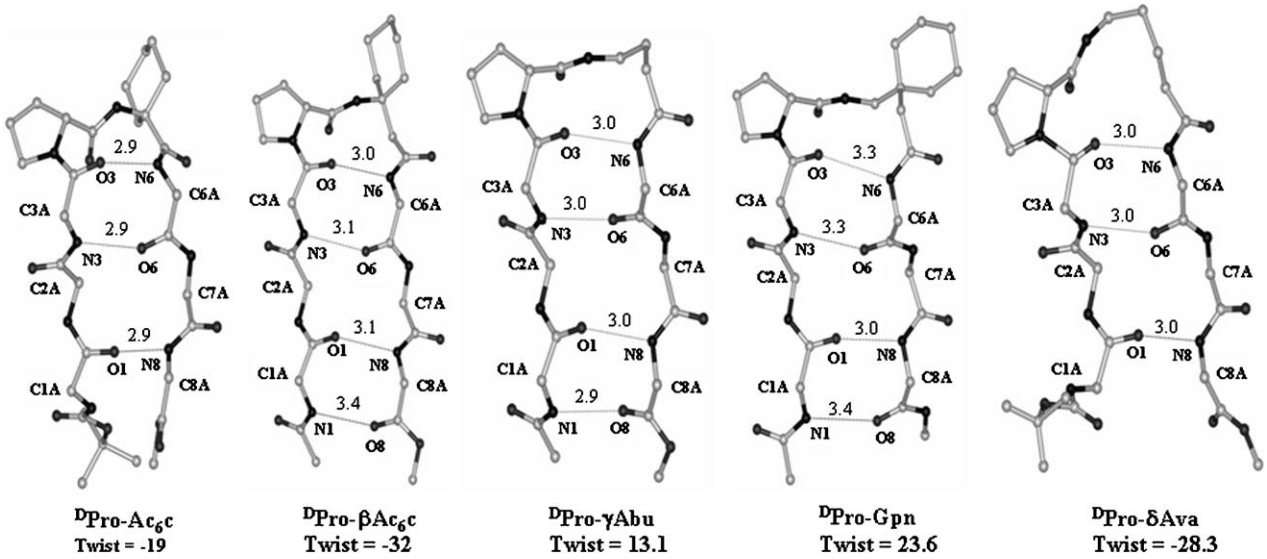

Figure 7. Hairpin conformations in designed octapeptides with central ^{D}Pro–Xxx segments. The structures for ^{D}Pro-Ac$_6$c[6i] and ^{D}Pro-δ-Ava have been determined in crystals. The structure for ^{D}Pro-β^{3}-Ac$_6$c, ^{D}Pro-γAbu and ^{D}Pro-Gpn have been determined using computer modeling, with turn conformations being derived from the crystals of peptides **5** and **6**. The average value of the interstrand facing C^{α}–C^{α} distance is 5.0 Å (±0.3 Å). The twist angle is the virtual torsion angle (θ) at the turn, $C3^{\alpha}$-$C4^{\alpha}$-$C5^{\alpha}$-$C6^{\alpha}$.

hanced stability of these modified backbones to proteolytic cleavage.[12c,24] The retention of biological activity in an analogue peptide requires that the functional three-dimensional structure be accessible and stable in the modified sequence. In this context, it is important to establish that hybrid peptide sequences can mimic the canonical secondary structure that is extensively characterized in all-α-amino acid containing peptides. The results presented above establish that β, γ- and δ-residues can be inserted into the *i*+2 position of hairpin facilitating turns. In a previous study, we have demonstrated that β- and γ-residues can be incorporated into type I/III helical turns.[13a] The β-, γ- and δ-residues may also be accommodated into extended strands.[6d–g] Therefore hybrid sequences can be designed that adopt either β hairpin or helical structures.

Naturally occurring proteins are polymers of all-α-amino acid sequences. It has long been an established tenet of biochemistry that the observed folding patterns in proteins are determined by the conformational limitations imposed by α peptide backbones. In principle, the higher homologues of α-amino acids can sample a much larger conformational space because of the greater number of degrees of torsional freedom in the backbone. The growing realization that folded structures are readily achieved, even in sequences with expanded backbones, suggests that new structural classes of folded heteropolypeptides remain to be experimentally characterized. Globularity is an attribute that appears to be achievable, even in hybrid sequences, in light of the successful demonstration of the construction of stable turns, helices, and hairpins in model hybrid peptides.

Experimental Section

Peptide synthesis: Peptides **1**–**3** were synthesized by conventional solution-phase chemistry by using a racemization-free fragment condensation strategy. Peptide **4** has been previously described.[17] The Boc group was used for N-terminal protection, and the C terminus was protected as a methyl ester. Deprotections were performed by using 98% formic acid, and by saponification for the N and C terminus, respectively. Couplings were mediated by *N*,*N*-dicyclohexylcarbodiimide (DCC) and 1-hydroxy-1*H*-benzotriazole (HOBT). The final step in the synthesis of the peptides was achieved by the fragment condensation of Boc-Leu-Phe-Val-OH and HN-^{D}Pro-Xxx-Leu-Phe-Val-OMe (Xx*x* = Ac_6c, β^3-Ac_6c, and Gpn). Peptide **5** was synthesized by the coupling of Piv-^{L}Pro-OH (Piv, pivaloyl) and the methyl ester of β^3-Ac_6c by using a mixed anhydride procedure that used ethylchloroformate. The dipeptide ester was dissolved in methanol and saturated with dry CH_3NH_2 gas. The solution was kept tightly stoppered for 24 h at room temperature. Evaporation of methanol yielded a white solid; this was subjected to chromatography on a silica gel column by using $CHC1_3$/MeOH (98:2) as the eluent. For peptide **6**, Boc-Ac_6c-Gpn-OH, which was synthesized from Boc-Ac_6c-OSu and H-Gpn-OH, was coupled to Ac_6c-OMe, which was obtained from its hydrochloride, by using DCC and *N*-hydroxysuccinimide in THF. All of the intermediates were characterized by ^{1}H NMR spectroscopy (80 MHz) and thin-layer chromatography (TLC) on silica gel, and were used without further purification. The final peptides were purified by reverse-phase, medium-pressure liquid chromatography (C_{18}, 40–60 μ) and HPLC on a reverse-phase C_{18} column (5–10 μ, 7.8 mm × 250 mm) by using methanol/water gradients. The final peptides were characterized by mass spectrometry on a HP-1100 electrospray ionization mass spectrometer (ESI-MS) and by complete assignment of the 500 MHz ^{1}H NMR spectra. The following mass spectrometry data was observed for peptides **1**: *m*/*z*: 1095 $[M+Na]^+$ (calcd: 1072); **2**: *m*/*z*: 1109 $[M+Na]^+$ (calcd: 1086); and **3**: *m*/*z*: 1123 $[M+Na]^+$ (calcd: 1100).

NMR spectroscopy: NMR experiments were carried out on a Bruker DRX 500 spectrometer. One-dimensional (1D) and 2D spectra were recorded at a peptide concentration of ≈3 mM in CD_3OH at 300 K. Intramolecular hydrogen bonding was probed by recording spectra at five different temperatures between 278–323 K at intervals of 10 K, and by determining the temperature coefficients of the amide proton chemical shifts. Resonance assignments were carried out with the help of 1D and 2D spectra. Residue-specific assignments were obtained from TOCSY[25] experiments, while ROESY[26] spectra permitted sequence-specific assignments. All 2D experiments were recorded in phase-sensitive mode by using the TPPI (time proportional phase incrementation) method. A data set of 1024 × 450 was used for acquiring the data. The same data set was zero-filled to yield a 2048 × 1024 data matrix before Fourier transformation. A spectral width of 6000 Hz was used in both dimensions. Mixing times of 100 and 200 ms were used for TOCSY and ROESY, respectively. Shifted square sine-bell windows were used while processing. All processing was done by using BRUKER XWINNMR software.

Structure solution and refinement: Crystals of the octapeptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe (**4**) were grown from methanol/water by slow evaporation in the form of flat, thin needles. After numerous trials, the "best" data set that was obtained with single diffraction spots was at −50 °C and had a resolution 1.15 Å. Crystal data are: $C_{49}H_{92}N_8O_{13}\cdot H_2O\cdot CH_3OH$, space group $P2_12_12_1$, a = 9.678(5), b = 11.967(3), c = 52.228 (14) Å, $\alpha=\beta=\gamma=90°$, V = 6048.9(38) Å^3, Z = 4, and ρ_{calcd} = 1.095 g cm^{-3}. Fifty thousand trials with the SHELXS program[27] yielded a fragment of the structure; this was expanded to the full structure by using the tangent formula[28] and difference maps. The limited number of observed diffraction data required the use of ISOR restraints[27] with full-matrix least-squares refinement of the F^2 data of the non-hydrogen atoms, and of the hydrogen atoms that were placed in idealized positions and riding on the carbon or nitrogen atoms to which they are bonded. The reliability factor R_1 is 9.0% for 1382 observed data $[F_o > 4.0\sigma(F_o)]$ and 522 parameters.

Single crystals of Piv-^{L}Pro-β^3-Ac_6c-NHMe (**5**) and Boc-Ac_6c-Gpn-Ac_6c-OMe (**6**) suitable for X-ray diffraction were grown from a methanol/water mixture by slow evaporation of the solvent. X-ray intensity data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer, by using $Mo_{K\alpha}$ radiation (λ = 0.71073 Å). An ω scan type was used for collecting the data. Crystal data for peptide **5**: $C_{19}H_{33}N_3O_3\cdot 2H_2O$, space group $P2_1$, a = 9.859(1), b = 8.532(1), c = 13.423(2) Å, β = 97.2(3)°, V = 1120.3(3) Å^3, Z = 2, and ρ_{calcd} = 1.137 g m^{-3}. Crystal data for peptide **6**: $C_{29}H_{49}N_3O_6$, space group $P2_1/c$, a = 10.408(2), b = 17.404(1), c = 17.751(2) Å, β = 103.3(2)°, V = 3128.6(6) Å^3, Z = 4, and ρ_{calcd} = 1.137 g cm^{-3}. Structures of **5** and **6** were solved by direct methods of phase determination by using the program SHELXS-97.[29] The structures were refined against F^2 with a full-matrix least-squares method by using SHELXL-97.[30] All of the hydrogen atoms, except for those connected to C^{α} (Pro), and the nitrogen atoms in peptide **5** 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. The hydrogen atoms that were bonded to N2, N3, and C_1^{α} were located from the difference Fourier map and were refined isotropically in the final cycle. For peptide **6**, except for the hydrogen atoms connected to Boc and OMe groups, all of the hydrogen atoms were located from the difference Fourier map. The R factor (R_1) for peptide **5** was 7.7% (wR_2 = 19.8%) for 1883 observed reflections with $F_o \geq 4\sigma|Fo|$ and for 256 variables. The final R factor (R_1) for peptide **6** was 5.1% (wR_2 = 16.1%) for 5028 observed reflections with $F_o \geq 4\sigma|Fo|$ and for 491 parameters. CCDC-608845 (**4**), CCDC-615467 (**5**), and CCDC-615468 (**6**) 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.

Energy minimization: The polypeptide β hairpin models were built in the model-builder module of INSIGHT II. The crystal structure coordinates of Piv-Pro-β^3-Ac_6c-NHMe (**5**) were used to model the octapeptide β hair-

pin with ^{D}Pro-β^3-Ac$_6$c at the turn region. The backbone torsion angles ϕ and ψ for the strand residues (Gly) were fixed at $-120°$ and $+120°$, respectively. The values of torsion angles for ^{D}Pro were $\phi=56°$, $\psi=-130°$ and that of β^3-Ac$_6$c were $\phi=-60°$, $\theta=-80°$, $\psi=85°$ in the starting model. The torsion angle ω was fixed at 180° for all the residues in the starting model. The model was then minimized in the DISCOVER module of INSIGHT II by using the AMBER force field and by keeping the dielectric constant at 1. N···O and H···O distances for the four possible interstrand hydrogen bonds were constrained during minimization. The ω of β^3-Ac$_6$c was constrained between $180\pm10°$ during the last 50 cycles of minimization, as the value of this dihedral angle dropped to 155° after the first 50 cycles. A similar model building and minimization procedure was employed to model hairpins with ^{D}Pro-γ-Abu and ^{D}Pro-Gpn as the turn residues. The values of torsion angles for ^{D}Pro were $\phi=60°$, $\psi=-120°$ and that of γ-Abu (or Gpn) were $\phi=-150°$, $\theta_1=57°$, $\theta_2=91°$, $\psi=-90°$ in the starting model. The coordinates of the minimized models along with the values of torsion angles and hydrogen bond parameters are provided in the Supporting Information.

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