

Hybrid Peptide Hairpins Containing α - and ω -Amino Acids: Conformational Analysis of Decapeptides with Unsubstituted β -, γ -, and δ -Residues at Positions 3 and 8

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Abstract: The effects of inserting unsubstituted ω -amino acids into the strand segments of model β -hairpin peptides was investigated by using four synthetic decapeptides, Boc-Leu-Val-Xxx-Val-D-Pro-Gly-Leu-Xxx-Val-Val-OMe: peptide **1** (Xxx = Gly), peptide **2** (Xxx = β Gly = β HGly = homoglycine, β -glycine), peptide **3** (Xxx = γ Abu = γ -aminobutyric acid), peptide **4** (Xxx = δ Ava = δ -aminovaleric acid). ¹H NMR studies (500 MHz, methanol) reveal several critical cross-strand NOEs, providing evidence for β -hairpin confor-

mations in peptides **2–4**. In peptide **3**, the NMR results support the formation of the nucleating turn, however, evidence for cross-strand registry is not detected. Single-crystal X-ray diffraction studies of peptide **3** reveal a β -hairpin conformation for both molecules in the crystallographic asymmetric unit, stabilized by four cross-strand

hydrogen bonds, with the γ Abu residues accommodated within the strands. The D-Pro-Gly segment in both molecules (A,B) adopts a type II' β -turn conformation. The circular dichroism spectrum for peptide **3** is characterized by a negative CD band at 229 nm, whereas for peptides **2** and **4**, the negative band is centered at 225 nm, suggesting a correlation between the orientation of the amide units in the strand segments and the observed CD pattern.

Keywords: amino acids • conformation analysis • crystal structure • peptides • protein folding

The higher homologues of the α -amino acids, in which additional carbon atoms are introduced into the residues, may be used to expand the range of folded polypeptide structures. Oligo- β -peptides have been shown to adopt variants of helical structures, with new patterns of backbone hydrogen bonds.^[1] Polypeptide sheets with altered polarity can also be constructed.^[2] Considerable recent work has reported the conformation of peptide oligomers of β -^[1] and γ -^[1m,3]

residues. The incorporation of β -, γ -, and δ -residues into defined secondary structures formed by α -amino acids has also been reported. Earlier studies from this laboratory have demonstrated the incorporation of β -, γ -, and δ -amino acid residues into polypeptide helices.^[4] Substituted β -residues have also been incorporated into the turn^[2a,e,5a] and strand segments of model β -hairpins.^[2] Homooligomers of (*R,R*)-2-aminocyclopentane carboxylic acid (*trans*-ACPC) adopt 12 helical structures^[1b], whereas homooligomers of (*1R,2S*)-2-aminocyclopentane carboxylic acid (*(1R,2S)-cis*-ACPC) adopt nonpolar, sheetlike structures.^[6a] The constrained γ -amino acid *trans*-3-ACPC has been incorporated into the strand segments of a parallel-sheet structure.^[6b] Results of NMR studies established the accommodation of 3-aminobenzoic acid into the strand segments of a model hairpin structure.^[6c] δ Ava has been shown to be accommodated at the *i*+2 position of a nucleating D-Pro-Xxx turn in a model octapeptide hairpin, Boc-Leu-Val-Val-D-Pro- δ Ava-Leu-Val-Val-OMe.^[5b] The incorporation of additional backbone atoms into the strand segments of β -hairpins permits alteration of the local polarity of β -sheets. In the case of unsubstituted ω -amino acids, lengthening of the spacer segment be-

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tween the amino and the carboxyl ends of the residue results in a considerable increase in the range of accessible backbone conformations. Nevertheless, the earlier demonstrations of accommodation of β -, γ -, and δ -residues into folded structures suggest that further exploration of the conformational space available to these residues may be useful in defining parameters for their use in peptide design.

Here, we analyze the effects of introducing β -, γ -, and δ -residues into the strand segments of peptide hairpins. The following peptides were investigated: Boc-Leu-Val-Xxx-Val-D-Pro-Gly-Leu-Xxx-Val-Val-OMe, in which Xxx = Gly (**1**), Xxx = β Gly (**2**) (β Gly = β hGly, homoglycine, betaglycine; note that the previously used common name β -alanine is misleading in the context of the rapidly developing literature on β -peptides),^[1a] Xxx = γ Abu (**3**) (γ Abu = γ -aminobutyric acid), and Xxx = δ Ava (**4**) (δ Ava = δ -aminovaleric acid).

The choice of sequences used was based on previous studies that established a β -hairpin conformation in solution and in crystals for the octapeptide Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe.^[7] In addition, insertion of β Phe residues ((S)- β^3 -homophenylalanine) into strand segments at the *non-hydrogen-bonding position* in the model peptide (Boc-Leu-Val- β Phe-Val-D-Pro-Gly-Leu- β Phe-Val-Val-OMe) results in a stable hairpin structure.^[2c] Figure 1 shows a schematic view of an anticipated β -hairpin fold and defines the degrees of backbone torsional freedom in ω -amino acid residues. In the sequences of peptides **2–4**, the unsubstituted ω -amino acid residues are inserted at two facing *non-hydrogen-bonding positions*. In the canonical β -hairpin that includes exclusively α -residues, peptide **1**, the achiral α -amino acid residue

(Gly) is incorporated at the facing *non-hydrogen-bonding position*. The results presented in this paper provide strong evidence for a predominant population of β -hairpins in solution in the cases of peptides **2** (Xxx = β Gly) and **4** (Xxx = δ Ava). X-ray diffraction studies of peptide **3** reveal the β -hairpin conformation for two independent molecules in the crystallographic asymmetric unit.

Experimental Section

Peptide synthesis: Peptides **1–4** were synthesized by conventional solution-phase methods by using a fragment-condensation strategy.^[2e] The *tert*-butyloxycarbonyl group was used for N-terminus protection, and the C-terminus was protected as a methyl ester. Deprotections were performed with 98% formic acid and saponification for the N- and C-termini, respectively. Couplings were mediated by dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT). At the final step, the tetrapeptide acid (Boc-Leu-Val-Xxx-Val-OH) was coupled to the N-terminus-deprotected hexapeptide (H-D-Pro-Gly-Leu-Xxx-Val-Val-OMe). The tetrapeptide Boc-Leu-Val-Xxx-Val-OMe was prepared by [1+3] condensation involving Boc-Leu-OH and H-Val-Xxx-Val-OMe. The tripeptide Boc-Val-Xxx-Val-OMe was prepared by [2+1] condensation involving an N-terminus dipeptide acid Boc-Val-Xxx-OH and H-Val-OMe, by using DCC/N-hydroxysuccinimide (HOSu). The hexapeptide Boc-D-Pro-Gly-Leu-Xxx-Val-Val-OMe was prepared by [2+4] coupling involving Boc-D-Pro-Gly-OH and H-Leu-Xxx-Val-Val-OMe. The tetrapeptide Boc-Leu-Xxx-Val-Val-OMe was prepared by [2+2] condensation involving an N-terminus dipeptide acid Boc-Leu-Xxx-OH and a C-terminus-deprotected dipeptide H-Val-Val-OMe, by using DCC/HOSu. Intermediates were characterized by 80 MHz NMR spectroscopy and/or by mass spectrometry (MALDI-TOF and ESI-MS). The target peptides were purified by reverse-phase medium-pressure liquid chromatography on a C₁₈ (40–63 μ m) column with methanol/water gradients. Peptides **1** and **3** were further purified by HPLC on a reverse-phase C₁₈ (5–10 μ m) column with methanol/water gradients.

Mass spectrometry: The purified peptides were analysed by mass spectrometry using a Kratos PC-Kompact MADLI-TOF mass spectrometer; *m/z*: calcd for **1**: 1022 Da; found: 1046.7 Da [*M*+Na⁺], 1063.0 Da [*M*+K⁺]; *m/z*: calcd for **2**: 1050 Da; found: 1074.0 Da [*M*+Na⁺], 1089.7 Da [*M*+K⁺]; *m/z*: calcd for **3**: 1079 Da; found: 1102.3 Da [*M*+Na⁺], 1118.1 Da [*M*+K⁺]; *m/z*: calcd for **4**: 1106 Da; found: 1129.4 Da [*M*+Na⁺], 1145.8 Da [*M*+K⁺]. Peptides **2–4** were fully characterized by 500 MHz ¹H NMR spectroscopy.

NMR spectroscopy: All NMR studies were carried out by using a Bruker DRX-500 MHz spectrometer at a probe temperature of 300 K. Resonance assignments were obtained by TOCSY and ROESY analysis. All two-dimensional data were collected in phase-sensitive mode, by using the time-proportional phase incrementation (TPPI) method. Sets of 1024 and 450 data points were used in the *t*₂ and *t*₁ dimensions, respectively. For TOCSY and ROESY analysis, 24 and 64 transients were collected, respectively. A spectral width of 6000 Hz

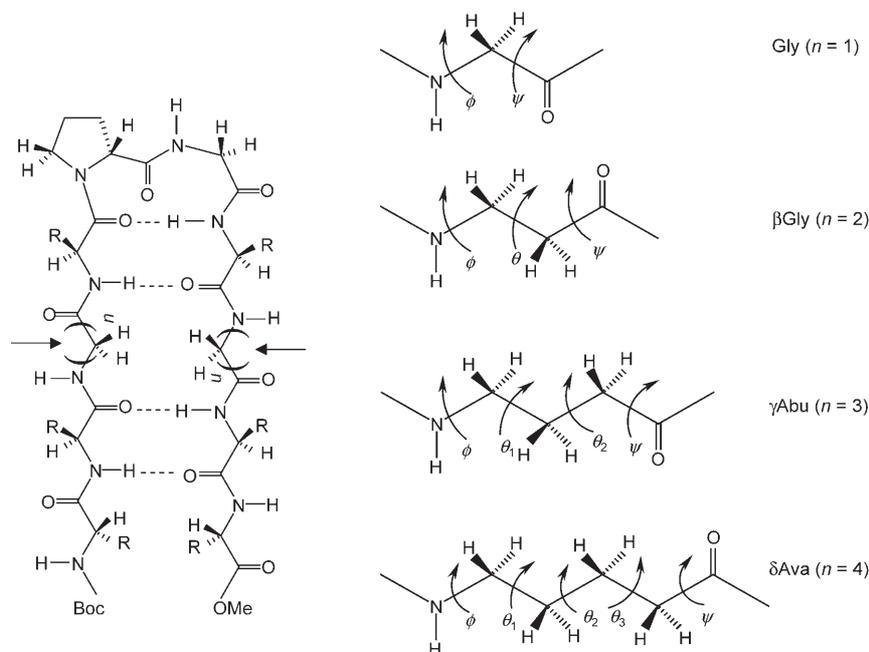


Figure 1. Schematic view of a hairpin (left) and definition of backbone torsional freedom in ω -amino acid residues (right). The arrows (left) represent the sites of insertion of ω -amino acid residues, and “*n*” refers to the number of methylene units in each strand segment.

was used in both dimensions. A spin-lock time of 250 ms was used to obtain ROESY spectra. Zero-filling was carried out to finally yield a data set of $2\text{ K} \times 1\text{ K}$. A shifted square-sine-bell window was used before processing.

Circular dichroism (CD): CD spectra were recorded by using a JASCO J-715 spectropolarimeter. The instrument was calibrated with (+)-10-camphor sulfonic acid. The path length used was 1 mm. The data were acquired in the wavelength scan mode, with a 2 nm band and a step size of 0.2 nm. Spectra were acquired at 300 K. Typically, four scans were acquired from 200–260 nm by using a scan speed of 50 nm min^{-1} . The resulting data were baseline-corrected and smoothed.

X-ray diffraction: Crystals of peptide **3**, in the form of thin, flat needles, were obtained from both methanol/water and methanol/dioxane/water solvent mixtures by slow evaporation. Numerous trials for X-ray data collection at several different temperatures and from different crystallization attempts resulted in a “best” data set, with single diffraction spots at ambient temperature. Decreasing the temperature resulted in fractured spots. The effective scattering resolution was 1.1 \AA , in which the ratio of mean intensity over σ was ≥ 1.8 . X-ray data were obtained from a colorless crystal, $0.50 \times 0.30 \times 0.10\text{ mm}$ in size, on a four-circle diffractometer (Bruker P4) by using $\text{Cu K}\alpha$ radiation ($\lambda = 1.54178\text{ \AA}$). The θ - 2θ scan mode was used, with a scan width of $1.5^\circ + 2\theta(\alpha_1 - \alpha_2)$ and a scan speed of 13° min^{-1} . There were no diffraction spots with measurable intensity beyond $2\theta = 105^\circ$. Crystal data for $\text{C}_{53}\text{H}_{94}\text{N}_{10}\text{O}_{13} \cdot \text{H}_2\text{O}$: space group $P1$, $a = 9.742(3)$ $b = 10.842(3)$ $c = 31.473(12)\text{ \AA}$, $\alpha = 89.46(2)$ $\beta = 83.28(4)$ $\gamma = 78.85(3)^\circ$, $V = 3238.8\text{ \AA}^3$, $Z = 2$, and $\rho_{\text{calcd}} = 1.120\text{ g cm}^{-3}$. The structure was solved by using a 25-atom fragment from a known type II' β -turn^[7b] in a vector-search procedure,^[8a] followed by the expansion of the partial structure by using the tangent formula^[8b] and difference maps. Full-matrix least-squares refinement of F^2 data of the non-hydrogen atoms, and of hydrogen atoms placed in idealized positions and riding on the carbon or nitrogen atoms to which they are bonded, resulted in a reliability factor of $R_1 = 10.2\%$ for 4028 observed data [$F_o > 4.0\sigma(F_o)$] and 1380 parameters. CCDC 262350 contains the supplementary crystallographic data for this paper (atomic coordinates, bond lengths, and bond angles). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Results and Discussion

The 500 MHz ^1H NMR spectrum for peptide **1** in methanol solution did not yield well-dispersed amide resonances. In addition, the presence of minor conformers, presumably corresponding to *cis-trans* isomerization about the Val4-D-Pro5 bond, was also observed. The overlap of amide resonances precluded a detailed analysis. The introduction of Gly residues into the strand segments may contribute to the destabilization of registered, antiparallel strands. Subsequent NMR studies were, therefore, confined to peptides **2–4**. All three peptides yielded sharp, well-resolved resonances, with adequate chemical-shift dispersion for the amide resonances. Resonances due to the presence of minor conformers, arising from *cis-trans* isomerization about the Val4-D-Pro5 bond, are observed. The subsequent analysis is restricted to the major *trans* conformer. Assignment of resonances was accomplished by using a combination of TOCSY and ROESY experiments.

Figure 2 shows a representative TOCSY spectrum of peptide **4** ($\text{Xxx} = \delta\text{Ava}$). Resonance assignments are indicated. Peaks arising from a minor conformation are marked “m”. Partial ROESY spectra of peptide **2**, indicating NOE connectivities for $\text{NH} \leftrightarrow \text{C}^\alpha\text{H}$ (for α -residues) and $\text{NH} \leftrightarrow \text{C}^\beta\text{H}$

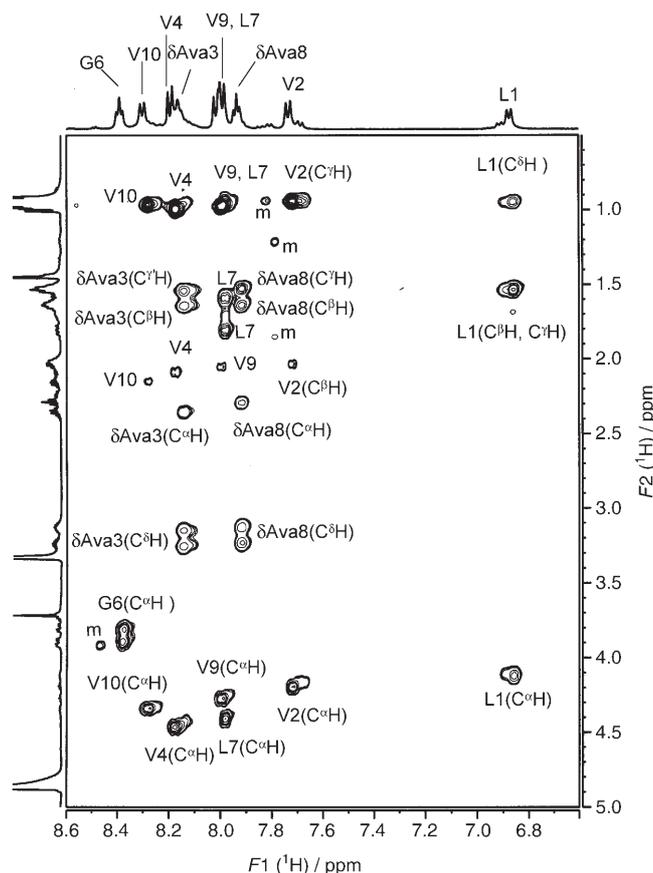


Figure 2. 500 MHz partial TOCSY spectrum of peptide **4** in CD_3OH at 300 K, illustrating the spin systems of individual amino acids.

(for β -residues) (top panel) and for $\text{NH} \leftrightarrow \text{NH}$ (bottom panel), are shown in Figure 3. Figure 4 illustrates NOEs for $\text{NH} \leftrightarrow \text{C}^\alpha\text{H}$ (for α -residues) and $\text{NH} \leftrightarrow \text{C}^\beta\text{H}$ (for δ -residues) (top panel) and for $\text{NH} \leftrightarrow \text{NH}$ (bottom panel) for peptide **4**. The NMR parameters for peptides **2–4** are provided as Supporting Information.

The observed NOE connectivities for the three peptides are indicated schematically in Figure 5. The NOEs compatible with the hairpin conformation are represented as filled arrows, and open arrows identify NOEs that are incompatible with the β -hairpin structure. The presence of both types of NOEs is a reflection of conformational heterogeneity. Notably, the hairpin-incompatible NOEs are observed towards the N- and C-termini of the peptides. Fraying of hairpins at the termini has been established earlier in model peptides, both in solution and in the solid state.^[2e] In all three peptides, strong $\text{D-Pro4}(\text{C}^\alpha\text{H}) \leftrightarrow \text{Gly5}(\text{NH})$, $\text{Gly5}(\text{NH}) \leftrightarrow \text{Leu6}(\text{NH})$, and $\text{Gly5}(\text{C}^\alpha\text{H}) \leftrightarrow \text{Leu6}(\text{NH})$ NOEs, consistent with the presence of type II' β -turns, are observed. The results shown in Figures 3–5 provide reasonably strong NMR evidence for a predominant population of a β -hairpin conformation in peptide **2** ($\text{Xxx} = \beta\text{Gly}$) and peptide **4** ($\text{Xxx} = \delta\text{Ava}$). Interstrand NOEs in the case of peptide **4** are observed between Val2 (C^αH) and Val9 (C^αH) (data not shown) and Leu1 (NH) and Val10 (NH) (Figure 4, bottom

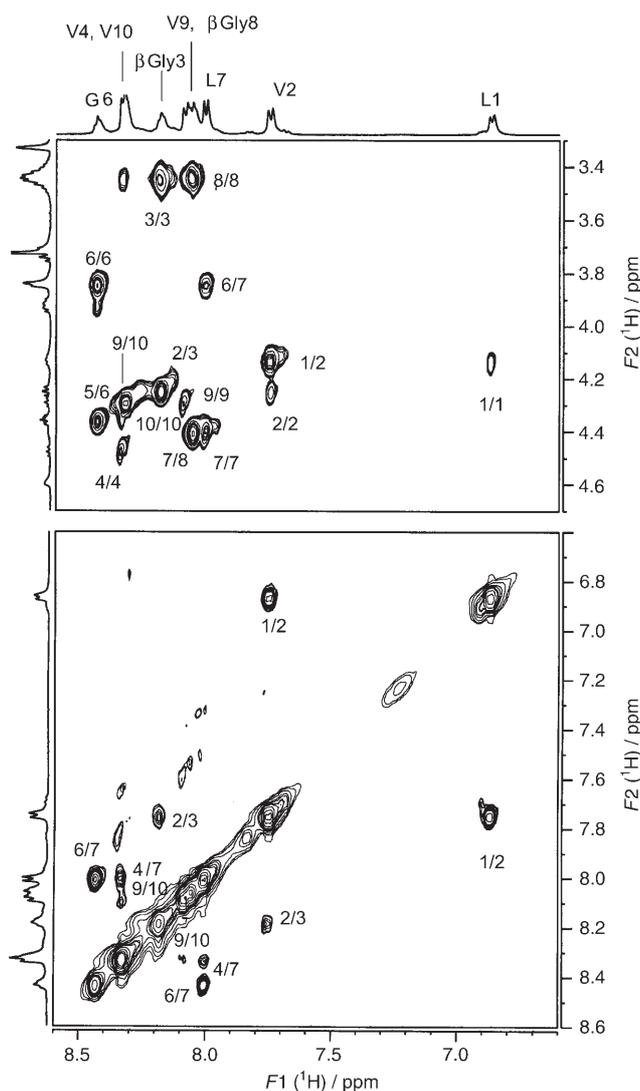


Figure 3. 500 MHz partial ROESY spectrum of peptide **2** in CD₃OH at 300 K, indicating NOEs for NH \leftrightarrow C^αH (for α -residues) and NH \leftrightarrow C^βH (for β -residues) (top) and for NH \leftrightarrow NH (bottom).

panel), indicating spatial proximity of the termini. In addition, a strong NOE between δ Ava3 (C^αH) and δ Ava8 (C^αH) is also detected (data not shown), providing support for chain reversal and strand registry. In the case of peptide **2**, NOEs between residues 1 and 10 and residues 2 and 9 were not detectable.

Nevertheless, NOE evidence for a hairpin fold encompassing residues 3 and 8 is observed. In peptide **3** (Xxx = γ Abu), NOE evidence supports β -turn formation at the D-Pro-Gly segment. However, the critical d_{NN} NOE between Val4 (NH) and Leu7 (NH) was not observed. This short d_{NN} distance, which is characteristic of the formation of the second hydrogen bond in a β -hairpin, is sometimes lengthened by solvation. In crystal structures of several model hairpins, solvent molecules often bridge the central peptide unit of the β -turn and one of the peptide units of the strand segments.^[9] All the observed NOEs correspond to short

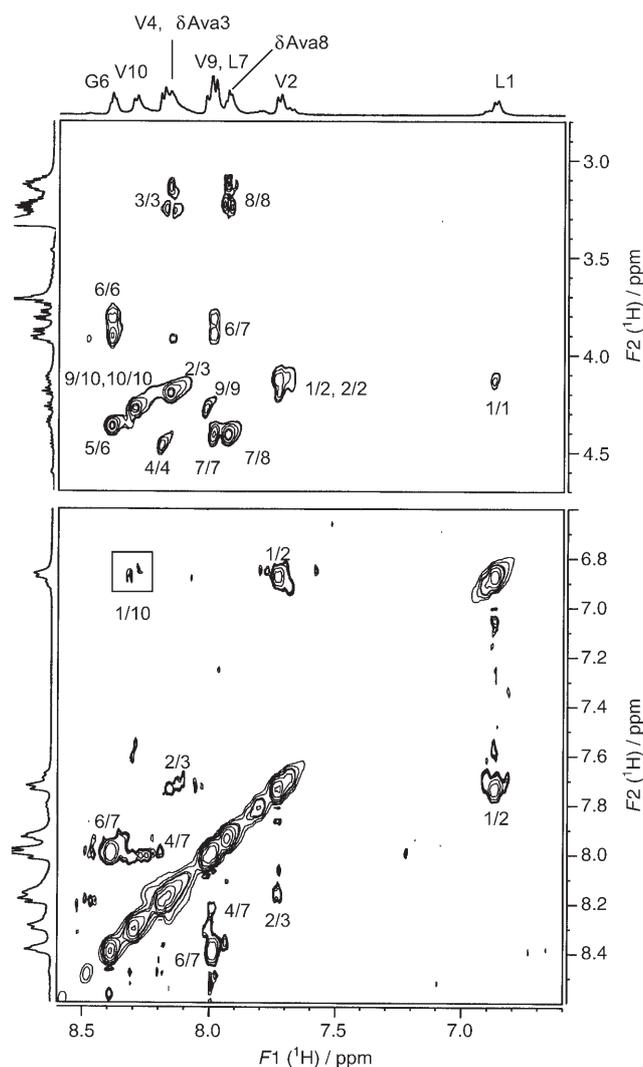


Figure 4. 500 MHz partial ROESY spectrum of peptide **4** in CD₃OH at 300 K, indicating NOEs for NH \leftrightarrow C^αH (for δ -residues) (top) and for NH \leftrightarrow NH (bottom).

inter-residue distances, suggestive of extended conformations in the strand segments of both the N- and C-termini. However, the absence of cross-strand NOEs precludes a firm conclusion regarding the population of β -hairpin conformations in peptide **3**. A notable difference between the peptides is in the schematic β -hairpin structure, as illustrated in Figure 5. In peptides **2** and **4**, five cross-strand hydrogen bonds may be anticipated in an ideal β -hairpin, whereas in peptide **3**, only four cross-strand hydrogen bonds are expected, with residues 1 and 10 not forming a part of the registered antiparallel-strand structure. This difference arises because the Xxx residues contain an even number of backbone methylene groups in peptides **2** and **4**, but an odd number in peptide **3**.

Interestingly, single crystals of peptide **3** were obtained and X-ray diffraction analysis, described below, establishes a β -hairpin conformation in the solid state. Ironically, despite several attempts, peptides **2** and **4**, which show strong evi-

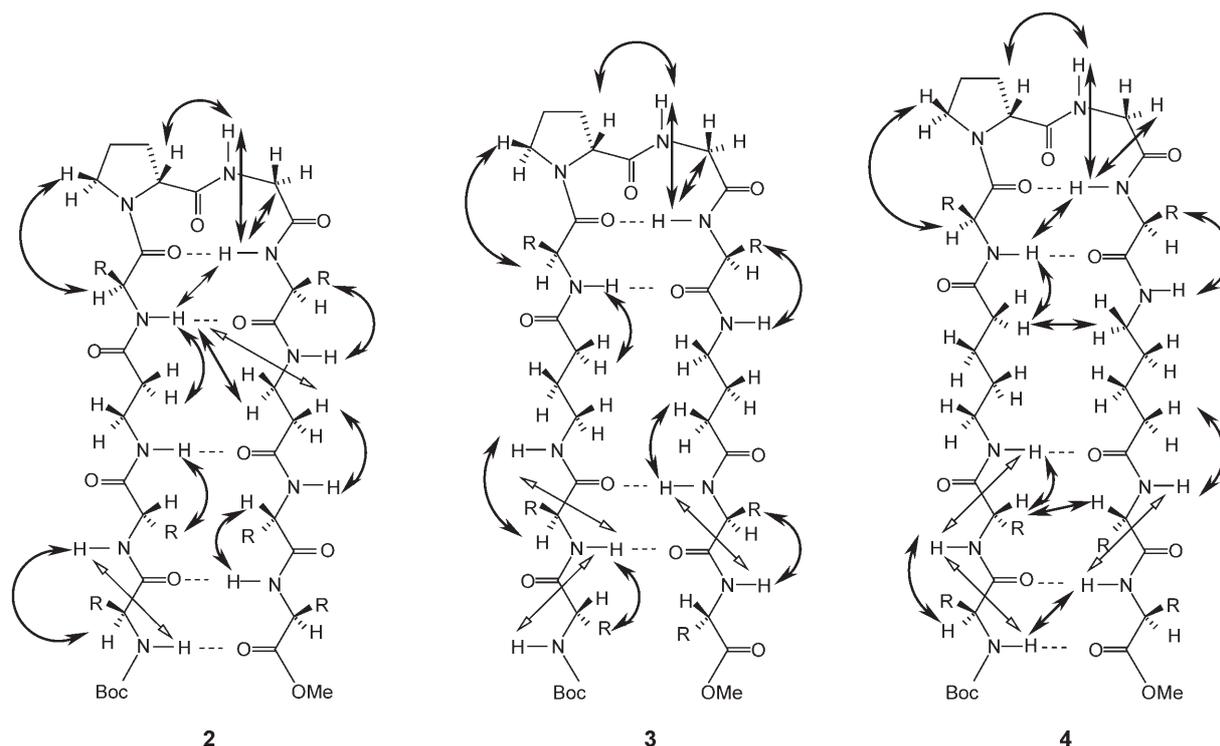


Figure 5. Schematic representation of hairpins **2–4**, showing all observed NOEs. Filled arrows indicate NOEs supportive of hairpin conformations and open arrows refer to NOEs that are inconsistent with the anticipated structure.

dence for β -hairpin structures in solution, did not yield single crystals.

X-ray diffraction: Single crystals of peptide **3** grown from both methanol/water and methanol/water/dioxane solvent mixtures diffracted to a resolution of approximately 1.1–1.2 Å. The crystallographic asymmetric unit contains two independent molecules, A and B. Figure 6 shows a view of the molecular conformation of molecule A and the associated intra- and intermolecular hydrogen bonds. The relevant torsion angles and hydrogen-bond parameters are summarized in Tables 1 and 2, respectively. The individual molecules A and B within the crystal of peptide **3** have very similar conformations, as seen in the superimposition of molecules A and B in Figure 7. Each γ -inserted molecule is folded into a β -hairpin conformation and stabilized by four cross-strand hydrogen bonds with appropriate dimensions. The directions of the cross-strand $\text{NH}\cdots\text{O}=\text{C}$ hydrogen bonds alternate in the same manner as in hairpin turns containing only α -residues in the strands. The β -turns encompassing Pro5-Gly6 and Pro15-Gly16 are type II' turns, such as those found in Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe.^[7b] Type I' turns have also been found in crystals of some of the β -hairpins containing β -amino acid residue inserts.^[2c,e]

The four $-(\text{CH}_2)_3-$ segments in the strands of molecules A and B (Figure 8) begin to show the type of conformational instability often observed for $-(\text{CH}_2)_n-$ chains. A greater torsional flexibility about C–C bonds has been observed in crystal structures as n increases from four to twelve in the

families of polymethylene-bridged cystine-based cyclobis-amides and cyclobisureas, hybrid peptides that assemble into

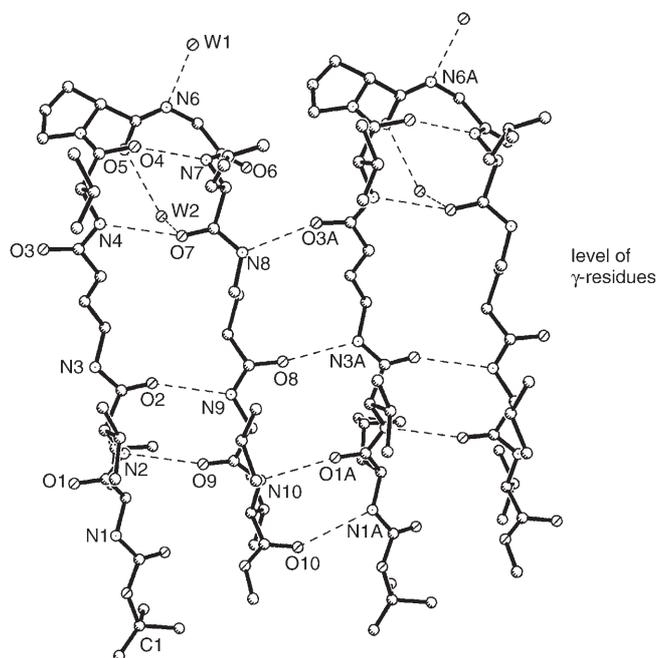


Figure 6. Hydrogen bonds in molecule A of peptide **3**. Molecules with hairpin turns assemble by lateral translation into a continuous β -sheet. A similar, but separate, β -sheet is formed by molecule B (not shown). The γ -residues in molecule A are in positions 3 and 8 and face each other across the strand.

Table 1. Torsion angles [°] in crystals of peptide 3.^[a]

Residue name	Torsion angles [°]					
	φ	θ_1	θ_2	ψ	χ_1	χ_2
Leu1	-161 (-158)			132 (122)	176 (-176)	169, 77 (-175, 60)
Val2	-130 (-118)			117 (125)	-173, -50 (-63, 173)	
γ Abu3	-119 (-116)	+107 (-173)	173 (176)	-158 (106)		
Val4	-131 (-128)			100 (90)	-55, 180 (-62, -174)	
D-Pro5 ^[b]	64 (64)			-127 (-134)	7 (-29)	-21 (39)
Gly6	-88 (-77)			-3 (-7)		
Leu7	-76 (-75)			138 (133)	-94 (-53)	179, 1 (176, -57)
γ Abu8	94 (179)	-175 (-171)	-65 (-135)	143 (132)		
Val9	-130 (-134)			127 (127)	177, -57 (-45, 171)	
Val10	-114 (-112)			-117 (-59)	-55, 180 (-57, 180)	

[a] Values are for two independent molecules, A and B, in the crystallographic asymmetric unit. Values in parentheses correspond to molecule B. [b] χ_3 : 25.2° (-33.2°); χ_4 : -20.3° (15.2°); χ_5 : 7.7° (8.6°); where χ is an additional torsion angle in the ring. The estimated standard deviations are ~3°.

Table 2. Hydrogen bonds in peptide 3.

Donor	Acceptor	D–A [Å]	H–A [Å]	C=O...N angle [°]
molecule A				
N1	O10 ^[a]	3.276	2.49	140
N2	O9	2.973	2.12	160
N3	O8 ^[a]	2.894	2.03	168
N4	O7	3.078	2.20	143
molecule B				
N5 (Pro)				
N6	W1	2.815	1.95	
N7	O4	2.881	2.03	139
N8	O3 ^[b]	2.930	2.04	149
N9	O2	2.957	2.09	157
N10	O1 ^[b]	2.932	2.05	151
solvent				
W1	O15	2.746		
W1	O17	2.882		
W2 ^[e]	O5	2.813		
W2	O7	2.914		

[a] Symmetry of acceptor, $1+x, y, z$. [b] Symmetry of acceptor, $-1+x, y, z$. [c] Symmetry of acceptor, $x, 1+y, z$. [d] Direct hydrogen bond between molecule A and molecule B. [e] The N16 atom does not participate in a hydrogen bond with W2 (N16...W2=3.76 Å). Compare to N6...W1=2.81 Å.

nanotubes.^[10] In the present peptide 3, the $-(CH_2)_3-$ groups are almost extended and do not disturb unduly the classic pleated-sheet conformation of β -hairpins (Figure 7). It is possible that peptide 4, with δ -amino acid residue inclusions, has sufficient disorder in the $-(CH_2)_4-$ moieties to preclude neat β -sheet formation, and, therefore, cannot easily form crystals.

The crystal packing in peptide 3, shown in Figure 9, has essentially the same features as the packing for an all- α -hairpin peptide,^[7b] except that the α -sheet stacks in peptide 3 have a V-shaped tilt, in contrast to the relatively flat ar-

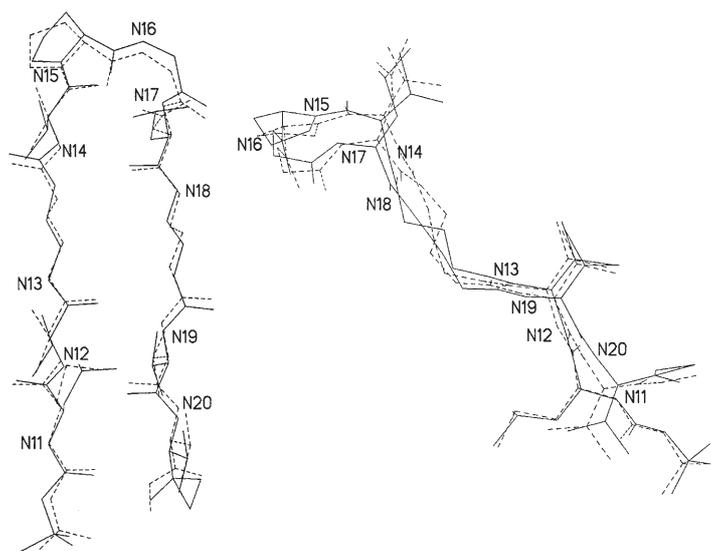


Figure 7. Face (left) and side (right) views of superimpositions, fitted by least-squares, of nitrogen atoms (1–10) in molecule A (dashed lines) to nitrogen atoms (11–20) in molecule B (solid lines).

rangement for the all- α -hairpin. In both crystals, the hydrogen bonds linking the heads of the left-side molecules to the heads of the right-side molecules are mediated by two water molecules, utilizing the carbonyl groups from D-Pro and Leu and the NH from Gly. The water molecules act as a scaffold that connects the β -sheets into a supramolecular assembly. Note, however, that in peptide 3, the W2–N16 distance is too large for a hydrogen bond, and instead, N16H is a donor to O6 directly. The packing of the stacks of β -sheets shown in Figure 9 is repeated along the horizontal direction (c axis), creating a hydrophobic boundary between the C- and N-termini of molecules A and B, as was also observed in the crystal of the all- α -hairpin.^[7b]

Circular dichroism: Model β -hairpin peptides containing only aliphatic α -amino acid residues with D-Pro-Gly as the nucleating turn segment have generally yielded CD spectra characterized by a negative CD band at ~218 nm. The CD spectra of model β -hairpin peptides may arise as a conse-

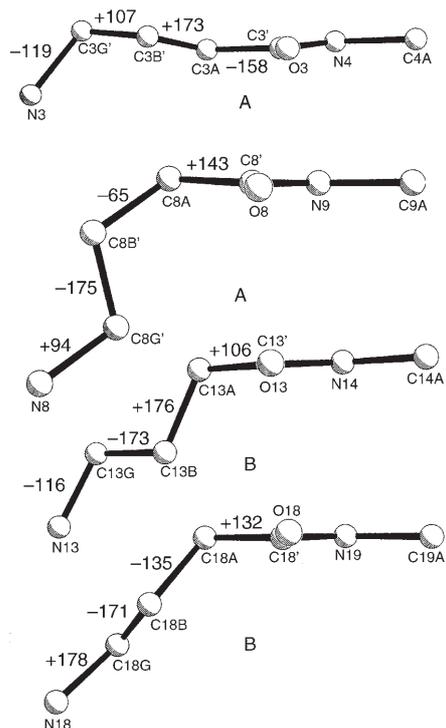


Figure 8. Comparison of four γ Abu residues, indicating the variations in the backbone torsion angles.

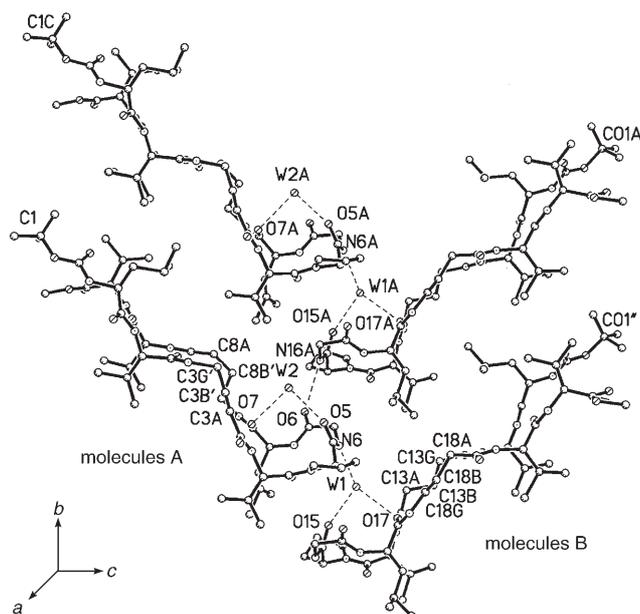


Figure 9. Crystal packing of the β -sheets (side view). The heads of the hairpin β -turns are connected in the vertical direction by one direct hydrogen bond, N16A...O6, and several hydrogen bonds mediated by water molecules, N6...W1, W1...O15, and W1...O17; and W2...O5 and W2...O7. If the assemblage is translated by one cell-length in the c direction, the tails of molecules A and B interdigitate with van der Waals distances between the hydrophobic tails and side chains.

quence of contributions from both the nucleating turn segment and registered antiparallel strands. Peptides **2–4** pro-

vide an example in which backbone peptide units of the strand segments have been systematically separated by an increasing number of methylene groups, thereby altering the spatial relationships between the amide chromophores. Figure 10 shows the far UV-CD spectra of peptides **2–4**. In

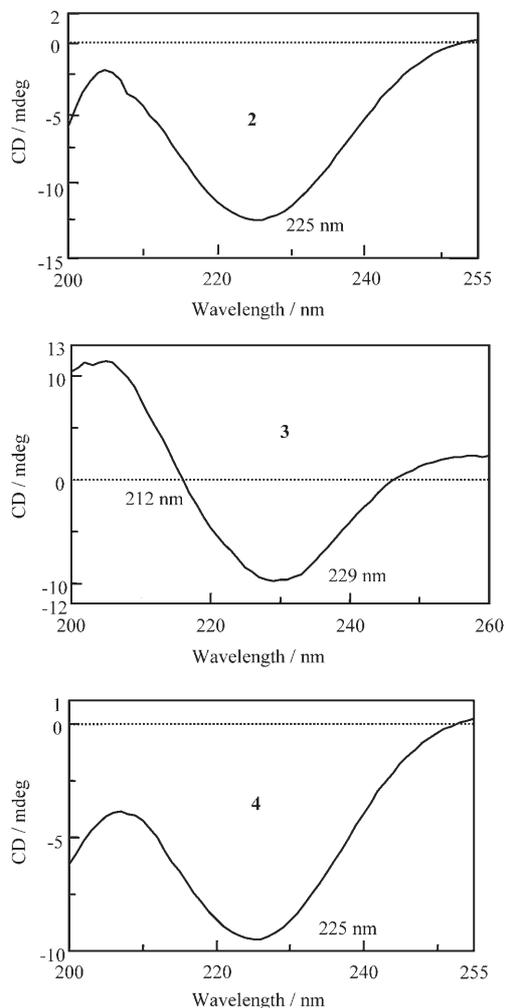


Figure 10. Far UV-CD spectra of peptides **2–4** in methanol at 300 K recorded at concentrations of 0.44–0.47 mM.

each case, a negative CD band is observed. Notably, in peptide **3**, the minimum is at 229 nm, whereas in peptides **2** and **4**, the minimum is blue-shifted to 225 nm. A crossover point at 212 nm for peptide **3** is indicative of a positive CD band at a shorter wavelength. In contrast, in peptides **2** and **4**, the crossover is not observed, suggestive of a short-wavelength, negative CD band. It is tempting to correlate the observed CD pattern with the insertion of methylene groups between the amide chromophores; peptide **3** corresponds to insertion of an odd number of methylene groups, whereas peptides **2** and **4** contain an even number of methylene groups. The effect of changing the local polarity of the sheet by inverting the orientation of amide units merits further investigation. It is not yet clear whether CD spectra are influ-

enced by transition from registered hairpin structures to frayed structures that retain the turn and strand segments, but have lost antiparallel hydrogen bonds.

Conclusion

The insertion of ω -amino acid residues into the strand segments of a model peptide hairpin can be accomplished without disruption of the overall fold of the molecule. Unsubstituted β , γ , and δ -amino acid residues were successfully inserted into facing positions of antiparallel strands in model decapeptides. This study establishes that hybrid (α,ω) peptide hairpins can be obtained by rational design.

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