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β-Turn Analogues in Model αβ-Hybrid Peptides: Structural Characterization of Peptides Containing $\beta^{2,2}Ac_6c$ and $\beta^{3,3}Ac_6c$ Residues

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Abstract: The effect of *gem*-dialkyl substituents on the backbone conformations of β-amino acid residues in peptides has been investigated by using four model peptides: Boc-Xxx-$\beta^{2,2}Ac_6c$(1-aminomethylcyclohexanecarboxylic acid)-NHMe (Xxx = Leu (**1**), Phe (**2**); Boc = *tert*-butyloxycarbonyl) and Boc-Xxx-$\beta^{3,3}Ac_6c$(1-aminocyclohexaneacetic acid)-NHMe (Xxx = Leu (**3**), Phe (**4**)). Tetrasubstituted carbon atoms restrict the ranges of stereochemically allowed conformations about flanking single bonds. The crystal structure of Boc-Leu-$\beta^{2,2}Ac_6c$-NHMe (**1**) established a C_{11} hydrogen-bonded turn in the αβ-hybrid sequence. The observed torsion angles ($\alpha(\phi\approx-60°, \psi\approx-30°)$, $\beta(\phi\approx-90°, \theta\approx60°, \psi\approx-90°)$) corresponded to a C_{11} helical turn, which was a backbone-expanded analogue of the type III β turn in αα sequences. The crystal structure of the peptide Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe (**4**) established a C_{11} hydrogen-bonded turn with distinctly different backbone torsion angles ($\alpha(\phi\approx-60°, \psi\approx120°)$, $\beta(\phi\approx60°, \theta\approx60°, \psi\approx-60°)$), which corresponded to a backbone-expanded analogue of the type II β turn observed in αα sequences. In peptide **4**, the two molecules in the asymmetric unit adopted backbone torsion angles of opposite signs. In one of the molecules, the Phe residue adopted an unfavorable backbone conformation, with the energetic penalty being offset by a favorable aromatic interaction between proximal molecules in the crystal. NMR spectroscopy studies provided evidence for the maintenance of folded structures in solution in these αβ-hybrid sequences.

Keywords: amino acids · aromatic interactions · beta-turn analogues · peptides · conformation analysis

Introduction

The conformational properties of α-amino acid residues in peptides and proteins are conveniently described by two torsional angle variables about the N–C^α (ϕ) and C^α–CO (ψ) bonds. The classical Ramachandran map describes the stereochemically allowed regions of ϕ–ψ space, delineated by using the simple principle of avoiding van der Waals clashes between atoms.[1] A dramatic reduction in the extent of allowed conformational space is observed in the case of the Ala residue compared with the Gly residue. The profound effect of substitution at C^α is further exemplified by the extremely limited regions of sterically allowed conformational space for α-aminoisobutyric acid (Aib), which is an α,α-dialkylated residue.[2] The stereochemical restraints imposed on C^α tetrasubstituted amino acid residues have been extensively exploited in the design of short peptide sequences with well-folded structures.[2c,d,e,3] The explosion of interest in recent years on the conformational properties of backbone homologated residues, especially β- and γ-amino acid residues,[4] has been stimulated by many elegant demonstrations of folded peptide structures in oligomeric sequences[5] and in hybrid sequences containing α residues together with higher homologues.[6] 1-Aminocycloalkane carboxylic acids (Ac_nc; in which *n* is the number of atoms in the cycloalkane ring) have been shown to stabilize specific β-turn conformations and helical structures in short peptides.[7,3c] In particular, 1-aminocyclohexanecarboxylic acid (Ac_6c; Figure 1) has been used in peptide design studies.[8] Conformational properties of the related γ-amino acid residue, 1-aminomethylcyclohexane acetic acid, gabapentin (Gpn; Figure 1), have also been extensively investigated.[9] In the case of the Gpn residue, the presence of the dialkyl group at the central β atom restricts the range of allowed conformations about C^β–C^γ (θ_1) and C^α–C^β (θ_2) bonds to *gauche–gauche* conformations ((g^+, g^+), (g^-, g^-); in which $\theta_1,\theta_2\approx\pm60°$), as demonstrated by crystal-structure determinations of a large number of Gpn peptides. Folding of a linear chain is facilitated by successive *gauche* conformations about single bonds.

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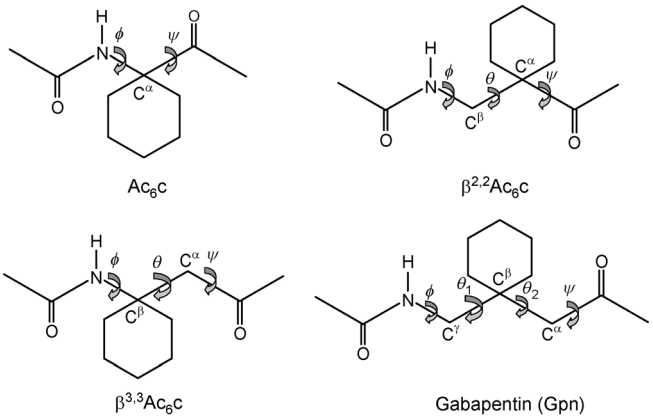

Figure 1. Definition of backbone torsion angles in α-, β-, and γ-amino acid residues.

In extending these studies to β-amino acid residues, we compared the structural effects of positioning the dialkyl substituents at the C^{α} and C^{β} atoms. An earlier study from our laboratory described the crystal structures of several protected derivatives and short peptides containing the β-residue 1-aminocyclohexaneacetic acid ($\beta^{3,3}Ac_6c$; Figure 1).[10] Interestingly, only one example of an intramolecularly hydrogen-bonded β-turn analogue was observed in the peptide Piv-Pro-$\beta^{3,3}Ac_6c$-NHMe [Piv = pivaloyl (tert-butyl carbonyl)].[10b] We turned, therefore, to the isomeric residue 1-aminomethylcyclohexanecarboxylic acid ($\beta^{2,2}Ac_6c$; Figure 1), for which structural analysis has been reported by the group of Seebach for the protected derivative Boc-$\beta^{2,2}Ac_6c$-OMe (Boc = *tert*-butyloxycarbonyl) and the tripeptide Boc-$\beta^{2,2}Ac_6c$-$\beta^{2,2}Ac_6c$-$\beta^{2,2}Ac_6c$-OMe. Notably, the tripeptide exhibits an unusual C_{10} intramolecular hydrogen bond with reversed polarity of the type $N{-}H_i{\cdots}O{=}C_{i+1}$.[11]

We describe herein conformational studies of the model sequences Boc-Xxx-$\beta^{2,2}Ac_6c$-NHMe (Xxx = Leu (**1**), Phe (**2**)) and Boc-Xxx-$\beta^{3,3}Ac_6c$-NHMe (Xxx = Leu (**3**), Phe (**4**)). Folded intramolecularly hydrogen-bonded conformations, which correspond to expanded analogues of canonical β turns in αα sequences, are established in these αβ-hybrid peptides. X-ray diffraction studies reveal that the precise nature of the αβ-hybrid turn conformation depends on the position of the dialkyl substituents.

Results

Peptide Crystal Structures

Single crystals were obtained for the peptides Boc-Leu-$\beta^{2,2}Ac_6c$-NHMe (**1**) and Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe (**4**). In both cases two independent molecules were observed in the crystallographic asymmetric unit. Backbone torsion angles and hydrogen-bond parameters are listed in Tables 1 and 2.

Boc-Leu-$\beta^{2,2}Ac_6c$-NHMe

Figure 2 shows a view of the molecular conformations observed in the two independent molecules. In both cases, a C_{11} (11-atom ring) hydrogen bond is observed, that is, Boc C=O···HNMe. The hydrogen-bond parameters (N3···O0) are N···O = 3.01 Å, ∢N–H···O = 145.0° for molecule 1 and N···O = 3.02 Å, ∢N–H···O = 139.5° in molecule 2.

Table 1. Backbone and side-chain torsion angles [°] in the crystal structures of **1** and **4**.[a]

Peptides	Amino acid residues[b]	ϕ	θ	ψ	ω	χ^1	χ^2
1 molecule 1	Leu(1)	−77.5		−14.2	176.2	−59.3	−61.3, 175.0
	$\beta^{2,2}Ac_6c(2)$	−93.9	64.9	−87.9	170.9		
molecule 2	Leu(1)	−72.8		−18.2	176.3	−57.3	−63.2, 172.9
	$\beta^{2,2}Ac_6c(2)$	−92.2	68.2	−90.8	170.4		
4 molecule 1	Phe(1)	55.5		−127.3	−167.8	−60.6	−27.3, 153.0
	$\beta^{3,3}Ac_6c(2)$	−55.7	−72.2	66.6	178.5		
molecule 2	Phe(1)	−64.1		145.1	173.4	−54.5	−62.9, 117.6
	$\beta^{3,3}Ac_6c(2)$	55.5	67.5	−74.3	−178.9		

[a] $\kappa(C'_{i-1}N_iC^{\alpha}_iC^{\beta}_i)$ for Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe, molecule 1, $\kappa_L = -80.4°$ (As, $\kappa_L \approx \phi - 120$) and for molecule 2, $\kappa_L = 175.8°$ (As, $\kappa_L \approx \phi - 120$). [b] (1) and (2) represent the amino acid residue number.

Table 2. Hydrogen-bond parameters in the crystal structures of **1** and **4**.

Peptides	Donor (D)	Acceptor (A)	D···A [Å]	H···A [Å]	∢D-H···A [°]
intramolecular hydrogen bonds					
1 molecule 1	N3	O0	3.01	2.28	145.0
molecule 2	N3'	O0'	3.02	2.31	139.5
4 molecule 1	N3	O0	2.92	2.18	145.9
molecule 2	N3'	O0'	2.93	2.23	138.4
intermolecular hydrogen bonds					
1	N1	O2 (−*x*, *y*+1/2, −*z*−1/2)	2.86	2.10	147.9
	N2	O1 (−*x*, *y*+1/2, −*z*−1/2)	3.26	2.50	146.9
	N1'	O2' (*x*+1/2, −*y*+1/2, −*z*)	2.90	2.09	153.1
	N2'	O1' (*x*+1/2, −*y*+1/2, −*z*)	3.18	2.40	154.0
4	N1	O1'	2.84	1.99	170.2
	N1'	O1	3.04	2.21	164.8
	N2'	O2'(−*x*, *y*−1/2, -*z*)	2.92	2.09	160.7
	N2	O2(−*x*−1, *y*+1/2, −*z*+1)	3.13	2.28	174.8

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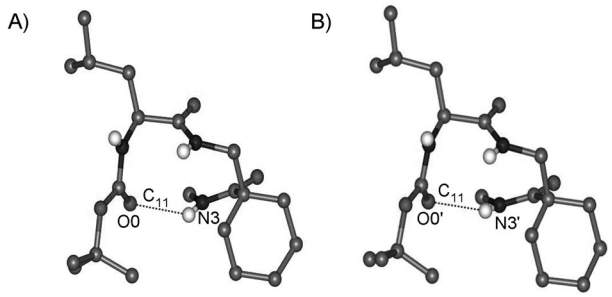

Figure 2. A view of two independent molecules (molecules 1 (A) and 2 (B)) in crystals of Boc-Leu-$\beta^{2,2}Ac_6c$-NHMe (**1**). Molecules are shown in the same orientation.

The α residue, Leu (**1**), adopts ϕ–ψ values in the helical region of conformational space. The $\beta^{2,2}Ac_6c$ residues adopt *gauche* conformations about the C^{α}–C^{β} bonds ($\theta \approx 60°$). The $\beta^{2,2}Ac_6c$ backbone conformation may be described by the torsion angles $\phi \approx -90°$, $\theta \approx 60°$, $\psi \approx -90°$. These values correspond closely to those previously characterized for helical C_{11} turns in hybrid αβ sequences. The following backbone torsion angles have been suggested for an ideal αβ C_{11} helix: α($\phi = -62°$, $\psi = -44°$), β($\phi = -105°$, $\theta = 80°$, $\psi = -73°$).[4d] These may be formally considered as a backbone-expanded analogue of the 3_{10} helix.[12] Molecule 1 forms intermolecularly hydrogen-bonded chains along the crystallographic *a* axis, whereas molecule 2 forms a similar chain in a perpendicular direction along the *b* axis. The two exposed N–H groups in each molecule are hydrogen bonded to exposed C=O groups of a symmetry-related neighbor (Figure S1 in the Supporting Information).

Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe

Figure 3 shows a view of the molecular conformation of both independent molecules. C_{11} hydrogen-bonded turns (Boc C=O···HNMe) are observed and the hydrogen-bond parameters are as follows: molecule 1: N···O = 2.92 Å, ∢N–H···O = 145.9° for N3···O0; molecule 2: N···O = 2.93 Å, ∢N–H···O = 138.4° for N3'···O0'.

Peptide **4** crystallized in the monoclinic space group $P2_1$. Surprisingly, the two independent molecules in the asymmetric unit revealed opposite signs for the backbone torsion angles. The identity of the configuration at both chiral Phe residues was established by measuring the dihedral angle $\kappa(C'_{i-1}N_iC^{\alpha}{}_iC^{\beta}{}_i)$ and using the relationship $\kappa_L \approx \phi - 120°$.[13] The L configuration was assumed because the synthetic peptide was derived from L-Phe. The Phe residues in both molecules adopt polyproline (P_{II})-like conformations of opposite handedness. This may be contrasted with the helical (α_R) conformation adopted by the Leu residue in peptide **1**.

The unusual backbone conformation for the Phe residue in one of the two molecules in the asymmetric unit may be a consequence of crystal packing forces. Figure 4 shows a view of two closely packed translationally related molecules and reveals an extremely close aromatic interaction between the two phenyl rings of adjacent molecules.[14] The centroid to centroid distance of 3.98 Å and the very small interplanar angle of 5.9° are clearly indicative of a strong aromatic interaction. The orientation, best described as a parallel displaced structure, has been suggested to be energetically favorable in the case of benzene dimers[15] (Figure 4). The energetic cost required for an unfavorable backbone conformation at the Phe residue is undoubtedly paid by the favorable aromatic interactions between the adjacent molecules.

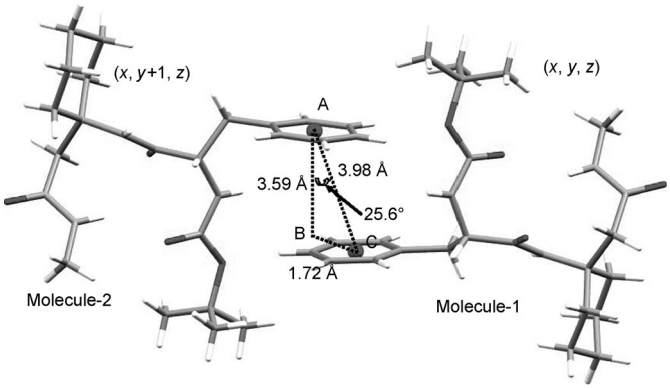

Figure 4. Aromatic interactions in crystals of **4**.The relevant parameters are as follows: centroid–centroid distance between the two phenyl group is 3.98 Å; interplanar angle = 5.9°; distance AB (3.59 Å) is the normal to the Phe plane of molecule 1(*x*, *y*, *z*), which passes through the centroid of the Phe ring of molecule 2(*x*, *y*+1, *z*).

Another interesting feature is the side-chain torsion angles observed for the Phe (**1**) residues in the two molecules: molecule 1: $\chi^1 = -60.6°$, $\chi^{2,1} = -27.3°$, $\chi^{2,2} = 153.0°$; molecule 2: $\chi^1 = -54.5°$, $\chi^{2,1} = -62.9°$, $\chi^{2,2} = 117.6°$. In both cases the χ^1 values are close to those normally observed for Phe residues in peptide crystal structures ($\chi^1 = 60° \pm 30°$, $-60° \pm 30°$, $180° \pm 30°$). However, there is a significant difference in the $\chi^{2,1/2,2}$ values observed for the Phe residues in the two independent molecules. In the case of molecule 1, the observed $\chi^{2,1/2,2}$ values ($\chi^{2,1} = -27.3°$, $\chi^{2,2} = 153.0°$) deviate considerably from that observed for Phe residues in peptides ($\chi^{2,1/2,2} = -90.0° \pm 30°$, $90.0° \pm 30°$).[16] In contrast, in mole-

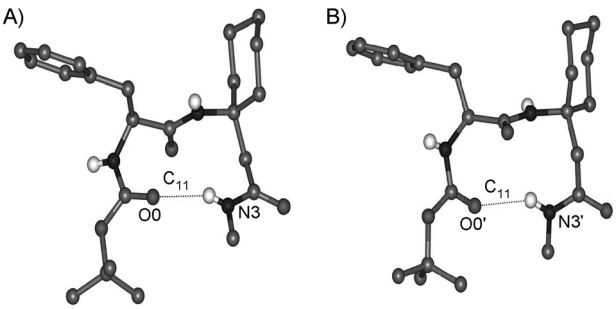

Figure 3. A view of two independent molecules (molecules 1 (A) and 2 (B)) in crystals of Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe (**4**). Molecules are shown in the same orientation.

cule 2, the observed $\chi^{2,1/2,2}$ values ($\chi^{2,1}=-62.9°$, $\chi^{2,2}=117.6°$) are in good agreement with those observed for Phe residues in peptides ($\chi^{2,1/2,2}=-90.0°\pm30°$, $90.0°\pm30°$).[16]

The $\beta^{3,3}Ac_6c$ residues adopt *gauche* conformations about the C^{α}–C^{β} bonds. In both molecules, the ϕ,ψ values at the $\beta^{3,3}Ac_6c$ residues lie close to 60°. This may be contrasted with the values observed for the $\beta^{2,2}Ac_6c$ residue, which are close to 90°. Most significantly, the relative signs of the dihedral angles ϕ, θ, and ψ differ in the cases of two kinds of β-amino acid residues. In $\beta^{2,2}Ac_6c$, the observed signs in peptide **1** are −, +, and −, whereas in $\beta^{3,3}Ac_6c$ they are −, −, and +. The nature of the hybrid αβ C_{11} turns formed by these two residues is thus different. In peptide **4**, the C_{11} turn may be considered as a backbone-expanded analogue of the type II β turn observed in αα segments.[10b] This is essentially a non-helical turn because repetition of the observed conformation does not lead to a continuous hydrogen-bonded chain. Figure 5 compares the backbone conformations of the two types of C_{11} turns characterized in peptides **1** and **4**.

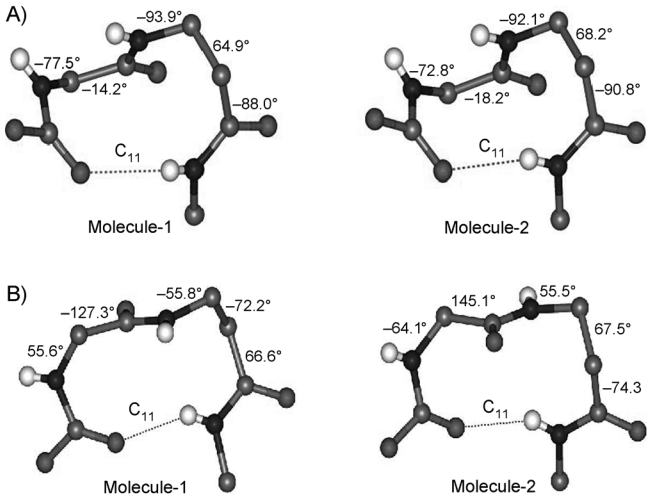

Figure 5. Conformation of αβ hybrid turns observed in **1** (A) and **4** (B). Backbone torsion angles are indicated.

Substituent Orientations on the Cyclohexane Ring

The cyclohexane rings of the $\beta^{2,2}Ac_6c$ and $\beta^{3,3}Ac_6c$ residues of peptides **1** and **4** adopt classical chair conformations. The endocyclic torsion angles are $\pm55.9°\pm1.5°$ for the $\beta^{2,2}Ac_6c$ residues and $\pm53.8°\pm4.9°$ for the $\beta^{3,3}Ac_6c$ residues with alternating signs. Figure 6 shows a view of the substituent orientation on the cyclohexane ring for the independent molecules in the crystallographic asymmetric unit for peptides **1** and **4**. In the case of the $\beta^{2,2}Ac_6c$ residue, the aminomethyl group in both molecules occupies an equatorial position (Figure 6). In the case of the $\beta^{3,3}Ac_6c$ residue, both molecules of peptide **4** reveal that the amino group occupies an axial position.

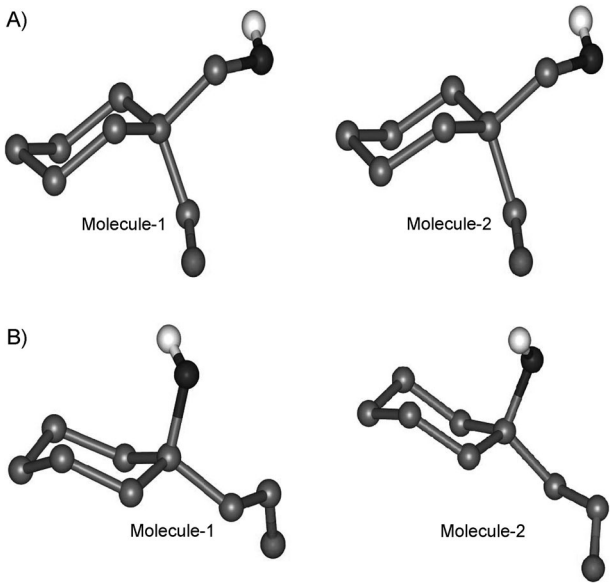

Figure 6. Cyclohexane conformations in **1** and **4**. A) The aminomethyl group in **1** is equatorial. B) The amino group in **4** is axial.

Conformations in Solutions

NMR spectroscopy studies were carried out in $CDCl_3$, which is a poorly interacting solvent that promotes intramolecularly hydrogen-bonded conformations. Specific NH resonance assignments were readily achieved by inspection of multiplet patterns in resolution-enhanced spectra. Delineation of the hydrogen-bonded NH groups in peptides **1** to **4** was probed by three methods: 1) Rates of hydrogen–deuterium (H/D) exchange in $CDCl_3/CD_3OD$ mixtures, 2) solvent dependence of NH chemical shifts upon addition of $[D_6]$DMSO to solutions in $CDCl_3$, and 3) free-radical-induced line broadening upon addition of 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO).

Boc-Leu-$\beta^{2,2}Ac_6c$-NHMe (1) and Boc-Phe-$\beta^{2,2}Ac_6c$-NHMe (2)

Figure 7 A illustrates the differential rates of H/D exchange observed in peptide **1** in $CDCl_3$ following the addition of CD_3OD. It is evident that the Leu(1) NH and $\beta^{2,2}Ac_6c(2)$ NH resonances exchange rapidly, while the methylamide(3) NH has a dramatically lower exchange rate. This is consistent with the involvement of the methylamide NH in intramolecular hydrogen bonding, as observed in the conformation characterized in crystals. The addition of $[D_6]$DMSO to solutions in $CDCl_3$ results in a downfield shift of NH resonances due to solvent–solute hydrogen bonding. Large solvent shifts ($\Delta\delta=\delta(CDCl_3+30\%\ [D_6]DMSO)-\delta(CDCl_3)$) are observed for exposed NH groups, whereas intramolecularly hydrogen-bonded or sterically shielded NH groups exhibit low $\Delta\delta$ values. In the case of peptide **1**, the observed $\Delta\delta$ values are as follows: Leu(1) NH: $\delta=1.58$ ppm; $\beta^{2,2}Ac_6c(2)$ NH: $\delta=0.95$ ppm, and NHMe: $\delta=0.75$ ppm.

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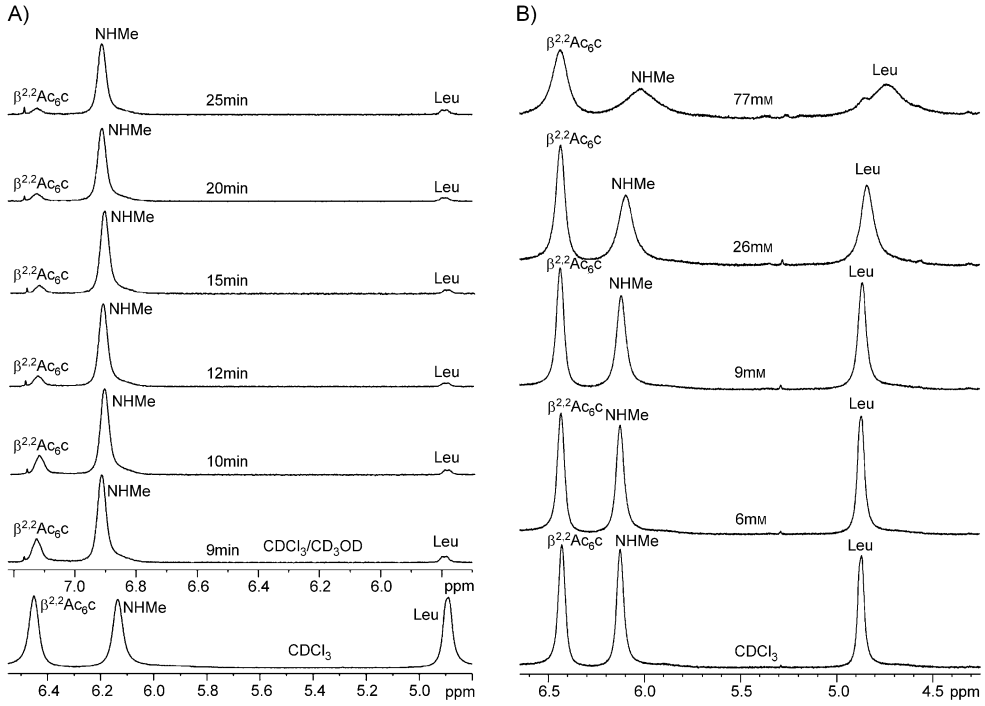

Figure 7.
A) H–D exchange of NH resonances in **1**. Exchange was initiated by addition of CD_3OD to a solution in $CDCl_3$. B) Broadening of the NH resonance in **1** on addition of the free radical TEMPO. Concentrations of the free radical are indicated.

The methylamide NH has a relatively low $\Delta\delta$ value compared with Leu(1) NH. Notably, the $\beta^{2,2}Ac_6c(2)$ NH also shows an appreciably lower $\Delta\delta$ value than that of Leu(1) NH. This may be a consequence of steric shielding because in the solid-state conformation the NH group points inwards into the αβ C_{11} turn (Figure 2). In this orientation, the approach of a [D_6]DMSO molecule may be impeded. Figure 7B shows the effect of the addition of the free radical TEMPO on the NH resonances in peptide **1**. Interestingly, the extent of line broadening follows the order Leu(1) NH ≈ NHMe(3) > $\beta^{2,2}Ac_6c(2)$ NH. The approach of the nitroxyl radical appears to be sterically less favorable in the case of $\beta^{2,2}Ac_6c(2)$ NH. Interestingly, H/D exchange rates are rapid for the $\beta^{2,2}Ac_6c(2)$ NH, which is consistent with the solid-state conformation. The results obtained by solvent perturbation and radical-induced broadening experiments are less definitive in delineating intramolecularly hydrogen-bonded NH groups. Similar results were obtained for Boc-Phe-$\beta^{2,2}Ac_6c$-NHMe (**2**).

Boc-Leu-$\beta^{3,3}Ac_6c$-NHMe (3) and Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe (4)

Figure 8 shows the H/D exchange spectra for the NH resonances in **4**. The order of exchange rates is Phe(1) NH > NHMe(3) ≫ $\beta^{3,3}Ac_6c(2)$. A comparison with **1** establishes a dramatic difference in exchange rate for the $Ac_6c(2)$ NH group, depending on the position of the cyclohexyl substituent. Whereas the relatively slow exchange rate for the

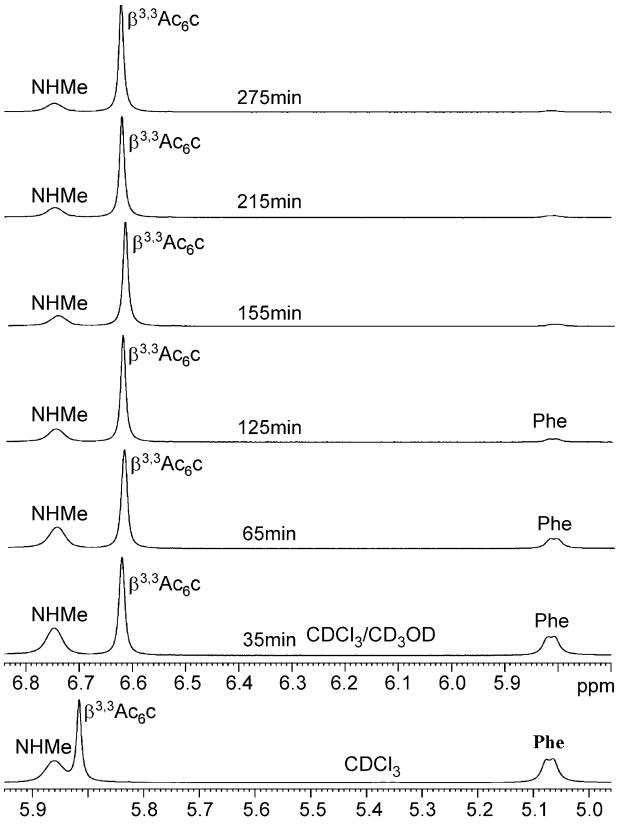

Figure 8. H–D exchange of NH resonances in **4**. Exchange was initiated by addition of CD_3OD to a solution in $CDCl_3$.

NHMe resonance relative to the Phe(1) NH may be attributed to its involvement in an intramolecular hydrogen bond, the behavior of the $\beta^{3,3}Ac_6c(2)$ NH group must be a consequence of steric shielding. The crystal structure of peptide **4** reveals that the amino group occupies an axial position on the cyclohexyl ring, with the NH hydrogen pointing inwards (Figure 6), an orientation in which solvation will be significantly hindered. The $\Delta\delta$ values observed in $CDCl_3$/[D_6]DMSO solvent titration experiments are as follows: peptide **3**: Leu(1) NH = 1.55 ppm, $\beta^{3,3}Ac_6c(2)$ NH = 0.95 ppm, NHMe(3) = 0.85 ppm; peptide **4**: Phe(1) NH = 1.50 ppm, $\beta^{3,3}Ac_6c(2)$ NH = 1.30 ppm, NHMe = 1.10 ppm. In the radical-induced broadening experiments, the observed order of line broadening is Phe(1) NH > NHMe (3) > $\beta^{3,3}Ac_6c(2)$ NH (results not shown). Similar results were obtained for Boc-Leu-$\beta^{3,3}Ac_6c$-NHMe (**3**).

Discussion

The introduction of *gem*-dialkyl substituents on backbone carbon atoms results in a dramatic restriction of allowed conformational space for the amino acid residues. The presence of a $C^{\alpha,\alpha}$-tetrasubstituted carbon atom limits the allowed torsion angles about the flanking single bonds.[2,3] In the case of β-amino acid residues, two distinct types of substitution patterns may be considered: $\beta^{2,2}$ and $\beta^{3,3}$ disubstituted derivatives. The results described herein establish the presence of folded conformations in model peptides containing $\beta^{2,2}Ac_6c$ and $\beta^{3,3}Ac_6c$ residues.

In the case of the $\beta^{2,2}$ substitution pattern, $\theta(N_i\text{-}C_i^{\beta}\text{-}C_i^{\alpha}\text{-}C_i')$ and $\psi(C_i^{\beta}\text{-}C_i^{\alpha}\text{-}C_i'\text{-}N_{i+1})$ are largely restricted to *gauche* values, whereas in the case of the $\beta^{3,3}$ substitution pattern, the torsion angles $\phi(C_{i-1}'\text{-}N_i\text{-}C_i^{\beta}\text{-}C_i^{\alpha})$ and $\theta(N_i\text{-}C_i^{\beta}\text{-}C_i^{\alpha}\text{-}C_i')$ are largely restricted to *gauche* values.[10] The crystal structures of **1** and **4** described above provide a view of two conformationally distinct C_{11} hydrogen-bonded turns for an αβ-hybrid backbone. The occurrence of two molecules in the asymmetric unit in both cases provides parameters for two sets of C_{11} turns. Figure 6 shows a view of the C_{11} turn characterized in these hybrid αβ sequences. Inspection of the backbone torsion angles revealed that the α residue adopted a conformation in the right-handed α-helical region (α_R) of Ramachandran space, in the case of peptide **1**. In contrast, the α residue adopts a P_{II}-like conformation in the case of peptide **4**. The $\beta^{2,2}Ac_6c$ residue in peptide **1** adopts a g^-, g^+, g^- conformation ($\phi \approx -90°$, $\theta \approx 60°$, $\psi \approx -90°$) with a distortion of about 30° at ϕ and ψ. In contrast, the $\beta^{3,3}Ac_6c$ residue in peptide **4** adopts a g^-, g^-, g^+ conformation with ϕ, θ, and ψ values lying very close to the ideal *gauche* value of about ±60°.

The two types of C_{11} turn structures observed in these αβ sequences may be related to the canonical β-turn structures in αα sequences. The C_{11} turn observed in **1** is best described as a backbone-expanded analogue of the classical type I/III β turn. This structure may be repeated to generate a continuous αβ C_{11} helix, which is a backbone-expanded analogue of the 3_{10} helix.[4d] The backbone torsion angle parameters described for an αβ C_{11} helix ($\alpha(\phi \approx -60°,\ \psi \approx -30°)$, $\beta(\phi \approx -90°,\ \theta \approx 60°,\ \psi \approx -90°)$)[4d] are in good agreement with those observed in peptide **1**, thus suggesting that the $\beta^{2,2}Ac_6c$ residue may be valuable in the design of helical folds, incorporating substituted β residues. In a peptide helix the cyclohexane rings project outwards, thereby providing a strongly apolar surface.

The C_{11} turn observed in **4** is a backbone-expanded analogue of the classical type II β turn in αα sequences. The $\beta^{3,3}Ac_6c$ residue adopts a g^+, g^+, g^- conformation with the preceding α residue in a P_{II} conformation. A similar C_{11} turn conformation has been previously established in the peptide Piv-Pro-$\beta^{3,3}Ac_6c$-NHMe.[10b] In peptide **4**, the two independent molecules adopt approximately enantiomeric conformations with a reversed sign of all the backbone torsion angles. As noted earlier, the unusual backbone conformation at the chiral Phe(1) residue in molecule 1 is likely to be a consequence of strong intermolecular aromatic interactions observed between translationally related molecules in the crystal. Previous studies of the $\beta^{3,3}Ac_6c$ residue established relatively few examples of intramolecular hydrogen-bonded conformations.[10] The model peptides described herein provide well-characterized examples of conformationally distinct hybrid C_{11} turns, which may be achieved in αβ sequences. Dialkylated β residues should prove valuable in the rational design of folded hybrid polypeptides.

Experimental Section

Peptide Synthesis

The Boc group was used for N-terminal protection, whereas the C terminus was protected as a methyl ester. Deprotection of the Boc group was achieved by using 98% formic acid and the methyl ester was removed by saponification with 2 N NaOH in methanol. Couplings were mediated by *N*,*N*-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The conversion of C-terminal methyl esters into N-methyl amides was carried out by saturating peptide ester solutions in dry tetrahydrofuran (THF) with methylamine gas. The final peptides were purified by reverse-phase medium-pressure liquid chromatography (MPLC; C_{18}, 40–60 μ) followed by high-performance liquid chromatography (HPLC; C_{18}, 10 μ, 7.8 mm x250 mm) by using methanol/water gradients.

X-ray Diffraction

Single crystals of dipeptides **1** and **4**, suitable for X-ray diffraction studies, were grown by slow evaporation from a methanol/water mixture. Peptide **1** crystallized in the orthorhombic space group $P2_12_12_1$ with two peptide molecules in the asymmetric unit, whereas peptide **4** crystallized in the monoclinic space group $P2_1$ with two peptide molecules in the asymmetric unit. For peptides **1** and **4**, X-ray data were collected on a Bruker AXS KAPPA APEXII CCD diffractometer with $Mo_{K\alpha}$ radiation (λ = 0.71073 Å) using phi and omega scans (crystal data and structure refinement parameters are listed in Table 3). For peptides **1** and **4**, the structures were solved by using direct method in SHELXS.[17] After the initial solution methods, all the structures were refined against F^2 isotropically followed by full-matrix anisotropic least-squares refinement by using SHELXL-97.[18] In the case of **1**, all hydrogen atoms attached to N atoms and to the atoms C1A, C1B, C1G, C2B, C2A1, C2A5, C1A′, C1B′, C2B′, and C7A′ were located from the difference Fourier map. In case of **4**, all hydrogen atoms attached to N atoms and to the atoms C1A, C2D2, C1A′, C2G", C2A′, and C2D" were located from the difference Fourier

Table 3. Crystal data and structure refinements for **1** and **4**.

Peptide	**1**	**4**
empirical formula	$C_{20}H_{37}N_3O_4$	$C_{23}H_{35}N_3O_4$
crystal habit	rectangular	rectangular
crystal size [mm]	(0.11×0.08×0.05)	(0.27×0.11×0.02)
crystallizing solvent	CH_3OH/H_2O	CH_3OH/H_2O
space group	$P2_12_12_1$	$P2_1$
a [Å]	10.6243(4)	14.143(2)
b [Å]	10.7162(4)	10.279(1)
c [Å]	41.358(2)	16.312(2)
β [°]		96.050(7)
V [Å^3]	4708.7(3)	2358.2(5)
Z	8	4
molecules/asymmetric unit	2	2
co-crystallized solvent	none	none
molecular weight	383.53	417.54
ρ_{calcd} [g cm^{-3}]	1.082	1.176
F (000)	1680	904
radiation	$Mo_{K\alpha}$	$Mo_{K\alpha}$
T [K]	296 (2)	296 (2)
2θ max [°]	59.20	54.30
measured reflns	34146	37676
R_{int}	0.0622	0.0458
unique reflns	7322	5507
observed refln [$\|F\| > 4\sigma(F)$]	3698	4069
final R [%]/$wR2$ [%]	5.10/14.89	4.05/10.99
GOF F^2 (S)	0.995	0.981
$\Delta\rho_{max}$ [e Å^{-3}]/$\Delta\rho_{min}$ [e Å^{-3}]	0.183/−0.157	0.120/−0.152
no. of restraints/parameters	6/579	10/605
data-to-parameter ratio	6.39:1	6.73:1

map. Suitable restraints were applied to get a chemically meaningful geometry. The remaining hydrogen atoms were fixed geometrically in the idealized position and allowed to ride with the C atoms to which they were bonded in the final cycles of refinement.

CCDC 861015 (**1**) and 861016 (**4**) 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.

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[1] a) G. N. Ramachandran, C. Ramakrishnan, V. Sasisekharan, *J. Mol. Biol.* **1963**, *7*, 95–99; b) G. N. Ramachandran, V. Sasisekharan, *Adv. Protein Chem.* **1968**, *23*, 283–437.

[2] a) G. R. Marshall, H. E. Bosshard, *Circ. Res.* **1972**, *30–31 (suppl. II)*, 143–150; b) A. W. Burgess, S. J. Leach, *Biopolymers* **1973**, *12*, 2599–2605; c) B. V. V. Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984**, *16*, 307–348; d) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756; e) C. Toniolo, E. Benedetti, *Macromolecules* **1991**, *24*, 4004–4009.

[3] a) S. Aravinda, N. Shamala, R. S. Roy, P. Balaram, *Proc. Indian Acad. Sci. Chem. Sci.* **2003**, *115*, 373–400; b) J. Venkatraman, S. C. Shankaramma, P. Balaram, *Chem. Rev.* **2001**, *101*, 3131; c) C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396–419.

[4] a) D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodiversity* **2004**, *1*, 1111–1239; b) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; c) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232; d) P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaram, *Chem. Rev.* **2011**, *111*, 657–687; e) F. Bouillère, S. Thétiot-Laurent, C. Kouklovsky, V. Alezra, *Amino Acids* **2011**, *41*, 687–707.

[5] a) D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, L. Huang, J. J. Barchi, S. H. Gellman, *Nature* **1997**, *387*, 381–382; b) D. Seebach, M. Overhand, F. M. N. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913–941; c) D. Seebach, M. Brenner, M. Rueping, B. Schweizer, B. Jaun, *Chem. Commun.* **2001**, 207–208; d) D. Seebach, M. Brenner, M. Rueping, B. Jaun, *Chem. Eur. J.* **2002**, *8*, 573–584; e) S. Hanessian, X. Luo, R. Schaum, S. Michnick, *J. Am. Chem. Soc.* **1998**, *120*, 8569–8570; f) P. G. Vasudev, K. Ananda, N. Shamala, P. Balaram, *Angew. Chem.* **2005**, *117*, 5052–5055; *Angew. Chem. Int. Ed.* **2005**, *44*, 4972–4975.

[6] a) I. L. Karle, A. Pramanik, A. Bannerjee, S. Bhattacharya, P. Balaram, *J. Am. Chem. Soc.* **1997**, *119*, 9087–9095; b) D. Seebach, D. F. Hook, A. Glättli, *Biopolymers* **2006**, *84*, 23–37; c) W. S. Horne, S. H. Gellman, *Acc. Chem. Res.* **2008**, *41*, 1399–1408; d) S. Chatterjee, R. S. Roy, P. Balaram, *J. R. Soc. Interface* **2007**, *4*, 587–606; e) S. K. Maji, R. Banerjee, D. Velmurugan, A. Razak, H. K. Fun, A. Banerjee, *J. Org. Chem.* **2002**, *67*, 633–639; f) P. G. Vasudev, S. Chatterjee, K. Ananda, N. Shamala, P. Balaram, *Angew. Chem.* **2008**, *120*, 6530–6532; *Angew. Chem. Int. Ed.* **2008**, *47*, 6430–6432; g) S. Chatterjee, P. G. Vasudev, S. Ragothama, N. Shamala, P. Balaram, *Biopolymers* **2008**, *90*, 759–771; h) S. Chatterjee, P. G. Vasudev, S. Ragothama, C. Ramakrishnan, N. Shamala, P. Balaram, *J. Am. Chem. Soc.* **2009**, *131*, 5956–5965.

[7] S. Vijayalakshmi, R. B. Rao, I. L. Karle, P. Balaram, *Biopolymers* **2000**, *53*, 84–98.

[8] a) R. Bardi, A. M. Piazzesi, C. Toniolo, M. Sukumar, P. Antony Raj, P. Balaram, *Int. J. Pept. Protein Res.* **1985**, *25*, 628–639; b) P. K. C. Paul, M. Sukumar, R. Bardi, A. M. Piazzesi, G. Valle, C. Toniolo, P. Balaram, *J. Am. Chem. Soc.* **1986**, *108*, 6363–6370; c) B. Di Blasio, A. Lombardi, F. Nastri, M. Saviano, C. Pedone, T. Yamada, M. Nakao, S. Kuwata, V. Pavone, *Biopolymers* **1992**, *32*, 1155–1161; d) M. Saviano, C. Isernia, F. Rossi, B. Di Blasio, R. Iacovino, M. Mazzeo, C. Pedone, E. Benedetti, *Biopolymers* **2000**, *53*, 189–199.

[9] a) P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaram, *Acc. Chem. Res.* **2009**, *42*, 1628–1639; b) L. Guo, W. Zhang, A. G. Reidenbach, W. M. Giuliano, I. A. Guzei, L. C. Spencer, H. S. Gellman, *Angew. Chem.* **2011**, *123*, 5965–5968; *Angew. Chem. Int. Ed.* **2011**, *50*, 5843–5846.

[10] a) P. G. Vasudev, R. Rai, N. Shamala, P. Balaram, *Biopolymers* **2008**, *90*, 138–150; b) R. Rai, P. G. Vasudev, K. Ananda, S. Raghothama, N. Shamala, I. L. Karle, P. Balaram, *Chem. Eur. J.* **2007**, *12*, 3295–3302.

[11] D. Seebach, S. Abele, T. Sifferlen, M. Hänggi, S. Gruner, P. Seiler, *Helv. Chim. Acta* **1998**, *81*, 2218–2243.

[12] a) C. Baldauf, R. Günther, H. J. Hofmann, *Biopolymers* **2006**, *84*, 408–413; b) S. H. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2008**, *130*, 6544–6650; c) M. A. Schmitt, S. H. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, *127*, 13130–13131; d) M. A. Schmitt, S. H. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2006**, *128*, 4538–4539; e) S. H. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2009**, *131*, 2917–2924; f) W. S. Horne, J. L. Price, J. L. Keck, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 4178–4180; g) K. Ananda, P. G. Vasudev, A. Sengupta, K. M. P. Raja, N. Shamala, P. Balaram, *J. Am. Chem. Soc.* **2005**, *127*, 16668–16674.

[13] I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Biopolymers* **1993**, *33*, 401–407.

[14] a) S. K. Burley, G. A. Petsko, *Science* **1985**, *229*, 23–28; b) L. Serrano, M. Bycroft, A. R. Fersht, *J. Mol. Biol.* **1991**, *218*, 465–475; c) J. B. O. Mitchell, R. A. Laskowski, J. M. Thornton, *Proteins Struct. Funct. Genet.* **1997**, *29*, 370–380; d) C. A. Hunter, K. M. Sanders, *J. Am. Chem. Soc.* **1990**, *112*, 5525–5534; e) C. Chipot, R. Jaffe, B. Maigret, D. A. Pearlman, P. A. Kollman, *J. Am. Chem. Soc.* **1996**, *118*, 11217–11224; f) L. M. Salonen, M. Ellermann, F. Diederich, *Angew. Chem.* **2011**, *123*, 4908–4944; *Angew. Chem. Int. Ed.* **2011**, *50*, 4808–4842; g) M. O. Sinnokrot, C. D. Sherrill, *J. Phys. Chem. A* **2006**, *110*, 10656–10668; h) K. S. Kim, P. Tarakeshwar, J. Y. Lee,

Chem. Rev. **2000**, *100*, 4145–4185; i) S. Marsili, R. Chelli, V. Schettino, P. Procacci, *Phys. Chem. Chem. Phys.* **2008**, *10*, 2673–2685.

[15] a) S. Tsuzuki, K. Honda, T. Uchimaru, M. Mikami, K. Tanabe, *J. Am. Chem. Soc.* **2002**, *124*, 104–112; b) M. O. Sinnokrot, E. F. Valeev, C. D. Sherrill, *J. Am. Chem. Soc.* **2002**, *124*, 10887–10893; c) C. A. Hunter, J. Singh, J. M. Thornton, *J. Mol. Biol.* **1991**, *218*, 837–846; d) G. B. McGaughey, M. Gagnes, A. K. Rappe, *J. Biol. Chem.* **1998**, *273*, 15458–15463; e) R. Podeszwa, R. Bukowski, K. Szalewicz, *J. Phys. Chem. A* **2006**, *110*, 10345–10354; f) C. D. Zeinalipour-Yazdi, D. P. Pullman, *J. Phys. Chem. B* **2006**, *110*, 24260–24265; g) P. Hobza, H. L. Selzle, E. W. Schlag, *J. Am. Chem. Soc.* **1994**, *116*, 3500–3506.

[16] A. Rajagopal, S. Aravinda, S. Raghothama, N. Shamala, P. Balaram, *Biopolymers* **2011**, DOI: 10.1002/bip.22003.

[17] G. M. Sheldrick, *SHELXS-97*, *A Program for Automatic Solution of Crystal Structures*, University of Göttingen, Göttingen (Germany), **1997**.

[18] G. M. Sheldrick, *SHELXL-97*, *A Program for Crystal Structure Refinement*, University of Göttingen, Göttingen (Germany), **1997**.

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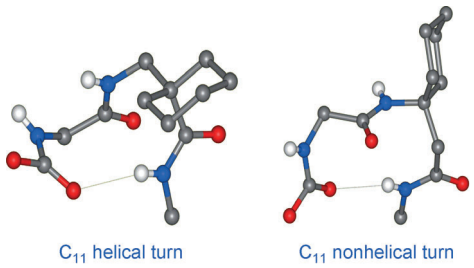

Twists and turns: Backbone-expanded analogues of β turns have been structurally characterized in peptides containing stereochemically constrained β-amino acid residues. The αβ-hybrid sequences yield analogues of both type I/III (helical) and type II (nonhelical) β-turn conformations (see picture).

Hybrid Peptides

Krishnayan Basuroy, Appavu Rajagopal, Srinivasarao Raghothama, Narayanaswamy Shamala, Padmanabhan Balaram** ____ ▮▮▮▮–▮▮▮▮

β-Turn Analogues in Model αβ-Hybrid Peptides: Structural Characterization of Peptides Containing $\beta^{2,2}Ac_6c$ and $\beta^{3,3}Ac_6c$ Residues

