Organic LETTERS

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C_{11}/C_9 Helices in Crystals of $\alpha\beta$ Hybrid Peptides and Switching Structures between Helix Types by Variation in the α-Residue

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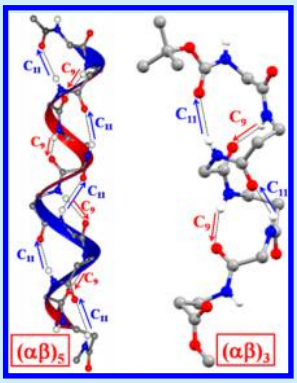
Supporting Information

ABSTRACT: Close-packed helices with mixed hydrogen bond directionality are unprecedented in the structural chemistry of α-polypeptides. While NMR studies in solution state provide strong evidence for the occurrence of mixed helices in $(\beta\beta)_n$ and $(\alpha\beta)_n$ sequences, limited information is currently available in crystals. The peptide structures presented show the occurrence of C_{11}/C_9 helices in $(\alpha\beta)_n$ peptides. Transitions between C_{11} and C_{11}/C_9 helices are observed upon varying the α-amino acid residue.



Canonical helical structures, such as the 3_{10} and α-helices found in proteins and polypeptides composed of α amino acids, are characterized by intramolecular hydrogen bonds that run in the same direction.[1] The two major helix types have hydrogen bonds of the type $C{=}O_i{\cdots}HN_{i+n}$ (n = 3 for the 3_{10} helix and n = 4 for the α-helix), which require orientation of peptide units in the same direction, resulting in the development of a macroscopic dipole moment, with a positive end at the N-terminus and negative end at the C-terminus.[2] Close-packed helical structures with mixed hydrogen bond directionality in which alternating C=O···HN hydrogen bonds run in opposite directions are unprecedented in the structural chemistry of α-polypeptides. The $\pi_{(L,D)}$ helix proposed for the gramicidin A sequence, where amino acid chirality alternates, has C_{16} and C_{14} hydrogen bonds in opposite directions but has a channel running through the body of the helix.[3]

The discovery of new hydrogen bonding patterns in polypeptide helices followed rapidly after the observation that helices with both hydrogen bond types $C{=}O_i{\cdots}HN_{i+n}$ and $NH_i{\cdots}C{=}O_{i+n}$ are possible in poly-β-peptides, which have an additional atom inserted into the backbone.[4] The novel C_{14} helix in which successive hydrogen bonds of type $NH_i{\cdots}CO_{i+2}$ are formed was the first member of this class to be structurally characterized in solution and the solid state.[5] Seebach and co-workers provided the first NMR evidence for helices with mixed hydrogen bonding patterns of the type $C_{12}/C_{10}/C_{12}$ (12/10/12 mixed helix) in their study of oligo-β-peptides with alternating substitution patterns (β^2 and β^3).[5c,6] Theoretical calculations by the groups of Hofmann[7] and Wu[8] provided a comprehensive evaluation of the energetics of helical structures in sequences containing β residues, permitting an assessment of relative stabilities of helices stabilized by unidirectional and mixed hydrogen bonding patterns. Most importantly, Wu and Wang recognized that mixed helices are preferred in sequences with alternating chirality of the β-residues.[8b] In considering the "chirality" of β-residues derived by backbone homologation of L-(S)-α-amino acids, it must be noted that the configuration specified at the asymmetric center is R.[4b] Molecular dynamics simulations have been used to compute the enthalpy difference between right-handed C_{12}/C_{10} helix and left-handed C_{14} helix for an oligo-β-peptide sequence.[9] Kessler and co-workers established the $C_{12}/C_{10}/C_{12}$ helix in an oligo-β-peptide, containing an alternating sequence of the unsubstituted residue β-homoglycine (β-hGly) and a sugar amino acid in which torsional freedom about the $C^{\beta}{-}C^{\alpha}$ (θ) bond was constrained by a furanoid sugar ring.[10] In an extensive series of investigations Sharma, Kunwar, and co-workers have characterized, by NMR, the C_{12}/C_{10} mixed helix in oligo-β-peptides containing a sugar amino acid, lacking covalent backbone constraints, in sequences with alternating residue chirality.[11] These authors demonstrated the occurrence of C_{11}/C_9 mixed helices in hybrid $\alpha\beta$ sequences, containing C-linked carbo-β^3-amino acids.[12] An *ab* initio MO study provides an indication of the intrinsic potential for formation of C_{11}/C_9 mixed helices in hybrid $\alpha\beta$ sequences.[13] The stereochemical patterning approach has been advanced for the design of novel helices in β-peptides and hybrid $\alpha\beta$ sequences.[14]

While studies in solution provide strong evidence for the occurrence of mixed helices in oligo-β-peptides and hybrid $\alpha\beta$ sequences, limited information is currently available in the crystalline state. Reported peptide crystal structures have been largely confined to short sequences of 3−4 residues, permitting characterization of isolated C_{11}/C_9 structures in $\alpha\beta$ peptides[15] and two examples of C_{12}/C_{10} structure in $\alpha\gamma\alpha\alpha$ and $\alpha\gamma\alpha\gamma$

Received: July 24, 2014
Published: August 21, 2014

dx.doi.org/10.1021/ol5021866 | *Org. Lett.* 2014, 16, 4614−4617

sequences[16]. Recently, Lee et al. have described mixed C_{11}/C_9 helices in peptides with the alternating D-Ala−*cis*-ACHC sequences [in the *cis*-ACHC residue the $C^{\alpha}-C^{\beta}$ torsion angle θ is constrained by covalent backbone cyclization].[17] During the course of studies of hybrid peptides containing the $\beta^{2,2}$-disubstituted residue $\beta^{2,2}Ac_6c$ (1-aminomethylcyclohexanecarboxylic acid), we observed formation of the mixed C_{11}/C_9 structure in the tripeptide Boc-Aib-$\beta^{2,2}Ac_6c$-Aib-OMe.[15d] Interestingly, in this example both of the $3_{10}/\alpha$-helix promoting Aib (α-aminoisobutyric acid) residues adopted the rarely observed semiextended polyproline (P_{II}, $\phi \approx -60°$, $\psi \approx 120°$; $P_{II'}$, $\phi \approx 60°$, $\psi \approx -120°$)-like conformations.[18] Extension of this sequence to 4 ($\alpha\beta\alpha\beta$) and 5 ($\alpha\beta\alpha\beta\alpha$) residue sequences yielded incipient C_{11} helices with conventional hydrogen bond directionality.[15d] In order to facilitate mixed helix formation by promoting P_{II} conformation at the α residues, we turned to $(\alpha\beta)_n$ sequences containing alternating chiral α-amino acid and $\beta^{2,2}Ac_6c$ residues.

We describe in this report the crystallographic characterization of two tripeptides, Boc-Leu-$\beta^{2,2}Ac_6c$-Aib-OMe (**1**) and Boc-Leu-$\beta^{2,2}Ac_6c$-Leu-OMe (**2**); one tetrapeptide, Boc-[Leu-$\beta^{2,2}Ac_6c]_2$-OMe (**3**); and one hexapeptide, Boc-[Leu-$\beta^{2,2}Ac_6c]_3$-OMe (**4**) (Figure 1). The tripeptides Boc-Leu-$\beta^{2,2}Ac_6c$-Aib-OMe (**1**) and

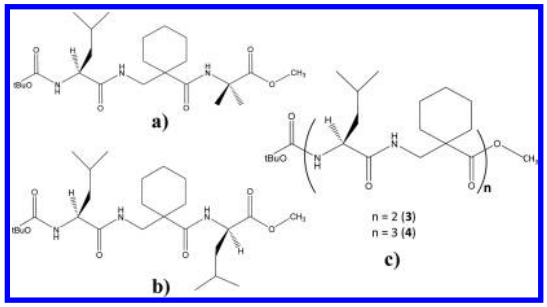


Figure 1. Structures of peptides. (a) Boc-Leu-$\beta^{2,2}Ac_6c$-Aib-OMe **1**. (b) Boc-Leu-$\beta^{2,2}Ac_6c$-Leu-OMe **2**. (c) Boc-[Leu-$\beta^{2,2}Ac_6c]_2$-OMe **3** and Boc-[Leu-$\beta^{2,2}Ac_6c]_3$-OMe **4**.

Boc-Leu-$\beta^{2,2}Ac_6c$-Leu-OMe (**2**) crystallized in the orthorhombic space group $P2_12_12_1$ with one peptide molecule in the crystallographic asymmetric unit (Supplemental Table S1). In both cases, mixed C_{11}/C_9 conformations are observed (Figure 2a,b). Extension to the tetrapeptide, Boc-[Leu-$\beta^{2,2}Ac_6c]_2$-OMe (**3**), revealed the persistence of the mixed C_{11}/C_9 hydrogen-bonded conformation (Figure 2c). This is in sharp contrast to the analogous tetrapeptide, Boc-[Aib-$\beta^{2,2}Ac_6c]_2$-OMe (**3**), in which Leu (1) and Leu (3) are replaced by Aib residues (Figure 2d) and two consecutive C_{11} hydrogen bonds are observed.[15d] The tetrapeptide **3** crystallized in the orthorhombic space group $P2_12_12_1$ with one peptide molecule and one co-crystallized solvent molecule ($CHCl_3$) in the crystallographic asymmetric unit (Supplemental Table S2).

Encouraged by these observations, we turned to an examination of longer (Leu-$\beta^{2,2}Ac_6c)_n$ sequences. Diffraction quality single crystals in the monoclinic space group *C*2 were obtained for the hexapeptide Boc-[Leu-$\beta^{2,2}Ac_6c]_3$-OMe (**4**) containing two peptide molecules in the crystallographic asymmetric unit along with one water molecule (Supplemental Table S2). Both molecules fold into a right-handed mixed C_{11}/C_9 helix containing four intramolecular hydrogen bonds, $C_{11}/C_9/C_{11}/C_9$ (Figure 3). The relevant backbone torsion angles are listed in Supplemental Table S3. The backbone torsion angles

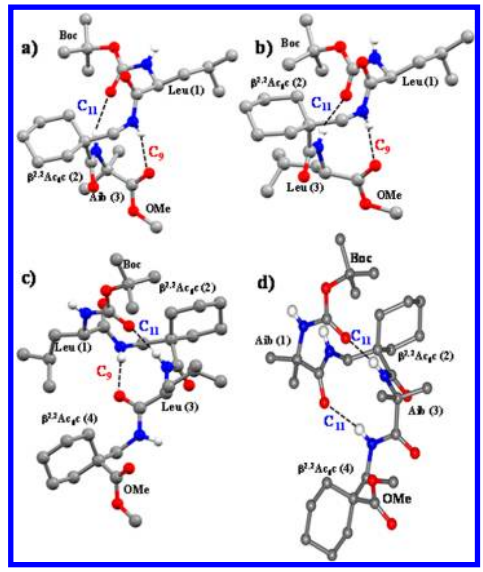


Figure 2. Molecular conformations of peptides in crystals. (a) Boc-Leu-$\beta^{2,2}Ac_6c$-Aib-OMe (**1**). (b) Boc-Leu-$\beta^{2,2}Ac_6c$-Leu-OMe (**2**). (c) Boc-[Leu-$\beta^{2,2}Ac_6c]_2$-OMe (**3**). (d) Boc-[Aib-$\beta^{2,2}Ac_6c]_2$-OMe.[15d]

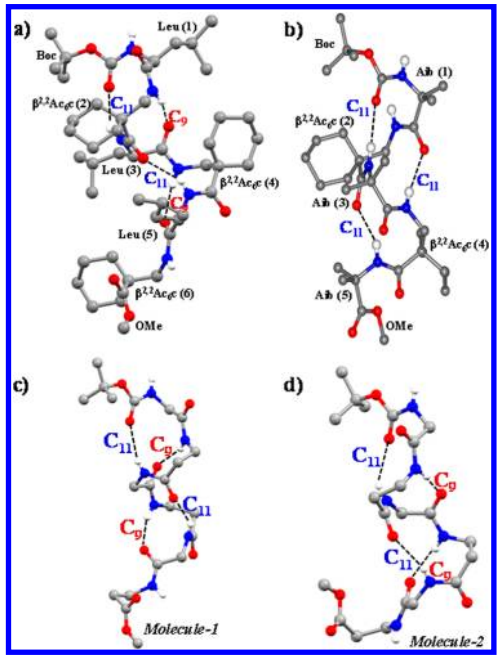


Figure 3. Molecular conformations of $(\alpha\beta)_n$ C_{11}/C_9 and C_{11} helices in the peptide sequences (a) Boc-[Leu-$\beta^{2,2}Ac_6c]_3$-OMe (**4**) and (b) Boc-[Aib-$\beta^{2,2}Ac_6c]_2$-Aib-OMe,[15d] respectively. (c, d) View of the backbone conformations of the two symmetry independent molecules in the crystallographic asymmetric unit of the hexapeptide Boc-[Leu-$\beta^{2,2}Ac_6c]_3$-OMe (**4**).

reveal that all six Leu residues, in the two independent molecules, adopt semiextended polyproline-like conformations (hydrogen bond parameters for all the peptides are listed in Supplemental Table S4). The structure of the hexapeptide **4** may be compared with a related $\alpha\beta\alpha\beta\alpha$ pentapeptide,[15d] containing Aib residues, which forms a C_{11} helical structure containing three successive C_{11} ($4 \rightarrow 1$) hydrogen bonds. The crystal structures described in this report provide definitive experimental support for the conformational requirement (P_{II}) at the α-residues in mixed helices. The β-residue necessarily requires a *gauche* conformation

dx.doi.org/10.1021/ol5021866 | *Org. Lett.* 2014, 16, 4614−4617

(g^+/g^-) about the $C^\beta-C^\alpha$ (θ) bond, depending on the chirality at the α-residue, in order to form hydrogen bonds with alternating directionality.

Figure 4a provides a distribution of backbone $\phi-\psi$ values from both α and β residues observed in the crystal structures of short

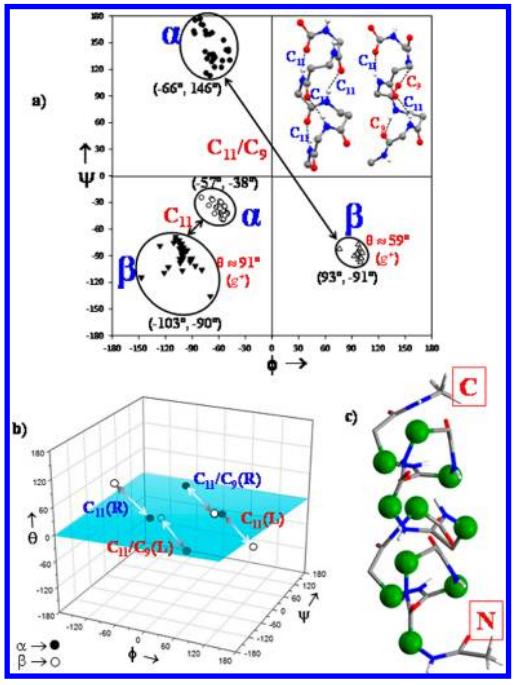


Figure 4. (a) Scatter plot in $\phi-\psi$ space for $(\alpha\beta)_n$ C_{11} and C_{11}/C_9 helices [θ average of β-residues for C_{11} and C_{11}/C_9 helices are 90.6°(9.0°) and 60.5°(4.1°), respectively]. Supplemental Tables S7 and S8 provide the listing of the structures used. (b) Schematic representation of *enantiomeric* helix types from both C_{11} and C_{11}/C_9 structures. Transitions between helices of the same handedness do not require significant changes in the values of θ. The points correspond to the average values determined from the analysis of the available crystal structures (see Supplemental Tables S7 and S8). (c) A view of the right-handed $(\alpha\beta)_n$ C_{11}/C_9 decapeptide helix, generated using the average backbone geometries (see Supplemental Tables S3, S5, and S6).

peptides containing a mixed C_{11}/C_9 hydrogen bonding pattern. In the case of observations made on peptides lacking chiral α-residues, two molecules of opposite handedness related by reflection or inversion symmetry are observed in crystals, characterized by achiral space groups. In such cases, only one set of torsion angles that corresponds to those observed for L-α-amino acid conformations has been chosen, resulting in right-handed helix types. For the β-residues the mean value of the torsion angle about the $C^\beta-C^\alpha$ (θ) is ~60°. The $\phi-\psi$ values for the β-residues cluster closely around 90°, with opposite signs. An alternative helical structure that may be considered for $(\alpha\beta)_n$ sequences is the continuous C_{11} helix, with conventional $C{=}O_i\cdots HN_{i+3}$ intramolecular hydrogen bonds, which is a backbone expanded analogue of the α-polypeptide 3_{10} helix. Cluster plots for observed $\phi-\psi$ values in $(\alpha\beta)_n$ C_{11} helices are also shown in Figure 4a, to permit direct comparison between two helix types. Figure 4b shows a representation of the right- and left-handed C_{11}/C_9 and continuous C_{11} helices on a 3D $\phi-\theta-\psi$ plot. Figure 4c shows a view of a right-handed, hybrid $\alpha\beta$ C_{11}/C_9 mixed helix generated for a model decapeptide using the average residue geometries derived from crystal structures of the peptides containing a $\beta^{2,2}$Ac$_6$c residue, with C_{11}/C_9 mixed hydrogen-bonding patterns (Supplemental Tables S3, S5, and S6 provide the listing of torsion angles, bond lengths, and bond angles used for making the model decapeptide). Figure 5 summarizes the

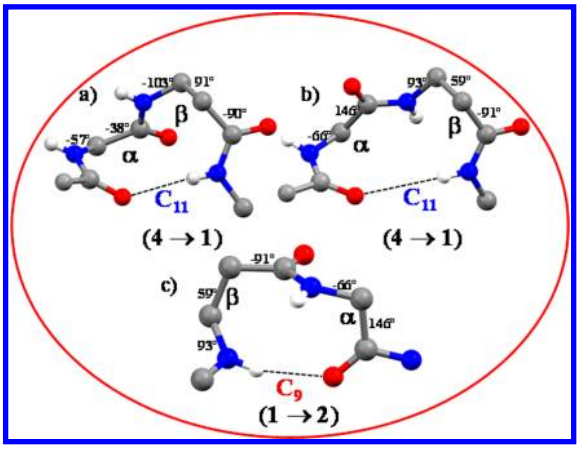


Figure 5. Average backbone torsion angles of the two types of hydrogen-bonded 4 → 1 ($\alpha\beta$) C_{11} turns and the 1 → 2 ($\beta\alpha$) C_9 turn. a) C_{11} turn in unidirectional C_{11} helices. b) C_{11} turn in alternate directionality C_{11}/C_9 helices. c) C_9 turn in alternate directional C_{11}/C_9 helices (Average backbone torsion angle values are shown).

backbone torsion angles characterizing two distinct C_{11} turn types and the C_9 hydrogen-bonded turn observed in structurally characterized $(\alpha\beta)_n$ hybrid peptides (Supplemental Tables S7 and S8 provide a listing of available structures). The two possible helix types, the C_{11} helix and the mixed C_{11}/C_9 helix characterized in $(\alpha\beta)_n$ sequences containing the $\beta^{2,2}$Ac$_6$c residue, differ only slightly in the observed values of θ ($C^\beta-C^\alpha$). It may be noted that the θ values in *unconstrained* β residues lie close to the ideal *gauche* value of 60°, while somewhat larger values of 80°–90° were observed in cyclically constrained residues.[19] As seen from Figure 3a,b, a transition between these two helix types may be achieved by major changes in the ψ value at the α residues ($P_{II} \rightarrow \alpha_R$, $\Delta\psi \approx 180°$) and ϕ values at the β residue (−90° → +90°, $\Delta\phi \approx 180°$). Transitions between helices of the same handedness do not involve changes in the sign of θ and can be achieved by 180° flips of alternate peptide units in $\alpha\beta$ segments, with relatively minor torsion angle change at $\beta\alpha$ segments. Further studies in solution are necessary to address the question whether a conformational equilibrium is indeed detectable. In $(\alpha\beta)_n$ sequences where both residues are chiral, mixed C_{11}/C_9 helices may be anticipated for sequences with alternating chirality (heterochiral), as demonstrated in earlier NMR studies.[12,20]

In the case of homochiral sequences, continuous helices (C_{11} or C_{14}/C_{15}) may be accessible in the absence of local conformational constraints. Indeed, Gellman and co-workers have provided NMR evidence for rapid interconversion between the 11-helix (C_{11}) and 14/15-helix (C_{14}/C_{15}) for a protected octapeptide composed of alternating sequences of (*S*,*S*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) and L-α-amino acids.[21] It is noteworthy that in the octapeptide Boc-[Aib-β^3(*R*)Val]$_4$-OMe a right-handed C_{14}/C_{15} helix, which is an analogue of the α-peptide α-helix (C_{13}) observed in crystals.[22]The backbone conformational parameters differ only slightly from those of the continuous C_{11} helix. Some general features of value in engineering specific helix types in $\alpha\beta$ hybrid peptides emerge from these structural results. In achiral $(\alpha\beta)_n$ sequences, as exemplified by (Aib-$\beta^{2,2}$Ac$_6$c)$_n$ peptides, both continuous C_{11} helices and mixed C_{11}/C_9 structures are indeed

dx.doi.org/10.1021/ol5021866 | *Org. Lett.* 2014, 16, 4614–4617

stereochemically feasible. The observed switch from the C_{11}/C_9 mixed helix to the C_{11} continuous helix on going from the tripeptides to the tetrapeptides may be attributed to the energy penalty that needs to be paid for the Aib residues to adopt the P_{II} conformation,[18] which is a requirement for C_{11}/C_9 helix formation. In principle, at the tetrapeptide level two intramolecular hydrogen bonds will be formed in both types of helices. The balance between these two helix types is then tilted by the conformational preferences of the Aib residue, which are determined by intraresidue nonbonded interactions. Hybrid $(\alpha\beta)_n$ sequences containing chiral α-residues and achiral β residues may provide an attractive model system for studying transitions in peptide helices between structures with unidirectional hydrogen bonds and mixed helices. Designed, model $(\beta\beta)_n$ peptides containing alternating chiral and achiral residues may be useful in experimentally probing the distribution of C_{14} helices (1 → 3, reverse hydrogen bond directionality), C_{12} helices (4 → 1, conventional hydrogen bond directionality), and C_{12}/C_{10} structures (mixed directionality). Notably the analogous C_{10}/C_8 structures in all α-polypeptides may be difficult to realize because of the requirement of a *cis* peptide bond to facilitate the C_8 hydrogen bond.[23]

The present study emphasizes the utility of the constrained β-residue $\beta^{2,2}Ac_6c$ in designing specific types of hydrogen-bonded helical structures in $(\alpha\beta)_n$ hybrid sequences, by varying the nature of the α residue.

■ ASSOCIATED CONTENT

Supporting Information

Details of synthetic procedure, X-ray diffraction data, structure solution, and refinement procedures and supplementary tables. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC deposition numbers for the peptides are 988391 (**1**), 988392 (**2**), 988393 (**3**), and 988394 (**4**), which 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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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported under the DBT-IISc partnership program. V.K. was supported by a UGC D. S. Kothari postdoctoral fellowship. We thank Dr. S. Raghothama for the ^{13}C NMR spectra.

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