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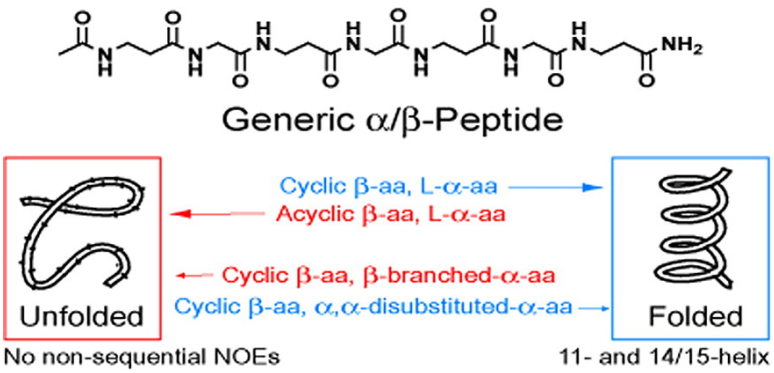

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Residue Requirements for Helical Folding in Short α/β-Peptides: Crystallographic Characterization of the 11-Helix in an Optimized Sequence

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Oligomers that are strongly predisposed to adopt specific conformations ("foldamers") have evoked widespread interest as scaffolds that can be used to generate new molecules with useful activities.[1] Enlarging the set of foldamer shapes should enhance our ability to achieve target functions, which will often require specific spatial arrangements of side chains. Reiser et al.[2] and we[3] have recently reported that short oligomers containing a 1:1 alternation of α- and β-amino acid residues ("α/β-peptides") adopt helical secondary structures in solution. Reiser et al. employed β-residues with a cyclopropyl constraint, while our α/β-peptides feature a five-membered ring β-residue constraint; these different constraints give rise to distinct helical shapes. The heterogeneous α/β-backbone is attractive for the design of functional foldamers because diverse side chains are supplied by readily available α-amino acid building blocks, while conformational stability and specificity are provided by the preorganized β-residues.[4]

Design of functional foldamers requires that one understand the conformational propensities of constituent residues. The propensities of α-residues have been extensively scrutinized in the context of pure α-backbones (i.e., conventional peptides and proteins).[5] More recently, the relationships between β-amino acid substitution pattern and β-peptide folding preferences have been elucidated.[6] Here, we explore the effects of variations in both α-residue and β-residue substitution on the favorability of helical folding among short α/β-peptides. Our results provide a foundation for structure-based design efforts involving these new foldameric scaffolds.

The new α/β-peptides described here (**2**−**7**) are based on octamer **1**, which was previously reported to display numerous *i*, *i* + 2 and *i*, *i* + 3 NOEs between backbone protons in CD_3OH (Figure 1).[3a] Rationalization of the complete NOE set for octamer **1** required us to propose rapid interconversion between two internally hydrogen-bonded helices.[3a] These conformations are designated the 11-helix and the 14/15-helix, based on the backbone C═O···H−N hydrogen bonding patterns (*i*, *i* + 3 and *i*, *i* + 4, respectively). Short α-peptides also oscillate between *i*, *i* + 3 and *i*, *i* + 4 C═O··· H−N hydrogen bonding patterns, that is, between the 3_{10}- and α-helical secondary structures.[7] (We subsequently found that lengthening the α/β-peptide backbone to 15 residues leads to predominance of the 14/15-helix.[3b]) We have now probed the effects of three changes in residue structure on α/β-peptide helicity: replacement of cyclically constrained with acyclic β-residues and introduction of a β-branched side chain or a second α-substituent into the α-residues.

The impact of cyclic β-residue constraint on α/β-peptide helicity was assessed by examining the four analogues of **1** in which one of the cyclic residues was replaced with an acyclic residue of comparable polarity; the cyclopentane residues were replaced with β^3-homoleucine (β^3hLeu; **2a** and **2c**), and the pyrrolidine residues were replaced with β^3hLys (**2b** and **2d**). In addition, we prepared **3**, in which all four cyclic β-residues were replaced. The maximally flexible α/β-peptide **3** displayed no NOEs in CD_3OH between

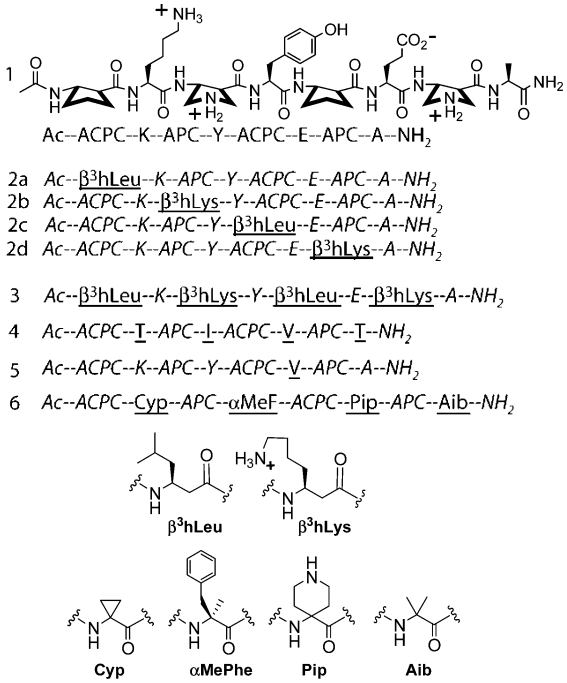

Figure 1. α/β-Peptides. Residues that have been altered to create analogues of **1** are underlined (and structures are shown at bottom).

residues that are not adjacent in sequence; the many *i*, *i* + 2 and *i*, *i* + 3 NOEs observed for **1** were absent for **3**. This stark difference clearly demonstrates that β-residue preorganization is essential for maximum α/β-peptide helix stability; a similar trend has been established within the pure β-peptide backbone.[8] NOE data for **2a**−**d** show that helical secondary structure propagates across a single β^3-residue, despite the decrease in conformational stability attending cyclic → acyclic β-residue substitutions. In each case, multiple NOEs involving nonadjacent residues emanate from and/or span the β^3-residue (in CD_3OH). These NOEs appear qualitatively to be less intense than the comparable NOEs from fully pre-organized **1** (see Supporting Information), which is consistent with the diminished folding propensity of β^3-residues relative to ring-constrained β-residues deduced from comparing **1** and **3**. The behavior of **2a**−**d** is promising with regard to our long-term interest in functional foldamers because insertion of an occasional β^3-residue will enhance our ability to generate specific constellations of side chains along helical α/β-peptide scaffolds.

α/β-Peptide **4** is an analogue of **1** in which the side chains of all four α-residues are β-branched; in contrast, none of the four α-residues of **1** has a branch point adjacent to the backbone. This α-residue change has a profound effect on folding: α/β-peptide **4** does not display any *i*, *i* + 2 or *i*, *i* + 3 NOEs in CD_3OH, in contrast to the extensive pattern of such nonsequential NOEs seen for **1**. Thus, we conclude that α-residues with β-branched side chains have

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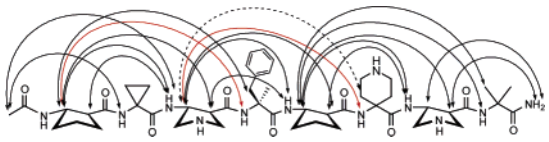

Figure 2. Medium-range NOEs observed in α/β-peptide **6**. Dotted line indicates an ambiguous NOE. Red NOEs were observed in both methanolic and aqueous solution.

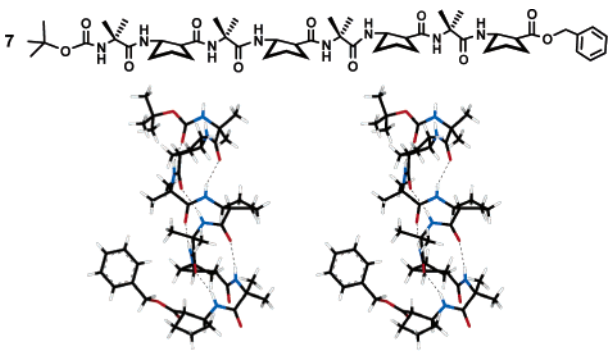

Figure 3. Stereoview of X-ray structure of **7**; view is perpendicular to the helical axis. Dotted lines indicate hydrogen bonds.

significantly lower propensity for α/β-peptide helix formation than do α-residues without such branching. This trend mirrors the low α-helical propensity of Val, Ile, and Thr,[5] but may reflect a departure from the impact of side chain branching on β-peptide helix formation.[9] The numerous *i*, *i* + 2 or *i*, *i* + 3 NOEs observed for **5** in CD_3OH show that α/β-peptide helices tolerate inclusion of isolated α-residues with β-branched side chains.

We used **6** to examine the effect of α,α-disubstituted α-amino acid residues on helical secondary structure. This α/β-peptide in CD_3OH displayed the largest number of *i*, *i* + 2 or *i*, *i* + 3 NOEs among **1**−**6** (Figure 2), which shows that α,α-disubstituted α-residues are tolerated within α/β-peptide helices and suggests that such residues may enhance helicity. Among α-peptides, α,α-disubstituted residues are well-known to promote helical folding,[10] but β,β-disubstituted residues discourage β-peptide helicity.[11] In our previous study of **1**, we found that nonsequential NOEs could not be detected in water,[3a] which indicates that this solvent is less conducive to folding than is methanol; comparable trends are well-established among α-peptides and β-peptides.[12] The strong NOE profile of **6** in methanol prompted NMR analysis in water. Although the number of NOEs was substantially diminished relative to methanol, **6** displayed two unambiguous *i*, *i* + 3 NOEs in water, which supports the conclusion that α,α-disubstituted α-residues are more conducive to α/β-peptide helicity than are α-monosubstituted residues.

The behavior of **6** led to crystallization trials with related octamer **7**, which contains alternating ACPC and Aib residues. This α/β-peptide adopts an 11-helical conformation in the solid state (Figure 3);[13] each of the six possible 11-membered ring hydrogen bonds is present. All of the *i*, *i* + 2 and *i*, *i* + 3 NOE patterns we predicted for an 11-helical conformation[3a] are consistent with the proton−proton distances observed along the backbone of **7** (see Supporting Information).

The results reported here provide guidelines for the design of helical α/β-peptides. Incorporation of acyclic β^3-residues or α-residues with a β-branched side chain leads to a diminution of α/β-peptide helix stability, while incorporation of α,α-disubstituted α-residues enhances helix stability. Perhaps it will be possible to use helix-stabilizing and helix-destabilizing substitutions to compensate for one another. These design rules are necessarily qualitative because we cannot determine folded populations for the α/β-peptide helices; indeed, there is no unnatural foldamer backbone for which reliable population analysis can yet be performed. Our findings will be useful for the generation of α/β-peptides that display specific side chain clusters. Foldamers of this type might mimic recognition surfaces on proteins and thereby disrupt specific protein−protein interactions[14] or perform multifunctional catalysis of chemical reactions.

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Supporting Information Available: Chemical shift assignments, NMR data collection procedures, and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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