β-Peptides are attracting increasing interest because they are able to adopt a variety of secondary structures, and choice of residue substitution pattern controls folding preference in a predictable way. In contrast to conventional peptides (i.e., peptides that consist of α-amino acid residues), β-peptides are not subject to proteolytic degradation, and they can display substantial conformational stability at relatively short lengths in water. These characteristics suggest that β-peptides are good scaffolds for the creation of biologically active molecules (see ref 4 for examples). Further development for biomedical applications requires efficient strategies for placing diverse sets of functional groups at specific sites along the β-peptide sequence; folding translates sequential relationships among side chains into spatial relationships.

Here we show that short β-peptide 14-helices, defined by 14-membered ring \( i \rightarrow i + 2 \) N–H–O=C backbone hydrogen bonds, can be easily decorated with diverse side chains without sacrificing conformational stability. Initial studies suggested that it was difficult to achieve high 14-helix stability and extensive side chain diversity simultaneously. The largest 14-helix propensity is displayed by residues preorganized by a six-membered ring constraint (e.g., trans-3-aminoacyclohexanecarboxylic acid (ACHC)), but these are difficult to functionalize. β-Substituted α-amino acids (“β-residues”), which can be prepared rapidly and enantioselectively from α-amino acids, support the 14-helix under some conditions, but oligomers containing only β-residues generally show little or no tendency to fold in water. Cheng and DeGrado and Seebach et al. have recently demonstrated that cyclic residues are not absolutely required for substantial 14-helicity in water: ion pairing between side chains of appropriately spaced residues can induce high 14-helix population in β-peptides containing only β-residues. Both groups recognized that i, i + 3 relationships between residues with complementary basic and acidic side chains (e.g., \( \beta^3 \)-homoornithine (\( \beta^3 \)-hOrn) and \( \beta^2 \)-hGlu) would allow intramolecular ion pair formation to promote 14-helical folding at neutral pH, because the 14-helix has ca. three residues per turn and a pitch of ca. 5.0 Å. The crucial structural role of the ion pairs was demonstrated by the precipitous decline or disappearance of 14-helicity at pH extremes. Dramatic pH effects led Cheng and DeGrado to conclude that the 14-helical propensity of β-residues in water is lower than the α-helical propensity of α-amino acid residues.

We began by comparing the zwitterionic hepta-β-peptide of Seebach et al. (I; Figure 1) with an analogue in which the three \( \beta^3 \)-hVal residues have been replaced with ACHC residues (2). Figure 2 juxtaposes the far-UV circular dichroism of 1 and 2 (0.2 mM) under four sets of conditions. Methanol is a strongly structure-promoting solvent for β-peptides, and the similarity of the CD signatures of 1 and 2 in this solvent (Figure 2a) suggests similar extents of 14-helicity. In pH neutral aqueous buffer (Figure 2b), CD data suggest that 1 retains considerable 14-helicity, as previously reported. The decrease in the intensity of the characteristic CD maximum of 1 in pH 7.2 aqueous buffer relative to methanol, however, indicates diminished 14-helical folding in water. In contrast, the CD spectrum of 2 is slightly more intense in pH 7.2 aqueous buffer relative to methanol. Thus, the 14-helical population of 2 may be maximal in both solvents. β-Peptide 1 displays little or no 14-helicity at pH extremes, but 2 retains a strong 14-helical CD signature (Figure 2c,d). Thus, the CD data suggest that alterations in pH exert relatively little effect on 14-helix population when three of the seven β-amino acid residues are preorganized.

We turned to two-dimensional NMR analysis for further insight on 2 because CD is an intrinsically low-resolution method. ROESY data for 2 in CD3OH (1.8 mM) and in 9:1 H2O:D2O...
half of the residues. It was not obvious from previous work that such a small proportion of preorganized residues would be so effective. Because this shape stability does not require attractive interactions between the side chains of the $\beta$-residues, these nonpreorganized residues are free to display functionality that serves other purposes such as catalysis or recognition. ACHC itself is readily prepared in either enantiomeric form, but access to functionalized derivatives is limited. Our results show that a few ACHC residues can compensate for the high intrinsic flexibility of $\beta$-residues, which allows one to take full advantage of the broad synthetic accessibility of $\beta$-residues in the design of $\beta$-peptides with defined shape and function in aqueous solution.

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Note Added in Proof. Schepartz et al. have recently reported (Hart, S. A.; Bahadour, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. J. Am. Chem. Soc. 2003, 125, 4022) that 14-helicity in $\beta$-peptides containing only $\beta$-residues can be promoted in water by combining favorable design features including side chain ion pairing and side chain branching adjacent to the $\beta$-peptide backbone. (For another recent example of side chain branching effects, albeit not in water, see: Raguse, T. L.; Lai, J. R.; Gellman, S. H. Helv. Chim. Acta 2002, 85, 4154.)

Supporting Information Available: A complete description of the NMR analysis and the equilibrium ultracentrifugation studies on 3 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(10) References 8 and 9 describe $\beta$-peptides containing $\beta$-residues with opposite configuration relative to those used here; therefore, our molecules display inverse CD signals.
(13) Ultracentrifugation data were acquired at several rotor speeds ranging from 25 to 60 k rpm. Linear least-squares fitting of ln(Abs275) versus (radial distance)3 data resulted in molecular weight estimates that were consistent with monomeric.

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