

Accommodation of α -Substituted Residues in the β -Peptide 12-Helix: Expanding the Range of Substitution Patterns Available to a Foldamer Scaffold

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Abstract: β -Amino acid oligomers composed exclusively of homochiral *trans*-2-aminocyclopentanecarboxylic acid (ACPC) residues and/or related pyrrolidine-based residues are known to favor a specific helical secondary structure that is defined by 12-membered ring C=O(*i*)-H-N(*i*+3) hydrogen bonds ("12-helix"). The 12-helix is structurally similar to the familiar α -helix and therefore represents a source of potential α -helix-mimics. The 12-helix will be most useful in this regard if this conformational scaffold can be employed to arrange specific sets of protein-like side chains in space. Here we examine whether the 12-helix tolerates insertion of acyclic β -amino acid residues bearing a substituent in the α -position (" β^2 -residues"). Seventeen homologous β -peptide heptamers have been prepared in which one to four β^2 -residues reside among ACPC and/or pyrrolidine residues. Circular dichroism comparisons suggest that β^2 -residues have a lower 12-helical propensity than do residues preorganized by a five-membered ring, as expected, but that β -peptides containing β^2 -residues at one or two of the seven positions retain a significant preference for 12-helix formation. These results indicate that a limited number of β^2 -residues can be used to introduce side chains at specific positions along the surface of a 12-helix.

Introduction

Interest in foldamers¹ (oligomers with well-defined folding propensities) is expanding in scope from control of molecular shape to control of function.² Engineering specific activities into foldamers is an attractive prospect because three-dimensional relationships among side chains in the folded conformation can be predicted on the basis of sequential relationships among monomer residues. The ability to design foldamers that perform specific tasks requires development of strategies for introducing side chains at defined positions along a given foldamer backbone. As the backbones of residues grow larger, there is

an increase in the number of positions within each residue at which side chains may be attached.

β -Peptides, oligomers of β -amino acids, are among the most thoroughly studied unnatural foldamers to date.³ The three types of regular secondary structure observed in α -amino acid peptides and proteins, reverse turn, sheet, and helix, have also been documented among β -peptides. β -Amino acids have two carbon atoms between the amino and carboxyl groups, which leads to a larger set of possible substitution patterns than is available for α -amino acids. Variation in residue substitution enables one to impose stronger and more diverse conformational propensities among β -peptides than are possible among α -peptides.³ For example, only two types of internally hydrogen bonded helix are commonly observed among α -peptides, the α -helix (13-membered ring C=O- H-N hydrogen bonds) and the 3_{10} -helix (10-membered ring hydrogen bonds). The α -helix seems intrinsically more favorable for most proteinogenic α -amino acid residues, but the factors that control helix preference among α -peptides are subtle.⁴ In contrast, four internally hydrogen bonded helices have been identified to date among β -peptides. These helices are named according to their internal hydrogen

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bonding pattern: the 14-helix, the 12-helix, the 10/12-helix, and the 10-helix.³ Formation of a particular β -peptide helix can be programmed by choice of residue substitution. 14-Helical folding is most strongly promoted by residues that incorporate a six-membered ring constraint, e.g., *trans*-2-aminocyclohexanecarboxylic acid (ACHC).⁵ The 14-helix is observed also in β -peptides composed of β -substituted β -amino acid residues (β^3 -residues)⁶ or in β -peptides composed of α -substituted β -amino acid residues (β^2 -residues).⁷ The 12-helix is promoted by residues that incorporate a five-membered ring constraint, e.g., *trans*-2-aminocyclopentanecarboxylic acid (ACPC) or *trans*-3-aminopyrrolidine-4-carboxylic acid (APC).^{8,9} The 10/12 helix is seen among β -peptide sequences in which β^2 - and β^3 -residues alternate.¹⁰ The 10-helix forms when residues have a four-membered ring constraint.¹¹

Increasing understanding of the relationship between β -amino acid substitution patterns and β -peptide conformational preferences has led to the development of β -peptides with interesting biological activities. Seebach et al., for example, have prepared amphiphilic 14-helices (i.e., 14-helices with lipophilic residues aligned along one side and hydrophilic residues aligned along the other), containing exclusively β^3 -residues, that inhibit fat absorption in a brush-border membrane model system.^{2a} De-Grado et al. have shown that amphiphilic 14-helices created with β^3 -residues display antimicrobial activity.^{2b,e,i} We have shown that amphiphilic 12-helices, generated by combining ACPC and APC in the proper sequence, also display antimicrobial activity.^{2d,f} Seebach et al. have used a reverse turn scaffold, constructed from β^2 - and β^3 -residues, to create potent somatostatin mimics.^{2g} A different β -peptide reverse turn unit has been used to replace a β -turn in ribonuclease A, generating a chimeric enzyme with nativelike stability and activity.^{2h}

The β -peptide 12-helix is a particularly interesting secondary structure because it resembles the α -helix in helical dimensions;^{8d} therefore, 12-helical scaffolds should allow one to mimic characteristic arrangements of side chains displayed by specific α -helices embedded within natural proteins. Achieving this goal might provide a general strategy for inhibiting protein–protein interactions that involve α -helix recognition. We have recently begun to develop strategies for placing specific functional groups at designated positions on a 12-helix. For example, we have shown that side chains can be introduced into the five-membered rings that preorganize β -amino acid residues for 12-helical

folding.^{8d,e} The use of functionalized cyclic residues is limited at present, however, because enantiospecific synthesis of the necessary monomers is time-consuming and because it is not yet possible to introduce side chains at all of the available positions of the five-membered ring. As a complementary approach to generating specifically functionalized 12-helices, we have interspersed β^3 -residues among ACPC and APC residues.¹² This strategy offers ready access to a wide array of side chain functionality because Fmoc- β^3 -amino acids can be rapidly and enantiospecifically prepared from the corresponding Fmoc- α -amino acids.¹³ At least one-third of the residues in a β -peptide may be acyclic without abolishing 12-helical folding propensity; we have observed potent antimicrobial activity in a 17-residue β -peptide that contains six β^3 -residues (along with 11 ACPC or APC residues) and was designed to form an amphiphilic 12-helix.¹²

The versatility of 12-helical scaffolds would be enhanced if β^2 -residues could be incorporated, since the side chains of these residues are positioned in a manner that is different from and complementary to the side chain positioning within β^3 -residues. β^2 -Amino acids are not as easily prepared as β^3 -amino acids,¹⁴ but recent developments offer significantly improved access to a broad range of side chain functionalities.¹⁵ Here we take advantage of this improved accessibility to examine the compatibility of β^2 -residues with the 12-helix secondary structure.

Results

Design. Hepta- β -peptides **1–17** (Chart 1) are related to a previously reported β -peptide, *p*-methoxyphenacyl-ACPC-APC-ACPC-APC-ACPC-APC-ACPC-NH₂, which displays 12-helical folding in methanol and, to a lesser extent, in water.¹² β -Peptides **1–17** contain (1*R*,2*R*)-ACPC, (3*S*,4*R*)-APC, and (*R*)- β^2 -residues. Among **1–7**, all possible replacements are made of cationic APC residues with cationic β^2 -hLys, including three single replacements (**1–3**), three double replacements (**4–6**), and one triple replacement (**7**). Among **8–17**, many of the possible replacements are made of ACPC residues with β^2 -hVal residues, including all four single replacements (**8–11**), three of the six double replacements (**12–14**), two of the four triple replacements (**15–16**), and the quadruple replacement (**17**). These systematic substitutions of cyclic residues with β^2 -residues of comparable polarity allow us to examine the impact on conformational stability of incrementally diminishing backbone preorganization. (The alternation of cationic and hydrophobic residues within these sequences was intended to discourage hydrophobically driven aggregation in aqueous solution.)

Circular Dichroism. Far-UV region CD data were acquired for hepta- β -peptides **1–17** in order to gain insight on conformational behavior. As is the case with conventional peptides,

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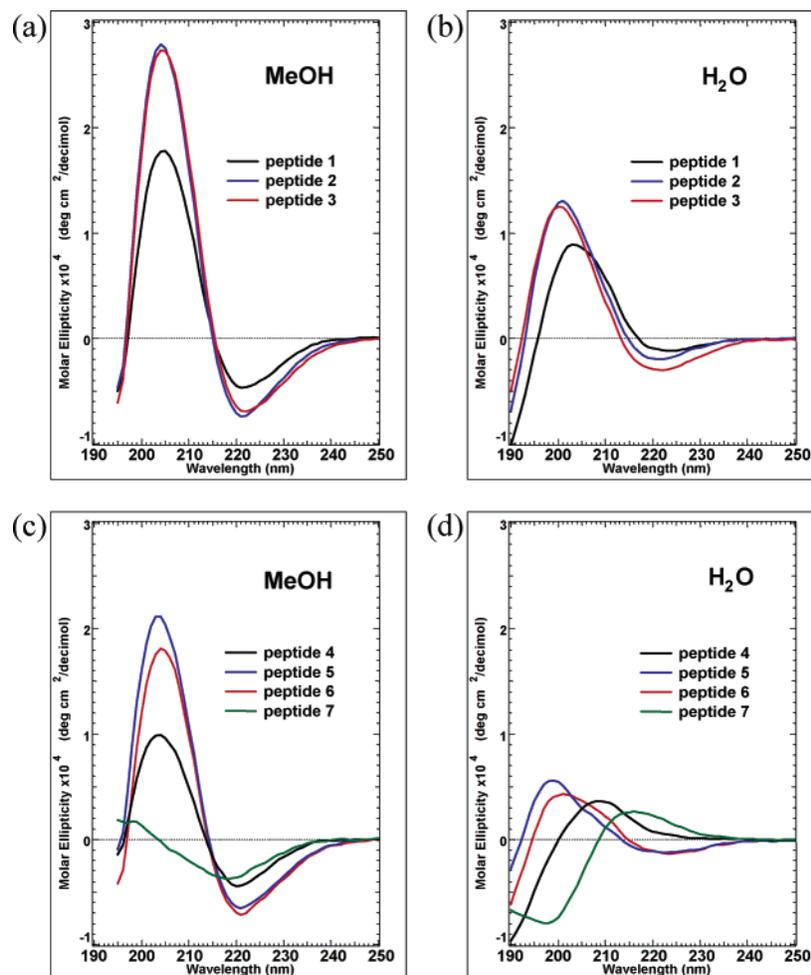


Figure 1. Circular dichroism data for β -peptides **1–7** (0.1 mM each) at 25 °C. The data are normalized for β -peptide concentration and number of residues (i.e., the vertical axis is mean residue ellipticity). (a) β -Peptides **1–3** in methanol. (b) β -Peptides **1–3** in water. (c) β -Peptides **4–7** in methanol. (d) β -Peptides **4–7** in water.

spectrum in both methanol and water, and in neither solvent does this spectrum correspond to the 12-helix. It is noteworthy that the shape of the CD spectrum of **7** changes fundamentally in water relative to methanol: there is a minimum at 220 nm in methanol, but a maximum at 218 nm in water. β -Peptide **4** displays a maximum at 208 nm, and both **5** and **6** have shoulders near this position, in addition to their maxima around 200 nm. Overall, these data suggest that **5** and **6** retain some 12-helical population in water, but that **4** and **7** do not.

Figure 2 shows CD data for **8–17** in methanol and in water. All four β -peptides with single β^2 -hVal replacements, **8–11**, show 12-helical CD signatures in methanol (Figure 2a) and in water (Figure 2b), although the signals are significantly weaker in water. Among the double replacements, **12** and **14** display 12-helical signatures in methanol, but **13** does not (Figure 2c). In water only **12** among the double replacement β -peptides appears to retain some 12-helical population (Figure 2d). Among the triple and quadruple replacements, **15–17**, none shows a clear 12-helical signature in either methanol (Figure 2e) or water (Figure 2f).

Nuclear Magnetic Resonance. Conformational analysis based on CD data must be regarded as tentative,²² and we

therefore examined several β -peptides among **1–17** via two-dimensional NMR. These studies were intended to determine whether the 12-helix can propagate across flexible β^2 -residues. CD-based conclusions are necessarily ambiguous on this point since partial 12-helix population could arise exclusively from the cyclic residues in these oligomers. Indeed, we have previously shown that a tetramer of ACPC displays the 12-helical CD signature in methanol;^{8b} a number of the oligomers discussed here contain blocks of four or more cyclic residues. In contrast to CD, NMR data provide insights on folding at specific positions along the β -peptide backbone. Since conformational transitions within these β -peptides, e.g., between 12-helical and unfolded states, are probably rapid on the NMR time scale, the data reflect conformational averaging.

β -Peptide **5** showed moderate proton resonance dispersion in CD₃OH at 14 °C. Two-dimensional NMR measurements were conducted at 4.2 mM; we conclude that no self-association occurs under these conditions because 10-fold dilution caused no change in the one-dimensional NMR spectrum (similar behavior was observed for the other oligomers examined by NMR (vide infra)). Resonance assignments for **5** were derived from COSY,²³ TOCSY,²⁴ and ROESY²⁵ measurements. Figure

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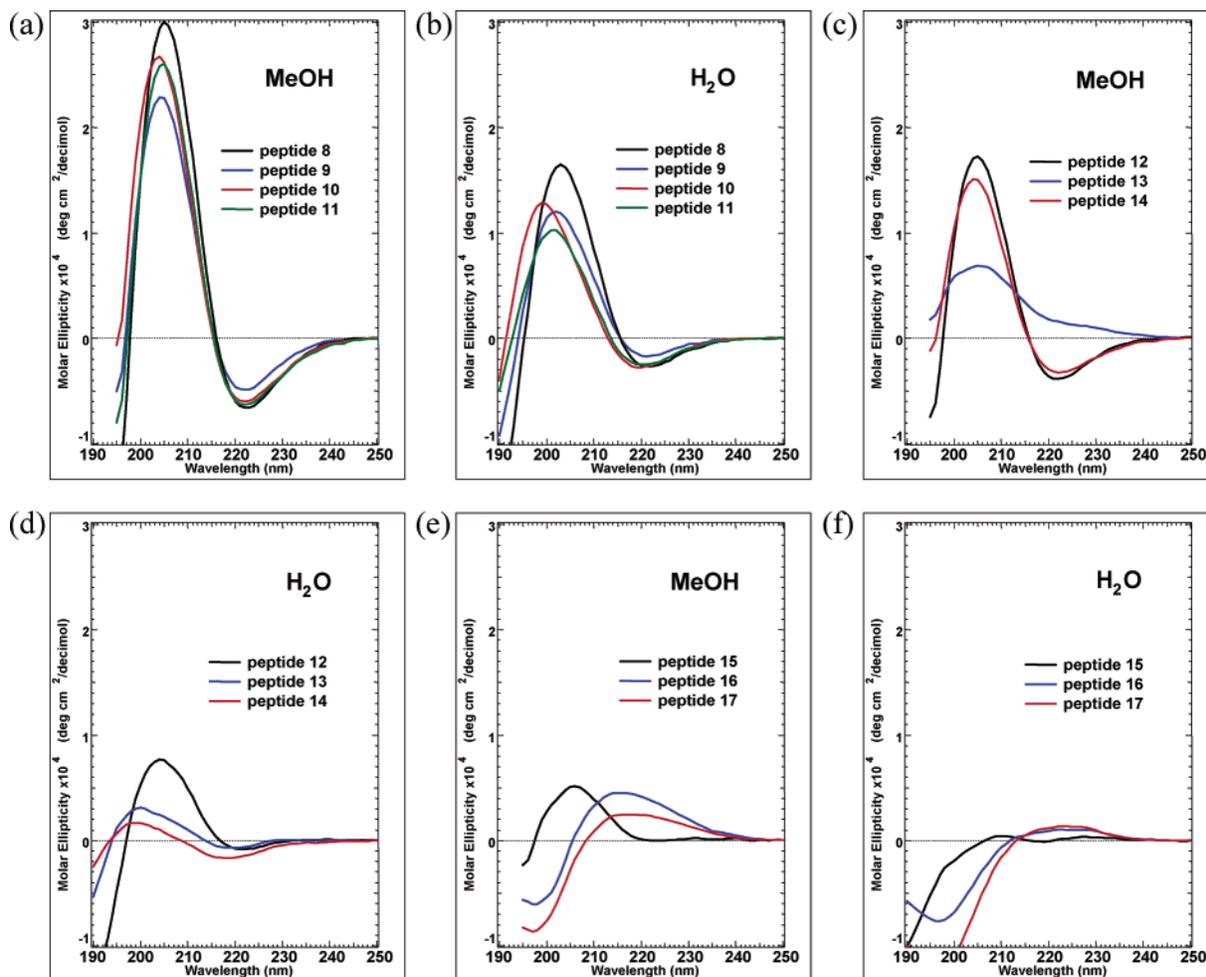


Figure 2. Circular dichroism data for β -peptides 8–17 (0.1 mM each) at 25 °C. The data are normalized for β -peptide concentration and number of residues (i.e., the vertical axis is mean residue ellipticity). (a) β -Peptides 8–11 in methanol. (b) β -Peptides 8–11 in water. (c) β -Peptides 12–14 in methanol. (d) β -Peptides 12–14 in water. (e) β -Peptides 15–17 in methanol. (f) β -Peptides 15–17 in water.

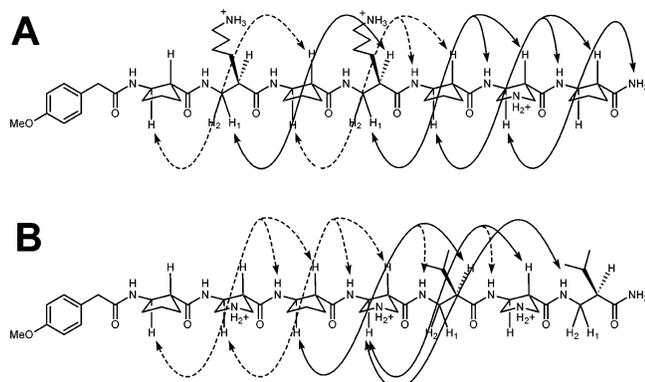


Figure 3. Graphical summary of NOEs involving sequentially nonadjacent residues observed in CD_3OH . The dotted lines indicate NOEs that may have been present but were obscured because of resonance overlap. The chemical shifts for diastereotopic C_βH protons on the acyclic β^2 -residues were not degenerate (as indicated), but these signals could not be assigned stereospecifically. (a) β -Peptide 5 (4.2 mM and 14 °C). (b) β -Peptide 12 (3.3 mM and 4 °C).

3a summarizes several of the NOEs detected between residues not adjacent in sequence. NOEs of this type constitute strong

evidence that compact conformations are at least partially populated. Assignment of some of the nonsequential NOEs observed for **5** was ambiguous because of resonance overlap.

The nonsequential NOEs for **5** include two types: $\text{C}_\beta\text{H}(i) \rightarrow \text{C}_\alpha\text{H}(i+2)$ and $\text{C}_\beta\text{H}(i) \rightarrow \text{NH}(i+2)$, as indicated in Figure 3a. Among the former, three of a possible total of five could be unambiguously identified, and among the latter three of a possible six were unambiguous. The other two possible $\text{C}_\beta\text{H}(i) \rightarrow \text{C}_\alpha\text{H}(i+2)$ NOEs may have been present, but their detection was obscured by overlapping signals between protons close in sequence (short-range signals). In addition, a fourth $\text{C}_\beta\text{H}(i) \rightarrow \text{NH}(i+2)$ NOE was obscured. Both of these nonsequential NOE patterns are consistent with 12-helical secondary structure. No nonsequential NOEs inconsistent with the 12-helix could be identified. Particularly important among the unambiguous NOEs is that between C_βH of β^2 -hLys-2 (i.e., the β^2 -hLys residue in position two) and C_αH of β^2 -hLys-4, which suggests that both of the β^2 -residues in **5** are incorporated into the 12-helix at least some of the time. This conclusion is further supported for the second β^2 -residue by the unambiguous NOEs between C_βH of β^2 -hLys-4 and both NH and C_αH of APC-6.

Proton resonance dispersion for β -peptide **12** in CD_3OH was optimal at 4 °C, but even under these conditions the dispersion was inferior to that seen for **5**. Nevertheless, three nonsequential NOEs could be unambiguously identified for **12** (Figure 3b).

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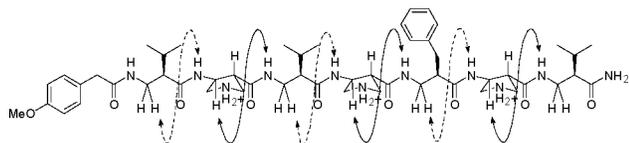


Figure 4. Sequential $C_{\beta}H(i) \rightarrow NH(i+1)$ NOE pattern observed in the ROESY spectra of **18** in CD_3OH . Signals between the $C_{\beta}H$ proton of APC residues and the NH of the following acyclic residue were of medium intensity and could be assigned unambiguously. However, the analysis NOEs between the $C_{\beta}H$ of acyclic residues and the NH of the following APC residue were obscured by overlap (indicated by dotted arrows). Although these $C_{\beta}H(i) \rightarrow NH(i+1)$ NOEs are between sequentially adjacent residues, the two protons are five bonds away from each other. Therefore, this NOE pattern may be indicative of partial folding.

All three span or involve β^2 -hVal-5: $C_{\beta}H$ of ACPC-3 $\rightarrow C_{\alpha}H$ of β^2 -hVal-5, $C_{\beta}H$ of APC-4 $\rightarrow C_{\alpha}H$ of APC-6, and $C_{\beta}H$ of APC-4 $\rightarrow NH$ of β^2 -hVal-7. These NOEs provide strong evidence that β^2 -hVal-5 is incorporated into the 12-helix at least part of the time. The last of these NOEs suggests that β^2 -hVal-7 may also participate in 12-helical folding, which is particularly interesting since helix termini tend to be frayed.²⁶

We were intrigued by the observation that β -peptide **17**, with alternating β^2 -hVal and APC residues, displays a CD spectrum in methanol that is quite distinct from the 12-helical signature. The CD spectrum of **17** in water is weaker but qualitatively similar to that in methanol. It seemed possible that this CD signature represents an alternative folding pattern, and we therefore analyzed related β -peptide **18** by NMR in CD_3OH (Figure 4). β -Peptides **17** and **18** differ only at residue 5, where β^2 -hVal in **17** has been replaced by β^2 -hPhe in **18**. Introduction of an aromatic side chain near the C-terminus was intended to enhance proton resonance dispersion without altering the intrinsic conformational propensity of the backbone. Far-UV region CD data for **17** and **18** were similar (not shown). The NMR spectrum of **18** displayed reasonable dispersion in CD_3OH at 14 °C, but only one unambiguous NOE was detected between sequentially nonadjacent residues, $C_{\beta}H$ of β^2 -hVal-1 $\rightarrow NH$ of β^2 -hVal-3. ($C_{\beta}H$ of β^2 -hVal-3 $\rightarrow NH$ of β^2 -hPhe-5 may have been present, but the cross-peak could not be unambiguously assigned because of resonance overlap.) The paucity of non-sequential NOEs suggests that **18** does not have a strong propensity to adopt a compact secondary structure.

Medium-intensity NOEs were observed for **18** in CD_3OH between $C_{\beta}H$ of each APC residue and NH of the following residue (Figure 4). Although these NOEs involve sequential neighbors, the protons involved are separated by five bonds, and their proximity is not necessarily enforced. Similar NOEs may have been present between the $C_{\beta}H$ of acyclic residues and the NH of the following cyclic residue (e.g., $C_{\beta}H$ of β^2 -hVal-1 $\rightarrow NH$ of APC-2), but detection of these signals was obscured by overlapping signals. $C_{\beta}H(i) \rightarrow NH(i+1)$ NOEs are observed in the 12-helical conformation, and their appearance in **18**, in the absence of CD data or multiple nonsequential NOEs consistent with 12-helix formation, hints that appropriately constrained β -amino acid residues such as APC and ACPC have an intrinsic conformational bias toward the 12-helix. Thus, β -peptides containing these residues may never be truly “unfolded.”

Discussion

The results presented here indicate that 12-helical secondary structure can propagate through flexible β^2 -amino acid residues,

at least when the β^2 -residues are flanked by cyclic residues preorganized for 12-helix formation. All of the hepta- β -peptides containing one β^2 -residue, and even a few with two β^2 -residues, show evidence by CD of partial 12-helicity in water, which is noteworthy since hepta- α -peptides composed of proteinogenic residues show no evidence of α -helix formation in water.²⁶ Although we cannot determine folded state populations for β -peptides, as discussed above, comparison of the present data with related data on β -peptides containing mixtures of residues constrained by five-membered rings and β^3 -residues¹² suggests qualitatively that β^2 - and β^3 -residues are tolerated to similar extents in the 12-helix.

The arrangement of residues has a significant impact on 12-helix formation within the series of homologous heptamers we examined. This point is seen most clearly by comparing isomers **12**, **13**, and **14**, all of which contain two β^2 -hVal residues. Both **12** and **14** show moderately strong 12-helical CD signatures in methanol, and the presence of a significant 12-helical population along the length of **12** in methanol is supported by two-dimensional NMR data. Isomer **13**, however, does not appear to fold to the 12-helix to a significant extent. This distinction can be explained by proposing that 12-helical secondary structure displays length-dependent cooperativity, i.e., that the 12-helix grows more stable as it grows longer. Length-dependent cooperativity is well-established in the α -helix formed by conventional peptides.²⁷ We have previously deduced that the 12-helix displays length-dependent cooperativity from observations that the extent of 12-helix population increases, on a per-residue basis, when ACPC heterooligomers or ACPC/APC heterooligomers are lengthened.^{8b,c} In the present case, the low tendency for 12-helix formation in **13** relative to isomers **12** or **14** presumably arises because the β^2 -residues have a much lower 12-helix propensity than do the cyclic residues, and placing the β^2 -residues near the center of the sequence (as in **13**) leads to smaller contiguous segments of cyclically preorganized residues than does placing the β^2 -residues near either end (as in **12** and **14**). The variation among isomers **12**–**14** shows that the ACPC and APC residues are not conformationally locked into the 12-helical conformation; if they were locked, the extent of 12-helicity would not vary among **12**–**14**. The energetic favorability of the 12-helical conformation must originate, in part, from favorable interactions between β -amino acid residues that are not sequentially adjacent. However, the short lengths at which 12-helicity becomes detectable, relative to α -helicity among conventional peptides,^{26,27a} suggests that the balance in helix-promoting factors between intrinsic conformational propensities of individual residues and favorable inter-residue interactions is skewed toward residue propensities in the 12-helix relative to the α -helix.

Our data show that 12-helix stability is substantially diminished by placement of two or three β^2 -residues in a heptamer otherwise composed of cyclically preorganized residues. Even when 12-helicity is undetectable, however, our findings suggest that the cost of adopting a 12-helical conformation will be relatively low for oligomers containing mixtures of cyclic and β^2 -residues. Neither **4** nor **14**, for example, shows evidence of

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12-helix formation in water, but both experience partial 12-helical folding in methanol. Similarly, we expect that a β -peptide containing a mixture of cyclic and acyclic residues could be readily induced to adopt a 12-helical conformation upon binding to an appropriate partner molecule, if the intermolecular interaction were sufficiently favorable. (Analogous coordinated binding/ α -helical folding events are well-known for interactions of peptides with other biopolymers or surfaces.²⁸) Thus, our results support the hypothesis, presented in the Introduction, that oligomers constructed from a mix of residues constrained by five-membered rings and acyclic β^2 - and/or β^3 -residues represent good starting points for the development of β -peptides that can mimic α -helical segments of natural proteins and thereby inhibit protein–protein interactions that involve α -helix recognition. Lead β -peptides generated in this way could presumably be improved by replacing some or all of the acyclic residues with derivatives of ACPC bearing an additional substituent, the side chain, at carbon-3, -4, or -5.

Experimental Section

Synthesis of β -Peptides. Fmoc-protected acyclic β^2 -amino acids (β^2 -hVal and β^2 -hLys), Fmoc-APC(Boc), and Fmoc-ACPC were synthesized by published methods.^{15c,29} All of the β -peptides were synthesized on a 25 μ mol scale with Fmoc-amide resin (AM^{NH2} PSC) by standard methods with HBTU activation on an Applied Biosystems Model 432A (Synergy) automated synthesizer. The module program for controlling the coupling–decoupling was modified to extend the reaction time automatically. Cleavage from the solid support and simultaneous deprotection of the side chain protecting groups were accomplished by shaking the resin with TFA/H₂O (95:5) for 3 h. The solid support was removed via filtration through glass wool and rinsed with additional TFA; the filtrate was then concentrated under a stream of nitrogen. The residue was dissolved in a minimum amount of methanol and then Et₂O was added to induce precipitation, which was maximized by cooling the mixture in an ice bath for 5 min. The mixture was centrifuged and the solution was decanted, to isolate the precipitate. Each crude β -peptide was purified by reverse-phase HPLC on a Vydac C₄-silica preparative column (10 μ m, 22 mm \times 250 mm), eluting with a linear gradient of acetonitrile in water (0.1% TFA in each) at a flow rate of 15 mL/min. Thus, each β -peptide was isolated as a triple trifluoroacetate salt. The purity of each β -peptide was established by observation of a single peak via analytical HPLC with a Vydac C₄-silica reversed-phase column (5 μ m, 4 mm \times 250 mm), and the identity of each β -peptide was verified by mass spectrometry (see below).

CD Spectroscopy. Circular Dichroism (CD) data were obtained on an Aviv 202SF spectrometer at 25 °C using a 1 mm path length quartz cell over a range of 185 to 260 nm for water solution and 190 to 260 nm for methanol solution. Samples were prepared by dissolving lyophilized β -peptides (after HPLC purification) in either water or methanol. Peptide concentrations were determined by mass, assuming one molecule of TFA per cationic charge. The data were normalized for β -peptide concentration and number of residues, which means the vertical axes in CD plots are mean residue ellipticity. HPLC-grade distilled water or methanol was used to prepare samples for CD analysis.

NMR Spectroscopy. NMR samples were prepared by dissolving the appropriate amount of β -peptide in CD₃OH to 4.2 mM for **5**, to 3.3 mM for **12**, and to 3.1 mM and 11 mM for **18** (two independent samples were prepared and analyzed for **18**; both samples resulted in identical spectra). Peptide concentrations were determined by mass, assuming one molecule of TFA per cationic charge. All spectra were acquired

on a Varian INOVA 600 MHz spectrometer with a 7000 Hz spectral window and a recycle delay of 1.0 s. Data were acquired at 14 °C for **5** and **18**, and at 4 °C for **12**. Solvent signal suppression was achieved by selective low-power irradiation for 0.6 to 1.5 s. All spectra were processed in standard VNMRSYS software and referenced to an internal 2-(trimethylsilyl)-1-propanesulfonic acid. Peak assignments were achieved from a combination of COSY, TOCSY (80 ms), and ROESY (200 ms) experiments. Typical TOCSY and ROESY data sets consisted of 500 to 600 free-induction decay (FID) increments of 12 to 28 transients each.

Mass Spectrometry. MALDI-TOF-MS (matrix-assisted laser desorption–ionization time-of-flight mass spectrometry) data were obtained on a Bruker REFLEX II spectrometer with a 337-nm laser using the α -cyano-4-hydroxycinnamic acid matrix. The instrument was calibrated to a standard mixture of Leu⁵-enkephalin ($M + H^+ = 556.28$), angiotensin I ($M + H^+ = 1296.7$), and neurotensin ($M + H^+ = 1672.9$).

β -Peptide 1. MALDI-TOF-MS *m/e* calcd for (C₅₀H₇₇N₁₁O₉) 975.6, found 976.6 ($M + H^+$), 998.6 ($M + Na^+$).

β -Peptide 2. MALDI-TOF-MS *m/e* calcd for (C₅₀H₇₇N₁₁O₉) 975.6, found 976.7 ($M + H^+$), 998.6 ($M + Na^+$), 1014.6 ($M + K^+$).

β -Peptide 3. MALDI-TOF-MS *m/e* calcd for (C₅₀H₇₇N₁₁O₉) 975.6, found 976.7 ($M + H^+$), 998.7 ($M + Na^+$), 1014.7 ($M + K^+$).

β -Peptide 4. MALDI-TOF-MS *m/e* calcd for (C₅₂H₈₃N₁₁O₉) 1005.6, found 1006.6 ($M + H^+$), 1028.6 ($M + Na^+$), 1044.6 ($M + K^+$).

β -Peptide 5. MALDI-TOF-MS *m/e* calcd for (C₅₂H₈₃N₁₁O₉) 1005.6, found 1006.7 ($M + H^+$), 1028.7 ($M + Na^+$), 1044.7 ($M + K^+$).

β -Peptide 6. MALDI-TOF-MS *m/e* calcd for (C₅₂H₈₃N₁₁O₉) 1005.6, found 1006.8 ($M + H^+$), 1028.8 ($M + Na^+$), 1044.8 ($M + K^+$).

β -Peptide 7. MALDI-TOF-MS *m/e* calcd for (C₅₄H₈₉N₁₁O₉) 1035.7, found 1036.4 ($M + H^+$), 1058.4 ($M + Na^+$).

β -Peptide 8. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₃N₁₁O₉) 947.6, found 948.2 ($M + H^+$), 970.2 ($M + Na^+$).

β -Peptide 9. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₃N₁₁O₉) 947.6, found 948.4 ($M + H^+$), 970.4 ($M + Na^+$), 986.4 ($M + K^+$).

β -Peptide 10. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₃N₁₁O₉) 947.6, found 948.1 ($M + H^+$), 970.1 ($M + Na^+$).

β -Peptide 11. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₃N₁₁O₉) 947.6, found 948.6 ($M + H^+$), 970.6 ($M + Na^+$), 986.6 ($M + K^+$).

β -Peptide 12. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₅N₁₁O₉) 949.6, found 950.3 ($M + H^+$), 972.3 ($M + Na^+$).

β -Peptide 13. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₅N₁₁O₉) 949.6, found 950.5 ($M + H^+$), 972.4 ($M + Na^+$), 988.4 ($M + K^+$).

β -Peptide 14. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₅N₁₁O₉) 949.6, found 950.3 ($M + H^+$), 972.3 ($M + Na^+$).

β -Peptide 15. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₇N₁₁O₉) 951.6, found 952.2 ($M + H^+$), 974.2 ($M + Na^+$).

β -Peptide 16. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₇N₁₁O₉) 951.6, found 952.3 ($M + H^+$), 974.3 ($M + Na^+$), 991.3 ($M + K^+$).

β -Peptide 17. MALDI-TOF-MS *m/e* calcd for (C₄₈H₇₉N₁₁O₉) 953.6, found 954.5 ($M + H^+$), 976.5 ($M + Na^+$), 992.5 ($M + K^+$).

β -Peptide 18. MALDI-TOF-MS *m/e* calcd for (C₅₂H₇₉N₁₁O₉) 1001.6, found 1002.6 ($M + H^+$), 1024.6 ($M + Na^+$), 1040.6 ($M + K^+$).

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