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Tolerance of Acyclic Residues in the β-Peptide 12-Helix: Access to Diverse Side-Chain Arrays for Biological Applications

Paul R. LePlae,† John D. Fisk,† Emilie A. Porter,† Bernard Weisblum,‡ and Samuel H. Gellman*,†

Departments of Chemistry and Pharmacology, University of Wisconsin-Madison, Madison, Wisconsin 53706

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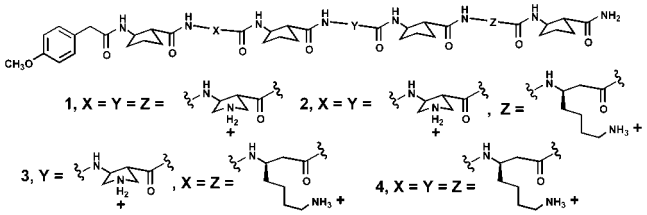
Oligomeric backbones with well-defined conformational propensities can serve as scaffolds for displaying sets of functional groups in specific three-dimensional arrangements. This approach has generated molecules that bind specifically to other molecules and/or manifest selective biological activity.[1,2] β-Peptides are particularly interesting as scaffolds because several distinct secondary structures can be induced by appropriate choice of β-amino acid substitution pattern.[3] The β-peptide 12-helix (defined by 12-membered ring C═O(*i*)- -H−N(*i* + 3) hydrogen bonds) merits special attention as this helix bears some resemblance to the α-helix commonly formed by conventional peptides.[4] We have previously shown that the 12-helix is promoted by residues containing a five-membered ring constraint, and that β-peptides containing as few as six appropriately constrained residues adopt 12-helical conformations in aqueous solution.[5]

Here we show that 12-helical propensity is maintained when some cyclic β-amino acid residues are replaced with acyclic residues. This result is important because use of acyclic residues greatly facilitates introduction of diverse side chains at specific sites along the 12-helical scaffold. We demonstrate the utility of this advance in the context of antibiotic design.



Initial studies involved hepta-β-peptides **1**−**4**, which contain three different residues: (1*R*,2*R*)-*trans*-2-aminocyclopentanecarboxylic acid (*trans*-2-ACPC),[6a] (3*S*,4*R*)-*trans*-3-aminopyrrolidine-4-carboxylic acid (*trans*-3,4-APC)[6b] and (3*R*)-β^3-homolysine (β^3-hLys).[7] Seebach et al. and others have reported extensive studies of β-peptides containing acyclic residues; β-peptides containing exclusively β^3-residues adopt the 14-helix (defined by 14-membered ring C═O(*i*)- -H−N(*i* − 2) hydrogen bonds).[2,8] Enantiomerically pure β^3-residues are easily prepared from the corresponding enantiomerically pure α-amino acids,[7] which provides ready access to a large set of side chains. In series **1**−**4**, the cationic *trans*-3,4-APC residues are progressively replaced by cationic β^3-hLys residues[7] (prepared from D-lysine, so that configuration at C3 matches *trans*-3,4-APC). β-Peptides **1**−**4** bear an N-terminal *p*-methoxyphenacyl group; the aromatic ring was intended to enhance ^{1}H NMR dispersion.

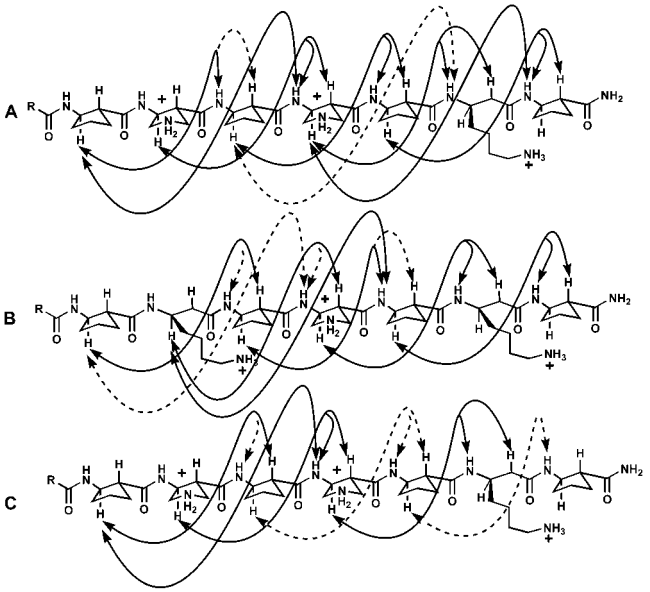


Figure 1. NOEs between nonadjacent residues for **2** (A) and **3** (B) in methanol, and for **2** in water (C). Unambiguous NOEs (solid); possible NOEs that are ambiguous because of resonance overlap (dotted). R = 4-MeOC$_6$H$_4$CH$_2$

Circular dichroism (CD) was used for preliminary conformational evaluation of **1**−**4**.[9] In methanol, which is very conducive to β-peptide helicity,[8b,e] all four β-peptides display a characteristic 12-helical signature:[10] maximum around 202 nm and weaker minimum around 222 nm. Water destabilizes β-peptide helices relative to methanol, especially for β-peptides composed solely of acyclic residues.[8b,e] CD data for **1**−**3** in water retain the 12-helical pattern, although the intensity is diminished relative to methanol in each case, and the maximum is blue-shifted 2−4 nm. For **4** the minimum completely disappears in water. The CD data suggest that 12-helix formation is possible in methanol when up to three cyclically constrained residues in a heptamer are replaced with β^3-residues. In water, on the other hand, 12-helix formation seems to require that at least five of the seven residues be constrained.

Previous studies have identified three types of NOE between backbone protons on nonadjacent residues that are characteristic of the β-peptide 12-helix: $C_\beta H_i \rightarrow NH_{i+2}$, $C_\beta H_i \rightarrow C_\alpha H_{i+2}$ and $C_\beta H_i \rightarrow NH_{i+3}$.[4,5b] For both **2** and **3**, numerous nonadjacent residue NOEs are observed in methanol; all are consistent with high population of the 12-helix (Figure 1A,B). These data show that the β^3-hLys residues have been incorporated into the 12-helix in methanol, because some of the characteristic NOEs involve or span these acyclic residues.

NMR analysis of β^3-hLys-containing oligomers in aqueous solution was hampered by low solubility; only **2** could be dissolved

* To whom correspondence should be directed. E-mail: gellman@chem.wisc.edu.
† Department of Chemistry.
‡ Department of Pharmacology.

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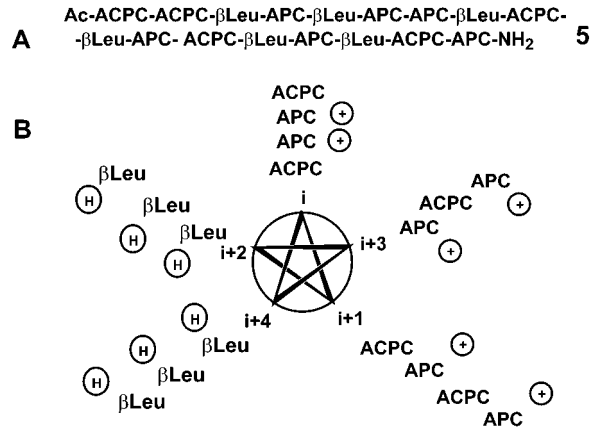

Figure 2. Sequence (A) and 12-helical wheel diagram (B) of **5**.

to >1 mM. Proton resonance dispersion for **2** was lower in water than in methanol, which further hampered analysis. Nevertheless, several characteristic backbone NOEs involving nonadjacent residues could be unambiguously identified (Figure 1C). Two of these NOEs involve β^3-HLys-6. Thus, the NMR data for **2** indicate that a β^3-residue can be incorporated into a short 12-helix in aqueous solution.

We turned to antimicrobial activity for an operational test of our conclusion that 12-helical propensity is retained after partial replacement of cyclic residues with acyclic residues. Conventional peptides (α-amino acid residues) that can adopt amphiphilic α-helical conformations and bear a net positive charge frequently display antimicrobial activity.[11] DeGrado et al.[2b,e] have shown that β-peptides containing exclusively β^3-residues are toxic to *Escherichia coli.* Independently, we have shown that a 17-residue β-peptide ("β-17") constructed from *trans*-1,2-ACPC and *trans*-3,4-APC, in a sequence that generates an amphiphilic 12-helix, displays antimicrobial activity toward four bacterial species.[2c] This spectrum of activity is comparable to that of natural host-defense peptides, like the magainins.[11] The parallel between β-17 and natural host-defense peptides includes a low tendency to cause human red blood cell rupture (low hemolytic activity).[2c,11]

β-Peptide **5** (Figure 2A) represents a new antimicrobial design in which the six β^3-hLeu residues are intended to form the hydrophobic surface of an amphiphilic 12-helix. Figure 2B shows a projection along the 12-helical axis, assuming 2.5 residues per turn.[5a] The six cationic *trans*-3,4-APC residues are distributed (along with the *trans*-2-ACPC residues) along 3/5 of the helix circumference, and the β^3-hLeu residues occupy the other 2/5. We evaluated antimicrobial activity against strains of *E. coli* (JM109, ref 12a), *Bacillus subtilis* (BR151, ref 12b), *Staphylococcus aureus* (1206, penicillin-, spectinomycin- and erythromycin-resistant, ref 12c) and *Enterococcus faecium* (A436, vancomycin-resistant, ref 12d). In all four cases,[9] mimimum inhibitory concentrations (MIC) were comparable to those previously determined with β-17.[2c] β-Peptide **5** is slightly more hemolytic than is β-17,[9] but **5** is comparable in hemolytic activity to a synthetic magainin II analogue.[9] CD data for **5** showed a strong 12-helix signature in MeOH, and a weaker 12-helix signature in water.[9]

Our results show that acyclic β^3-amino acid residues can be incorporated into the β-peptide 12-helix if most of the residues are appropriately preorganized for 12-helical folding. This result is important in terms of β-peptide conformational preferences, adding to previous evidence that β^3-residues are quite malleable.[8d] β-Peptides constructed exclusively from β^3-residues adopt the 14-helix rather than the 12-helix.[2a,b] Alternation of β^3-residues and β^2-residues (side chain at the α-carbon) can generate a third helical secondary structure, the 10/12-helix.[13] All three helices require *gauche*-type (O═)CC$_\alpha$−C$_\beta$N torsion angles, although the precise torsion angles vary. β^3-Residues can also be incorporated into sheet secondary structure, where they may display either *gauche* or *anti* torsion angles.[14] Our findings are of practical importance because they delineate an efficient path to creating 12-helical β-peptides with diverse arrays of surface functionality.[15]

Supporting Information Available: CD, NMR, MIC and hemolysis data for **5** and reference peptides (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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