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Use of Parallel Synthesis To Probe Structure−Activity Relationships among 12-Helical β-Peptides: Evidence of a Limit on Antimicrobial Activity

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Abstract: We report structure−activity trends among helix-forming β-amino acid oligomers that are intended to mimic α-helical host-defense peptides. Parallel synthesis of two small, focused β-peptide libraries allowed us to identify relatively short (11-residue) β-peptides that display antimicrobial activity. These β-peptides exhibit selectivity for bacteria relative to human red blood cells. A large hydrophobic helical surface is necessary for antimicrobial activity. Longer analogues (16 residues) of the most active library members were prepared and evaluated. Some of these longer β-peptides showed very good antimicrobial activity, but none was more active than a previously reported β-peptide [Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565]. The extensive literature on α-helical host-defense peptides and related α-peptides indicates that such molecules are seldom active at concentrations below 1 μg/mL, and our results suggest that amphiphilic helical β-peptides are subject to a comparable limit.

Introduction

The increasing prevalence of pathogenic bacteria resistant to clinical antibiotics has prompted extensive effort to identify new antimicrobial agents. Host-defense peptides, components of the innate immune system, represent a potential source of new therapeutic antibiotics.[1] These peptides are ribosomally synthesized and widespread among eukaryotes.[1] Magainins, the first host-defense peptides to be isolated from a vertebrate, were reported by Zasloff in 1987.[2] A wide array of host-defense peptides has subsequently been reported from many organisms, including humans.[1]

Molecular shape appears to be crucial to host-defense peptide function. The 23-residue magainins, for example, are unfolded in aqueous solution, but they can be induced to adopt an α-helical conformation by an organic solvent (e.g., an alcohol) or by the presence of micelles or vesicles.[1] The latter are thought to mimic the surface of a bacterium, and the α-helical form is presumed to be the biologically active conformation. This helical conformation is globally amphiphilic: hydrophilic (cationic) side chains are arrayed along one side of the helix, and lipophilic side chains are arrayed along the other side. The magainins' mechanism of antimicrobial action is a subject of debate, but the predominant view is that these peptides manifest their toxicity by disrupting bacterial membranes.[3] Magainins (and other host-defense peptides) disrupt bacterial membranes at much lower concentrations than are required to disrupt eukaryotic cell membranes, a selectivity that seems essential for the biological role of these peptides. In contrast, peptide toxins such as melittin, which also adopt globally amphiphilic α-helical conformations, are not selective, killing both prokaryotic and eukaryotic cells at low peptide concentrations.[4] The cell-selectivity of magainins and other host-defense peptides is attributed to their positive charge; bacterial cells generally have a greater negative charge density on their outer surface than do eukaryotic cells.[5] Melittin, too, is cationic, and the origin of the selectivity differences between melittin and magainins is not completely clear.

The magainins and other helix-forming host-defense peptides have inspired numerous efforts to develop analogues with improved biological activity. Many of these efforts have involved modification of one or more of the residues in the α-amino acid sequence.[6] A more profound alteration has been achieved by constructing analogues from D-α-amino acids rather than from the natural L-α-amino acids.[7] The success of this approach constitutes strong evidence that antibacterial activity does not require the peptide to interact with a specific bacterial protein. In the past few years, more dramatic departures from the natural prototype peptides have been explored, involving

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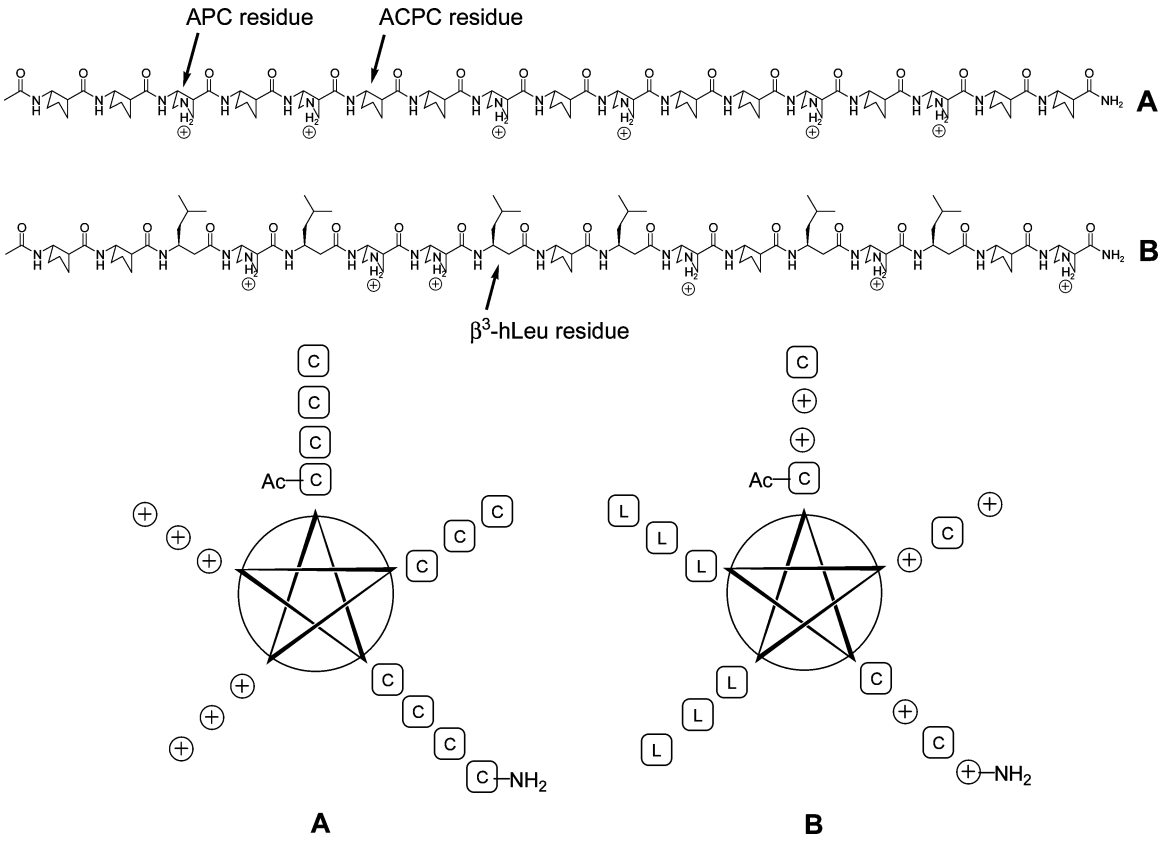

Figure 1. Structures and helical wheel diagrams of **A** and **B**. "C" denotes ACPC; "L" denotes β^3-hLeu; "+" denotes APC.

oligomers constructed from building blocks other than α-amino acids. Initial efforts, from DeGrado et al.[8] and from our group,[9] made use of β-amino acid oligomers ("β-peptides").[10] This oligomer class is attractive because discrete folding rules are available, which allows one to design β-amino acid sequences that will adopt globally amphiphilic helical conformations. Indeed, three different helical β-peptide secondary structures have been used for development of antimicrobial agents, the 14-helix, the 12-helix, and the 10/12-helix (names of these β-peptide helices are based on the characteristic ring size(s) of hydrogen bonds between backbone amide groups in the helical conformation).[8,9,11] Mechanistic analysis suggests that the β-peptides' mode of antimicrobial action is comparable to that of magainins and related α-peptides.[11c] Other unnatural amide-based oligomers designed to display antimicrobial activity have been constructed from *N*-alkyl-glycine residues ("peptoids"),[12] from aromatic subunits,[13] and from mixtures of α- and β-amino acid residues ("α/β-peptides").[14]

The large literature on helix-forming antimicrobial α-peptides suggests that it is difficult or impossible to find members of this class that display a minimum inhibitory concentration (MIC) significantly below 1 μg/mL.[6] Such a "floor" in activity might be intrinsic to a membrane-disruption mode of action. For example, increasing net hydrophobicity beyond a certain point might be counterproductive because of decreased peptide solubility or increased peptide self-association. The studies described below were motivated by our desire to know whether helix-forming β-peptides are subject to a lower MIC limit comparable to that displayed by antimicrobial α-helical α-peptides.

Oligomeric species lend themselves to solid-phase synthesis, which, in turn, is conducive to library preparation via parallel or combinatorial methods. The solid-phase methodology developed for α-peptide synthesis has been routinely applied to β-peptides, in both manual and automated formats. We decided to use the exploration of structure−antimicrobial activity relationships as an opportunity to begin to evaluate parallel synthesis of small β-peptide libraries.

The studies reported here focus on β-peptides designed to adopt the 12-helix secondary structure. Our original efforts showed that 17-residue β-peptide **A** (Figure 1) displays antimicrobial activity comparable to that of a derivative of the host-defense peptide magainin II.[9] In this benchmark α-peptide, referred to below as "magainin derivative", residues 8, 13, and 18 have been changed to alanine, and the C-terminus has been

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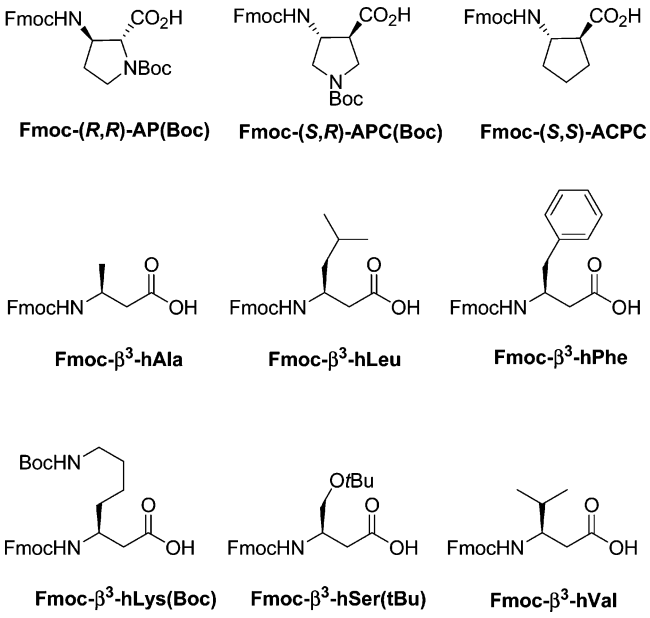
Chart 1. Examples of Protected β-Amino Acids Employed in Parallel Synthesis



capped as a primary amide. This magainin derivative was reported by Chen et al. to display enhanced antimicrobial activity relative to magainin II itself.[15] The 12-helix formed by **A** should be similar in length to the α-helix formed by the 23-residue magainin derivative. In addition to comparable MIC values against several bacterial species, **A** and the magainin derivative display comparable selectivity toward bacteria relative to human red blood cells (RBCs);[9] RBCs are commonly used to evaluate a peptide's ability to disrupt eukaryotic cell membranes. Cell-leakage studies indicate that **A** causes large-scale disruptions in bacterial membranes, as does the magainin derivative.[11c] Model studies with lipid vesicles, however, suggest that the details of membrane interaction vary between **A** and helix-forming α-peptides.[16]

The 12-helix, defined by 12-membered ring *i*,*i*+3 backbone C═O--H−N hydrogen bonds, has ca. 2.5 residues per turn.[17] The helical wheel diagram shown for **A** (Figure 1) reveals that this β-peptide is expected to display global amphiphilicity in the 12-helical conformation. This design hypothesis was supported by the lack of antimicrobial activity observed for a sequence isomer of **A** that, in the 12-helical conformation, should have hydrophilic and lipophilic residues distributed around the entire periphery of the helix (i.e., an isomer that is not expected to adopt a globally amphiphilic conformation).[11c]

The 12-helical conformation is strongly promoted by β-amino acid residues having a five-membered ring constraint, with the amino and carboxy substituents trans on the ring. These features are shared by *trans*-2-aminocyclopentanecarboxylic acid (ACPC) and *trans*-3-aminopyrrolidine-4-carboxylic acid (APC), the two residues in **A**. At the time of our initial studies, it appeared that only appropriately constrained residues could be used in 12-helix-forming β-peptides, and our first efforts to examine the relationship between β-peptide structure and biological activity were therefore limited to a small number of molecules. In **A**, cationic residues occupy 40% of the helix circumference, and lipophilic residues occupy 60%, as indicated by the 12-helix wheel diagram. An analogue in which these proportions were reversed was significantly less active.[11c] We replaced APC with the isomeric *trans*-3-amino proline (AP) residue, as the positive charge-bearing constituent, but this replacement led to a modest drop in activity.[11c]

In more recent studies, we have found that 12-helical folding can be maintained when some constrained residues are replaced by more flexible acyclic residues bearing a substituent adjacent to the backbone nitrogen atom ("β^3-amino acid residues").[11b] Seebach et al. have shown that β^3-residues intrinsically prefer to adopt a different type of β-peptide secondary structure, the 14-helix (defined by 14-membered ring *i*,*i*-2 backbone C═O--H−N hydrogen bonds).[18] However, these acyclic residues have lower inherent folding propensities than do cyclic residues such as ACPC and APC, and the 12-helix can propagate across β^3-residues when they are surrounded by residues with the five-membered ring constraint.[11b] We showed that 17-residue β-peptide **B** (Figure 1) displays antibacterial activity comparable to that of **A** against a set of four species; **B** is slightly more hemolytic than is **A**.[11b] As shown by the helical wheel diagram for **B** (Figure 1), this β-peptide is expected to adopt a globally amphiphilic 12-helix conformation is which the lipophilic surface (40% of the helix circumference) is composed of β^3-homoleucine (β^3-hLeu) residues. A mixture of ACPC and APC residues define the hydrophilic surface. β-Peptide **B** displays a weak 12-helical circular dichroism (CD) signature in water and a stronger 12-helical signature in methanol.[11b]

The observation that β^3-residues can be incorporated into the 12-helix raises the prospect of exploring a broader structure−antibiotic activity relationship than is currently possible if we are confined to cyclically constrained residues. Appropriately protected β^3-amino acids with a wide range of side chains are readily available, in enantiomerically pure form, from the corresponding α-amino acids.[19] In contrast, it is challenging to incorporate side chains into cyclopentane-based β-amino acids.[20]

Results and Discussion

Design of the First Library. We used a β-peptide length of 12-residues for the first library. The most active β-peptides identified in our previous work (e.g., **A** and **B**) contain 17 residues, but preliminary studies with a 12-residue homologue of **A** indicated that this molecule displays modest antibacterial activity.[21] Construction of a 12-mer library (rather than a 17-mer library) allowed us to conserve valuable β-amino acid building blocks. We reasoned that it would be easy to recognize 12-mers showing improved activity relative to the 12-mer homologue of **A**, given the limited efficacy of this 12-mer. Longer versions of these "hits" could then be prepared and evaluated more thoroughly.

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Table 1. Sequences of β-Peptides in Library **1**[a]

	1	2	3	4	5	6	7	8	9	10	11	12
1	ACPC	ACPC	βLeu	APC	βLeu	APC	APC	βLeu	APC	βLeu	APC	APC
2	ACPC	ACPC	βAla	APC	βAla	APC	APC	βLeu	APC	βLeu	APC	APC
3	ACPC	ACPC	βPhe	APC	βPhe	APC	APC	βLeu	APC	βLeu	APC	APC
4	ACPC	ACPC	βVal	APC	βVal	APC	APC	βLeu	APC	βLeu	APC	APC
5	ACPC	ACPC	βLeu	APC	βLeu	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
6	ACPC	ACPC	βAla	APC	βAla	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
7	ACPC	ACPC	βPhe	APC	βPhe	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
8	ACPC	ACPC	βVal	APC	βVal	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
9	ACPC	ACPC	βLeu	APC	βLeu	βLeu	βSer	βLeu	APC	βLeu	βSer	APC
10	ACPC	ACPC	βAla	APC	βAla	βLeu	βSer	βLeu	APC	βLeu	βSer	APC
11	ACPC	ACPC	βPhe	APC	βPhe	βLeu	βSer	βLeu	APC	βLeu	βSer	APC
12	ACPC	ACPC	βVal	APC	βVal	βLeu	βSer	βLeu	APC	βLeu	βSer	APC
13	ACPC	ACPC	βLeu	APC	βLeu	βLeu	βSer	ACPC	APC	ACPC	βSer	APC
14	ACPC	ACPC	βAla	APC	βAla	βLeu	βSer	ACPC	APC	ACPC	βSer	APC
15	ACPC	ACPC	βPhe	APC	βPhe	βLeu	βSer	ACPC	APC	ACPC	βSer	APC
16	ACPC	ACPC	βVal	APC	βVal	βLeu	βSer	ACPC	APC	ACPC	βSer	APC
17*	ACPC	ACPC	βLeu	APC	βLeu	AP	AP	βLeu	APC	βLeu	AP	AP
18*	ACPC	ACPC	βPhe	APC	βPhe	AP	AP	βLeu	APC	βLeu	AP	AP
19*	ACPC	ACPC	βLeu	APC	βLeu	AP	AP	β-Phe	APC	β-Phe	AP	AP
20*	ACPC	ACPC	ACPC	APC	ACPC	AP	AP	ACPC	APC	ACPC	AP	AP

[a] Numbers across the top of the table designate residue position; numbers in the first column designate library member number. Member number is the suffix in the compound name; for example, member **3** of library **1** has the compound name **1.3**. Members with an asterisk (*) are composed of "*R*" amino acids. "βAla" denotes β^3-homoalanine (not to be confused with β-homoglycine, which is commonly called "β-alanine"). All β-peptides have a free N-terminus and an amidated C-terminus.

Some of the protected β-amino acids used for library construction are shown in Chart 1. The β^3-amino acids drawn are derived from L-α-amino acids, and the cyclic β-amino acids ACPC and APC drawn have the corresponding absolute configuration, as required for right-handed 12-helix formation. Both of these constrained building blocks are available in either enantiomeric series. In contrast, the building block leading to the AP residue is currently available only in the (*R*,*R*) configuration;[22] preparation of 12-helical β-peptides containing AP requires β^3-amino acids derived from D-α-amino acids and the corresponding forms of ACPC and APC. (The handedness of the 12-helix does not affect antimicrobial efficacy: **A** and its enantiomer have comparable activity.[21])

Table 1 shows the sequences of the first library, which contains 20 12-mers (compounds **1.1**−**1.20**) and is divided into 5 sets of 4 compounds (**1.1**−**1.4**, **1.5**−**1.8**, **1.9**−**1.12**, **1.13**−**1.16**, **1.17**−**1.20**). All 20 β-peptides have some features in common in addition to length: all have a free N-terminus and an amide-capped C-terminus, all have two ACPC residues at the N-terminus, and all are designed to adopt a globally amphiphilic 12-helical conformation. 12-Helix wheel diagrams for the five sets are shown in Figure 2, with X indicating the positions that are variable within each set. The first set (**1.1**−**1.4**) is based on β-peptide **B**. Members of this set have a lipophilic face that is defined by β^3-residues and comprises 40% of the helix circumference. These four β-peptides each contain six side chains (APC) that are expected to be cationic at neutral pH. β-Peptide **1.1** is a shortened homologue of **B**, and **1.2**−**1.4** have alternative lipophilic residues (β^3-hAla, β^3-hPhe, or β^3-hVal) at positions 3 and 5.

The remaining four sets of four β-peptides represent alternative variations on set 1. The second set (**1.5**−**1.8**) has an expanded lipophilic surface (60% of the 12-helix circumference), and only four side chains that should be cationic. The third set (**1.9**−**1.12**) reverts to the lipophilic surface of set 1, but the net positive charge is reduced because two APC residues in the hydrophilic surface are replaced by β^3-hSer residues. The fourth set (**1.13**−**1.16**) retains the hydrophilic surface of set 3, but two of the four lipophilic residues, fixed as β^3-hLeu in **1.1**−**1.12**, are replaced with ACPC. The fifth set reverts to the basic plan embodied among **1.1**−**1.4**, but now the cationic residues are all AP rather than APC.

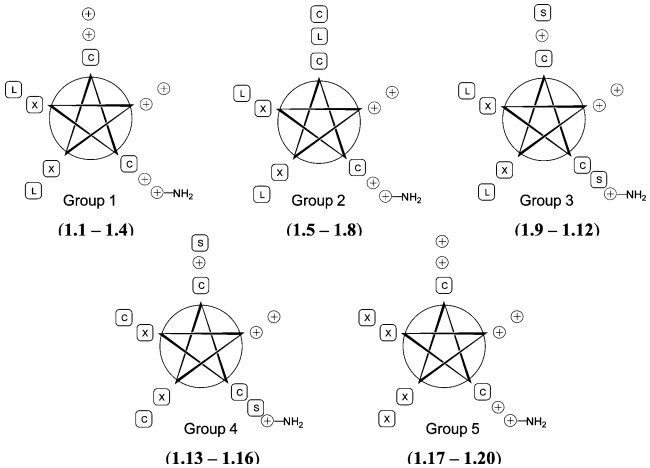

Figure 2. Helical wheel diagrams for library **1**. "C" denotes ACPC; "L" denotes β^3-hLeu; "S" denotes β^3-hSer; "X" denotes β^3-hLeu, β^3-hAla, β^3-hPhe, or β^3-hVal (or sometimes ACPC in the case of group 5); "+" denotes APC, or in the case of group 5, APC or AP.

Synthesis and Biological Evaluation of the First Library. The first β-peptide library was synthesized on a 15 μmol scale on an Argonaut Quest parallel synthesizer (Table 2). The synthesis employed Rink amide MBHA resin as the solid support, HBTU as the coupling agent, and 90 min coupling periods. After synthesis was complete, the β-peptides were cleaved from the resin on the Quest and precipitated. The precipitates were dissolved in H_2O/CH_3CN mixtures and lyophilized to give crude β-peptides, which were analyzed by HPLC. The major peak in each chromatogram was examined by MALDI-TOF mass spectrometry; additional peaks were examined as well in some cases (HPLC monitored via absorbance at 220 nm). A fraction containing the molecular weight of the desired compound was identified in 18 of the 20 cases, and this fraction, assumed to be the desired compound, corresponded to the largest HPLC peak in 12 of the 20 cases. The initial purity of each library member was assessed by

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Table 2. Analysis of Library **1**

β-peptide	initial purity (%)	major peak	β-peptide	initial purity (%)	major peak
1.1	28	yes	**1.11**	35	yes
1.2	16	no	**1.12**	21	yes
1.3		no	**1.13**	40	yes
1.4	24	yes	**1.14**		no
1.5	25	yes	**1.15**	41	yes
1.6	22	no	**1.16**	57	yes
1.7	48	yes	**1.17**	6	no
1.8	43	yes	**1.18**	10	no
1.9	40	yes	**1.19**	13	no
1.10	38	yes	**1.20**	10	no

Table 3. Antimicrobial Activities (MIC) of Selected Members of β-Peptide Library **1** (μg/mL)[a]

β-peptide	*E. coli*	*B. subtilis*
1.5	100	12.5
1.6	>200	>200
1.7	25−50	12.5
1.8	200	25−50

[a] All other members of library **1** displayed MIC values of ≥100 μg/mL against both bacteria, except for **1.3** and **1.14**, which could not be evaluated.

measuring the absorbance of the HPLC peak corresponding to the correct molecular weight relative to total UV absorbance for the chromatogram. Among the APC-containing library members (**1.1**−**1.16**), initial purities varied between 16% and 54% for the 14 cases that provided the expected product. Among the AP-containing library members (**1.17**−**1.20**), initial purities were much lower, varying between 6% and 13%; net yields were also much lower for these four cases. The origin of these substantially poorer synthetic results for the AP-containing library members is not clear.

The desired 12-mer (as identified by mass spectrometry) was purified by HPLC for all but **1.3** and **1.14**, and the 18 purified β-peptides were tested against two bacteria, *E. coli* JM109 (Gram negative)[23] and *B. subtilis* BR151 (Gram positive).[24] Most of the β-peptides failed to display significant antimicrobial activity. The three most active β-peptide 12-mers, **1.5**, **1.7**, and **1.8**, all belong to the second four-member set among the first library (Table 3). Thus, these results suggest that a lipophilic surface comprising 60% of the 12-helix circumference (as in **A** but not **B**) is optimal for antibacterial activity.

Design of the Second Library. The second library of 20 β-peptides (Table 4) was designed to explore the 12-helical architecture identified as most promising by the results from the first library. Some features are common to all members of the second library: (1) a length of 11 residues (this length was chosen to facilitate the extension of active sequences to 16 residues, as discussed below), (2) 4 positive charge-bearing side chains, and (3) an acetyl cap at the N-terminus and a primary amide cap at the C-terminus. Each sequence was designed so that if a 12-helical conformation were adopted, this helix would be globally amphiphilic, with a lipophilic surface covering 60% of the circumference. The variations among library members were intended to explore the significance of conformational rigidity and side chain identity on biological activity.

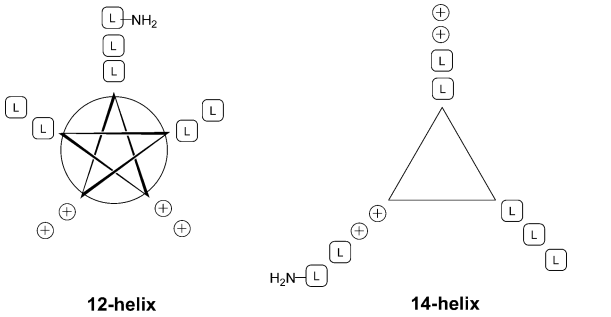

Figure 3. The 12-helix wheel diagram for β-peptides **2.1** and **2.11** and the 14-helix wheel diagram for β-peptide **2.11**. "L" denotes β^3-hLeu, and "+" denotes APC (for **2.1**) or β^3-hLys (for **2.11**).

Library member **2.1** contains only two types of residue, APC and β^3-hLeu. The seven β^3-hLeu residues would be segregated along one side of a 12-helical conformation of **2.1** (Figure 3). β-Peptides **2.2** and **2.3** are analogues of **2.1** in which two or three of the β^3-hLeu residues, respectively, are replaced with ACPC. Because ACPC is lipophilic but more preorganized than β^3-hLeu, these changes are intended to influence conformational stability more than hydrophilic/lipophilic balance. Compound **2.4** is an analogue of **2.1** in which every β^3-hLeu has been replaced by β^3-hPhe, and **2.5** and **2.6** contain β^3-hPhe → ACPC changes analogous to those in **2.2** and **2.3**. β-Peptide **2.7** is a hybrid of **2.2** and **2.5**, with a combination of β^3-hLeu and β^3-hPhe residues.

β-Peptides **2.8**−**2.10** can be viewed as derivatives of **2.2**, with one or two modifications that should decrease 12-helix stability. In **2.8**, residue 4 is β-hGly (conventionally referred to as "β-

Table 4. Sequences of β-Peptides in Library **2**[a]

	1	2	3	4	5	6	7	8	9	10	11
1	βLeu	APC	βLeu	βLeu	APC	βLeu	APC	βLeu	βLeu	APC	βLeu
2	βLeu	APC	βLeu	ACPC	APC	βLeu	APC	ACPC	βLeu	APC	βLeu
3	βLeu	APC	βLeu	ACPC	APC	ACPC	APC	ACPC	βLeu	APC	βLeu
4	βPhe	APC	βPhe	βPhe	APC	βPhe	APC	βPhe	βPhe	APC	βPhe
5	βPhe	APC	βPhe	ACPC	APC	βPhe	APC	ACPC	βPhe	APC	βPhe
6	βPhe	APC	βPhe	ACPC	APC	ACPC	APC	ACPC	βPhe	APC	βPhe
7	βPhe	APC	βLeu	ACPC	APC	βLeu	APC	ACPC	βPhe	APC	βLeu
8	βLeu	APC	βLeu	βGly	APC	βLeu	APC	ACPC	βLeu	APC	βLeu
9	βLeu	APC	βLeu	ACPC*	APC	βLeu	APC	ACPC	βLeu	APC	βLeu
10	βLeu	APC	βLeu*	ACPC*	APC	βLeu	APC	ACPC	βLeu	APC	βLeu
11	βLeu	βLys	βLeu	βLeu	βLys	βLeu	βLys	βLeu	βLeu	βLys	βLeu
12	βPhe	βLys	βPhe	βPhe	βLys	βPhe	βLys	βPhe	βPhe	βLys	βPhe
13	βLeu	βLys	βLeu	ACPC	βLys	ACPC	βLys	ACPC	βLeu	βLys	βLeu
14	βPhe	βLys	βPhe	ACPC	βLys	ACPC	βLys	ACPC	βPhe	βLys	βPhe
15	ACPC	βLys	ACPC	ACPC	βLys	ACPC	βLys	ACPC	ACPC	βLys	ACPC
16	ACPC	APC	ACPC	ACPC	APC	ACPC	APC	ACPC	ACPC	APC	ACPC
17	ACPC	βLeu	APC	βLeu	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
18	ACPC	βPhe	APC	βPhe	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
19	ACPC	βVal	APC	βVal	βLeu	APC	βLeu	APC	βLeu	ACPC	APC
20	ACPC	βLeu	APC	βLeu	APC	APC	βLeu	ACPC	βLeu	APC	ACPC

[a] Numbers across the top of the table designate residue position; numbers in the first column designate library member number. Member number is the suffix in the compound name; member **1** of library **2** has the compound name **2.1**. β-Amino acids with an asterisk (*) are of the opposite "R" configuration. "βGly" denotes β-homoglycine (commonly called "β-alanine"). All β-peptides have an acetylated N-terminus and an amidated C-terminus.

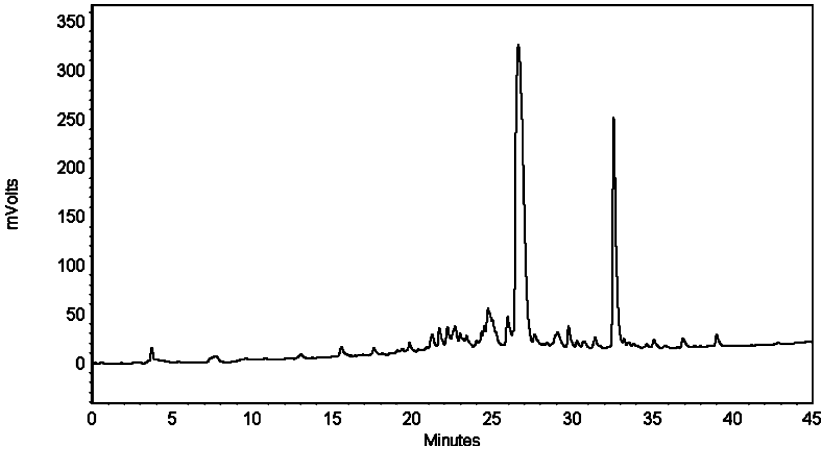

Figure 4. HPLC chromatogram of β-peptide **2.16** immediately after synthesis. The desired β-peptide is represented by the major peak at 27 min. An unidentifiable impurity is present at 33.5 min in all library **2** samples.

alanine"), while the corresponding residue in **2.2** is ACPC. β-Peptide **2.9** is a stereoisomer of **2.2** in which ACPC-4 has the opposite configuration, and **2.10** is a stereoisomer of **2.2** in which both β^3-hLeu-3 and ACPC-4 have opposite configurations. The motivation to examine these conformationally destabilizing changes comes from remarkable results reported by Shai et al. for α-peptides that contain a few D-residues.[3d,7] Such heterochiral peptides retain good antimicrobial activity relative to all-L stereoisomers, and the heterochiral peptides can display better selectivity for bacteria over RBCs than do the homochiral isomers.

In β-peptides **2.11**−**2.15**, the positive charge-bearing residues are all β^3-hLys (rather than APC in other library members). Each of the residues in **2.11** and **2.12** is acyclic; the lipophilic residues are β^3-hLeu in the former and β^3-hPhe in the latter. β-Peptides **2.13** and **2.14** are analogues of **2.11** and **2.12** in which the central three lipophilic residues are replaced by ACPC. In **2.15**, each of the seven of the lipophilic positions is occupied by ACPC. Compounds **2.11** and **2.12** should have very low 12-helical propensities, because they lack constrained residues. β^3-Residues intrinsically prefer the 14-helix, which has ca. three residues per turn, although β-peptides composed exclusively of β^3-residues typically do not fold in water.[25,26] Figure 3 compares the 12-helix and 14-helix wheel diagrams for **2.11**; the 12-helix displays clear global amphiphilicity, but this characteristic is attenuated in the 14-helix.

Compounds **2.16**−**2.20** were included for correlation with previously examined β-peptides. β-Peptide **2.16** is an 11-residue fragment of **A**; any library members with activity superior to that of **2.16** are of particular interest. Compounds **2.17**−**2.19** are analogues of the three most active compounds from the first library; they were included to determine whether the decrease from 12 to 11 residues has a significant effect on antimicrobial activity. β^3-Peptide **2.20** is an 11-residue fragment of **B**.

Synthesis and Biological Evaluation of the Second Library. The second library was synthesized in a completely manual fashion, involving fritted polypropylene tubes, a process that required ca. 4 days. The synthesis employed Rink amide MBHA resin as the solid support, HBTU as the coupling agent, and 120 min coupling periods. The initial purity of each library member was analyzed by HPLC as described above, and the samples used for biological and physical characterization were purified by HPLC.

Table 5. Analysis of Library **2**

β-peptide	initial purity (%)	major peak	β-peptide	initial purity (%)	major peak
2.1	25	yes	**2.11**	18	yes
2.2	34	yes	**2.12**	11	yes
2.3	39	yes	**2.13**	17	yes
2.4	36	yes	**2.14**	11	yes
2.5	51	yes	**2.15**	54	yes
2.6	51	yes	**2.16**	50	yes
2.7	36	yes	**2.17**	35	yes
2.8	32	yes	**2.18**	40	yes
2.9	44	yes	**2.19**	33	yes
2.10	22	yes	**2.20**	40	yes

Overall, the initial purities of β-peptides from the second library (Table 5) were significantly better than those observed for the first library, which may be due to longer coupling times (120 min vs 90 min). Every member of the second library was present as the major peak in its HPLC chromatogram. Each HPLC chromatogram contained a substantial peak at 33.5 min that could not be identified by MALDI-TOF mass spectrometry. The HPLC trace for crude β-peptide **2.16** is shown as an example in Figure 4. We suspect that the material giving rise to the 33.5 min peak is not β-peptide-related, and that this material is generated during the cleavage step (possibly from breakdown of the resin or linker). This impurity peak accounted for 15−20% of the total UV absorbance in most of the crude product HPLC chromatograms, uniformly depressing the initial purities reported in Table 5.

Table 6 summarizes antimicrobial activity of all members of the second library, except for **2.4**, which was insoluble in water. Most of these β-peptides displayed significant activity against *E. coli* and/or *B. subtilis*. Each library member, other than **2.4**, was evaluated also for hemolytic activity using hRBC (data not shown). In all but two cases, no hemolysis was detected at 200 μg/mL. β-Peptide **2.6** displayed weak hemolytic activity at 200 μg/mL, and **2.12** was significantly more hemolytic.

β-Peptide **2.16**, the 11-residue fragment of **A**, was regarded as a benchmark in these initial screens of the second library.

(23) Yanisch-Perron, C.; Viera, J.; Messing, J. *Gene* **1985**, *33*, 103−119.
(24) Young, F. E.; Smith, C.; Reilly, B. E. *J. Bacteriol.* **1969**, *98*, 1087−1097.
(25) (a) Abele, S.; Guichard, G.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 2141. (b) Appella, D. H.; Barchi, J. J.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309.
(26) Exceptions: (a) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 5162. (b) Arvidsson, P. I.; Rueping, M.; Seebach, D. *J. Chem. Soc., Chem. Commun.* **2001**, 649. (c) Hart, S. A.; Bahadoor, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022.

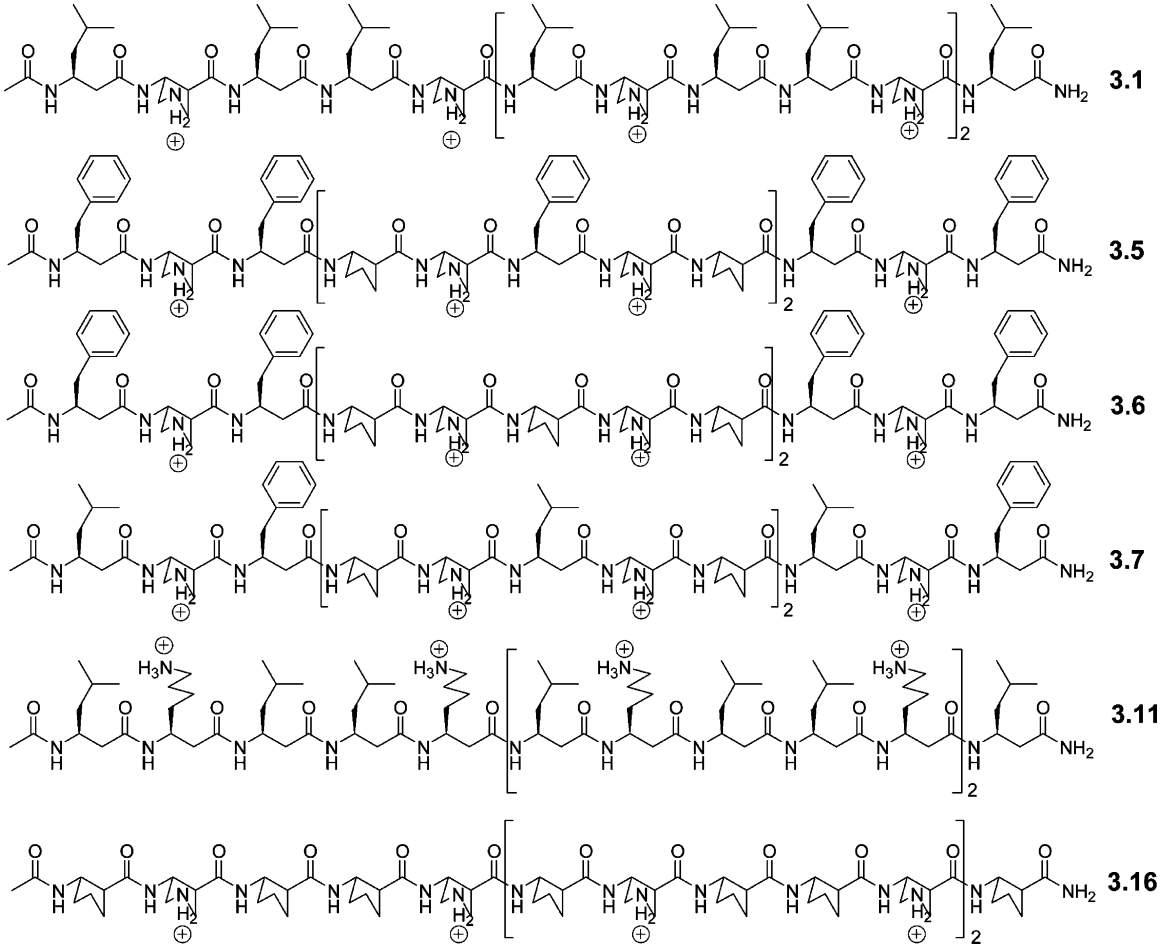

Figure 5. Extended versions of antimicrobial β-peptides from library **2**.

Table 6. Antimicrobial Activities (MIC) of Library **2** (μg/mL)

β-peptide	*E. coli*	*B. subtilis*	β-peptide	*E. coli*	*B. subtilis*
2.1	50	12.5	**2.11**	50	6.3
2.2	100	6.3	**2.12**	>200	25
2.3	100	6.3	**2.13**	>200	>200
2.5	50	3.2	**2.14**	>200	100
2.6	50	<1.6	**2.15**	>200	>200
2.7	50	<1.6	**2.16**	100	12.5
2.8	>200	50	**2.17**	200	12.5
2.9	>200	100	**2.18**	100	12.5
2.10	200	50	**2.19**	>200	100
			2.20	100	50

Several library members showed activity against both bacteria that was as good as or better than the activity of **2.16**. Of these promising β-peptides, five were selected as the basis for additional studies: **2.1**, **2.5**, **2.6**, **2.7**, and **2.11**. The subsequent analysis included synthesis of longer homologues of these five candidates and **2.16**, designated **3.1**, **3.5**, **3.6**, **3.7**, **3.11**, and **3.16** (Figure 5). These 16-mers were prepared on an automated synthesizer.

Biological Evaluation of Selected 16-Residue β-Peptides. The elongated β-peptides were evaluated against four bacteria: in addition to the *E. coli* and *B. subtilis* strains mentioned above, we examined *E. faecium* (A634, a clinical isolate that is vancomycin resistant)[27] and *S. aureus* (1206, a clinical isolate that is methicillin-resistant).[28] Table 7 compares the activities of the 16-mers against all four bacteria and the activities of the corresponding 11-mers. Also shown are data for our original antimicrobial β-peptide, **A**. As expected, the slight length difference between 16 residues (**3.16**) and 17 residues (**A**) does not lead to significant variation in antimicrobial activity.

Comparison of each 16-mer with the analogous 11-mer reveals that increased length leads to improved activity (lower MIC) for each bacterium except *B. subtilis*; most of the 11-mers are already quite effective at inhibiting *B. subtilis* growth. The correlation between increased length and improved activity is consistent with our original observations for **A** versus shorter analogues. DeGrado et al. have reported an analogous trend among β-peptides designed to form globally amphiphilic 14-helices.[8,11a]

The trend toward slight apparent improvement in activity of 11-mers **2.1**, **2.5**, **2.6**, **2.7**, and **2.11** relative to benchmark 11-mer **2.16**, detected in the initial screen (Table 6), is maintained in the more extensive comparison summarized in Table 7. Among the 16-mers, however, none is superior to **3.16** (or to **A**), and three, **3.5**, **3.6**, and **3.7**, are indistinguishable from **3.16**, all showing MIC values in the 1.6−6.3 μg/mL range. Thus, these data suggest that there is a lower limit on the MIC value that can be achieved with amphiphilic β-peptides, analogous to the lower limit observed among amphiphilic α-helical α-peptide antibiotics.

Hemolytic activity toward hRBCs was evaluated for 16-mers **3.1**, **3.5**, **3.6**, **3.7**, **3.11**, and **3.16** (Figure 6), to determine whether

(27) Nicas, T. I.; Wu, C. Y. E.; Hobbs, J. N.; Preston, D. A.; Allen, N. E. *Antimicrob. Agents Chemother.* **1989**, *33*, 11121−11124.

(28) Weisblum, B.; Demohn, V. *J. Bacteriol.* **1969**, *98*, 447−452.

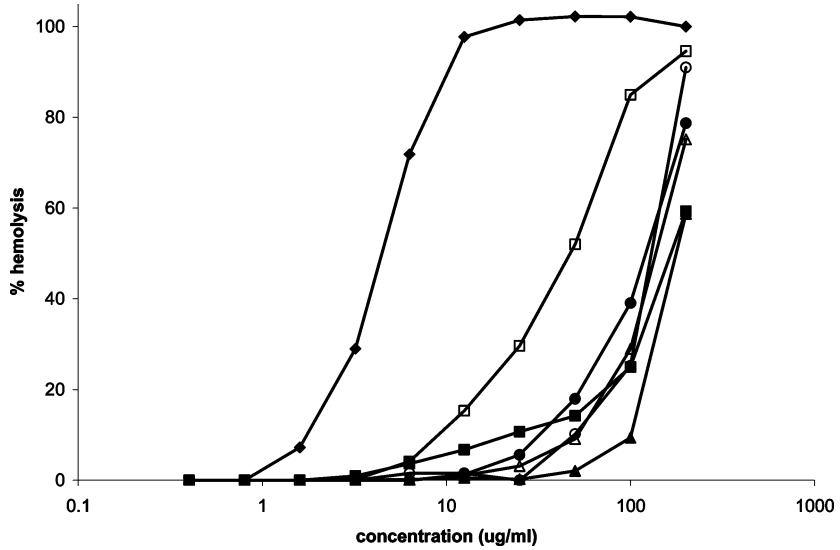

Figure 6. Hemolytic activities of 16-residue β-peptides. Melittin was used as a positive control. The value for melittin at 200 μg/mL is taken to represent complete hemolysis. Curves correspond to melittin (◆), **3.1** (■), **3.5** (●), **3.6** (□), **3.7** (△), **3.11** (▲), and **3.16** (○).

Table 7. Antimicrobial Activities (MIC, μg/mL)[29]

β-peptide	*E. coli*	*B. subtilis*	*E. faecium*	*S. aureus*
2.1	50	12.5	n.t.[a]	n.t.[a]
3.1	25	12.5	6.3	12.5
2.5	50	3.2	100	12.5
3.5	6.3	3.2	6.3	3.2
2.6	50	1.6	50	12.5
3.6	3.2	1.6	3.2	3.2
2.7	50	3.2	25	12.5
3.7	6.3	3.2	3.2	3.2
2.11	50	6.3	25	50
3.11	50	6.3	12.5	12.5
2.16	100	6.3	>100	50
3.16	3.2	1.6	3.2	3.2
***β*-17 (A)**	3.2	1.6	6.3	3.2

[a] "n.t." = not tested.

these β-peptides retain magainin-like selectivity for bacteria relative to eukaryotic cells. The behavior of **3.16** is similar to that of **A**, and **3.1**, **3.5**, **3.7**, and **3.11** are comparable to these benchmarks. β-Peptide **3.6** is significantly more hemolytic than the other 16-mers, although not as hemolytic as melittin. The distinctive hemolytic activity of **3.6** in this set matches a similar distinction noted above for 11-mer **2.6**. The origin of this enhanced hemolysis is unclear.

Circular Dichroism of Selected *β*-Peptides. All of the β-peptides described here were designed to be globally amphiphilic in a 12-helical conformation. Because, however, some of the β-peptides contain high proportions of acyclic residues (up to 100%), it is not clear which of them actually adopt the 12-helical conformation. We addressed this structural question by obtaining far-UV circular dichroism (CD) data for the 16-mers and the homologous 11-mers. Far-UV CD is widely employed to assess the secondary structure of α-peptides, because the backbone amide groups absorb in this spectral region; variations in the far-UV CD signature of an α-peptide can be empirically correlated with specific conformations (e.g., α-helix, β-sheet). CD has been used for analogous studies of β-peptides,[10c] but data interpretation is less secure in these cases. Relative to α-peptides, there are considerably fewer high-resolution structural data for folded β-peptides (from multidimensional NMR or X-ray crystallography) that can be correlated with CD data. Recent reports have highlighted the potential perils of β-peptide conformational analysis via CD.[30]

The far-UV CD signature of the 12-helix has been qualitatively established by correlating CD data for numerous β-peptides with two-dimensional NMR data, theoretical predictions, and, indirectly, X-ray crystal structures.[11b,17,20,22,31] In all of these cases, well over one-half of the residues have had the appropriate cyclic constraint (usually all residues have been cyclic). This body of information provides a basis for qualitative interpretation of the CD data for 16-mers **3.1**, **3.5**, **3.6**, **3.7**, **3.11**, and **3.16** and the corresponding 11-mers.

Figure 7A provides CD data for **2.5**, **2.6**, **2.7**, **2.11**, and **2.16** in methanol, which is known to be conducive to secondary structure formation in both α-peptides and β-peptides. β-Peptide **2.16**, comprised entirely of cyclically constrained residues, displays a typical 12-helical signature (minimum at 206 nm, maximum at 223 nm).[17,31] In **2.6**, four residues that were ACPC in **2.16** have been switched to β^3-hPhe, two replacements at or near each terminus. These replacements lead to a dramatic drop in CD intensity (relative to **2.16**), which is consistent with our previous observations that β^3-residues have a much lower 12-helical propensity than does ACPC.[11b] β-Peptide **2.5** has one additional β^3-residue relative to **2.6**: the central residue has been changed from ACPC in the latter to β^3-hPhe in the former. This change leads to a small further decrease in CD intensity. β-Peptide **2.7** differs from **2.6** in that three β^3-hPhe residues of the latter have been replaced by β^3-hLeu residues. This change leads to a substantial increase in the intensity and a slight blue-shift in the minimum; the significance of the β^3-hLeu-induced changes is not clear. The most puzzling CD signature is that of **2.11**, which is comprised entirely of β^3-residues. This β-peptide displays the second most intense CD spectrum among this set,

(29) The results for the 11-mers in Table 7 differ slightly from the results in Table 6; the results in each table are from different days, and there is a ±2-fold error in the MIC measurement.

(30) Glattli, A.; Daura, X.; Seebach, D.; van Gunsteren, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 12972−12978.

(31) (a) Applequist, J.; Bode, K. A.; Appella, D. H.; Christianson, L. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4891. (b) Wang, X.; Espinosa, J. F.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 4821−4822. (c) Lee, H.-S.; Syud, F. A.; Wang, X.; Gellman, S. H. *J. Am. Chem. Soc.* **2001**, *123*, 7721−7722.

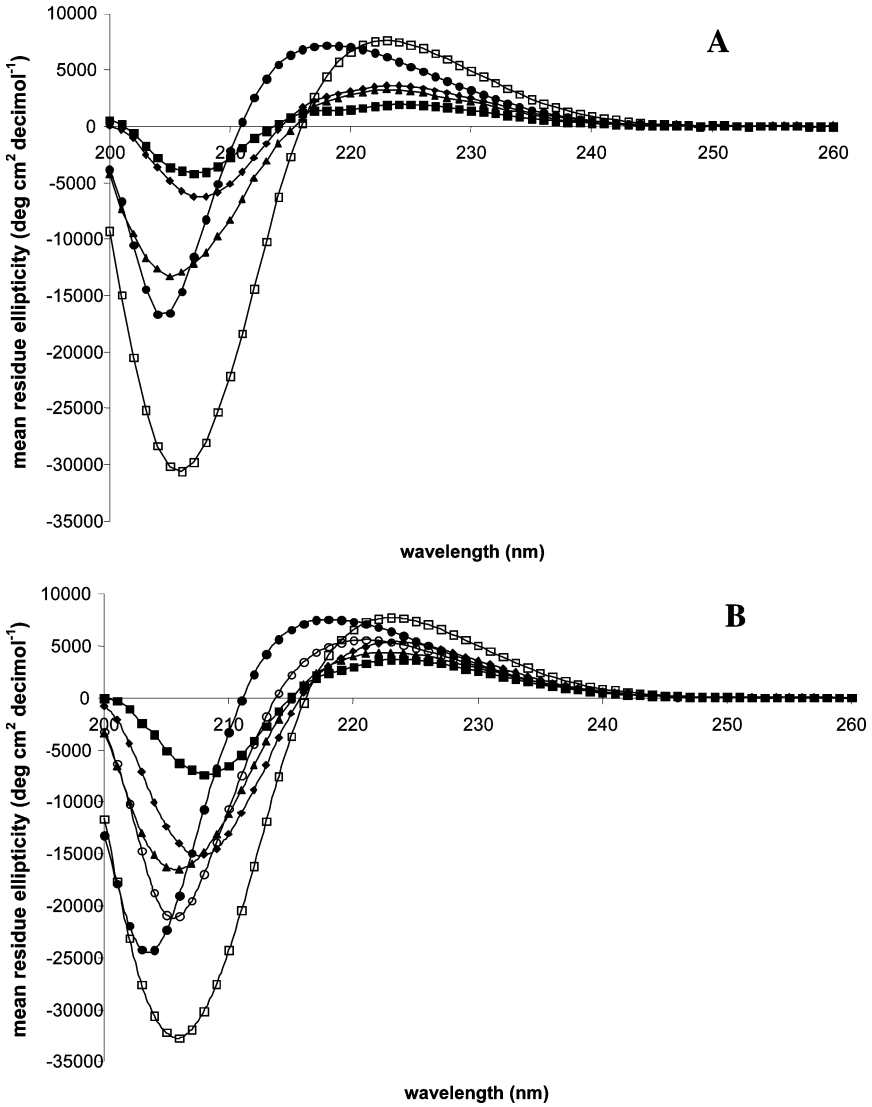

Figure 7. (A) CD spectra of β-peptides from library **2** in methanol. Curves represent **2.5** (■, 0.50 mM), **2.6** (◆, 0.52 mM), **2.7** (▲, 0.053 mM), **2.11** (●, 0.51 mM), and **2.16** (□, 0.57 mM). (B) CD spectra of 16-residue β-peptides in methanol. Curves represent **3.1** (○, 0.37 mM), **3.5** (■, 0.35 mM), **3.6** (◆, 0.37 mM), **3.7** (▲, 0.37 mM), **3.11** (●, 0.35 mM), and **3.16** (□, 0.40 mM).

but both the minimum and the maximum are blue-shifted relative to the 12-helical signature displayed by **2.16**. This spectrum is reminiscent of the mirror image of the 14-helical CD spectrum typically observed for β-peptides composed entirely of β^3-residues derived from L-α-amino acids.[18] We have previously observed such a "mirror image" effect with a β-peptide composed largely of β^3-hLys and β^3-hLeu residues.[32] The origin of this CD signature is unclear. van Gunsteren, Seebach, et al. have shown that β-peptides unable to form a 14-helix can nevertheless display a 14-helical CD signature;[30] perhaps the behavior of **2.11** and related β-peptides indicates that the converse is true as well, that is, that a β-peptide that adopts the 14-helical conformation can fail to display the conventional 14-helical CD signature. Alternatively, **2.11** may fold in some other way.

Figure 7B shows CD signatures for six 16-residue β-peptides, the analogues of the five 11-mers documented in Figure 5A (i.e., **3.5**, **3.6**, **3.7**, **3.11**, and **3.16**) along with **3.1**, all in methanol. The trends observed among the 11-mers (Figure 7A) are largely maintained among these longer analogues. For example, **3.16**, composed entirely of cyclically constrained residues, again shows the most intense CD signature, and this signature matches that previously assigned to the 12-helix. The four 16-mers that contain a mix of cyclic and β^3-residues also display 12-helical signatures, but with diminished intensity relative to **3.16**. β-Peptide **3.11**, containing entirely β^3-residues, displays an unusual CD signature analogous to that seen for shorter analogue **2.11**.

Figure 8A shows CD data for β-peptide 11-mers **2.5**, **2.6**, **2.7**, **2.11**, and **2.16** in aqueous buffer, and Figure 8B shows CD data for the analogous 16-mers plus **3.1** in this solvent. We have previously observed that most β-peptides, including all intended to form a 12-helix, display diminished CD intensity in aqueous solution relative to methanol;[11c,31b,c] a similar trend has been noted for β-peptides composed exclusively of β^3-residues.[25a] Comparison of the CD data in Figures 7 and 8 shows the expected diminution of intensity in aqueous solution. Among the 11-mers, only **2.16**, composed entirely of cyclically preorganized residues, displays a 12-helical signature (Figure 8A). Both the minimum (204 nm) and the maximum (222 nm) are

(32) Raguse, T. R.; Lai, J. R.; Gellman, S. H. *Helv. Chim. Acta* **2002**, *85*, 4154−4164.

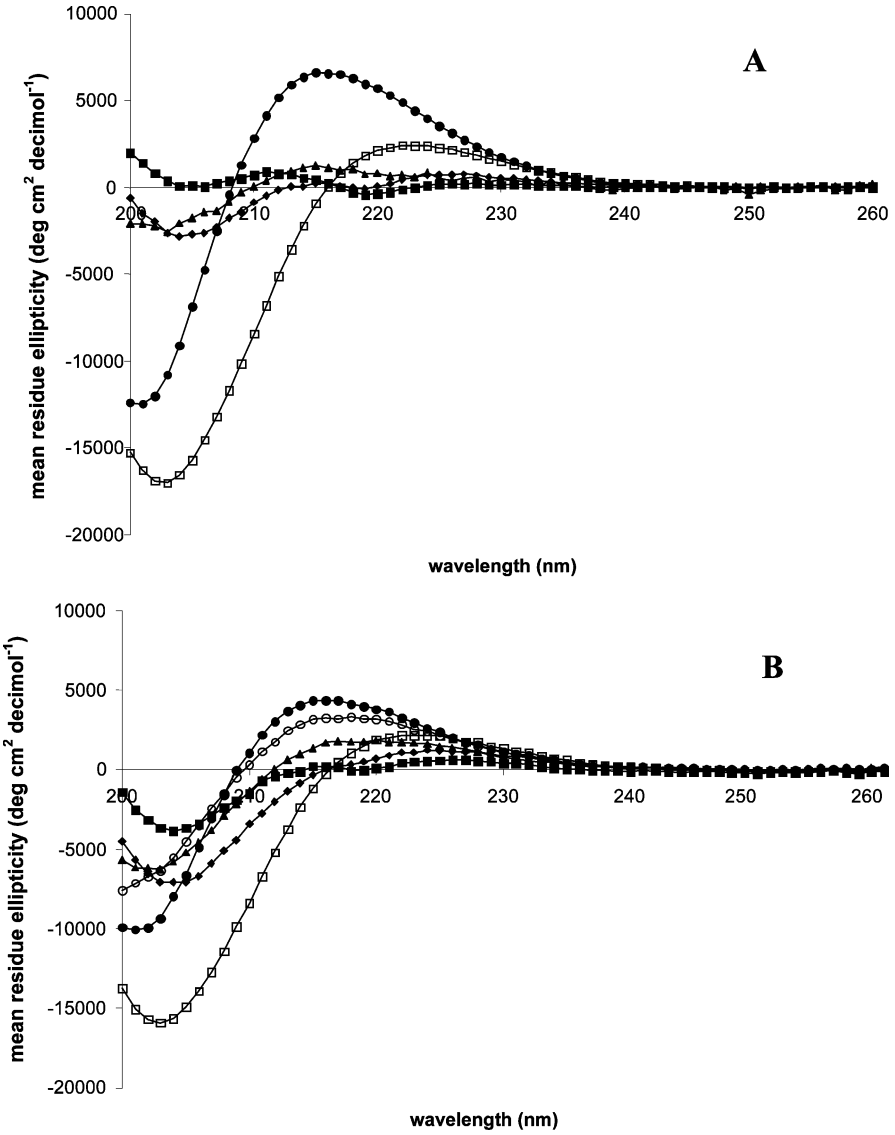

Figure 8. (A) CD spectra of β-peptides from library **2** in Tris buffer (10 mM, pH 7.2). Curves represent **2.5** (■, 0.25 mM), **2.6** (◆, 0.26 mM), **2.7** (▲, 0.053 mM), **2.11** (●, 0.25 mM), and **2.16** (□, 0.29 mM). (B) CD spectra of 16-residue β-peptides in Tris buffer (10 mM, pH 7.2). Curves represent **3.1** (○, 0.093 mM), **3.5** (■, 0.088 mM), **3.6** (◆, 0.092 mM), **3.7** (▲, 0.093 mM), **3.11** (●, 0.087 mM), and **3.16** (□, 0.099 mM).

slightly blue-shifted relative to the positions observed for these extrema in methanol, which is consistent with prior observations.[31b,c] β-Peptides **2.5**, **2.6**, and **2.7**, which contain a combination of cyclic and β^3-residues, display little CD intensity, which suggests that these molecules may not be folded to any significant extent in aqueous solution. The CD signature of **2.11**, containing exclusively β^3-residues, maintains the unusual features noted above. That this signature remains strong in water heightens the mystery of its origin. Similar trends are observed among the analogous 16-mers in water (Figure 8B), except that in this set the mixed cyclic/acyclic sequences (**2.5**−**2.7**) show weak CD signals reminiscent of the 12-helical signature.

We examined 16-mer **3.11** in aqueous buffer containing DPC micelles to determine whether binding to a membrane-like surface could induce a conformational change in this all-β^3-residue oligomer. This study was motivated by the fact that magainins and related α-peptides do not adopt α-helical conformations until they are presented with a membrane or membrane-like surface;[6] similar behavior has been documented with all-β^3-residue oligomers designed to form globally amphiphilic 14-helices.[8,11a,d] Figure 9 compares the CD signatures of **3.11** in aqueous Tris buffer, with and without DPC micelles. The micelles cause only a small red-shift in the minimum (204 to 202 nm) and maximum (218 to 217 nm). Thus, the micelle surface does not appear to induce a new secondary structure in **3.11**.

Overall, the CD results suggest that there is little correlation between the β-peptide folding propensity, as manifested by CD signatures in water or methanol, and antibacterial activity. Thus, for example, **3.5**−**3.7** display a lower 12-helical propensity than does **3.16** (composed exclusively of preorganized ACPC and APC residues), but these four 16-mers have nearly identical activities against the four bacteria we examined. β-Peptide **3.11**, which contains only β^3-residues and does not seem to adopt a 12-helical conformation in water or methanol, is nevertheless moderately active against these bacteria.

Conclusions

We have reported the first library-based approach to exploring structure−activity relationships among β-peptides. β-Peptides

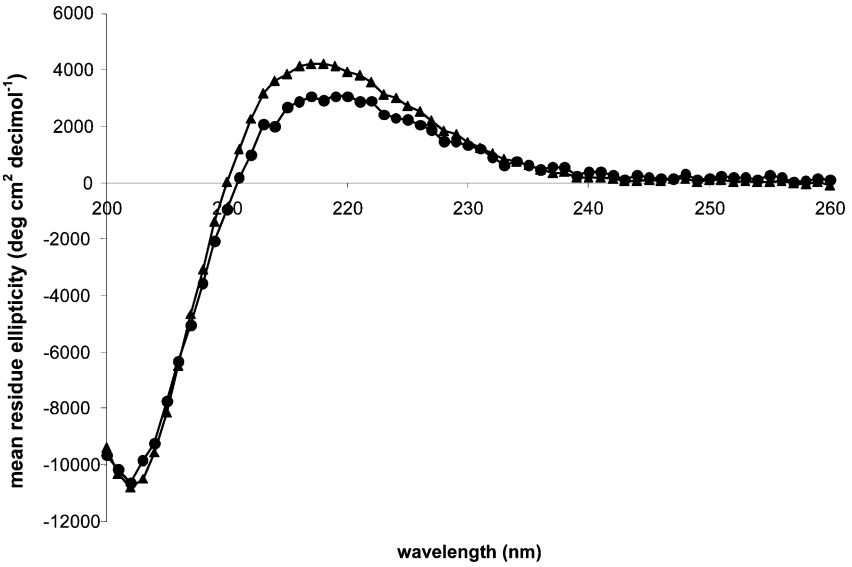

Figure 9. Circular dichroism of **2.11** (●, 51 μM) and **3.11** (▲, 70 μM) in Tris buffer (10 mM, pH 7.2) with DPC micelles (5 mM monomer).

lend themselves naturally to the design and synthesis of sets of analogous compounds, because of their oligomeric nature. We have examined two formats for parallel β-peptide synthesis, one semi-automated and the other fully manual; we find the latter approach, which is simple but somewhat labor-intensive, to be particularly useful. The libraries examined are relatively small, but our results suggest that 12-helical β-peptides are subject to a lower limit on MIC values in the 1 μg/mL range, as has previously been observed for α-peptides designed from host-defense peptide prototypes.[6]

Experimental Section

Fmoc-α-amino acids used as precursors for Fmoc-β^3-amino acids and Fmoc-β-alanine were purchased from Novabiochem. The side chain of lysine was Boc-protected. The side chain of serine was protected as the corresponding *tert*-butyl ether. *N*-Methylpyrrolidinone (NMP) was purchased from Advanced Chemtech. *N*,*N*-Dimethylformamide (DMF) was purchased as HPLC grade and stored over Dowex 50W-X8 ion-exchange resin. Dichloromethane (DCM) was distilled or HPLC grade. Ether was anhydrous. Rink amide MBHA resin (4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl-(4-methylbenzhydryl-amide)-polystyrene resin; loading = 0.78 mmol/g) was purchased from Novabiochem. Rink amide NovaGel resin (loading = 0.67 mmol/g) was purchased from Novabiochem. Fmoc amide resin (4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-ethyl-polystyrene resin; loading = 0.63 mmol/g) was obtained from Applied Biosystems. Diisopropylethylamine (DIEA) was distilled from CaH_2. [Ala^{8,13,18}]-Magainin II amide, melittin, and Tris were purchased from Sigma. All other reagents were purchased from Aldrich.

The 24-port solid-phase extraction vacuum manifold for manual solid-phase synthesis was purchased from VWR. The manifold consists of a lid with 24 female ports with stopcocks, a glass chamber, and a vacuum regulator that can be attached to an aspirator. The polypropylene solid-phase extraction tubes and caps used for manual solid-phase synthesis were purchased from Alltech. The female caps for the bottom of the solid-phase extraction tubes (syringe pressure caps) were purchased from Aldrich. The rocker used for agitation of the tubes in manual solid-phase synthesis was purchased from Fisher. All consumables used on the Quest 210 were purchased from Argonaut.

The β-peptide library members were purified by reversed-phase HPLC on a C_4-silica reversed-phase preparative column (10 μm, 22 × 250 mm; Vydac) with a flow rate of 15 mL/min. The longer β-peptides were purified by reversed-phase HPLC on a C_4-silica reversed-phase semipreparative column (5 μm, 10 × 250 mm; Vydac) with a flow rate of 3 mL/min. The A solvent and B solvent were TFA/H_2O (0.1%, v/v) and CH_3CN/H_2O/TFA (80/20/0.1, v/v/v), respectively. The purified β-peptides displayed one peak by analytical HPLC using a C_4-silica reversed-phase analytical column (5 μm, 4 mm × 250 mm, Vydac), a gradient of 5−95% B over 45 min, and a flow rate of 1 mL/min. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a Bruker REFLEX II spectrometer with a 337-nm laser using α-cyano-4-hydroxycinnamic acid matrix and calibrated with a standard mixture of angiotensin I ($M + H^+ =$ 1296.7) and neurotensin ($M + H^+ = 1672.9$).

Synthesis of β-Amino Acids. Fmoc-ACPC, Fmoc-APC(Boc), and Fmoc-AP(Boc) were synthesized as previously described.[22,33] Fmoc-β^3-amino acids were synthesized by Arndt-Eistert homologation of Fmoc-α-amino acids. Diazoketone formation was carried out as reported by Seebach and co-workers,[19a] and silver-promoted Wolff rearrangement was performed as described by Sewald and co-workers.[19b]

General Procedure for β-Peptide Synthesis on the Argonaut Quest. Twenty β-peptides (15 μmol scale) were synthesized at once on the Argonaut Quest 210 in 5-mL Teflon-lined reaction vessels with 30 μm frits. The mixing control was set at "mix every: 3.0 s," "upstroke: 1.8 s," "% upward: 60%." Solvent A was DMF, solvent B was piperidine/DMF (1/4, v/v), and solvent D was DCM. The Argonaut Quest 210 was equipped with an automated solvent wash (ASW) module that allows for unattended solvent addition and washing steps. For the ASW module to deliver solvents, the upper manifold membrane switch was set to "Open RVs" on side A and side B. The upper manifold valves were set to "Solvent delivery" and "Autowash." While a program was being run, the lower manifold drain valves were set to "Open RVs" as required for the ASW to drain solvent into the waste reservoir during washing steps.

MBHA resin (15 μmol, calculated from resin loading) was weighed into each of the reaction vessels (RVs). In preparation for β-peptide synthesis, the resin was swelled and deprotected using Program 8 (Table S1, Supporting Information). The C-terminal β-amino acids (45 μmol) were weighed into vials and dissolved in 2 mL of DMF. If more than one β-peptide required a particular β-amino acid, then the masses and volumes were multiplied as necessary and split among the RVs. The luer plugs were removed from the upper manifold for reagent addition, and the lower manifold drain valves were closed. The β-amino acid

(33) (a) LePlae, P. R.; Umezawa, N.; Lee, H.-S.; Gellman, S. H. *J. Org. Chem.* **2001**, *66*, 5629−5632. (b) Lee, H.-S.; LePlae, P. R.; Porter, E. A.; Gellman, S. H. *J. Org. Chem.* **2001**, *66*, 3597−3599.

solutions were added to the RVs through the luer ports, followed by 90 μL of *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 45 μmol, 0.5 M in DMF), 90 μL of 1-hydroxybenzotriazole (HOBt, 45 μmol, 0.5 M in DMF), and 180 μL of DIEA (90 μmol, 0.5 M in DMF). The luer plugs were replaced. The general coupling program (Program 9, Table S2, Supporting Information) was started, and the lower manifold drain valves were opened.

When the resin-bound β-peptide intermediate had to sit overnight until the next coupling reaction, Program 10 (Table S3, Supporting Information) was used, which includes only the coupling steps and not the deprotection steps. The next morning Program 11 (Table S4, Supporting Information) was run to deprotect the Fmoc group before any additional couplings. After the final coupling and deprotection were complete, the resin was washed and dried thoroughly using Program 12 (Table S5, Supporting Information). After completion of Program 12, the lower manifold valves were closed, the upper manifold membrane switch was set to "Closed", and the upper manifold valves were set to "Closed". The luer plugs were removed, and cleavage solution (2 mL, TFA/TIS/H_2O, 95/2.5/2.5) was added through the luer ports to each RV. The luer plugs were replaced, and the reactions were agitated via the controller for 2 h. Agitation was stopped, and the cleavage solutions were drained into test tubes. The cleavage solutions were evaporated under a stream of nitrogen. The β-peptides were dissolved in a minimal amount of methanol and precipitated with cold anhydrous ether. The test tubes were centrifuged, and the supernatants were decanted. The pellets were dissolved in H_2O/CH_3CN and lyophilized.

General Procedure for Manual Solid-Phase β-Peptide Synthesis. This method employs a 24-port solid-phase extraction manifold. Rink amide MBHA resin (10 μmol, calculated from resin loading) was weighed into 20 polypropylene solid-phase extraction tubes (1.5-mL size). Dichloromethane (0.5 mL) was added to each tube, and the resin was swelled for 15 min. The tubes were transferred to the manifold and washed (3 × DCM, 3 × DMF). The tubes were removed from the manifold and fitted with syringe pressure caps. Deprotection solution (0.5 mL, 1/4 piperidine/DMF) was added to the resin, and the tubes were fitted with the appropriate caps. The tubes were rocked for 15 min. The deprotection solution was drained on the manifold, and the resin was washed (3 × DCM, 3 × DMF).

The C-terminal β-amino acids (30 μmol) were weighed into vials. *O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 30 μmol) was placed in the vials. DMF (0.5 mL) was added to the vials, and the mixtures were allowed to dissolve. The amino acid/HBTU solutions were added to the appropriate tubes, followed by 60 μL of HOBt (30 μmol, 0.5 M in DMF) and 120 μL of DIEA (60 μmol, 0.5 M in DMF). The tubes were rocked for 2 h. The coupling solutions were drained on the manifold, and the resin was washed (3 × DCM, 3 × DMF). The deprotection/coupling protocol was repeated until the syntheses were complete.

After the final coupling, the N-terminal β-amino acids were deprotected using the procedure described above. The deprotection solution was drained on the manifold, and the resin was washed (3 × DMF, 3 × DCM). An acetylation cocktail (0.5 mL, Ac_2O/NEt_3/DCM, 5/1/14) was added to each tube, and the tubes were rocked for 2 h. The acetylation cocktail was drained on the manifold, the resin was washed (3 × DCM, 3 × MeOH), and the tubes were left on the aspirator to dry for a few minutes. Cleavage cocktail (0.5 mL, TFA/H_2O, 95/5, v/v) was added to the resin, and the tubes were rocked for 2 h. The cleavage solutions were drained into vials, and the resin beds were washed with methanol (1 mL). The TFA/methanol was evaporated on a Speedvac, H_2O/CH_3CN was added and evaporated on a Speedvac (some loss of material due to bumping), and the β-peptides were purified by HPLC.

General Procedure for Automated β-Peptide Synthesis. β-Peptides were synthesized on a 25 μmol scale by standard Fmoc/*t*-Bu methods on Fmoc amide resin using a Synergy 432A automated peptide synthesizer (Applied Biosystems). HBTU and HOBt were used as coupling reagents. Two-hour couplings and extended deprotections were employed. After β-peptide synthesis was complete, the N-terminus was acetylated using an acetic anhydride/NEt_3/CH_2Cl_2 solution (2 mL, 0.25/0.05/1.7, v/v/v) and shaking for 2 h. The resin-bound β-peptides were cleaved from the solid support and deprotected simultaneously using TFA/H_2O (1 mL, 95/5, v/v) and shaking for 2 h. The cleavage solutions were drained into vials, and the resin beds were washed with methanol (1 mL). The TFA/MeOH solutions were evaporated under a stream of nitrogen.

Purification of β-Peptide Libraries. The β-peptide libraries were analyzed for initial purity by analytical HPLC using a gradient of 5−95% B over 45 min (initial purity is measured by the absorbance of the HPLC peak corresponding to the correct molecular weight relative to total UV absorbance for the chromatogram). After initial purity analysis, β-peptides (each dissolved in 1 mL of H_2O and 0.5 mL of CH_3CN) were purified in two injections each (500 μL injection followed by a 1000 μL injection) by prep HPLC. The purified β-peptides were characterized by MALDI-TOF MS (values given in characterization section) and determined to be >95% pure by analytical HPLC using a gradient of 5−95% B over 45 min.

Characterization of β-Peptides. β-Peptide 1.1. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−65% B over 35 min, 8% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{70}H_{121}N_{19}O_{12}$ (M) 1419.9, obsd 1420.7 ($M + H^+$), 1442.7 ($M + Na^+$).

β-Peptide 1.2. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 15−48% B over 33 min, 3% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{64}H_{109}N_{19}O_{12}$ (M) 1335.9, obsd 1336.7 ($M + H^+$), 1358.7 ($M + Na^+$). A deletion β-peptide was also observed in the mass spectrum at 1225, which corresponds to M − ACPC.

β-Peptide 1.4. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 25−55% B over 30 min, 7% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{68}H_{117}N_{19}O_{12}$ (M) 1391.9, obsd 1393.3 ($M + H^+$), 1415.2 ($M + Na^+$), 1431.2 ($M + K^+$).

β-Peptide 1.5. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−50% B over 20 min, 8% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{73}H_{127}N_{17}O_{12}$ (M) 1434.0, obsd 1435.1 ($M + H^+$), 1457.1 ($M + Na^+$). A deletion β-peptide was also observed in the mass spectrum at 1308.1 and 1330.1, which corresponds to M − β^3-hLeu.

β-Peptide 1.6. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−65% B over 35 min, 7% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{67}H_{115}N_{17}O_{12}$ (M) 1349.9, obsd 1350.7 ($M + H^+$), 1372.7 ($M + Na^+$). A deletion β-peptide was also observed in the mass spectrum at 1127.7 and 1149.7, which corresponds to M − ACPC − APC.

β-Peptide 1.7. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−50% B over 20 min, 13% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{79}H_{123}N_{17}O_{12}$ (M) 1502.0, obsd 1502.4 ($M + H^+$), 1524.4 ($M + Na^+$).

β-Peptide 1.8. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−45% B over 15 min, 12% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{71}H_{123}N_{17}O_{12}$ (M) 1406.0, obsd 1406.4 ($M + H^+$), 1428.5 ($M + Na^+$).

β-Peptide 1.9. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−65% B over 30 min, 12% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{68}H_{119}N_{17}O_{14}$ (M) 1397.9, obsd 1398.5 ($M + H^+$), 1420.5 ($M + Na^+$).

β-Peptide 1.10. The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 15−30% B over 15 min, 13% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{62}H_{107}N_{17}O_{14}$ (M) 1313.8, obsd 1314.7 ($M + H^+$), 1336.7 ($M + Na^+$).

***β*-Peptide 1.11.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−65% B over 35 min, 9% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{74}H_{115}N_{17}O_{14}$ (M) 1465.9, obsd 1466.9 (M + H^+), 1489.0 (M + Na^+).

***β*-Peptide 1.12.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 20−40% B over 20 min, 9% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{66}H_{115}N_{17}O_{14}$ (M) 1369.9, obsd 1371.3 (M + H^+), 1393.4 (M + Na^+).

***β*-Peptide 1.13.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−65% B over 35 min, 14% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{66}H_{111}N_{17}O_{14}$ (M) 1365.9, obsd 1367.4 (M + H^+), 1389.4 (M + Na^+).

***β*-Peptide 1.15.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 25−45% B over 20 min, 13% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{72}H_{107}N_{17}O_{14}$ (M) 1433.8, obsd 1435.3 (M + H^+), 1457.2 (M + Na^+).

***β*-Peptide 1.16.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 15−35% B over 20 min, 8% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{64}H_{107}N_{17}O_{14}$ (M) 1337.8, obsd 1339.1 (M + H^+), 1361.1 (M + Na^+), 1377.1 (M + K^+). A deletion *β*-peptide was also observed in the mass spectrum at 1227, which corresponds to M − APC.

***β*-Peptide 1.17.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−50% B over 20 min, 1% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{70}H_{121}N_{19}O_{12}$ (M) 1419.9, obsd 1420.9 (M + H^+), 1443.0 (M + Na^+). A *β*-peptide was also observed in the mass spectrum at 1305, which corresponds to M + AP/APC − β^3-hLeu.

***β*-Peptide 1.18.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 35−75% B over 20 min, 1% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{76}H_{117}N_{19}O_{12}$ (M) 1487.9, obsd 1488.2 (M + H^+), 1510.2 (M + Na^+), 1526.2 (M + K^+). An unidentified impurity was also observed in the mass spectrum at 1398.

***β*-Peptide 1.19.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 30−50% B over 20 min, 1% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{76}H_{117}N_{19}O_{12}$ (M) 1487.9, obsd 1488.2 (M + H^+), 1510.1 (M + Na^+).

***β*-Peptide 1.20.** The synthesis was performed as described on the Argonaut Quest. Prep HPLC purification: 25−45% B over 20 min, 1% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{68}H_{113}N_{19}O_{12}$ (M) 1387.9, obsd 1388.2 (M + H^+), 1410.2 (M + Na^+).

***β*-Peptide 2.1.** The synthesis was performed manually as described. Prep HPLC purification: 30−70% B over 40 min, 3% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{71}H_{128}N_{16}O_{12}$ (M) 1397.0, obsd 1397.8 (M + H^+), 1419.7 (M + Na^+), 1436.8 (M + K^+). A deletion *β*-peptide was also observed in the mass spectrum at 1158.7 and 1180.7, which corresponds to M − APC − β^3-hLeu.

***β*-Peptide 2.2.** The synthesis was performed manually as described. Prep HPLC purification: 30−52% B over 22 min, 22% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{69}H_{120}N_{16}O_{12}$ (M) 1364.9, obsd 1366.0 (M + H^+), 1388.1 (M + Na^+), 1404.1 (M + K^+).

***β*-Peptide 2.3.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, rt = 17.4 min, 19% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{68}H_{116}N_{16}O_{12}$ (M) 1348.9, obsd 1349.8 (M + H^+), 1371.8 (M + Na^+).

***β*-Peptide 2.4.** The synthesis was performed manually as described. Prep HPLC purification: 30−52% B over 22 min, 5% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{92}H_{114}N_{16}O_{12}$ (M) 1634.9, obsd 1635.7 (M + H^+), 1657.8 (M + Na^+), 1673.7 (M + K^+). A deletion *β*-peptide was also observed in the mass spectrum at 1362.7 and 1384.6, which corresponds to M − APC − β^3-hPhe.

***β*-Peptide 2.5.** The synthesis was performed manually as described. Prep HPLC purification: 30−45% B over 15 min, 10% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{84}H_{110}N_{16}O_{12}$ (M) 1534.9, obsd 1535.8 (M + H^+), 1557.8 (M + Na^+).

***β*-Peptide 2.6.** The synthesis was performed manually as described. Prep HPLC purification: 30−52% B over 22 min, 19% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{80}H_{108}N_{16}O_{12}$ (M) 1484.8, obsd 1486.0 (M + H^+), 1508.0 (M + Na^+), 1523.9 (M + K^+).

***β*-Peptide 2.7.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 11% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{69}H_{120}N_{16}O_{12}$ (M) 1364.9, obsd 1366.1 (M + H^+), 1388.1 (M + Na^+), 1404.1 (M + K^+). An unidentified impurity was present in the mass spectrum at 1314.0 and 1337.0.

***β*-Peptide 2.8.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 12% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{69}H_{120}N_{16}O_{12}$ (M) 1364.9, obsd 1365.5 (M + H^+), 1387.5 (M + Na^+).

***β*-Peptide 2.9.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 6% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{75}H_{116}N_{16}O_{12}$ (M) 1432.9, obsd 1433.3 (M + H^+), 1455.4 (M + Na^+).

***β*-Peptide 2.10.** The synthesis was performed manually as described. Prep HPLC purification: 30−45% B over 15 min, 9% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{66}H_{116}N_{16}O_{12}$ (M) 1324.9, obsd 1325.8 (M + H^+), 1347.9 (M + Na^+), 1363.9 (M + K^+). An unidentified impurity was present in the mass spectrum at 1260.9 and 1282.8.

***β*-Peptide 2.11.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 9% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{79}H_{152}N_{16}O_{12}$ (M) 1517.2, obsd 1517.8 (M + H^+), 1539.8 (M + Na^+), 1555.8 (M + K^+).

***β*-Peptide 2.12.** The synthesis was performed manually as described. Prep HPLC purification: 30−55% B over 25 min, 5% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{100}H_{138}N_{16}O_{12}$ (M) 1755.1, obsd 1755.8 (M + H^+), 1777.8 (M + Na^+), 1793.8 (M + K^+).

***β*-Peptide 2.13.** The synthesis was performed manually as described. Prep HPLC purification: 30−45% B over 15 min, 3% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{76}H_{140}N_{16}O_{12}$ (M) 1469.1, obsd 1469.8 (M + H^+), 1491.8 (M + Na^+), 1507.8 (M + K^+).

***β*-Peptide 2.14.** The synthesis was performed manually as described. However, HPLC revealed that the crude product contained many components, so **2.14** was resynthesized using Rink amide NovaGel as the resin, 1/4 DCM/NMP as the solvent, and 2 h couplings. Prep HPLC purification: 30−50% B over 20 min, 3% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{88}H_{132}N_{16}O_{12}$ (M) 1605.0, obsd 1606.0 (M + H^+), 1628.0 (M + Na^+), 1644.0 (M + K^+). A *β*-peptide was also observed in the mass spectrum at 1718.1 and 1741.1, which corresponds to M + APC.

***β*-Peptide 2.15.** The synthesis was performed manually as described. Prep HPLC purification: 20−37% B over 17 min, 7% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{72}H_{124}N_{16}O_{12}$ (M) 1405.0, obsd 1405.9 (M + H^+), 1427.9 (M + Na^+).

***β*-Peptide 2.16.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 25% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{64}H_{100}N_{16}O_{12}$ (M) 1284.8, obsd 1285.6 (M + H^+), 1307.6 (M + Na^+), 1323.6 (M + K^+).

***β*-Peptide 2.17.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 12% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{69}H_{120}N_{16}O_{12}$ (M) 1364.9, obsd 1365.9 (M + H^+), 1387.9 (M + Na^+), 1403.8 (M + K^+).

***β*-Peptide 2.18.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 13% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{75}H_{116}N_{16}O_{12}$ (M) 1432.9, obsd 1434.2 (M + H^+), 1456.2 (M + Na^+), 1472.1 (M + K^+).

***β*-Peptide 2.19.** The synthesis was performed manually as described. Prep HPLC purification: 30−45% B over 15 min, 14% yield (after purification). MALDI-TOF-MS *m/z* calcd for $C_{67}H_{116}N_{16}O_{12}$ (M) 1336.9, obsd 1338.0 (M + H^+), 1360.0 (M + Na^+), 1375.9 (M + K^+).

***β*-Peptide 2.20.** The synthesis was performed manually as described. Prep HPLC purification: 30−50% B over 20 min, 18% yield (after purification). MALDI-TOF-MS *m*/*z* calcd for $C_{68}H_{116}N_{16}O_{12}$ (M) 1348.9, obsd 1349.7 ($M + H^+$), 1371.7 ($M + Na^+$). A deletion β-peptide was also observed in the mass spectrum at 1237.6, which corresponds to M − ACPC.

***β*-Peptide 3.1.** The automated β-peptide synthesis was performed as described above. Semi-prep HPLC purification: 40−58% B over 18 min, ca. 14% yield (after purification, purified ca. 1/2 of sample). MALDI-TOF-MS *m*/*z* calcd for $C_{102}H_{183}N_{23}O_{17}$ (M) 2002.4, obsd 2003.5 ($M + H^+$), 2025.5 ($M + Na^+$), 2041.4 ($M + K^+$).

***β*-Peptide 3.5.** The automated β-peptide synthesis was performed as described above. Semi-prep HPLC purification: 20−60% B over 40 min, ca. 50% yield (after purification, purified ca. 1/2 of sample). MALDI-TOF-MS *m*/*z* calcd for $C_{116}H_{155}N_{23}O_{17}$ (M) 2142.2, obsd 2143.7 ($M + H^+$), 2165.7 ($M + Na^+$).

***β*-Peptide 3.6.** The automated β-peptide synthesis was performed as described above. Semi-prep HPLC purification: 40−55% B over 15 min, ca. 60% yield (after purification, purified ca. 1/2 of sample). MALDI-TOF-MS *m*/*z* calcd for $C_{108}H_{151}N_{23}O_{17}$ (M) 2042.2, obsd 2043.6 ($M + H^+$), 2065.7 ($M + Na^+$).

***β*-Peptide 3.7.** The automated β-peptide synthesis was performed as described above. Semi-prep HPLC purification: 40−60% B over 20 min, ca. 34% yield (after purification, purified ca. 3/4 of sample). MALDI-TOF-MS *m*/*z* calcd for $C_{104}H_{163}N_{23}O_{17}$ (M) 2006.3, obsd 2007.4 ($M + H^+$), 2029.5 ($M + Na^+$).

***β*-Peptide 3.11.** The automated β-peptide synthesis was performed as described above. Semi-prep HPLC purification: 40−56.5% B over 16.5 min, ca. 40% yield (after purification, purified ca. 1/2 of sample). MALDI-TOF-MS *m*/*z* calcd for $C_{114}H_{219}N_{23}O_{17}$ (M) 2182.7, obsd 2184.8 ($M + H^+$), 2205.9 ($M + Na^+$).

***β*-Peptide 3.16.** The automated β-peptide synthesis was performed as described above. Semi-prep HPLC purification: 30−55% B over 25 min, ca. 40% yield (after purification, purified ca. 3/4 of sample). MALDI-TOF-MS *m*/*z* calcd for $C_{92}H_{143}N_{23}O_{17}$ (M) 1842.1, obsd 1843.1 ($M + H^+$), 1865.2 ($M + Na^+$), 1881.1 ($M + K^+$).

Antibacterial Activity of *β*-Peptides. The antibacterial activity of the β-peptides was determined in sterile 96-well plates (Falcon 3075 microtiter plate) by a microdilution method. A bacterial suspension of approximately 10^6 CFU/ml brain-heart infusion (BHI) medium was added in 50 μL aliquots to 50 μL of medium containing the β-peptide in 2-fold serial dilutions for a total volume of 100 μL in each well. The plates were incubated at 37 °C for 6 h. Growth inhibition was determined by measuring optical density at 650 nm with a microplate reader. Antibacterial activity is stated as the minimum inhibitory concentration (MIC), the concentration at which growth of the bacteria was totally inhibited. A synthetic magainin analogue, [$Ala^{8,13,18}$]-magainin II amide,[15] was used for comparison.

Hemolytic Activity of *β*-Peptides. Freshly drawn human red blood cells (hRBC, blood type A, with EDTA or heparin anticoagulant) were washed several times with Tris-buffered saline (50 mM Tris, pH 7.2, 150 mM NaCl) and centrifuged until a clear supernatant was observed. A suspension of hRBC in Tris-buffered saline (1% v/v) was used. Two-fold serial dilutions of β-peptide in Tris-buffered saline were added to each well in a sterile 96-well plate (Falcon 3075 microtiter plate), for a total volume of 20 μL in each well. The 1% hRBC suspension (80 μL) was added to each well. Melittin at 200 μg/mL was used as the 100% hemolysis point, and Tris-buffered saline containing no peptide was used as the 0% hemolysis point. The plate was incubated at 37 °C for 1 h and then centrifuged at 3500 rpm for 5 min. The supernatant (80 μL) was diluted with Millipore water (80 μL), and hemoglobin was quantified by measuring optical density at 450 nm with a microplate reader.

Circular Dichroism. CD data were obtained on an Aviv 202SF instrument at 25 °C using a quartz cell with a 1-mm path length, between 200 and 260 nm (10 s averaging times). The baseline spectrum of the appropriate solvent was subtracted from CD spectrum of each β-peptide. The data were normalized for β-peptide concentration and number of residues (i.e., the vertical axis in CD plots is mean residue ellipticity). The raw data were converted to mean residue ellipticity using the following equation,

$$[\Theta] = \psi M_r/100lc$$

where ψ is the CD signal in degrees, M_r is the molecular weight divided by the number of amide chromophores in the β-peptide, l is the path length in decimeters, and c is the concentration in g/mL.

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Supporting Information Available: Tables showing the results of running Programs 8−12 on the resin. This material is available free of charge via the Internet at http://pubs.acs.org.

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