Design, Synthesis and Characterization of Cationic Peptide and Steroid Antibiotics

Paul B. Savage[a]

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Novel cationic antibiotics have been developed to mimic the cationic, facial amphiphilic structures of naturally occurring peptide antibiotics. Several series of designed peptide antibiotics have been reported, as well as examples based on β-peptides and steroids. The antibacterial activities of these compounds are derived from the disruption and/or permeabilization of bacterial membranes, and many display potent antibacterial activity and selectivity for bacterial cells. These antibiotics may prove valuable in combating bacterial infections, especially those from drug-resistant organisms.

I. Introduction

We live in a world that is dominated, numerically, by bacteria. Compared to the roughly ten trillion cells in the human body, it is estimated that up to 100 trillion bacteria can live in and on a human body.[1] Because of the number of bacteria existing in our environment, organisms have necessarily developed means of controlling bacterial growth. In higher organisms, aspects of innate immunity control bacterial populations, and an essential component of this immunity consists of endogenous cationic peptide antibiotics. Antibacterial peptides have been isolated from a wide range of organisms including mammals,[2] amphibians,[3] insects,[4] plants[5] and even bacteria.[6] In general, these peptides are cationic, facially amphiphilic molecules,[7] and they display broad-spectrum antibacterial activity, rapid killing times, selectivity for bacteria over host cells, and are unlikely to induce the formation of resistant strains of bacteria.[8] The antibacterial activity of peptide antibiotics is a result of the permeabilization or disruption of bacterial membranes. Cationic peptide antibiotics have been classified according to their structure, and detailed mechanisms for their activity have been proposed.[9] Several excellent reviews have recently been published describing classes of peptide antibiotics and their mechanisms of action.[10,11]

Peptide antibiotics are relatively large (> 20 amino acids) and can be difficult to derivatize and purify.[11] In addition, many of these peptides have limited lifetimes in the presence of proteases. Consequently, attention has been paid to developing simpler and smaller compounds that display activities similar to those of endogenous peptide antibiotics for potential clinical use and to aid in the elucidation of how small molecules disrupt bacterial membranes. Three approaches have been used in the development of cationic peptide antibiotic mimics: (1) the rational design of cationic peptides, often smaller than endogenous examples, containing cationic and hydrophobic amino acids, (2) the use of cationic, helical β-peptides which are not substrates for proteases, and (3) the modification of steroids with amine

[a] Department of Chemistry and Biochemistry, Brigham Young University
Provo, UT 84602, USA
Fax: (internat.) + 1-801/378-5474
E-mail: paul_savage@byu.edu

Paul B. Savage was born in Arizona, USA. He received his bachelors degree in chemistry from Brigham Young University in 1988. During his undergraduate education he worked in the laboratory of Professor Jerald S. Bradshaw. He then joined the group of Professor Samuel H. Gellman at the University of Wisconsin working with carbohydrate receptors based on phosphane oxides and sulfoxides and received his Ph.D. in 1993. After a period of postdoctoral research in the laboratories of Professor Leo A. Paquette at The Ohio State University, he joined the faculty at Brigham Young University in the Department of Chemistry and Biochemistry as an Assistant Professor in 1995. He was promoted to Associate Professor in 2000. His current research interests include the development of cationic steroid antibiotics, the elucidation of the roles of non-covalent interactions in lipid A-small molecule association, the preparation of fluorescent chemosensors for metal ions, and glycolipid immunology.

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groups. This review will provide examples from the first approach and detailed descriptions of the second and third.

II. General Design Approaches

As a consequence of secondary and/or tertiary structure, cationic peptide antibiotics adopt facially amphiphilic conformations. That is, one face of the molecule presents cationic groups (protonated amines or guanidines) and the other face contains hydrophobic groups. Two major classes of these peptides include examples that adopt amphiphilic α-helices or those that form β-sheets.[10,11] For example, a helix wheel of magainin I,[3b] an antibiotic isolated from an amphibian skin secretion, demonstrates the segregation between hydrophobic and cationic amino acid side chains (Figure 1). The putative lipopolysaccharide-binding portion of bactericidal permeability-increasing protein, a bactericidal protein found in humans, is comprised of an amphiphilic anti-parallel β-sheet (Figure 1).[12]

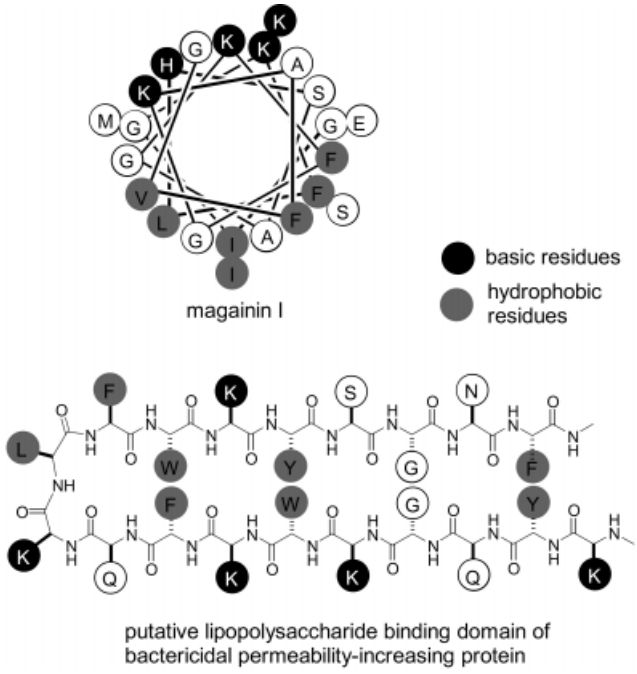


Figure 1. Helix wheel demonstrating the facial amphiphilicity of magainin I and a schematic drawing of the putative lipopolysaccharide binding domain of bactericidal permeability increasing protein; the one letter code for amino acids is used

The facial amphiphilicity common to cationic peptide antibiotics suggests that for antimicrobial activity mimics must display a similar morphology. Consequently, designed peptide antibiotics contain cationic residues appropriately spaced among hydrophobic amino acids to allow the segregation of both types of residues upon α-helix or β-sheet formation. Similarly, cationic antibiotics derived from helical β-peptides have been designed to adopt facially amphiphilic conformations. Constraints of the relatively inflexible steroid ring system and the stereochemistry of the attachment points of tethered cationic groups enforce the separation of cationic and hydrophobic groups in steroid-derived antibiotics.

III. Characterization of Antimicrobial Compounds

The primary measure of the efficacy of an antibiotic is its minimum inhibition concentration (MIC), traditionally described in units of μg/mL.[13] An MIC is the concentration of an antibiotic required to inhibit population growth in a bacterial culture, typically for 24 h.[14] Antibiotics often display different MIC values with different strains of bacteria, especially between Gram-negative and Gram-positive, with Gram-negative strains often being less susceptible to antibiotics due to the permeability barrier provided by their outer membrane. Consequently, a full characterization of antibacterial activity should be assessed using both types of organisms. Examples of commonly found Gram-negative bacteria are *Escherichia coli, Salmonella typhimurium* and *Pseudomonas aeruginosa*, and examples of Gram-positive strains are *Staphylococcus aureus* and *Streptococcus pyogenes*.

Because cationic peptide and steroid antibiotics interact with bacterial membranes, they can disrupt the organization of the outer membranes of Gram-negative bacteria and sensitize them to hydrophobic antibiotics that ineffectively traverse the outer membrane. This ability to disorganize the outer membrane is typically observed as synergism between the cationic antibiotic and a hydrophobic antibiotic, and can be quantified using fractional inhibition concentrations (FICs) (FIC = $[A]/MIC_A + [B]/MIC_B$, where MIC_A and MIC_B are the MIC values of compounds A and B, respectively, and [A] and [B] are the concentrations at which compounds A and B, in combination, inhibit bacterial growth. Synergism is defined by FIC < 0.5).[15]

An important aspect in the characterization of cationic peptide and steroid antibiotics is their selectivity for bacterial (prokaryotic) membranes over host (eukaryotic) membranes. A measure of membrane selectivity can be obtained by comparing the MICs of an antibiotic to its minimum hemolytic concentration (MHC, minimum concentration required to lyse red blood cells). Compounds that display similar MIC and MHC values offer little or no membrane selectivity; compounds that are selective for prokaryotic membranes have low MIC values and high MHC values.

IV. Designed Cationic Peptide Antibiotics

A. Classes of Antibiotics and Antibacterial Properties

Many designed cationic peptide antibiotics have been developed based upon the structures of naturally occurring examples. In general, these designed antibiotics fall into the major classes of naturally occurring cationic antibiotics: those that form α-helices in the presence of bacterial membranes and those that are constrained to adopt β-sheet conformations via cyclization mediated by disulfide and/or amide bonds (for examples see Figure 2).

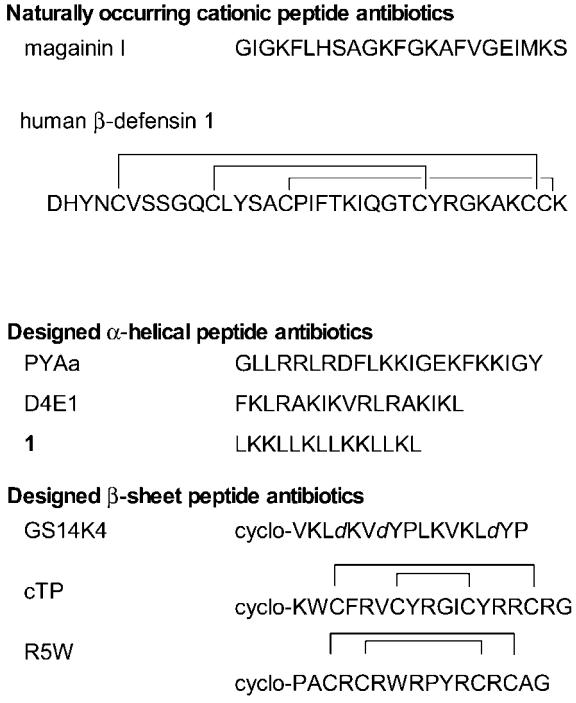


Figure 2. Examples of naturally occurring and designed peptide antibiotics; lines connecting cystein residues indicate the formation of disulfide bonds

1. Helix Forming Antibiotics

Helix forming antibiotics are generally comprised of lysines and arginines interspersed with hydrophobic amino acids in such a way that when an α-helix forms, the basic side chains are oriented on one face of the helix. By determining the most frequent amino acids in the first 20 positions for more than 80 α-helical peptide antibiotics, a series of antibiotics were designed including PYAa (Figure 2).[16] Members of this series have broad-spectrum activity against Gram-negative and Gram-positive bacteria; PYAa displays MIC values of less than 5 μg/mL against both classes of bacteria. Similar to many naturally occurring peptide antibiotics, this compound is unstructured in water and only forms an α-helix in the presence of a bacterial membrane. Two other designed antibiotics are also shown in Figure 2. D4E1 was reported among a series of peptides designed to be similar to naturally occurring tracheal antimicrobial peptides, defensins and magainins.[17] This compound gives MIC values of approx. 1 μg/mL against *S. aureus* and *P. aeruginosa* and is more active against these strains than tracheal antimicrobial peptide. Even very simple peptides comprised only of an appropriate sequence of leucine and lysine display antimicrobial activity, although less than other designed peptides. For example, **1** (Figure 2) gives an MIC of 25 μg/mL against *E. coli*.[18]

2. β-Sheet Forming Antibiotics

Designed peptide antibiotics that adopt amphiphilic β-sheet conformations have been developed that mimic portions of the structures of peptide antibiotics including gramicidin S, tachyplesin and lipopolysaccharide binding protein. GS14K4 (Figure 2), a ″second generation″ antibiotic derived from gramicidin S gives MIC values of ≤16 μg/mL with Gram-negative and positive bacteria.[19] Cyclic peptides containing additional disulfide bond constraints were designed based on the structure of tachyplesin. These compounds (e.g., cTP Figure 2) display low MIC values, with many below 1 μg/mL with Gram-negative and -positive bacteria.[20] In an effort to develop antibiotic peptides that are selective for Gram-negative bacteria, a series of cyclic peptides with sequences similar to the lipopolysaccharide binding site of lipopolysaccharide binding protein were prepared (e.g., R5W Figure 2).[21] Members of this series are very active against Gram-negative organisms (MIC values < 1 μg/mL) while only weakly active against Gram-positive bacteria. This selectivity was proposed to originate from selective binding of the lipopolysaccharide of the outer membranes of Gram-negative bacteria.

B. Permeabilization of the Outer Membranes of Gram-Negative Bacteria

The abilities of designed peptide antibiotics to permeabilize the outer membranes of Gram-negative bacteria and sensitize them to other antibiotics have been determined in a few cases.[22] Compounds as simple as the nonapeptide KFFKFFKFF display good synergism (FIC values ≤ 0.10) with rifampin against *E. coli*.[23]

C. Hemolytic Properties

Most of the designed peptide antibiotics display a degree of hemolytic activity, although many are selective for prokaryotic membranes. A thorough study of cyclic peptide antibiotics related to GS14K4 suggested that limiting the amount of hydrophobic residues improves selectivity; however, if too many hydrophobic groups are eliminated, the compounds lose their antibacterial activity.[24] Peptides that predominantly form α-helices in membranes were also investigated to determine the optimal ratio of cationic to hydrophobic residues to impart the highest amount of prokaryote over eukaryote cell selectively. This study gave similar conclusions to the previously mentioned investigation. That is, incorporation of too many hydrophobic residues results in hemolytic peptides.[25]

V. β-Peptide Antibiotics

A. Design of Antibiotics

Through the pioneering work of Gellman[26] and Seebach,[27] the propensities of appropriately substituted β-peptides to adopt helical conformations came to light, and the potential for β-peptide use in antimicrobial compounds soon became apparent. An advantage offered by β-peptides over ″natural″ α-peptides is that the former are not substrates for proteases, and consequently they would be expected to have longer lifetimes in biological environments.

Antibacterial β-peptides have been designed to yield a morphology similar to that of α-helical peptide antibiotics (i.e., cationic amphiphilic helices). By appropriately spacing hydrophobic and cationic β-amino acid residues, facially amphiphilic conformations are achieved upon helix formation. β-Amino acids can be coupled to form β-peptides using standard peptide bond-forming techniques. Efficient means of preparing enantiomerically pure β-amino acids have been reported,[28] including homologation of α-amino acids[29] and the use of chiral auxiliaries.[30]

B. Antibiotics Based on Homologated Natural Amino Acids

DeGrado and co-workers first reported the preparation and characterization of a series of antibacterial β-peptides (e.g., **2** Figure 3).[31] A few of the compounds described in this initial report display good activity against *E. coli* with MIC values near 1 μg/mL. However, the most active compounds are hemolytic and display little selectivity for prokaryotes over eukaryotes. In a subsequent report,[32] the preparation and characterization of less hydrophobic compounds (**3** and **4**, Figure 3) was described. These latter compounds are slightly less potent antibiotics against *E. coli* than **2**, but they are much less hemolytic. Conformational studies with **3** and **4** by circular dichoism revealed that, analogous to many helical peptide antibiotics,[10,11] the compounds adopt random conformations in solution and helical forms in the presence of micelles.

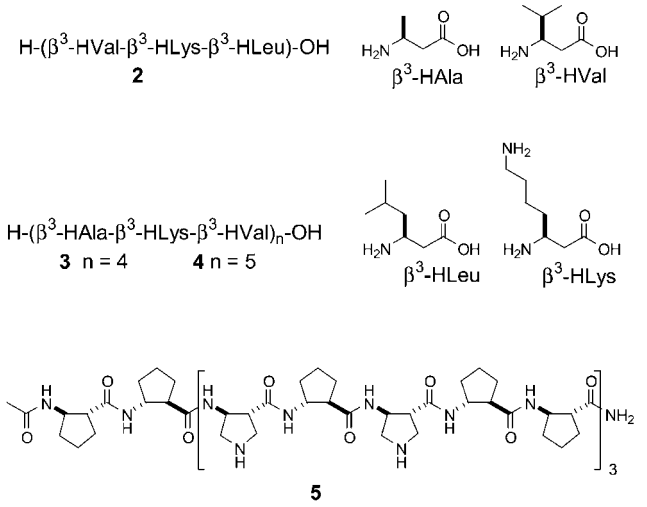


Figure 3. β-Peptide antibiotics **2**–**5**

C. Antibiotics Based on Aminopentanecarboxylic Acids

Gellman and co-workers[33] reported the preparation and characterization of a β-peptide antibiotic derived from (*R,R*)-*trans*-aminocyclopentanecarboxylic acid and (*3R,4S*)-*trans*-4-aminopyrrolidine-3-carboxylic acid (**5**, Figure 3) that displays antimicrobial activity comparable or better than a derivative of magainin II against Gram-negative and -positive organisms. In addition, compound **5** shows only very weak hemolytic activity.

The fact that compounds prepared from "unnatural" amino acids display activities similar to cationic peptide antibiotics suggests that the key requirement for the development of cationic antibiotics is facial amphiphilicity with regard to hydrophobic vs. cationic ratios to provide cell selectivity. Also, that the scaffolding from which hydrophobic and cationic groups may be effectively appended is not limited to α-helical- or β-sheet-forming peptides.

VI. Cationic Steroid Antibiotics

A. Design of Antibiotics

Two types of cationic steroid antibiotics have been developed: those designed to mimic the properties of squalamine and those intended to behave as peptidomimetics[34] of polymyxin B (PMB).[35] Squalamine (Figure 4) is an antibiotic first isolated from the dogfish shark.[36] Although squalamine may not appear to be a facial amphiphile and its relation to cationic peptide antibiotics questioned, if as proposed[37] the polyamine group becomes strapped across one face of the steroid when inserted in a membrane, a facial amphiphile is created. In addition, the antibacterial activity of squalamine has been likened to melittin, a cationic peptide antibiotic.[36] PMB (Figure 4) is a cationic peptide antibiotic that selectively kills Gram-negative bacteria and contains a lipophilic acyl chain and a heptapeptide ring that is responsible for lipopolysaccharide binding.[6b] Derivatives of PMB lacking the acyl chain retain the ability to bind lipopolysaccharides, and as a consequence of this association these truncated versions of PMB disrupt the organization of the outer membrane of Gram-negative bacteria and sensitize them to hydrophobic antibiotics that ineffectively traverse the outer membrane.[38]

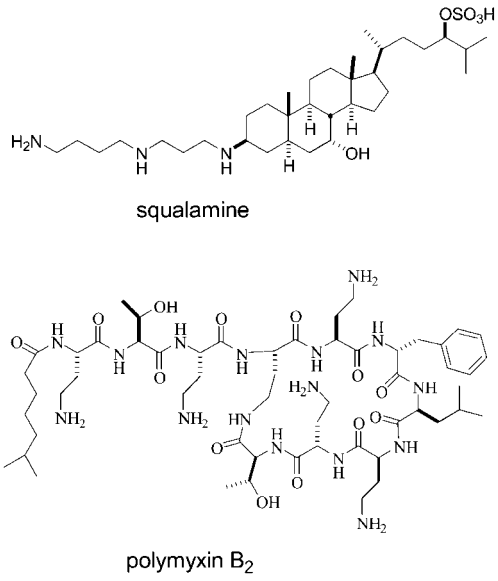


Figure 4. Structures of squalamine and polymyxin B

B. Squalamine Mimics

Squalamine displays broad-spectrum activity against bacteria;[36] however its synthesis is challenging[39] compared to

those of mimics of squalamine developed by other groups.[40] These mimics (e.g., **6** and **7**, Figure 5) were designed by exchanging the positions occupied by the sulfate and polyamine groups, and many examples display antibacterial activities that rival those of squalamine.[41] Interestingly, among the squalamine mimics compounds lacking the sulfate group are more active than those with the group. Compound **7** has good activity against a broad spectrum of bacteria with some MIC values below 1 μg/mL. In contrast, **6** is active only against a few strains, and even against these it is less active than **7**.

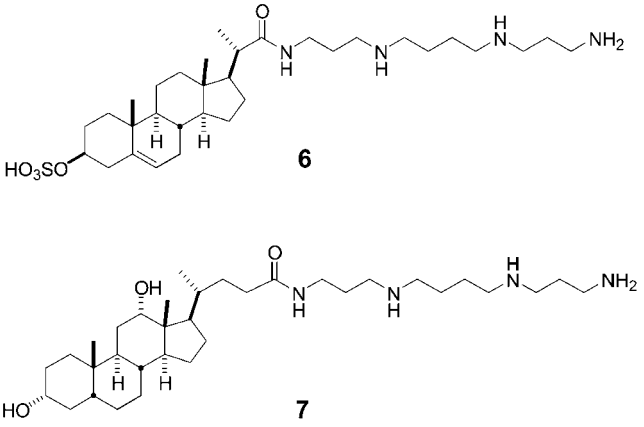
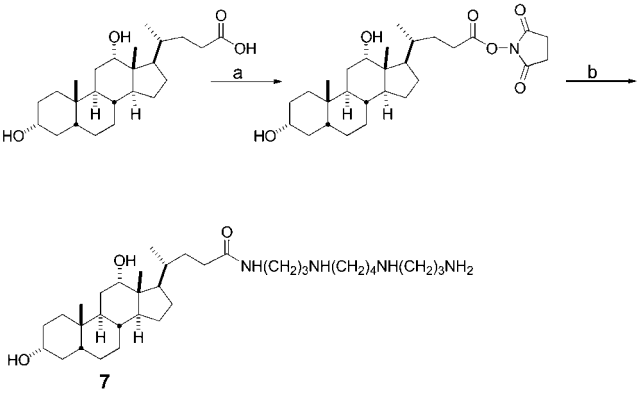


Figure 5. Structures of squalamine mimics **6** and **7**

In addition to its bactericidal activity, **7** permeabilizes the outer membranes of Gram-negative bacteria. In combination with rifampin, **7** gives FIC values as low as 0.05.[41]

Many of the squalamine mimics demonstrate a selectivity for prokaryotic cells, although the most active compounds are, in general, the most hemolytic. For example, **7** has an MHC of 12.5 μg/mL, while that of **6** is over 100 μg/mL. Nevertheless, among this series of compounds, there are examples that display good antibacterial activity against selected strains and relatively high MHC values (≥ 50 μg/mL).



Scheme 1

The squalamine mimics can be prepared in a few steps from commercially available steroid starting materials. For example, the synthesis of **7** (Scheme 1)[41] requires only two steps. Activation of the acid functionality in deoxycholic acid via formation of the *N*-hydroxysuccinimidyl ester is followed by reaction with spermine to give the antibiotic in good overall yield.

C. Polymyxin B Mimics

1. Description and Preparation of Active Compounds

Initial steroid-based mimics of PMB were designed based on simple modeling of PMB,[42] which resulted in the identification of conformations in which the amine groups on the macroring were oriented on one face of the molecule. Fur-

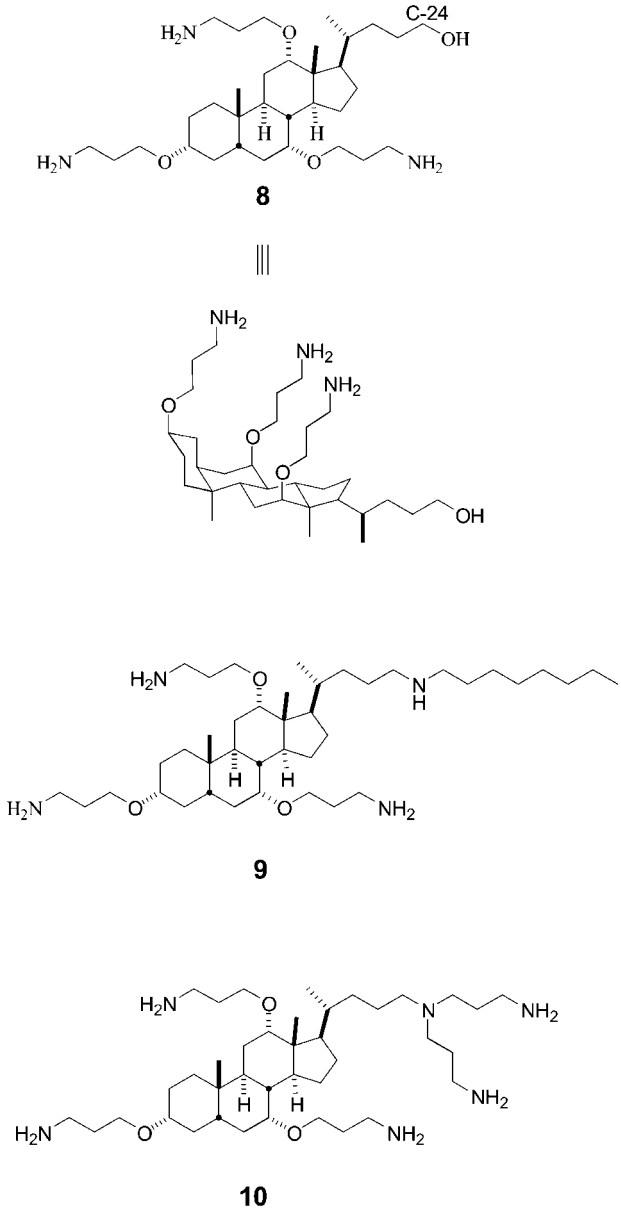


Figure 6. Structures of PMB mimics **8**, **9** and **10**, including a perspective drawing of **8** showing its facial amphiphilicity

ther studies have also suggested that there is a segregation between the amine and hydrophobic groups in PMB.[43] Mimics were prepared by tethering amine groups to a cholic acid scaffolding by ether linkages generating compounds such as **8** and **9** (Figure 6). The stereochemical orientations of the oxygen atoms on the steroid ring system force the tethered amine groups to occupy only one face of the steroid (see the perspective drawing in Figure 6). Consequently, appropriately appended cholic acid derivatives exhibit the facial amphiphilicity common to cationic peptide antibiotics.

PMB mimics derived from cholic acid have potent antibacterial properties[44] rivaling and exceeding those of PMB, with MIC values of less than 1 μg/mL with multiple strains of bacteria. These compounds also display this level of bactericidal activity against multidrug resistant Gram-negative and -positive bacteria.[45]

Cationic steroid mimics of PMB can be prepared in a few steps from cholic acid or from a reduced form of the acid (C-24 alcohol). For example, the preparation of **8** (Scheme 2) involves protection of the primary alcohol of the starting steroid, followed by allylation of the remaining alcohol groups. Hydroboration and oxidation is followed by the conversion of the resulting alcohols into azides. Deprotection of the C-24 alcohol and reduction of the azides yields compound **8** in 48% overall yield.

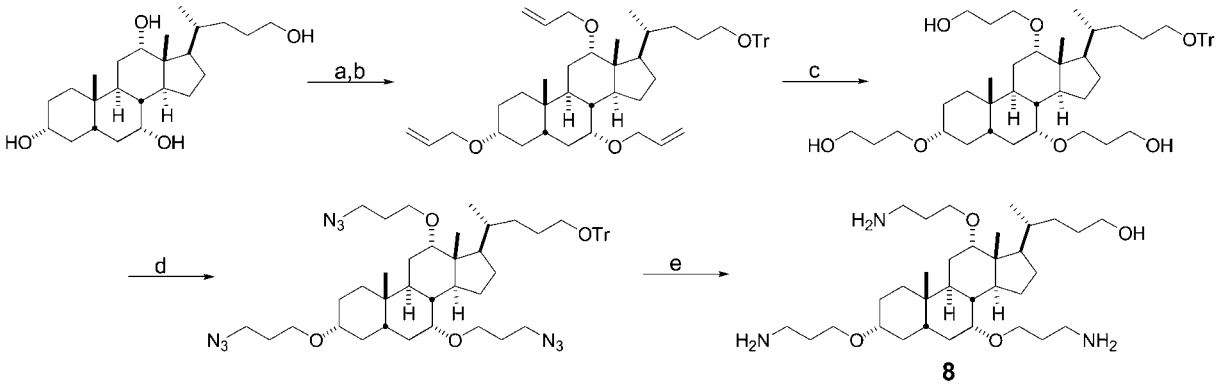
2. Separation of Bactericidal and Outer Membrane Permeabilizing Activities

During the study of a series of related cationic steroid antibiotics, the influence of the group attached to the steroid at C-24 was discovered. Cationic steroids with hydrophobic chains extending from C-24 (e.g., **9**) display potent bactericidal activity against Gram-negative and -positive bacteria, while compounds with shorter chains (e.g., **8**) are only weakly active against Gram-negative bacteria while retaining strong activity against Gram-positive organisms.[46] It was proposed that the reason for the difference in activities against Gram-negative bacteria is that a hydrophobic chain is necessary for the molecule to be able to pass through the outer membranes of these organisms. Similarly, the hydrophobic chain of PMB is required for it to pass through the outer membrane, and this process of traversing the outer membrane is termed "self-promoted transport".[47] Is should be noted that access to the inner membrane is required to cause cell death with Gram-negative bacteria, and Gram-positive organisms do not possess a comparable outer membrane.

Compounds lacking a hydrophobic chain extending from C-24 (such as **8**), while not bactericidal against Gram-negative bacteria, demonstrate the ability to permeabilize effectively the outer membranes of these organisms. This behavior parallels that of the PMB and its derivatives: with a hydrophobic chain, PMB is bactericidal, without a hydrophobic chain, PMB derivatives lose bactericidal activity yet retain the ability to permeabilize the outer membranes of Gram-negative bacteria. The synergism of cationic steroid derivatives with a number of hydrophobic antibiotics is notable. FIC values of **8** and related compounds with erythromycin, novobiocin and rifampin are very low (< 0.10) with some below 0.030. For example, **8** at a concentration of 0.3 μg/mL lowers the MIC of novobiocin from 41 μg/mL to 1 μg/mL against *E. coli*.[45] Direct comparisons with the PMB derivative PMBN[48] showed that the cationic steroids are more effective permeabilizers of the outer membrane.[46]

3. Hemolytic Activity

As is the case with other membrane active compounds, cationic steroid mimics of PMB demonstrate various levels of hemolytic activity.[45] In general, compounds with hydrophobic chains are the most hemolytic. For example, the MHC values of **8** and **9** are 100 and 29 μg/mL, respectively. Due to the charge differences between prokaryotic and eukaryotic cells, cationic compounds preferentially associate with bacteria.[49] To improve cell selectivity, a polyamine was attached at C-24 to yield **10** (Figure 6).[50] This compound does not show hemolytic activity up to a concentration of 200 μg/mL yet retains antibacterial activity (MICs ca. 5 μg/mL with Gram-negative and positive organisms).



Reagents (yields in parentheses): (a) trityl chloride, Et_3N, DMF (70%). (b) allyl bromide, NaH, THF (96%). (c) 9-BBN, THF; H_2O_2, NaOH (80%). (d) MsCl, Et_3N, CH_2Cl_2; NaN_3, DMSO (94%). (e) TsOH, MeOH; $LiAlH_4$, THF (94%).

Scheme 2

This result suggests that simple charge recognition can enhance selectivity for bacterial cells.

4. Comparison of Activities to Those of Cationic Peptide Antibiotics

Although the cholic acid derivatives were designed to mimic the antibacterial activities of PMB, it was noted that the cationic steroids are active against Gram-positive organisms,[45] while PMB is only weakly active against these organisms. This result suggests that there may be a divergence in the mechanism of action of PMB and cationic steroid antibiotics. Jain and co-workers have postulated a mechanism of action for PMB that is distinct from those of other cationic peptide antibiotics,[43a] and the antimicrobial action of the cationic steroids may be more closely related to general mechanisms for cationic peptide antibiotics (e.g., the carpet model).[9]

To correlate the activities of cationic steroid and peptide antibiotics, direct comparisons were performed between steroids **8** and **9** and cationic peptides.[50] Compounds **8** and **9** cause depolarization of bacterial membranes[51] at rates very similar to magainin I, although the steroids cause depolarization at much lower concentrations. Compound **8** stimulates the activation of the same bacterial promoters[50] that the cecropin peptide antibiotics activate,[52] indicating that there are similarities in the way bacteria respond to both types of antibiotics. Finally, electron micrographs of bacteria treated with **8** and **9** show clear indications of membrane disruption, with entire segments of membrane material removed from bacteria.[50] This result is consistent with the carpet model of antibacterial action.[53]

5. Incorporation of Amino Acids onto a Steroid Scaffolding

One means of improving the selectivity of membrane active compounds is through charge recognition; cationic antibiotics preferentially associate with negatively charged bacterial membranes (vide supra). A further method of achieving cell selectivity is to target compounds toward a specific component of a cell wall. For example, the cationic peptide antibiotic nisin displays selectivity for Gram-positive bacteria due to its high affinity for lipid II.[54] Similarly, PMB selectively binds the lipid A portion of lipopolysaccharide[55] and is selective for Gram-negative bacteria. The affinities of nisin and PMB for lipid II and lipid A are presumably due to more than charge recognition: there are probably interactions between uncharged amino acid side chains and the lipid binding partners.

As a potential means of increasing the cell selectivity of cationic steroid antibiotics, amino acids have been attached to a steroid scaffolding. The rationale for using amino acids is that the amine groups will provide the cationic recognition and the side chains may provide additional associative interactions with a specific binding target, thereby increasing the affinity and, potentially, selectivity.

Amino acids can be incorporated onto cholic acid by ester linkages to yield antimicrobial agents such as **11** (Figure 7).[56] However, due to steric constraints imposed by the steroid backbone, only triesters formed from non-α-branched amino acids yield stable compounds. Nevertheless, compounds such as **11** demonstrate that the amine groups of amino acids provide sufficient amphiphilicity to cause disruption of bacterial membranes. Because **11** and related compounds within the series contain both amine and ester functionalities, it is possible to control the stabilities of the ester linkages by changing the pH. Under acidic conditions, the compounds are stable in aqueous solution for weeks; however, under mildly basic conditions the compounds decompose in minutes. Decomposition by ester cleavage yields compounds that are either endogenous or relatively non-toxic. That is, decomposition of **11** yields cholic acid (a common bile acid), choline and γ-aminobutyric acid. Consequently, cationic antibiotics with amino acids attached by ester linkages may be useful in preventing microbial growth in food as, once ingested, the compounds should decompose before they reach the natural flora of the gastrointestinal tract.

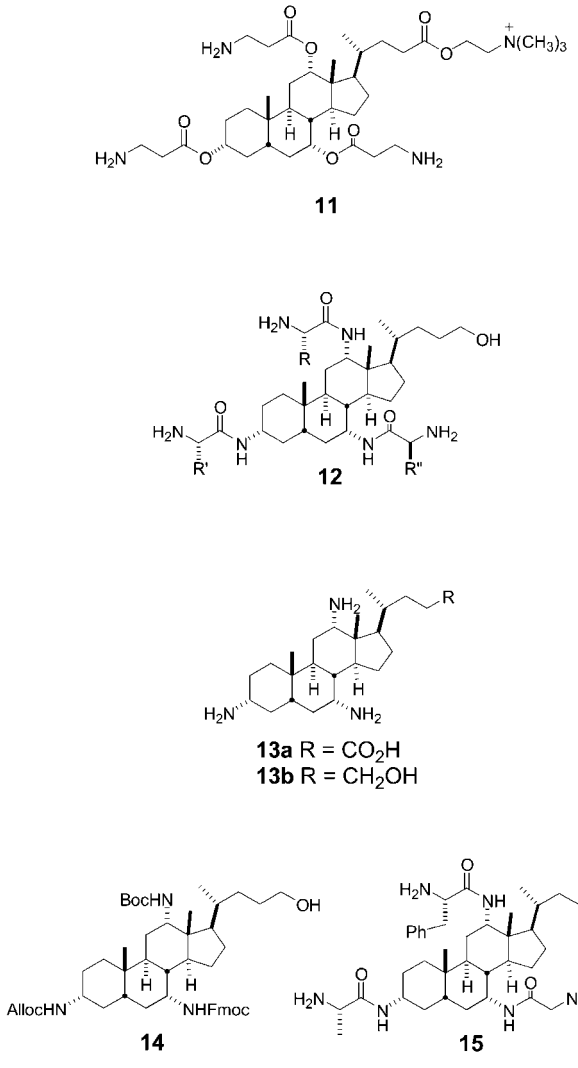


Figure 7. Structures of **11**−**15**

Because incorporation of three α-branched amino acids by ester linkages on a cholic acid scaffolding appears not

to be possible, it was necessary to use a more stable linking functionality. Linking amino acids to cholic acid by amide bonds allows the incorporation of three α-branched amino acids yielding compounds represented by **12** (Figure 7). Formation of the desired triamide requires the use of a triamine analogue of cholic acid (**13** Figure 7) in which the amine groups are all oriented on the α face of the steroid, as are the hydroxyl groups in cholic acid.

Davis and co-workers were the first to publish the synthesis of a triamine analogue of cholic acid.[57] Through incremental incorporation of amine groups at C-3, C-7, and C-12, they were able to prepare **13a** in low overall yield with stereocontrol at each amine-appended stereocenter. Later they refined their synthesis to fewer steps and a greater overall yield.[58] A less elegant method of preparing a similar analogue of cholic acid was described in which a reduced form of cholic acid can be converted into **13b** in only two steps in >30% yield.[59] Attachment of amino acids to **13b** yields antimicrobial compounds with activities similar to their ester counterparts.[60]

6. Development of Combinatorial Methods

A well recognized means of generating large numbers of compounds with potential for desired activities is the use of combinatorial synthetic methods. Steroid scaffoldings have been prepared[61] and used for combinatorial synthesis with some success.[62] Stability considerations of amides vs. esters dictate that an optimal scaffolding for antibiotic development includes three orthogonally protected amine groups on a steroid. Compound **14** (Figure 7) can be prepared in moderate overall yield from cholic acid.[63] The orthogonal protecting groups allow the incremental deprotection and incorporation of amino acids in good yields, giving triamides such as **15**. Compound **14** can also be immobilized on a solid support, and **15** was prepared without the need for purification of intermediates. Scaffolding **14** is well suited for "split-mix" generation of combinatorial libraries of potential antimicrobial steroids. Screening of the libraries for binding to specific targets unique to bacterial membranes or cell walls may provide cationic steroid antibiotics with improved antibacterial activities and cell selectivities.

VII. Conclusion

Considering the fact that cationic, facially amphiphilic antimicrobial agents can be prepared from a number of building blocks suggests that the requirements for disrupting bacterial membranes are relatively straightforward (for a summary of activities, see Table 1). Generating compounds that display high activity against a broad spectrum of bacteria and are selective for bacterial cells is more complicated. As series of this type of antimicrobial agent are prepared and characterized, factors contributing to activity and selectivity are revealed. For example, independent of the building blocks used (i.e., peptides, β-peptides or steroids) it has been demonstrated that a balance of hydrophobicity and cationic character provides a measure of cell selectivity. Also, considering the naturally occurring antibiotics nisin and PMB, and the work reported by Tam and co-workers,[21] an improved selectivity may be achieved by increasing the affinity for unique bacterial components.

While much has been accomplished in the development of new cationic antimicrobial compounds, the field is relatively young and there is much to be improved upon and learned. The fact that membrane active compounds are unlikely to induce the formation of resistant organisms, considered with the emergence of many multidrug resistant bacteria, underscores the importance of continued work in this area,[64] the end goals being a better understanding of how small molecules can selectively permeabilize and disrupt bacterial membranes and clinically useful antimicrobial agents that will improve the human condition.

Table 1. Summary of properties of designed cationic antibiotics

Compound	Description	Antibacterial Properties (MIC Values) Gram-Negative Bacteria	Gram-Positive Bacteria[a]	Hemolytic Activity (MHC)[a]
PGYa	α-helical peptide	2−8 μg/mL	1−8 μg/mL	N. R.
D4E1	α-helical peptide	1−16 μg/mL	1−16 μg/mL	N. R.
1	α-helical peptide	25 μg/mL	N. R.	N. R.
GS14K4	β-sheet peptide	8−16 μg/mL	2−16 μg/mL	200 μg/mL
cTP	β-sheet peptide	≤1 μg/mL	≤1 μg/mL	>200 μg/mL
R5W	β-sheet peptide	<1−10 μg/mL	>60 μg/mL	N. R.
4	helical β-peptide	20 μg/mL	N. R.	>200 μg/mL
5	helical β-peptide	2−6 μg/mL	3−12 μg/mL	>100 μg/mL
6	cationic steroid	3→100 μg/mL	3→100 μg/mL	>100 μg/mL
7	cationic steroid	3−25 μg/mL	<1−6 μg/mL	12.5 μg/mL
8	cationic steroid	21−43 μg/mL	2−4 μg/mL	100 μg/mL
9	cationic steroid	2−3 μg/mL	<1−3	29 μg/mL
10	cationic steroid	6−25 μg/mL	3−5 μg/mL	>200 μg/mL

[a] N. R. = not reported.

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