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Antibacterial Activities of Aminoglycoside Antibiotics-Derived Cationic Amphiphiles. Polyol-Modified Neomycin B-, Kanamycin A-, Amikacin-, and Neamine-Based Amphiphiles with Potent Broad Spectrum Antibacterial Activity

Smritilekha Bera,[†] George G. Zhanel,[‡] and Frank Schweizer*[,†,‡]

[†]*Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada, and* [‡]*Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, R3E 3P4, Canada*

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Cationic amphiphiles containing multiple positively charged amino functions define a structurally diverse class of antibacterials with broad-spectrum activity and different modes of action. Oligocationic amphiphiles have been used as antibiotics to treat infections and as antiseptics and disinfectants for decades with little or no occurrence of resistance. We have prepared a novel class of cationic amphiphiles termed aminoglycoside antibiotics-derived amphiphiles in which the polyol scaffold of the aminoglycosides neomycin B, kanamycin A, amikacin, and neamine has been uniformly decorated with hydrophobic residues in the form of polycarbamates and polyethers. Our results show that the nature of the polyol modification as well as the nature of the aminoglycoside antibiotics has a strong effect on the antibacterial potency. The most potent antibacterials are polyol-modified neomycin B-based amphiphiles containing unsubstituted aromatic rings. These analogues exhibit up to 256-fold enhanced antibacterial activity against resistant strains when compared to neomycin B while retaining most of their activity against neomycin B-susceptible strains.

Introduction

The explosive growth of multidrug-resistant bacteria in hospitals and the community have led to an emerging crisis where an increasing number of antibiotics cease to be of microbiological and clinical usefulness.[1] As a result, there is a pressing need for novel classes of antibacterial agents with new or combined mechanisms of action that are active against multidrug-resistant bacteria and possess reduced likelihood for the development of resistance. Oligocationic antibacterials (OCAs[a]) containing multiple positively charged amino functions or other cationic groups define a structurally diverse class of antibacterials with broad-spectrum activity and different modes of action.[2,31] This class of antibacterial agents can be further subdivided into nonamphiphilic OCAs such as aminoglycosides[3] but also amphiphilic OCAs comprising the naturally occurring cationic antimicrobial peptides,[4] synthetic mimics of antimicrobial peptides (SMAMPs),[5] synthetic oligocationic lipopeptides,[6] oligocationic lipids,[7] and polymers.[8] The cationic charges of the OCA ensure accumulation at polyanionic microbial cell surfaces that contain acidic polymers, such as lipopolysaccharides, and wall-associated teichoic acids in Gram-negative and Gram-positive bacteria, respectively.[4a] Several OCAs including aminoglycoside antibiotics (gentamicin) and antimicrobial peptides (polymyxin B, defensins, gramicidin S variants, and others) transit the outer membrane by interacting at sites at which divalent cations cross-bridge adjacent polyanionic polymers. This causes a destabilization of the outer membrane that is proposed to lead to self-promoted uptake of the OCAs and/or other extracellular molecules.[4a,9] After transit through the outer membrane OCAs contact the anionic surface of the cytoplasmic membrane. Here depending on the structure of the OCA, several scenarios can be envisaged. Amphiphilic OCAs can insert themselves into the cytoplasmic membrane, thereby either disrupting the physical integrity of the bilayer, via membrane thinning, transient poration, and/or disruption of the barrier function, or translocating across the membrane and acting on internal targets.[4a] This mode of action has been shown to limit the risk of cross-resistance,[4,10] and several amphiphilic OCAs including chlorhexidine and polymyxins are in use as antiseptics, disinfectants, and antibiotics for several decades with little or no occurrence of resistance.[11,30] Nonamphiphilic OCAs such as aminoglycoside antibiotics must cross the bacterial membrane in order to bind to intracellular targets such as RNA, DNA, and proteins. In this case coadministration with membrane permeabilizing agents such as ionic lipids can result in synergistic enhancements of the antibacterial action.[12,13] It is generally believed that the selective bacterial cytotoxicity of OCAs is caused by the affinity of the net negative charge found on bacterial cell membranes in contrast to eukaryotic lipid bilayers which are typically made up of zwitterionic phospholipids.[4a]

Aminoglycoside antibiotics constitute a large family of clinically important nonamphiphilic OCAs used in the treatment of bacterial infections.[3] Aminoglycosides effect their antibacterial activity by interfering with ribosomal function (via binding to the A-site region on the 16S subunit of rRNA), which ultimately results in the disruption of protein

*To whom correspondence should be addressed. Phone: + 204-474-7012. Fax: + 204-474-7608. E-mail: schweize@cc.umanitoba.ca.

[a] Abbreviations: ATCC, American Type Culture Collection; AMPs, antimicrobial peptides; Boc, *tert*-butyl carbamate; CAN-ICU, Canadian Intensive Care Unit; CLSI, Clinical Laboratory Standards Institute; MIC, minimal inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; OCAs, oligocationic antibacterials; SMAMPs, synthetic mimics of antimicrobial peptides; TFA, trifluoroacetic acid.

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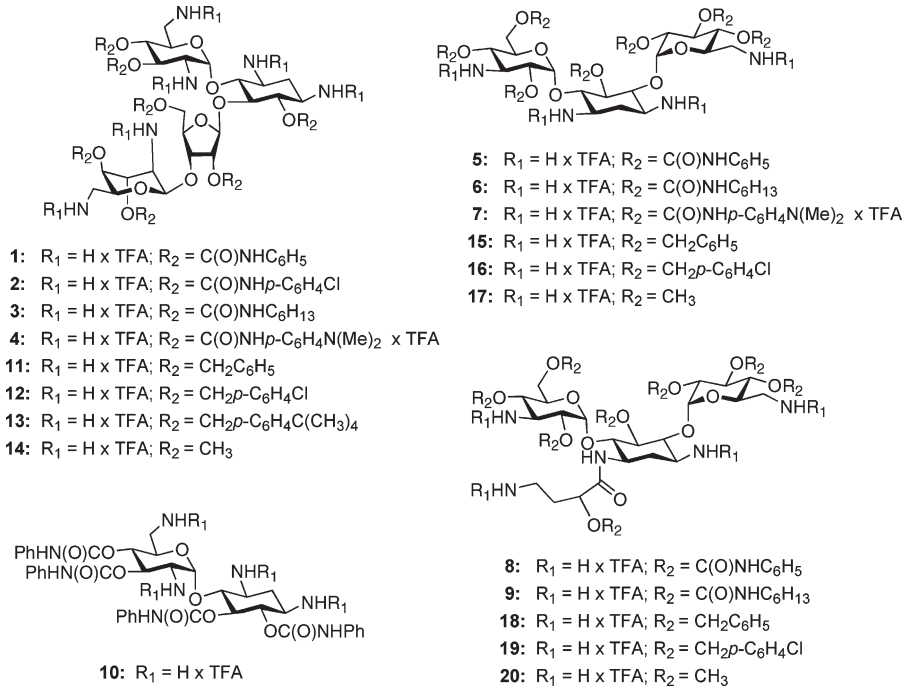


Figure 1. Amphiphilic aminoglycoside antibiotics-derived polycarbamates **1**−**10** and polyethers **11**−**20**.

biosynthesis.[14,15] Although aminoglycoside antibiotics exhibit potent bactericidal activity, their widespread use has been compromised by the worldwide emergence and spread of aminoglycoside-resistant strains[1] and toxicity.[16,17] Several mechanisms cause resistance including decreased uptake into cells, as a result of activation of drug efflux pumps, modified membrane potential, changes in membrane composition, covalent modification of the drug, and others.[18−20] Recently, we and others have shown that chemical modifications at the single primary C5″-hydroxyl group in neomycin B via attachment of hydrophobic substituents including lipid chains, aromatic residues, hydrophobic amino acids, and steroids resulted in the formation of potent antibacterials.[21−23] Analogues of this type display an amphiphilic surface comprising an oligocationic neomycin B-derived headgroup and a segregated lipid tail. Inspired by this unexpected result, we became interested in exploring whether hydrophobic modifications could be extended to other hydroxyl groups in neomycin B. Because of the synthetic difficulties associated with the regioselective differentiation of all remaining six secondary hydroxyl groups in neomycin B, we focused on neomycin B analogues in which all hydroxyl groups of the polyol are derivatized with hydrophobic polycarbamates or polyethers. Compounds of this type are expected to display polyamphiphilic surfaces when compared to the monoamphiphilic surface of cationic head-and-tail amphiphiles such as C5″-modified neomycin B−lipid conjugates. Subsequently, we were interested in extending our study to other members of aminoglycoside antibiotics including kanamycin A, amikacin, and neamine that differ in size, cationic charge, and structure of the polyol scaffold.

Results

In this paper we report on the synthesis and antibacterial activities of neomycin B-, kanamycin A-, amikacin-, and neamine-derived amphiphilic cationic polycarbamates and polyethers (Figure 1). Neomycin B and kanamycin A (Figure 2) were selected because of their commercial availability in multigram quantities and low price. Moreover, the

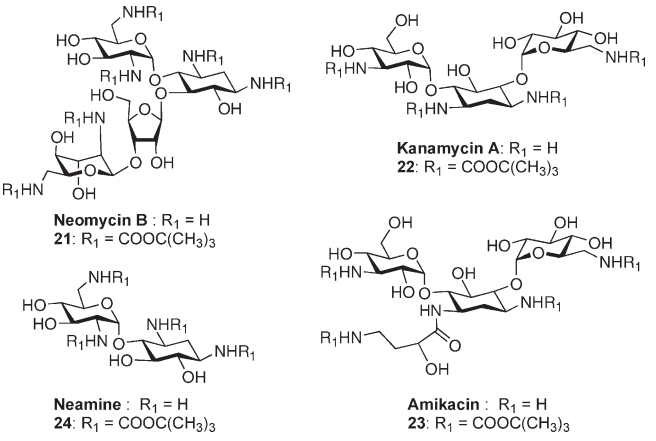


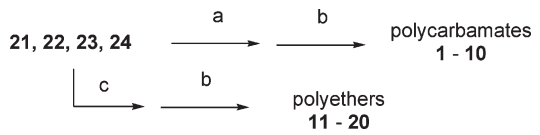
Figure 2. Structures of unprotected and Boc-protected aminoglycoside antibiotics used for conversion into amphiphilic polycarbamates and polyethers.

low antibacterial activity of neomycin B and kanamycin A toward several multidrug resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* was an additional incentive to develop novel analogues with reduced resistance and increased activity. Amikacin was selected because of its potent Gram-positive and Gram-negative activity, while neamine was chosen as a minimal neomycin B mimetic to explore size effects (Figure 2). The amphiphilic neomycin B-derived polycarbamates **1**−**4**, the kanamycin A-derived polycarbamates **5**−**7**, the amikacin-derived polycarbamates **8** and **9**, and the neamine-derived polycarbamate **10** were prepared from the corresponding amino protected *tert*-butyl carbamates (Boc) of the corresponding aminoglycosides **21**−**24**[35−38] (Figure 1) via carbamoylation of the hydroxy groups with various commercially available hydrophobic isocyanates including phenyl isocyanate, 4-chlorophenyl isocyanate, hexyl isocyanate, and 4-*N*,*N*-dimethylphenyl isocyanate in pyridine (Scheme 1).[33] Deblocking of the Boc

group using trifluoroacetic acid afforded the cationic and amphiphilic polycarbamates **1**−**10** in the form of their TFA salts. Analogously, the cationic and amphiphilic polyethers **11**−**20** were prepared form the corresponding Boc-protected aminoglycosides **21**−**23** via O-alkylation with reactive alkyl bromides such as benzyl bromide, 4-chlorobenzyl bromide, 4-*tert*-butylbenzyl bromide, and methyl iodide using barium hydroxide in DMF[34] (Scheme 1) followed by deblocking of the amino protecting groups to produce neomycin B-derived polyethers **11**−**14**, kanamycin A-derived polyethers **15**−**17**, and amikacin-derived polyethers **18**−**20** (Figure 1).

All compounds were tested against American Type Culture Collection (ATCC) reference strains as well as clinical isolates from the Canadian Intensive Care Unit (CAN-ICU) study.[25] Isolates tested included *S. aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33592, *S. epidermidis* ATCC 14990, methicillin-resistant *Staphylococcus epidermidis* (MRSE) (cefazolin-CZ MIC > 32 μg/mL) CAN-ICU 61589, *Enterococcus faecalis* ATCC 29212, *E. faecium* ATCC 27270, *Streptococcus pneumoniae* ATCC 49619, *E. coli* ATCC 25922, *E. coli* (gentamicin-resistant MIC > 32 μg/mL) CAN-ICU 61714, *E. coli* (amikacin MIC 32 μg/mL) CAN-ICU 63074, *Pseudomonas aeruginosa* ATCC 27853 and *P. aeruginosa* (Gent-R MIC > 32 μg/mL) CAN-ICU 62308, *Stenotrophomonas maltophilia* (CAN-ICU 62585), *Acinetobacter baumanni* (CAN-ICU 63169), and *S. pneumoniae* ATCC 13883. Antibacterial activity against Gram-positive and Gram-negative microorganisms was performed via broth macrodilution tests using CLSI methodology.[24] The minimum inhibitory concentrations (MICs) in μg/mL of the aminoglycoside-derived amphiphilic polycarbamates and polyethers were determined using established methods[24] and are shown in Tables 1 and 2, respectively. Our results show that the nature of the cationic scaffold (neomycin B, kanamycin A, amikacin, and neamine) and the nature of the polyol substituent (hydrophobic carbamate or ether) influence the antibacterial activity and strain specific susceptibility. The most potent neomycin B-derived heptaphenyl carbamate **1** exhibits excellent Gram-positive activity against *S. aureus*, MRSA, *S. epidermidis*, and MRSE (MIC < 1 μg/mL). A remarkable 256-fold enhancement of **1** when compared to unmodified neomycin B against MRSA is observed, while at the same time the potent activity against neomycin B-susceptible strains such as *S. aureus*, *S. epidermidis*, MRSE, and *S. pneumoniae* is maintained and also the molecular weight of **1** is more than doubled. Introduction of *p*-chloro and *p*-dimethylamino substituents into the phenyl ring of the polycarbamates results in significant loss of Gram-positive activity as observed for compounds **2**−**4**. Slightly reduced activities are observed for **1** against Gram-negative *E. coli*, while very little improvements are seen against *P. aeruginosa*. A very intriguing result is the potent activity of **1** against multidrug-resistant *S. maltophilia* (MIC = 4 μg/mL) demonstrating an over 128-fold increased susceptibility over neomycin B and a slightly increased activity against *A. baumannii* (MIC = 32 μg/mL).

Scheme 1[a]



[a] Reagents and conditions: (a) R_2NCO, pyridine, DMF; (b) TFA, 0 °C, 3 min; (c) $Ba(OH)_2$, DMF, benzyl bromide or MeI.

Replacement of the neomycin B-based hexacationic scaffold by a tetracationic kanamycin A-based scaffold results in reduced antibacterial activity. Kanamycin A-based amphiphilic heptacarbamates **5**−**7** exhibit significantly reduced Gram-positive activity (up to 32-fold) and little Gram-negative activity (MIC > 64 μg/mL) for most strains (except MRSA for compound **8**) when compared to neomycin B-based carbamates **1**−**4**. Moreover, modifications on the kanamycin A-based tetracationic scaffold influence the antibacterial activity. For instance, amikacin-based tetracationic octaphenyl carbamate **8** and octahexyl carbamate **9** exhibit significantly improved Gram-positive activity (2- to 16-fold) when compared to their respective kanamycin A-based heptacarbamates **5** and **6**. Amikacin-derived tetracationic octacarbamates **8** and **9** are structurally related to kanamycin A-derived tetracationic heptacarbamates **5** and **6** via amidation of N-3 of kanamycin A with a L-hydroxyaminobuteroyl substituent. In addition, tetracationic octahexylcarbamate **9** shows 8- to 32-fold improvements against the Gram-negatives *E. coli* and *S. maltophilia* when compared to **6**, indicating that subtle modifications on the kanamycin A-based tetracationic scaffold can lead to significant improvements in the antibacterial activity. Moreover, reduction in the size of the

Table 1. Minimal Inhibitory Concentrations (MIC) in μg/mL for Various Bacterial Strains against Neomycin B-, Kanamycin A-, Amikacin-, and Neamine-Derived Oligocationic Polycarbamates **1**−**11**

control organism	genta-micin	neomy-cin B	**1**	**2**	**3**	**4**	kanamy-cin A	**5**	**6**	**7**	amika-cin	**8**	**9**	**10**
S. aureus[a]	1	1	1	64	2	16	4	32	32	16	4	8	4	16
MRSA[b]	2	256	1	64	256	16	>512	32	64	32	8	16	4	16
S. epidermidis[c]	0.25	0.25	0.5	16	1	8	2	4	16	16	1	2	4	8
MRSE[d]	32	0.5	0.5	32	2	8	128	8	32	32	2	2	4	8
S. pneumoniae[e]	4	32	16	128	8	32	8	64	128	>256	8	32	32	>256
E. coli[f]	1	4	32	>256	8	128	8	>256	128	256	4	256	4	32
E. coli[g]	128	8	16	>256	8	128	16	>256	64	256	2	256	8	32
E. coli[h]	8	nd[n]	16	256	32	64	32	>256	64	256	32	256	4	32
P. aeruginosa[i]	8	512	256	>256	>256	128	>512	>256	128	>256	4	>256	64	128
P. aeruginosa[j]	128	512	128	256	256	128	>512	>256	32	256	128	256	16	128
S. maltophilia[k]	>512	>512	4	128	>256	256	>512	>256	>256	>256	>512	128	16	64
A. baumannii[l]	128	64	32	>256	>256	>256	32	>256	>256	>256	128	256	>128	256
S. pneumoniae[m]	0.25	1	256	256	4	>256	1	>256	>256	>256	0.5	256	>128	64

[a] ATCC 29213. [b] Methicillin-resistant *S. aureus* ATCC 33592. [c] ATCC 1490. [d] Methicillin-resistant *S. epidermidis* (ATCC 14990). [e] ATCC 49619. [f] ATCC 25922. [g] ATCC 6174 (gentamicin resistant). [h] CAN-ICU 63074. [i] ATCC 27853. [j] CAN-ICU 62308. [k] CAN-ICU 62584. [l] CAN-ICU 63169. [m] ATCC 13883. [n] nd = not determined.

Table 2. Minimal Inhibitory Concentrations (MIC) in μg/mL for Various Bacterial Strains against Neomycin B-, Kanamycin A-, and Amikacin-Derived Oligocationic Polyethers **12**−**21**

control organism	genta-micin	neomy-cin B	**11**	**12**	**13**	**14**	kanamy-cin A	**15**	**16**	**17**	amika-cin	**18**	**19**	**20**
S. aureus[a]	1	1	4	64	128	>256	4	8	32	>256	4	4	128	>256
MRSA[b]	2	256	4	64	128	>256	>512	8	32	>256	8	4	128	>256
S. epidermidis[c]	0.25	0.25	2	32	64	256	2	4	32	256	1	1	128	>256
MRSE[d]	32	0.5	2	64	64	>256	128	8	16	>256	2	1	64	>256
S. pneumoniae[e]	4	32	16	128	256	>256	8	32	64	>256	8	32	128	>256
E. coli[f]	1	4	8	128	128	>256	8	32	128	>256	4	16	>256	>256
E. coli[g]	128	8	16	>128	128	>256	16	32	128	>256	2	16	>256	>256
E. coli[h]	8	nd[n]	4	64	128	>256	32	16	64	>256	32	16	256	>256
P. aeruginosa[i]	8	512	32	>128	256	>256	>512	>64	>128	>256	4	128	>256	>256
P. aeruginosa[j]	128	512	64	>128	128	>256	>512	>64	>128	>256	128	64	>256	>256
S. maltophilia[k]	>512	>512	8	>128	>128	>256	>512	>64	>128	>256	>512	32	>256	>256
A. baumannii[l]	128	64	16	>128	>128	>256	32	>64	>128	>256	128	32	>256	>256
S. pneumoniae[m]	0.25	1	8	128	>128	>256	1	>64	>128	>256	0.5	16	>256	>256

[a]ATCC 29213. [b]Methicillin-resistant *S. aureus* ATCC 33592. [c]ATCC 1490. [d]Methicillin-resistant *S. epidermidis* (ATCC 14990). [e]ATCC 49619. [f]ATCC 25922. [g]ATCC 6174 (gentamicin resistant). [h]CAN-ICU 63074. [i]ATCC 27853. [j]CAN-ICU 62308. [k]CAN-ICU 62584. [l]CAN-ICU 63169. [m]ATCC 13883. [n]nd = not determined.

aminoglycoside antibiotic does not abolish antibacterial activity. For instance, the neamine-based tetracationic tetraphenyl carbamate **10** displays improved antibacterial activity against most Gram-positive strains and all Gram-negative strains when compared to kanamycin A-based tetracationic hexaphenyl carbamate analogue **5**. However, **10** shows significantly reduced Gram-positive activity when compared to related hexacationic heptaphenyl carbamate **1** (16-fold reduction in MIC). This indicates that the neamine portion of neomycin and the lower portion of neomycin are required for optimal antibacterial activity. However, the relative potent antibacterial activity of **10** may indicate that the upper portion of neomycin is more important for antibacterial activity.

A similar trend in the antibacterial activities is observed for the neomycin B-, kanamycin A-, and amikacin-based oligocationic polyethers **11**−**20** (Table 2). A variety of substituents including methyl, benzyl, *p*-chlorobenzyl, and *p*-tertbutyl ethers were evaluated. The most potent cationic polyether analogues in the order of activity are the neomycin B-based hexacationic heptabenzyl ether **11**, the amikacin-based tetracationic octabenzyl ether **18**, and the kanamycin A-based tetracationic heptaphenyl ether **15**. Similar to our previous observation with cationic polycarbamates, the introduction of a chloro or *tert*-butyl substituent into the para-position of the phenyl ring greatly diminishes (16- to 32-fold increase in MIC) antibacterial activity. The most potent cationic polyether analogue **11** exhibits broad-spectrum activity against Gram-positive and most Gram-negative strains tested. Of particular interest are the potent activities of **11** against the emerging multidrug resistant superbugs such as MRSE (MIC = 2 μg/mL), MRSA (MIC = 4 μg/mL) *S. maltophilia* (MIC = 8 μg/mL), *A. baumannii* (MIC = 16 μg/mL), and *P. aeruginosa* (two strains; MIC = 32−64 μg/mL) strains while retaining good activity against nonresistant strains such as *S. epidermidis* (MIC = 2 μg/mL), *S. aureus* (MIC = 4 μg/mL), *E. coli* ATCC25922 (MIC = 8 μg/mL), and *S. pneumoniae* (MIC = 16 μg/mL). Moreover, the requirement for a hydrophobic ether substituent on the oligocationic aminoglycoside scaffold is due to the fact that cationic polymethyl ethers **14**, **17**, and **20** do not exhibit antibacterial activities below an MIC of 256 μg/mL.

Discussion

In this paper we explored the antibacterial activities of 20 aminoglycoside antibiotics-derived cationic amphiphiles. Four different oligocationic aminoglycoside-based polyol scaffolds (neomycin B, kanamycin A, amikacin, and neamine) were selected in order to study how chemical modifications on aminoglycoside-based polyol scaffolds affect the antibacterial activity against Gram-positive, Gram-negative, and multidrug-resistant strains of bacteria. We focused on chemically easily accessible polyol modifications such as single-step conversion into hydrophobic polycarbamates and polyethers. Inspired by the antibacterial activity of other cationic amphiphiles (short cationic antimicrobial peptides, synthetic mimics of antimicrobial peptides, cationic lipids and polymers), we hypothesized that conversion of polar aminoglycoside antibiotics into oligocationic amphiphiles provides a general tool to restore antibacterial activity in this old class of antibiotics. Previous results obtained by us[21] and others[22,23] have shown that C5″-modified neomycin B-based polycationic lipids bearing C_{16}- or C_{20}-lipid chains exhibit strong Gram-positive but reduced Gram-negative activity. The most potent neomycin B−C_{16}-lipid conjugate in the form of the hexacationic TFA salt exhibited antibacterial activities against *S. aureus* (MIC = 8 μg/mL), MRSE (MIC = 2 μg/mL), *E. coli* ATCC 25922 (MIC = 32 μg/mL), and *P. aeruginosa* (MIC = 128 μg/mL).[21] However, exposure of this polycationic lipid to human red blood cells resulted in significant hemolytic activity (50% hemolysis at 200 μg/mL).[23] The high hemolytic activity of this cationic lipid very likely does not permit systemic use, and potential antibacterial applications are limited to use as disinfectants and antiseptics and as antibacterial agent in topical applications. Interestingly, combinational studies using neomycin B−C_{16}-lipid conjugate in combination with amikacin and vancomycin demonstrate synergistic effects against certain Gram-positive and Gram-negative strains indicating a different mode of action.[23] Because of the structural similarity of the neomycin B−C_{16}-lipid conjugate and the here described cationic polycarbamates and polyethers with other oligocationic amphiphiles, a membranolytic mode of action appears to be likely. Previous studies on (oligo)cationic surfactants, peptides, peptidomimetics, lipopeptides, and lipids have shown that cationic amphiphiles target the lipid bilayer of bacteria, resulting in enhanced permeability of the bacterial cell wall,[4,6g,8,11] although other mechanisms such as enzyme inactivation, denaturation of cell proteins, and RNA-binding have been proposed.[26] Evidence for a different antibacterial mode of action of polyol substituted aminoglycoside antibiotics-derived

amphiphiles when compared to their parent aminoglycosides is provided by the inactivity of the cationic polymethyl ethers **14**, **17**, and **20**. The necessity for a hydrophobic polyol substituent is consistent with the amphiphilic nature of other cationic antibacterials with proven membranolytic mode of actions such as cationic antibacterial peptides, lipopeptides, and cationic lipids and surfactants. However, because of the polycationic nature of aminoglycoside antibiotics-derived amphiphiles, RNA-mediated interactions are likely especially after enhanced permeability of the bacterial cell membrane. Synergistic effects of aminoglycoside antibiotics with membrane permeabilizing agents are well documented.[12,13]

Without any doubt a very intriguing finding of our study is the potent antibacterial activity of neomycin B-based hexacationic heptaphenyl carbamate **1** against multidrug-resistant Gram-positive MRSE (MIC = 0.5 μg/mL) and MRSA (MIC = 1 μg/mL). In contrast, the tetracationic kanamycin A (**5**)-, amikacin (**8**)-, and neamine (**10**)-based polyphenyl carbamates exhibit up to 64-fold reduced Gram-positive activity, indicating that the nature of the polyol scaffold, the number of positive charges, and number of hydrophobic groups influence the antibacterial Gram-positive activity. Compound **1** (MW = 2132.6) also exhibits good Gram-negative activity against three strains of *E. coli* (MIC 16−32 μg/mL) especially when considering the high molecular weight. Although the MIC is 2- to 8-fold reduced when compared to neomycin B sulfate (MW = 908.9) susceptible *E. coli*, the activity is comparable to neomycin B when the increase in molecular weight is taken into account. A rather surprising result is the observed potent activity of **1** against neomycin B-resistant Gram-negative *S. maltophilia* (MIC = 4 μg/mL) which is 32- to 64-fold lower when compared to amphiphilic di- and tetracationic lipopeptides.[27] In addition, good activity is also seen against *A. baumannii* (MIC = 32 μg/mL). *A. baumannii* and *S. maltophilia* are opportunistic nosocomial pathogens found mostly in intensive care units.[28] These microorganisms are known to be frequently resistant to many commonly used antimicrobial classes including the β-lactams and fluoroquinolones, and as a result, new chemotherapeutic options are in high demand.[29]

Replacement of the polyphenyl carbamate substituent by polybenzyl ethers also produces highly potent antibacterials. The most potent hexacationic neomycin B-based heptabenzyl ether **11** displays slightly decreased Gram-positive activity (MIC 2−4 μg/mL) against *S. aureus*, MRSA, *S. epidermidis*, and MRSE when compared to its neomycin B-based polycarbamate analogue **1**. However, enhanced Gram-negative activity is observed against *E. coli*, *P. aeruginosa* and *A. baumannii* while a slight 2-fold decrease is observed against *S. maltophilia*. A rather unexpected result is the observed aromatic substitution effect on the antibacterial activity in both the cationic polycarbamates and polyethers. For instance, we consistently observed that incorporation of a para-substituent into the aromatic ring of polyol-substituted phenyl carbamates and benzyl ethers in the neomycin B, kanamycin A, and amikacin scaffolds results in a 4- to 64-fold decrease in antibacterial activity. The cause for this surprising substituent effect is not understood and requires additional work including time-dependent cell death studies, studies on bacterial cell wall permeability, combinational studies with other classes of antibiotics, and extended structure activity relationships in order to provide a deeper understanding of this effect. Nevertheless, the observed substituent effect is rather unexpected and indicates that other factors besides the cationic charges and amphiphilicity contribute to the observed antibacterial activity in this class of compounds. On the basis of the potent antibacterial activity of neomycin B-based amphiphiles **1** and **11** against neomycin B-susceptible and resistant strains relative to other aminoglycoside-based and polyol-modified amphiphiles such as kanamycin A, amikacin, and neamine, it appears that the neomycin B-based scaffold exhibits superior antibacterial properties.

Conclusion

In the present study, we have established that polyol-substituted aminoglycoside antibiotics-derived cationic amphiphiles form a novel class of antibacterial agents with broad spectrum Gram-positive and Gram-negative activity. The amphiphilic nature of the oligocationic aminoglycoside antibiotics-derived amphiphile is crucial for their antibacterial activity, while O-methylation of the polyol scaffold abolishes antibacterial activity. Neomycin B and amikacin-based polyol-modified cationic amphiphiles display consistently higher antibacterial activities when compared to kanamycin A, indicating that the nature of the aminoglycoside antibiotics-derived polyol influences the antibacterial potency. The most potent compounds **1**, **9**, and **11** exhibit significantly improved antibacterial activity against resistant strains when compared to their parent compounds neomycin B and amikacin while retaining their antibacterial activity against most neomycin B- and amikacin-susceptible bacterial strains. Unsubstituted phenyl rings in the form of polyphenyl carbamates or polyphenyl ether consistently exhibit higher antibacterial activities when compared to their para-substituted analogues. The observed aromatic substitution effects suggest that the antibacterial mode of action of polyol-substituted aminoglycoside antibiotics-derived amphiphiles involves other factors besides polycationic charges and amphiphilicity. Other potential modes of action may involve selective binding to lipid A and other lipopolysaccharides,[32] as well as wall-associated teichoic acids, and RNA-mediated interactions.[3]

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Supporting Information Available: Synthetic procedures, spectral and analytical data for new compounds, and antibacterial testing protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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