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Tuning the Antibacterial Activity of Amphiphilic Neamine Derivatives and Comparison to Paromamine Homologues

Louis Zimmermann,[†] Antoine Bussière,[†] Myriam Ouberai,[‡] Isabelle Baussanne,[†] Claude Jolivalt,[§] Marie-Paule Mingeot-Leclercq,*[,‡] and Jean-Luc Décout*[,†]

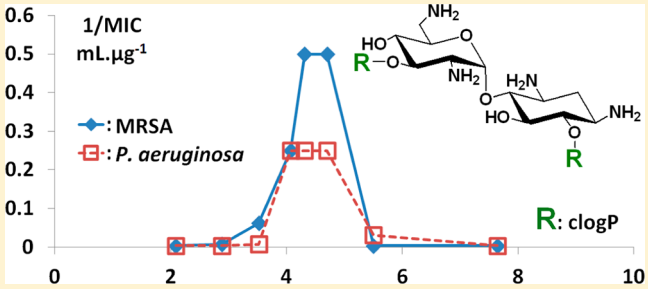
[†]Département de Pharmacochimie Moléculaire, Université de Grenoble I/CNRS, UMR 5063, ICMG FR 2607, 470 rue de la Chimie, BP 53, F-38041 Grenoble, France

[‡]Unité de Pharmacologie Cellulaire et Moléculaire, Louvain Drug Research Institute, Université Catholique de Louvain, Avenue E. Mounier 73, B1.73.05, B-1200 Brussels, Belgium

[§]Ecole Nationale de Chimie de Paris/CNRS, UMR 7573, 11 rue Pierre et Marie Curie, F-75231 Paris cedex 05, France

Supporting Information

ABSTRACT: Aminoglycosides are antibiotic drugs that act through binding to rRNA. In the search for antimicrobial amphiphilic aminoglycosides targeting bacterial membranes, we report here on the discovery of three dialkyl derivatives of the small aminoglycoside neamine active against susceptible and resistant Gram-positive and Gram-negative bacteria. One of these derivatives (R = 2-naphthylpropyl), which has good activity against MRSA and VRSA, showed a low toxicity in eukaryotic cells at 10 μM. The synthesis of amphiphilic paromamine and neamine homologous derivatives pointed out the role of the 6′-amine function of the neamine core in the antibacterial effects. The optimal number of lipophilic substituents to be attached to the neamine core and the corresponding required lipophilicity determined here should permit a more selective targeting of bacterial membranes relative to eukaryotic membranes. This work revealed the existence of windows of lipophilicity necessary for obtaining strong antibacterial effects that should be of interest in the field of antibacterial amphiphilic aminoglycosides.



INTRODUCTION

Aminoglycosides (AG) such as neomycin B (**1**) and paromomycin (**2**) (Figure 1) are potent and broad-spectrum antibiotics that act through binding to the A site of 16S rRNA, causing mRNA decoding errors, mRNA and tRNA translocation blockage, ribosome recycling inhibition, and in fine protein synthesis alteration.[1−9] Decades of widespread clinical use of AG strongly reduced their clinical efficacy through the selection of resistant bacteria. Three modes of bacterial resistance to AG have been identified: (i) reduction in the intracellular concentration of the antibiotics by efflux pump proteins or through reduced membrane permeability, (ii) deactivation by AG-modifying enzymes, and (iii) structural modifications of the 16S rRNA that lead to reduced target affinity.[8−16] The AG-inactivating enzymes that modify the hydroxyl and/or amine functions are classified in three families: AG nucleotidyltransferases (ANTs), AG phosphotransferases (APHs), and AG acetyltransferases (AACs).[8−13] Regarding the modifications of 16S rRNA, the methylation of specific nucleotides within the A site hampers the binding of aminoglycosides and increasingly appears to be a serious threat to the aminoglycoside antibiotics through the action of plasmid-mediated methyltransferases.[14−16] In many cases, AG-resistant bacteria have selected combinations of resistance mechanisms that render them very difficult to eradicate.

X-ray crystallography and NMR spectrometry have revealed that rings I and II of neomycin B (**1**) and paromomycin (**2**) (Figure 1), corresponding to neamine (**3**) and paromamine (**4**), respectively, can be the minimum scaffolds necessary for binding to 16S rRNA.[5,6] Thus, in the search for new antibiotic drugs acting on resistant bacteria and targeting rRNA through chemical modifications of AG, the small AG neamine **3** appears to be an attractive building block.[17−26]

In a recent approach, the conjugation of lipophilic groups to AGs has led to cationic amphiphiles (CA) that should be more difficult to modify by bacterial resistance-causing enzymes. Like the cationic amphiphiles used as antibacterial drugs, amphiphilic AGs could produce a membrane destabilization effect, either in place of or in addition to their inhibiting activity toward protein synthesis, through interactions with negatively charged lipids and/or lipopolysaccharides (LPS) present in the outer membrane of Gram-negative bacteria because the binding of AG to LPS is related to their self-promoted uptake mechanism.[27,28]

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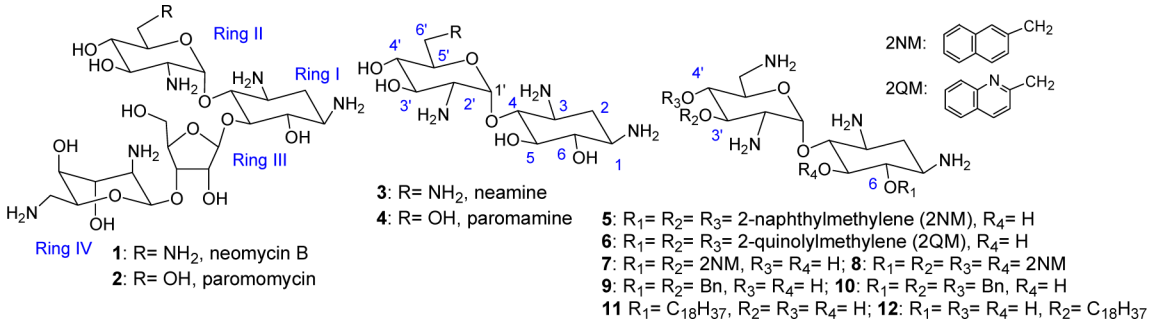

Figure 1. Structure of the natural antibiotic aminoglycosides neomycin B and paromomycin and of neamine and paromamine and their amphiphilic derivatives previously prepared. Compound **12** was synthesized for this study.

Antibacterial cationic amphiphilic drugs acting on the bacterial membranes and/or LPS are receiving a renewal of interest,[29,30] for example, the cyclopeptide colistin (polymyxin E), which is used today as an antipseudomonal compound and acts through binding to LPS of Gram-negative bacteria.[30−34] Several families of cationic amphiphiles (CA) have been identified as potent antimicrobial agents.[35−46] Unlike most mammalian cell membranes, bacterial membranes are rich in negatively charged lipids, such as cardiolipins and phosphatidylglycerol, which could be selectively recognized by CA through ionic interactions and hydrophobic effects.[40−43,47] Little in vitro resistance to these amphiphiles has been observed because of their multiple modes of action and ability to form pores in the bacterial membranes.[48] However, their clinical use is presently severely limited because of their toxicity and protease susceptibility.[41] Their therapeutic efficiency has been improved by reducing their ability to lyse red blood cells as well as by increasing their selectivity toward Gram-positive and Gram-negative bacteria.[49] The nonspecific binding of CA to human serum proteins can also be a limitation in their use, resulting in loss of antibacterial activity in vivo.[50] Despite these drawbacks, targeting bacterial membrane function with cationic derivatives remains an underexploited mechanism for treating persistent infections.[51]

In recent years, several studies demonstrated the potential of exploiting AG for the development of cationic amphiphilic antimicrobial agents by converting part or all of their amine and hydroxyl functions into alkyl- or aryl-amide and -ether groups, respectively. Some of these amphiphilic analogues showed improved activities against several bacterial strains with resistance to the parent AG antibiotics.

The groups of S. Hanessian and E. Westhof first reported the preparation and the study of paromomycin derivatives carrying small lipophilic substituents at the 2″ position of ring III targeting the bacterial A site of rRNA.[52−54] Chang et al. described lipophilic neomycin B amides resulting from the acylation of an amino group introduced at the 5″ position of ring III that are active against resistant strains of *Staphylococcus aureus* and *Escherichia coli*, and they showed that one of the derivatives disrupts the *S. aureus* membrane.[55−57] The groups of S. Bera and F. Schweizer synthesized and evaluated a collection of lipophilic aminoglycosides, such as neomycin, kanamycin A, amikacin, neamine, and, more recently, tobramycin derivatives, which were found to be active against susceptible and resistant Gram-positive bacteria and less active against Gram-negative bacteria.[58−63] Amphiphilic 6″-thioether tobramycin analogues that are less prone to deactivation by aminoglycoside-modifying enzymes than tobramycin and that have potent antibacterial activity against tobramycin-resistant bacteria have also been described by the groups of Garneau-Tsodikova and Fridman.[64,65] These compounds were shown to target the bacterial membrane rather than the ribosome, and one of them, active against several of the tobramycin-resistant bacterial strains, caused little to no measurable hemolysis at the tested concentrations.

In our approach in this field,[66,67] we assumed that the presence of a large number of amine functions in AG derivatives such as neomycin (**1**), which carries six amine functions, can be a source of toxicity[68−70] through nonspecific binding to other targets. Neamine **3**, which carries four amine functions, is less toxic than neomycin, and, as already mentioned, the neamine core corresponds to the minimum scaffold necessary for binding to 16S rRNA.[5,6] Therefore, for obtaining amphiphilic AG targeting rRNA, we have modified selectively the small aminoglycoside neamine **3** at one or more of the hydroxyl functions to keep the four amine functions potentially protonated at physiological pH unchanged, at least partially, with regard to their major role in the binding to rRNA[5,6] and potentially to bacterial membranes. This approach also benefited from our work on neamine in the search for anti-HIV[71,72] and gene-delivery vectors.[73]

In the search for antimicrobial agents, we have synthesized *O*-mono- and *O*-polyalkylated neamine derivatives and have identified two antibacterial derivatives. One of them, amphiphilic 3′,4′,6-tri2-naphthylmethylene (3′,4′,6-tri2NM) neamine derivative **5** (Figure 1), has shown a unique strong antibacterial activity against both susceptible and resistant Gram-positive and Gram-negative bacteria such as resistant MRSA, VRSA, *Pseudomonas aeruginosa*, and *E. coli* strains.[66] For the first time for an amphiphilic aminoglycoside, we have shown that compound **5** is unable to bind to 16S rRNA in vitro[66] and is unable to inhibit *P. aeruginosa* protein synthesis.[67] We also demonstrated the binding of **5** to LPS as well as its ability to induce *P. aeruginosa* membrane depolarization.[67] Both effects are probably related to its antibacterial effects. These results suggested a mechanism for the antibacterial activity that is related to the amphiphilic character, which allows binding to the bacterial membranes, leading to their destabilization. The replacement of the 2NM groups in **5** by 2-quinolylmethylene (2QM) groups to lead to compound **6**, which corresponds to the replacement of only three carbon atoms by three nitrogen atoms, has led to the loss of activity, suggesting a crucial role in the activity of the lipophilic substituents introduced.[66] Unfortunately, compound **5** appeared to be cytotoxic in contrast to the previously reported less lipophilic dialkylated 3′,6-di2NM neamine derivative **7** active against susceptible and resistant *S. aureus* strains but not against Gram-negative bacteria.[66,67] Thus, a decrease in the number of lipophilic groups and/or in the corresponding lipophilicity of the amphiphilic neamine deriva-

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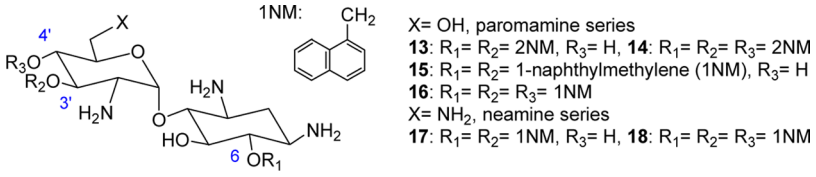

Figure 2. Structure of the naphthylmethylene paromamine (**13**–**16**) and neamine (**17** and **18**) derivatives prepared.

Table 1. clogP Values Calculated Using MarvinSketch Software 5.11.4 for Amphiphilic Neamine Derivatives in Comparison to Reference Compounds, and for the Substituent Attached to the Neamine Core (clogP of the Corresponding Alkane)

Antibacterials								Substituents Attached to the Neamine Core	
Reference antibacterials and 2NM Neamine selected as comparators		Dialkyl Neamine Derivatives		Trialkyl Neamine Derivatives		OcD Neamine Derivatives			
Compounds	clogP	Compounds	clogP	Compounds	clogP		clogP	Substituents	clogP
Colistin	-29.7	DiBu (**37**)	-15.8	Tri2QM (**6**)[66]	-11.6	DiocD (**40**)	-4.7	Bu	2.09
Neomycin	-29.9	DiBn (**9**)[66]	-14.7	TriBu (**43**)	-14.0	6-mono-ocD (**11**)[80]	-12.1	Bn	2.51
Neamine (**3**)	-19.4	DiHx (**38**)[74]	-14.2	TriBn (**10**)[66]	-12.3	3'-mono-ocD (12)	-12.1	Hx	2.88
Paromamine (**4**)	-15.9	DiNn (**39**)	-11.9	TriHx (**44**)[74]	-11.7			NM	3.52
Tri2NM Nea (**5**)[66]	-9.3	Di2NP (**34**)	-11.4	Tri2NP (**41**)	-7.4			Nn	4.07
Di2NM Nea (**7**)[66]	-12.7	Di2NB (**35**)	-10.6	Tri2NB (**42**)	-6.2			NP	4.31
Tetra2NM (**8**)[66]	-6.0	Di2NH (**36**)	-9.0					NB	4.70
								NH	5.50
								ocD	7.64

tives prepared could reduce the affinity for eukaryotic cell membranes and lead to less cytotoxic antibacterial agents.

Herein, we report on the tuning of the antibacterial activity of amphiphilic neamine derivatives through variations in their lipophilicity. We assumed that a critical lipophilicity is required for obtaining amphiphilic neamine derivatives strongly active against Gram-negative bacteria by targeting LPS. The lipophilicity of the active compounds should be lower than the previously found inactive tetra2NM derivative **8** and higher than the lipophilicity of 3′,6-di2NM **7**, which is only active against Gram-positive bacteria.[66] To reduce the lipophilic surfaces in the active compounds, we shifted the structure of the antibacterial derivatives from 3′,4′,6-trialkylated derivatives to 3′,6-dialkylated neamine derivatives and identified three dialkyl derivatives that are more active than the previously described compound **5** against susceptible and resistance strains of both Gram-positive and/or Gram-negative bacteria and, for one of them, strongly less cytotoxic than **5**. The determination of the critical clogP values necessary for targeting both Gram-positive and Gram-negative bacteria strains is here described in relationship with the first measurements of the cytotoxicity.

To investigate the role of the number of amine functions in the antibacterial activity of small amphiphilic AG, we synthesized naphthylmethylene (NM) paromamine derivatives that carry one less amine function than the corresponding NM neamine derivatives because of the replacement of the 6′-amine function of neamine ring II by a hydroxyl group in paromamine **4** (Figure 1). Here, we also show the key role of the 6′-amine function of the neamine core in the antibacterial activity.

SYNTHESIS

To compare the antibacterial activities of amphiphilic neamine derivatives to those of the corresponding paromamine derivatives, the 3′,6-di2NM (**13**) and 3′,4′,6-tri2NM (**14**) paromamine derivatives (Figure 2) have been prepared through (i) protection of the amine and the 5′-hydroxyl functions of paromamine by tetratritylation, (ii) alkylation, and (iii) detritylation in the presence of TFA.[74] For a concomitant evaluation of the role in the antibacterial activity of the attachment position of the naphthyl ring to the methylene group, the 3′,6-di-(1-naphthyl)methylene (1NM) (**15**) and 3′,4′,6-di1NM (**16**) paromamine derivatives as well as the corresponding neamine derivatives, **17** and **18**, were prepared according to the methods previously described (Figure 2).[74−76]

To tune the antibacterial activity of the neamine derivatives through the assumption of the key role of the lipophilicity in the antibacterial activity, we synthesized new neamine derivatives designed from clogP values calculated using the MarvinSketch software 5.11.4 (Table 1). The lipophilicity could be also characterized by calculated clogD values taking into account the theorical deprotonation of some amine functions at pH 7.4 (through theorical calculation of pK_a values). We chose to characterize the lipophilicities through clogP values because (i) clogD values at pH 7.4 calculated using the MarvinSketch software for 3′,6-dialkyl or 3′,4′,6-trialkylated derivatives are simply zero shifted from the clogP values and (ii) the theoretical calculations of clogD at pH 7.4 are very approximative because of the presence of four amine functions with pK_a values close to 7.[77−79] For example, the pK_a values of neamine determined at 25 °C by ^{1}H NMR titration experiments are pK_{a1} 6.44 ± 0.13 for the *N*3 of ring I, pK_{a2} 7.23 ± 0.09 for the *N*2′ of ring II, pK_{a3} 7.77 ± 0.19 for the *N*1 of ring I, and pK_{a4} 8.08 ± 0.15 for the *N*6′ of ring II.[79]

Therefore, the clogP values calculated for the tetraprotonated compounds were used for the comparison of the global lipophilicity of the synthesized compounds (Table 1) because of the lack of information about the protonation degree of the active form. In Table 1, the lipophilicities of the previously synthesized tri2QM (**6**),[66] 3′,6-dibenzyl (diBn)[66] (**9**), 3′,4′,6-triBn[66] (**10**), diHx (**38**),[74] triHx (**44**),[74] and 6-mono-octadecyl (ocD) (**11**)[80] derivatives (Figure 1) that were used in this study are also mentioned.

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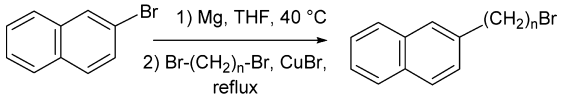
In regard to the loss of activity observed previously resulting from the replacement of the 2NM groups by 2-quinolylmethylene groups (2QM) (compound **5** versus **6**), we limited the modifications made to the substitution of the NM groups for lipophilic substituents that do not incorporate a heteroatom.

In a first approach, the naphthyl ring was progressively moved away from the oxygen atoms of the neamine core carrying the lipophilic substituents for obtaining the 3′,6-di- or the 3′,4′,6-tri-2-naphthylalkyl neamine derivatives (Schemes 1 and 2).

Scheme 1. Synthesis of the 2-(ω-Bromoalkyl)naphthalene Reagents Used for the Preparation of the Corresponding *O*-Alkyl Neamine Derivatives



Through the preparation of 3-(2-naphthyl)propyl (2NP), 4-(2-naphthyl)butyl (2NB), and 6-(2-naphthyl)hexyl (2NH) derivatives, the antibacterial activities of the corresponding 3′,6-di and 3′,4′,6-trialkylated neamine derivatives having similar or different lipophilicities could be compared (Table 1).

To synthesize such derivatives, the corresponding 2-(ω-bromoalkyl)naphthalene reagents, **19**, **20**, and **21**, were prepared from 2-bromonaphthalene through Grignard reactions (Scheme 1). In the synthesis of α,ω-bis(vinylaryl)alkanes using coupling reactions of Grignard reagents with α,ω-dibromoalkanes in the presence of copper(I) bromide and HMPA, compound **19** has been previously obtained in 53% yield.[81] We used this procedure and replaced the toxic solvent HMPA with THF, and we obtained **19**, **20**, and **21** in 33, 23, and 18% yields, respectively. The formation of 2,2′-binaphthyl and the difficult removal of 1,ω-dibromoalkane, especially 1,6-dibromohexane that was used in excess, made compounds **19**, **20**, and **21** difficult to purify. Compound **19** has been also synthesized through the preparation of 2-(3′-hydroxypropyl)naphthalene from 2-methylnaphtalene,[82] and compound **20** has been obtained from 2-bromonaphthalene and *n*-butyllithium (44%).[83]

The 3′,6-di2NP and 3′,4′,6-tri2NP (**34** and **41**, respectively), 3′,6-di2NB and 3′,4′,6-tri2NB (**35** and **42**, respectively), and 3′,6-di2NH (**36**) neamine derivatives were then prepared in two steps: (i) alkylation of tetra-*N*-tritylneamine **22**[75] with reagents **19**, **20**, and **21**, respectively, for obtaining the corresponding tetratritylated derivatives **23**−**25**, **30**, and **31** and (ii) removal of the trityl protecting groups in TFA/anisole (Scheme 2). Two methods of alkylation were used, leading to the similar results (i) under phase-transfer conditions[74] (toluene, 30% aqueous NaOH, TBAI, 50 °C) and (ii) with NaH in DMF.[75,76] Both methods of alkylation led, as previously, to mixtures containing mainly the tritylated 3′,6-di- and 3′,4′,6-trialkyl derivatives, which were separated. The yields appeared to be low because of the successive chromatographies necessary for removing the 3′,4′-dialkylated isomers formed as minor products. We did not isolate the 3′,4′,6-triNH derivative because of its expected high lipophilicity and insolubility in water.

We also synthesized neamine derivatives bearing linear alkyl chains to cover a large range of lipophilicity (Table 1). The 3′,6-dibutyl (Bu, **37**) and 3′,4′,6-triBu (**43**), 3′,6-dihexyl (Hx, **38**),[74] 3′,4′,6-triHx (**44**),[74] and 3′,6-dinonyl (Nn, **39**) derivatives as well as the more lipophilic 3′,6-dioctadecyl (ocD) aminoglycoside **40** were prepared through the preparation of the corresponding tetratritylated derivatives **26**−**29**, **32**, and **33**, respectively (Scheme 2). The 3′,4′,6-triNn neamine derivative was not prepared because of its high lipophilicity and its expected insolubility in water. The formation of the 3′,4′-dialkylated neamine isomers during the synthesis also made the purification of the tritylated compounds, which required further chromatographies on silica gel, difficult. The 3′-mono-ocD derivative **12** was prepared as a reference compound similar to the previously synthesized 6-mono-ocD **11**[80] (Figure 2).

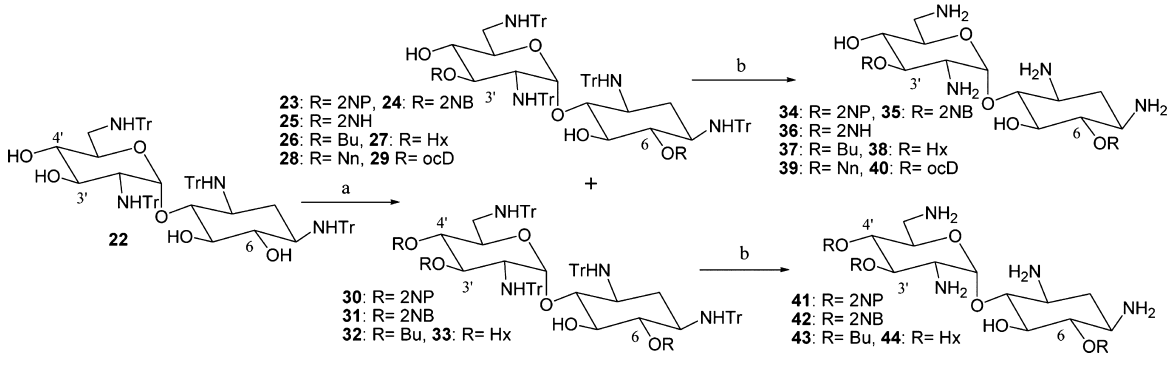
ANTIBACTERIAL ACTIVITY

The synthesized derivatives were evaluated against a large panel of *S. aureus* bacteria, including susceptible and resistant strains surexpressing resistance pumps (NorA or MsrA) and inactivating enzymes like APH2″-AAC6′, APH3′, and ANT4′ as well as against MRSA and VRSA strains, respectively (Table 2). Their effects against susceptible and resistant strains of Gram-negative bacteria *Acinetobacter lwoffi*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *E. coli* surexpressing aminoglycoside-modifying enzymes, efflux pumps, or r-methylase were also determined (Table 3).

Comparison between Paromamine and Neamine Derivatives and between 1NM and 2NM Derivatives. First, the antibacterial activities of the paromamine (Par) and the corresponding neamine (Nea) derivatives bearing two or three 1NM and 2NM groups (Figure 2) were compared against susceptible and resistant *S. aureus* strains (Table 2) and Gram-negative bacteria (Table 3).

Against *S. aureus* strains, all synthesized derivatives showed MIC values lower than or equal to 32 μg/mL except for the

Scheme 2. Preparation of the 3′,6-Di- and 3′,4′,6-Tri-alkylated Neamine Derivatives[a]



[a](a) Method 1:[72] 30% aq NaOH/toluene, TBAI, RX, 50 °C, 24 h. Method 2:[73] NaH, DMF, RX, rt, 2 h; (b) TFA/CH_2Cl_2, anisole, rt.

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Table 2. Minimum Inhibitory Concentrations (MIC) of the Naphthylmethylene (NM), Neamine (Nea), and Paromamine (Par) Derivatives Synthesized as Well as Some Representative AG against Susceptible and Resistant *Staphylococcus aureus* Strains[a]

	MIC (μg/mL)							
aminoglycosides	ATCC 25923	1199B pump NorA	pump MsrA	enzyme APH2″-AAC6′	enzyme APH3′	enzyme ANT4′	ATCC 33592 HA-MRSA	VRSA-VRS-2
gentamicin	0.5	ND	ND	ND	ND	ND	1−2	32
neomycin B **1**	2	1	2	1	>128	32	>128	128
neamine **3**	32	32	16	16	>128	>128	>128	>128
paromamine **4**	32−64	>128	>128	>128	>128	>128	>128	>128
3′,6-di2NM Nea **7**	8	8−16	8−16	8−32	4−8	8−16	16	16
3′,6-di2NM Par **13**	8	32	32	32	16	16	16	16
3′,6-di1NM Nea **17**	8	16	16	32	8	16	8	8
3′,6-di1NM Par **15**	32	64	64	64	32	32	32	ND
3′,4′,6-tri2NM Nea **5**	4	4	4	4	2	4	2	4
3′,4′,6-tri2NM Par **14**	2	16	32	16	4	8	64	128
3′,4′,6-tri1NM Nea **18**	1	4	2	4	2	4	4	16
3′,4′,6-tri1NM Par **16**	2	8	8	8	4	16	32	32

[a]Average of at least three determinations. ND, not determined.

Table 3. Minimum Inhibitory Concentrations (MIC) of the Naphthylmethylene (NM), Neamine (Nea), and Paromamine (Par) Derivatives Synthesized as well as Some Representative AG against Selected Bacterial Gram-Negative Susceptible and Resistant Strains[a]

	MIC (μg/mL)								
	A. lwoffi		*P. aeruginosa*			*K. pneumoniae*	*E. coli*		
aminoglycosides	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
gentamicin	0.5	4−8	1	>128	4	8	0.5	1	64
neomycin B **1**	0.5	>128	64	128	32	16−32	2	4	1
neamine **3**	2	>128	>128	>128	>128	32−64	32	>128	32
paromamine **4**	ND	ND	>128	>128	>128	ND	>128	>128	>128
3′,6-di2NM Nea **7**	64	>128	128	128	>128	128− >128	64	64	64
3′,6-di2NM Par **13**	64	>128	128	64−128	128	>128	128	64	64
3′,6-di1NM Nea **17**	16	>128	32−64	64−128	>128	>128	32	16	32
3′,6-di1NM Par **15**	128	>128	64	128	>128	128	128	64	128
3′,4′,6-tri2NM Nea **5**	4	32	8	8	4	16	16	4	4
3′,4′,6-tri2NM Par **14**	1	>128	32	32	32	32	64	16	32
3′,4′,6-tri1NM Nea **18**	2	128	8	8	8	128	8	2	16
3′,4′,6-tri1NM Par **16**	1−2	128	32	32	32	128	16	16	16

[a]Average of at least three determinations. ND, not determined. [b]ATCC 17925. [c]AI.88-483 APH3′-VIA. [d]ATCC 27853. [e]Psa.F03 AAC6′-IIA. [f]PA22 (PT629) surexp MexXY. [g]ATCC 700603. [h]ATCC 25922. [i]PAZ505H8101 AAC6′-IB. [j]L58058.1 ANT2″-IA.

3′,4′,6-tri2NM (**14**) and 3′,6-di1NM (**15**) paromamine derivatives (Table 2). The activities of the triNM compounds (**5** and **18** in the neamine series and **14** and **16** in the paromamine series) appeared to be higher than the ones of the diNM derivatives (**7** and **17** in the neamine family and **13** and **15** in the paromamine family), as was previously observed with the 3′,4′,6-tri2NM (**5**) and 3′,6-di- (**7**) neamine derivatives.[66] Slightly lower MIC values against most of strains were mainly obtained with the neamine derivatives in comparison to the paromamine compounds. The difference was higher for the triNM derivatives against MRSA and VRSA, with the neamine derivatives showing much lower MIC values (2−16 μg/mL) than the paromamine derivatives (32−128 μg/mL).

Against Gram-negative bacteria (Table 3), as was previously observed with the 3′,6-di2NM derivative **7**, the diNM compounds were inactive except for the weak effects observed with the 3′,6-di1NM neamine derivative **17** against susceptible *A. lwoffi* ATCC 17925 and *E. coli* strains. The triNM derivatives (**5** and **18** in the neamine series and **14** and **16** in the paromamine series) appeared to be much more active than the diNM derivatives, especially the neamine derivatives. The tri2NM neamine derivative **5** showed the lowest MIC values (≤32 μg/mL) against all Gram-negative strains with the exception of the susceptible *A. lwoffi* strain, against which the most active derivative was the 2NM paromamine derivative **14**. However, against this strain, the four triNM derivatives led to low MIC values (1−4 μg/mL). Against the resistant *A. lwoffi* strain, only **5** was active (MIC 32 μg/mL). A lack of activity of the tri1NM paromamine (**16**) and neamine (**18**) derivatives can also be observed against the susceptible *K. pneumonia* strain (MIC 128 μg/mL).

Against most of Gram-positive and Gram-negative strains used, the 1NM and 2NM isomers showed similar effects in the neamine series as well as in the paromamine series. However, against susceptible *K. pneumonia*, 2NM derivatives **5** and **14** both gave MIC values much lower than those of 1NM derivatives **16** and **18** (16, 32 and 128, 128 μg/mL, respectively).

The lower MIC values obtained with the neamine derivatives in comparison to the corresponding paromamine derivatives point out the role of the 6′-amino group in the antibacterial activity. The change in the attachment position of the methylene group on the naphthyl ring appeared to not significantly affect

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Table 4. Minimum Inhibitory Concentrations (MIC) against Susceptible and Resistant *Staphylococcus aureus* Strains Measured for the Alkyl Neamine Derivatives as well as for Some Representative AG[a]

	MIC (μg/mL)							
aminoglycosides	ATCC 25923	1199B pump NorA	pump MsrA	enzyme APH2″-AAC6′	enzyme APH3′	enzyme ANT4′	ATCC 33592 HA-MRSA	VRSA-VRS-2
gentamicin	0.5	ND	ND	ND	ND	ND	1−2	32
neomycin B **1**	2	1	2	1	>128	32	>128	128
neamine **3**	32	32	16	16	>128	>128	>128	128
3′,6-di2NM **7**	8	8 (16)	8 (16)	8 (32)	4 (8)	8 (16)	16	16
3′,6-di2NP **34**	2	2	0.5	2	1	1	2	1
3′,6-di2NB **35**	2	2−4	0.25	2−4	1	1	2	2
3′,6-di2NH **36**	64	>128	>128	128	64	>128	>128	>128
3′,6-diBu **37**	>128	>128	>128	128	>128	>128	>128	>128
3′,6-diBn **9**	>128	>128	>128	>128	>128	>128	ND	ND
3′,6-diHx **38**	128	128	128	>128	>128	>128	128	128
3′,6-diNn **39**	2	8	4−8	4−8	2−4	4	4	2
3′,6-di-ocD **40**	>128	>128	>128	256	128	256	>128	>128
3′,4′,6-tri2NM **5**	4	4	4	4	2	4	2	4
3′,4′,6-tri2QM **6**	128	>128	>128	128	64	>128	64	64
3′,4′,6-tri2NP **41**	>128	>128	32	64	32	128	128	>128
3′,4′,6-tri2NB **42**	>128	>128	128	128	128	128	>128	>128
3′,4′,6-triBu **43**	>128	>128	>128	>128	>128	>128	>128	>128
3′,4′,6-triBn **10**	>128	>128	>128	>128	>128	>128	>128	64
3′,4′,6-triHx **44**	4	4	8	8	4	8	4	4
6-mono-ocD **11**	>128	>256	>256	>256	>256	>256	>128	ND
3′-mono-ocD **12**	>128	>128	>128	>128	>128	>128	ND	ND

[a]Average of at least three determinations. ND, not determined.

Table 5. Minimum Inhibitory Concentrations (MIC) of the Alkyl Neamine Derivatives Synthesized as well as Some Representative AG against Selected Bacterial Gram-Negative Susceptible and Resistant Strains[a]

	MIC (μg/mL)								
	A. lwoffi		*P. aeruginosa*			*K. pneumoniae*	*E. coli*		
aminoglycosides	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
gentamicin	0.5	4−8	1	>128	4	8	<0.5−1	1	64−128
amikacin	0.5	>128	2−4	4	8−16	0.5	4	64	2
tobramycin	0.5	1	0.5	128	1	4	0.5	32	64
neomycin B **1**	0.5	>128	64	128	32	16−32	2	4	1
neamine **3**	2	>128	>128	>128	>128	32−64	32	>128	32
3′,6-di2NM **7**	64	>128	128	128	>128	128- >128	64	64	64
3′,6-di2NP **34**	ND	ND	4	16	16−32	ND	16	8	16
3′,6-di2NB **35**	4	64	4	8	8	32	8	4	8
3′,6-di2NH **36**	ND	ND	32	64	64	ND	128	64	128
3′,6-diBu **37**	64	>128	>128	>128	>128	>128	>128	>128	>128
3′,6-diHx **38**	>128	>128	>128	>128	>128	>128	128	128	128
3′,6-diNn **39**	ND	ND	4	4	4	ND	4−8	2−4	4
3′,6-di-ocD **40**	ND	ND	>128	>128	>128	ND	>128	>128	>128
3′,4′,6-tri2NM **5**	4	32	8	8	4	16	16	4	4
3′,4′,6-tri2QM **6**	128	>128	>128	>128	>128	>128	>128	>128	>128
3′,4′,6-tri2NP **41**	ND	ND	128	64	128	ND	128	32	>128
3′,4′,6-tri2NB **42**	ND	ND	128	128	128	ND	128	128	>128
3′,4′,6-triBu **43**	>128	>128	>128	>128	>128	>128	>128	>128	>128
3′,4′,6-triBn **10**	>128	>128	>128	>128	>128	>128	>128	>128	>128
3′,4′,6-triHx **44**	4	64	8	8	8	8−16	4	4	4

[a]Average of at least three determinations. ND, not determined. [b]ATCC 17925. [c]AI.88−483 APH3′-VIA. [d]ATCC 27853. [e]Psa.F03 AAC6′-IIA. [f]PA22 (PT629) surexp MexXY. [g]ATCC 700603. [h]ATCC 25922. [i]PAZ505H8101 AAC6′-IB. [j]LS8058.1 ANT2″-IA.

the antibacterial activities and thus we continued our approach with neamine derivatives carrying 2-naphthylalkyl substituents.

Antibacterial Activities of the 3′,6-Dialkyl and 3′,4′,6-Trialkyl Neamine Derivatives. With the 3′,4′,6-tri2NM neamine derivative **5** being the best antibacterial derivative previously identified,[66,67] we synthesized and evaluated the 3′,4′,6-tri2-naphthylalkyl neamine derivatives and the corresponding 3′,6-di2-naphthylalkyl analogues to study the role of the lipophilicity on the antibacterial effect and cytotoxicity.

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Among the dialkyl and trialkyl derivatives prepared, the most lipophilic 3′,4′,6-tri2-naphthylpropyl (2NP, **41**) and tri2-naphthylbutyl (2NB, **42**) derivatives (Table 1) were inactive or very weakly active against *S. aureus* strains (Table 4) and Gram-negative bacteria (Table 5). In contrast, the corresponding less lipophilic 3′,6-di2NP (**34**) and -di2NB (**35**) derivatives appeared to be active against susceptible and resistant Gram-positive and Gram-negative bacteria. In the 3′,6-disubstituted derivatives, the more lipophilic diNH derivative **36** rather than **34** and **35** showed weak effects, and the much less lipophilic diBu (**37**) derivative was inactive. These results taken together with the previously observed weak activity of the 3′,6-di2NM derivative **7** against *S. aureus* strains as well as its lack of activity against Gram-negative bacteria[66] suggested the existence of windows of optimal lipophilicities corresponding to observed significant antibacterial effects.

Regarding the antibacterial activities of the neamine derivatives bearing three linear alkyl chains (Tables 4 and 5), the 3′,4′,6-triHx derivative **44** showed low MIC values near those of the corresponding tri2NM derivative **5** and was active against Gram-positive and Gram-negative bacteria. The triBn (**10**) and triBu (**43**) derivatives possessing lower lipophilicities than the triHx derivative **44** were inactive.

Among the four derivatives carrying two linear chains, **37**−**40** (Bu, Hx, Nn, and ocD, respectively), only the 3′,6-dinonyl (Nn) derivative **39** showed good activity against both susceptible and resistant *S. aureus* strains as well as against Gram-negative bacteria, whereas the 3′,6-diBu (**37**), diHx (**38**), and diocD (**40**) derivatives were inactive.

Thus, among the 3′,6-dialkyl derivatives prepared, the most active against susceptible and resistant *S. aureus* strains were the 3′,6-di2NP (**34**) and 3′,6-di2NB (**35**) derivatives. The 3′,6-diNn derivative **39** appeared to be slightly less active. Against susceptible and resistant Gram-negative bacteria, the most active compound was the 3′,6-di2Nn derivative **39**, which was not far from the 3′,6-di2NB derivative **35** followed by **34**. Regarding the activities of the synthesized 3′,4′,6-trisubstituted neamine derivatives, the best compounds are the tri2NM (**5**) and triHx (**44**) derivatives that are active against susceptible and resistant *S. aureus* strains and Gram-negative bacteria. However, these compounds are less active against Gram-positive bacteria than the most active antibacterial 3′,6-dialkyl derivatives.

The 6- (**11**) and 3′-monooctadecyl (ocD) (**12**) derivatives, with a lipophilicity (clogP = −12.1, Table 1) close to those of the 3′,6-di2NP (**34**) and the 3′,6-diNn (**39**) derivatives (−11.4 and −11.9, respectively), evaluated against *S. aureus* strains were found to be inactive (Table 4). This result suggests that for neamine derivatives possessing comparable lipophilicities the presence of at least two lipophilic substituents on the neamine core is necessary for a strong antibacterial activity.

Structure−Activity Relationships. The results reported in Tables 4 and 5 point out the critical role of the number of substituents as well as the lipophilicity for an optimal antibacterial activity. To delineate more accurate structure−activity relationships, in each series of neamine derivatives, the di- and the trialkyl series, we plotted the MIC values of the 3′,6-di- and 3′,4′,6-trialkylated derivatives as a function of the corresponding clogP for two representative Gram-positive and Gram-negative bacteria, MRSA and susceptible *P. aeruginosa* ATCC 27853, respectively. In this analysis, we used MIC values in μg/mL and not in mol/L because of the similar high molecular weights of the compounds. Figure 3 shows the graphs obtained against MRSA with the di- and the trialkyl derivatives as an example.

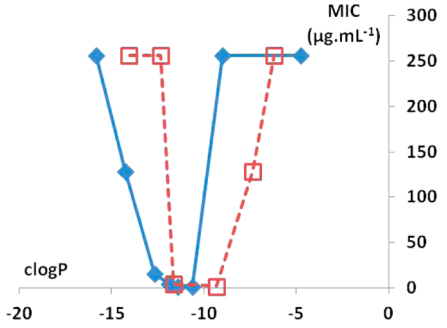

Figure 3. MIC values (μg/mL) against ATCC 33592 HA-MRSA as a function of clogP for the amphiphilic 3′,6-dialkyl (blue diamonds) and 3′,4′,6-trialkyl (open red squares) neamine derivatives prepared.

Clearly, the existence of a range of lipophilicities corresponding to significant antibacterial effects is observed in Figure 3. However, only strong variations in the MIC values are evident from such a graph because of the large differences observed in the MIC values.

To refine the analysis, the graphs of (1/(MIC values)) versus clogP of the compounds were drawn in Figure 4 (when the MIC value is higher than 128 μg/mL, a value of 256 μg/mL was used for the calculation of 1/MIC). The graphs corresponding to the values obtained against MRSA and susceptible *P. aeruginosa* ATCC 27853 for the dialkyl (Figure 4A) and the trialkyl (Figure 4B) derivatives, respectively, confirm the existence of critical ranges of lipophilicities necessary for obtaining significant antibacterial effects. The mimimum and maximum clogP values characterizing these ranges in the same series of derivatives, di- or trialkyl derivatives, appear to be close against MRSA and susceptible *P. aeruginosa* ATCC 27853, with values between −12.7 and −9.0 for the dialkyl derivatives (Figure 4A) and −12.0 and −7.4 for the trialkylated derivatives (Figure 4B). The ranges of lipophilicities leading to a significant effect against MRSA and *P. aeruginosa* are larger in the trialkyl series than in the dialkyl series.

This difference between the dialkyl and the trialkyl series was also observed in the 1/(MIC) graphs of the dialkyl derivatives and the trialkyl derivatives versus clogP against the same bacteria, MRSA or *P. aeruginosa* (in the Supporting Information). The lowest MIC values were obtained for dialkyl derivatives that are less lipophilic than the best trisusbtituted derivative **5**.

The graphs of 1/MIC as a function of the clogP values of the lipophilic substituent introduced on the neamine core plotted for all derivatives against MRSA (Figure 5A) and susceptible *P. aeruginosa* ATCC 27853 (Figure 5B) pointed out the difference between the antibacterial effects produced by the same substituent introduced on the neamine core twice at the 3′,6-positions in dialkyl derivatives and three times at the 3′,4′,6-positions. Clearly, Figure 5 revealed that for obtaining significant antibacterial effects more lipophilic substituents have to be used in the dialkyl series in comparison to the trialkyl series, resulting in a decrease of the global lipophilicity of the compounds in the dialkyl series compared to the trialkyl one (Figure 5). From Figure 5, it can also be concluded that the MIC values are related to the clogP values of the substituents, as expected from the calculation method of clogP.

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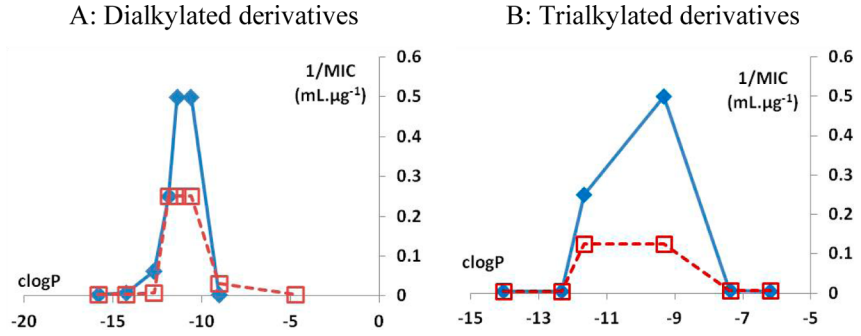

Figure 4. Values of 1/MIC (μg/mL) against ATCC 33592 HA-MRSA (blue diamonds) and *P. aeruginosa* (open red squares) ATCC 27853 as a function of clogP for 3′,6-dialkyl (A) and 3′,4′,6-trialkyl (B) neamine derivatives.

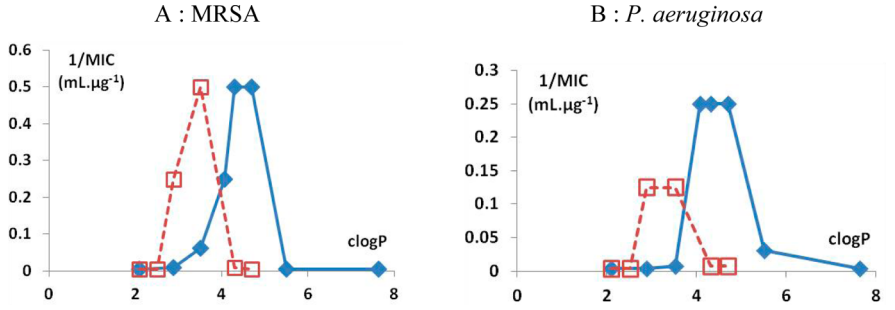

Figure 5. Values of 1/MIC (μg/mL) against ATCC 33592 HA-MRSA (A) and *P. aeruginosa* ATCC 27853 (B) as a function of clogP of the lipophilic substituent carried by the 3′,6-dialkyl (blue diamonds) and 3′,4′,6-trialkyl (open red squares) neamine derivatives (clogP of the corresponding alkanes).

These results pointed out the existence of windows of optimal lipophilicities necessary for obtaining significant antibacterial effects. In these windows, the less lipophilic active compounds are the dialkyl derivatives.

CYTOTOXICITY

The decrease in the global lipophilicity for an optimal antibacterial activity observed with the shift from tri- to dialkyl amphiphilic derivatives could result in a better selectivity for bacterial membranes and in a decrease in measured cytotoxicity.[40,43,51]

One of our objectives was reached with the identification of more active and less lipophilic neamine derivatives compared to the first broad spectrum antibacterial compound identified previously, the 3′,4′,6-tri2NM derivative **5**. In regard to their lower lipophilicities and the number of substituents, these compounds could be less cytotoxic than compound **5** through a decreased nonspecific binding to eukaryotic membranes. The cytotoxicities of the 3′,4′,6-tri- (**5**, **10**, and **41**−**44**) and 3′,6-dialkyl (**7** and **34**−**39**) neamine derivatives were evaluated through measurements of the viability of murine J774 macrophages using the MTT assay in the presence of 10 μM of the compounds.

As shown in Figure 6, the measured viability of the cells incubated with all trialkyl derivatives was lower than 75%. The newly identified active triHx derivative **44** induced a strong loss of cell viability (40%). Among the dialkyl derivatives evaluated, only the most lipophilic di2NHx derivative **36** was responsible for cytotoxicity (viability less than 50%).

Among the good antibacterial dialkyl derivatives, the 3′,6-diNP **34** appeared to be the least cytotoxic, with an observed 90% viability, followed by 3′,6-diNB **35** (80% viability) and 3′,6-diNn **39** (75% viability). In regard to the 75% viability observed with the previously identified 3′,4′,6-tri2NM derivative **5**, the

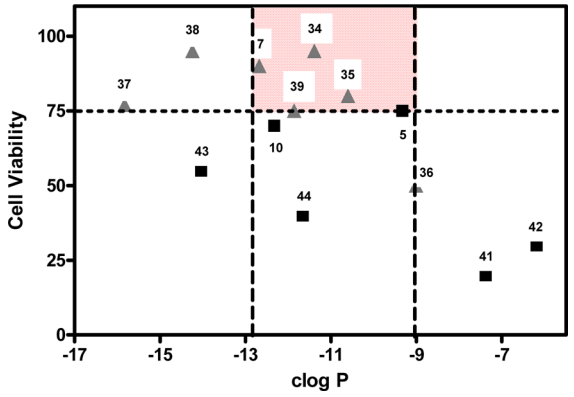

Figure 6. Viability (percent) of murine J774 macrophages in the presence of 10 μM of the prepared 3′,6-dialkyl neamine (gray triangles) and 3′,4′,6-trialkyl (black squares) derivatives after a 24 h incubation (average of three experiments). The trialkyl derivatives (increasing clogP) are **43** (triBu), **10** (triBn), **44** (triHx), **5** (tri2NM), **41** (tri2NP), and **42** (tri2NB), and the dialkyl derivatives (increasing clogP) are **37** (diBu), **38** (diHx), **7** (di2NM), **39** (diNn), **34** (di2NP), **35** (di2NB), and **36** (di2NH). The vertical dashed lines show the clogP limits at which the antibacterial activities can be observed against the *S. aureus* and/or *P. aeruginosa* strains.

cytotoxicity of the 3′,6-diNP derivative **34**, which is less lipophilic and more active against Gram-positive bacteria, appeared to be significantly lower.

In Figure 6, the cytotoxicity appears to be correlated to the number of substituents, increasing from dialkyl to trialkyl derivatives and, in the same series, is not well correlated to the lipophilicity.

Therefore, we have identified three antibacterial 3′,6-dialkyl neamine derivatives active against susceptible and resistant Gram-positive and Gram-negative bacteria: the di2NP (**34**),

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di2NB (**35**), and diNn (**39**) derivatives. These compounds showed a broad spectrum of activity and a low cytotoxicity at their MIC against *P. aeruginosa* ATCC 27853. For the di2NP derivative **34**, a low cytotoxicity at 10 μM was observed (90% viability) that corresponds to 5.5 times the MIC against MRSA and 11 times the MIC against VRSA. The diNBu (**35**) and diNn (**39**) derivatives showed lower viabilities than **34**, not far from 75% viability at 10 μM, corresponding to more than 5 times the MIC against VRSA. The most active diNn derivative, **39**, against Gram-negative bacteria showed 75% viability at 2.5 times the MIC against the *P. aeruginosa* strains.

DISCUSSION AND CONCLUSIONS

In the first part of this work, the comparison of the antibacterial activities of 1NM and 2NM paromamine and neamine derivatives pointed out the higher antibacterial activities, mainly against Gram-negative bacteria, of the neamine derivatives in comparison to their paromamine homologues and, therefore, the role in the antibacterial effects of the 6′-amine function of the neamine core that is protonated at physiological pH.[77−79] Electrostatic interactions are probably critical at the initial stage of bacterial recognition. The positive charges of the amphiphilic aminoglycosides conceivably target the anionic environment of Gram-negative bacteria because of the presence of anionic diphosphorylated sugar head groups of LPS as well as the negatively charged lipids like cardiolipin and phosphatidylglycerol in the bacterial membranes.

In the second part of this work devoted to the tuning of the antibacterial activities of amphiphilic neamine derivatives, our objectives were reached with the synthesis and identification of more active derivatives against Gram-positive and/or Gram-negative bacteria than the 3′,4′,6-tri2NM neamine derivative **5** previously identified as a broad spectrum antibacterial agent.[66] Increasing the lipophilicity in the dialkyl series in comparison to the di2NM derivative **7**, which is inactive against Gram-negative bacteria, led to broad spectrum antibacterial dialkyl derivatives: 3′,6-di2NP (**34**), 3′,6-di2NB (**35**), and 3′,6-diNn (**39**). The shift from trialkyl to dialkyl derivatives should increase the specificity for the bacterial membrane targets through the reduction in the number of lipophilic sites for binding to the membranes. The lack of activity against *S. aureus* strains of the 6- (**11**) and 3′-monooctadecyl (ocD) (**12**) derivatives possessing lipophilicities close to that of the active diNn derivative **39** observed here suggests that the presence of at least two linear lipophilic substituents are necessary for a strong and broad antibacterial activity.

Regarding more quantitative structure−activity relationships, the delineation of lipophilicity−activity relationships 1/MIC = f(clogP) clearly revealed the existence of optimal ranges of lipophilicity for obtaining significant antibacterial effects. The ranges determined against MRSA and susceptible *P. aeruginosa* ATCC 27853 are close, between −12.7 and −9.0 for the dialkyl derivatives and between −12.0 and −7.4 for the trialkylated derivatives, with the ranges of lipophilicities being sharper in the dialkyl series than in the trialkyl series.

We have assumed that the decrease of the lipophilicity and of the number of lipophilic substituents should decrease the nonspecific binding to eukaryotic membranes and could reduce the cytotoxicity. Clearly, at 10 μM, the highest cytotoxicity on murine J774 macrophages was observed with the trialkylated derivatives. For the same substituent, the trialkylated derivative appeared to be significantly more cytotoxic than the corresponding dialkylated derivative.

Regarding the potential cytotoxicity of the less lipophilic active dialkyl derivatives in comparison to the tri2NM derivative **5**, the 3′,6-di2NP derivative **34** at 10 μM showed a weak effect on the viability of murine J774 macrophages, with 90% viability at 5.5 times the MIC against MRSA and 11 times the MIC against VRSA. Against Gram-negative bacteria, the most active diNn derivative **39** at 10 μM showed a cytotoxicity similar to that of **5**, with 75% viability.

We have identified three amphiphilic 3′,6-dialkyl neamine derivatives, **34**, **35**, and **39**, active against susceptible and resistant Gram-positive and Gram-negative bacteria. These compounds are more active against Gram-positive bacteria than the previously identified 3′,4′,6-tri2NM derivative **5**.[66] The latter has been shown to bind to LPS in the *P. aeruginosa* membrane, inducing their depolarization.[67] The diNn derivative **39**, is also more active against Gram-negative bacteria. The linear alkyl chain introduced in **39** could be more favorable for binding to LPS than the alkylaryl chains found in the di2NP and di2NB derivatives.

This work allowed the determination of the optimal number of lipophilic substituents to be attached to the neamine core as well as the corresponding optimal lipophilicity necessary for obtaining good antibacterial effects. The decrease in the number of lipophilic groups carried by the neamine core should permit the more selective targeting of bacterial membranes relative to eukaryotic membranes. One feature that distinguishes the membranes of prokaryotic organisms from those of eukaryotic organisms is that the former harbor more negatively charged lipids in the outer leaflet of the plasma membrane. Most Gram-negative bacteria contain ∼25% negatively charged lipids such as phosphatidylglycerol or cardiolipin and ∼75% phosphatidylethanolamine as their most common zwitterionic lipids.[40,43,51,84]

The matching between the molecular shapes of the amphiphilic aminoglycosides and the lipids mostly found in bacterial membranes could be improved. The limits of lipophilicity determined here in the neamine family as well as the study of the effects of the identified active dialkyl derivatives on bacterial and eukaryotic membranes will help us in the design of more efficient and less cytotoxic derivatives.

The existence of optimal windows of lipophilicities necessary for obtaining strong antibacterial effects revealed by this work should be of interest in the field of antibacterial amphiphilic aminoglycosides.

EXPERIMENTAL SECTION

Calculation of the clogP Values. The lipophilicity character of the neamine derivatives prepared was estimated through the calculation of clogP values (octanol/water partition coefficients) using MarvinSketch software (Marvin 5.11.4, 2012, ChemAxon (http://www.chemaxon.com)). The clogP plug-in in this software calculates the octanol/water partition coefficient, which is used in QSAR analysis and rational drug design as a measure of molecular hydrophobicity. The calculation method used here is based on a modification of the method published by Viswanadhan and Ghose et al. (VG method).[85] The lipophilicities of the substituents were determined through a calculation of the lipophilicities of the corresponding alkanes with the same method.

Synthesis. *Procedure I.* General procedure used for the 3′,6-di-*O*-alkylation of the tetra-*N*-tritylated neamine derivative **22** under phase-transfer conditions.[74] To a solution of compound **22**[75] (1 g) in toluene (30 mL) were added TBAI (1.5 equiv), the halide (3 equiv), and an aqueous solution of NaOH (50% w/w, 15 mL). The resulting mixture was heated at 50 °C and stirred vigorously. After 5 h, another portion of halide (0.6 equiv) was added to the reacting mixture. After 24 h, the organic phase was diluted with ethyl acetate and washed twice with an aqueous saturated ammonium chloride solution before being dried over

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$MgSO_4$. After filtration and evaporation to dryness, the dialkylated product was purified by chromatography on alumina or silica gel with a gradient mixture of toluene/ethyl acetate.

Procedure II. General procedure used for the 3′,6-di-*O*-alkylation and the 3′,4′,6-tri-*O*-alkylation of **22** (homogeneous phase). To a solution of **22** (1 equiv) in dry DMF under argon was added NaH (60%, 10 equiv). After 30 min at rt, the halide was then added (4 equiv). After 1 h stirring at rt, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (×4). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with toluene/ethyl acetate to give the tetratrityl 3′,6-*O*- and 3′,4′,6-*O*-alkyl derivatives.

Procedure III. General procedure used for the 3′,4′,6-tri-*O*-alkylation of **22** under phase-transfer conditions.[74] To a solution of compound **22** (1 g) in toluene (30 mL) were added TBAF·$3H_2O$ (1.5 equiv), the halide (4 equiv), and an aqueous solution of NaOH (50% w/w, 15 mL). The resulting mixture was stirred vigorously for 24 h at rt. The organic solution was diluted with ethyl acetate and then washed twice with an aqueous saturated ammonium chloride solution before being dried over $MgSO_4$ and evaporated to dryness. The trialkylated product was purified by chromatography on alumina or silica gel, eluting with mixtures of toluene/ethyl acetate.

Procedure IV. General procedure for the deprotection of the alkylated tetra-*N*-tritylated neamine derivatives. The protected compound was dissolved at 0 °C or at room temperature in TFA/CH_2Cl_2 (1:4, v/v) in the presence of anisole (0.1 mL/mL). After 2 h of stirring, the solvents were evaporated under reduced pressure. H_2O and Et_2O were added, and the aqueous phase was washed twice with Et_2O before being evaporated to dryness. The residue was then chromatographed on C18 reversed-phase column, eluted with a H_2O/MeOH gradient, and obtained pure as the tetraTFA salt.

Procedure V. General procedure for the synthesis of the 2-alkylnaphthyl bromide derivatives. 2-Bromonaphthalene (25 g, 1 equiv) was dissolved in anhydride THF (125 mL) under an argon atmosphere. Mg (finely divided) (3 g, 1 equiv) was added to the solution and stirred at 40 °C for 45 min. The solution was filtered under an argon atmosphere before being added dropwise to a THF solution (35 mL) of α,ω-dibromoalkane (3 equiv) with CuBr (370 mg) in suspension. After refluxing for 10 h under an argon atmosphere, the reaction mixture was evaporated to dryness. The crude product was dissolved in toluene (200 mL) and washed with 10% aqueous HCl (2 × 100 mL) and water (2 × 100 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue obtained was chromatographed on silica gel, eluting with cyclohexane.

Purification. The aminosugar purity of the evaluated compounds was ≥95%. The tritylated 3′,4′-isomers formed in the alkylation steps were carefully removed by chromatography on silica gel. After removal of the trityl protective groups, the purity was measured by HPLC for the derivatives carrying chromophores and was controlled by ^{1}H NMR spectrometry and TLC on silica gel. Eluent: EtOH/H_2O/NH_3,H_2O (20%) 80:10:10. TLC visualizations: sulphuric acid spray (5 mL in 100 mL EtOH) and ninhydrin spray (0.3 g, 3 mL AcOH, 100 mL EtOH). For example, under these TLC conditions, the retardation factors of the 3′,6-dinonyl derivative **39** and its 3′,4′-isomer that was isolated and characterized were 0.5 and 0.3, respectively.

3′-Mono-*O*-octadecyl Neamine 12. To a solution of compound **22** (150 mg, 0.12 mmol) in toluene (4 mL) were added TBAF·$3H_2O$ (1.5 equiv, 55 mg, 0.17 mmol), 1-bromooctadecane (1.2 equiv, 48 μL, 0.14 mmol), and an aqueous NaOH solution (50% w/w, 2 mL). After 8 h of stirring vigorously at rt, another portion of halide (2.6 equiv, 100 mg, 102 μL, 0.30 mmol) was added, and the resulting mixture was stirred for 24 h. The organic solution was diluted with ethyl acetate and washed twice with a saturated aqueous ammonium chloride solution before being dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue obtained was chromatographed on alumina gel, eluting with a gradient of cyclohexane/dichloromethane (70:30 to 50:50) to give the protected compound with 46% yield (white solid). LRMS (MALDI, DHB) *m/z*: 1703 $[M + K]^+$, 1663 $[M + H]^+$, 1461 $[M\text{-}Tr + K]^+$, 1218 $[M\text{-}2Tr + K]^+$. The deprotection was achieved following procedure IV. **12**: 70% yield (48 mg, white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 5.94 (d, *J*= 3.6 Hz, 1H, H-1′), 4.03 (m, 1H, H-5′), 4.02−3.89 (m, 2H, H-3′, CH_2O), 3.82 (dd, *J*= 8.8, 10.4 Hz, 1H, H-3′), 3.69 (m, 1H, CH_2O), 3.60 (dd, *J*= 9.1 Hz, 1H, H-5), 3.45−3.35 (m, 4H, H-3, H-6, H-4′, H-6′), 3.25 (dd, *J*= 3.7, 10.5 Hz, 1H, H-2′), 3.17 (m, 1H, H-1), 3.07 (dd, *J*= 8.8, 13.2 Hz, 1H, H-6′), 2.42 (m, 1H, H-2), 1.93 (m, 1H, H-2), 1.65 (m, 2H, CH_2CH_2O), 1.40−1.21 (m, 30H, CH_2), 0.90 (t, *J*= 7.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz) δ 97.0 (C-1′), 79.5 (C-4), 78.0 (C-3′), 77.4 (C-5), 74.9 (CH_2O), 74.6 (C-6), 73.8 (C-4′), 71.5 (C-5′), 54.9 (C-2′), 51.7 (C-1), 50.4 (C-3), 42.0 (C-6′), 33.2−23.9 (16CH_2, C-2), 14.6 (CH_3). HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd, 597.4567; found, 597.4563. HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd, 575.4748; found, 575.4760.

3′,6-Di-*O*-1″-naphthylmethylene Paromamine 15. Compound **15** was synthesized following procedure I from tetratritylated paromamine[74] (1.0 g, 0.77 mmol) and 1-chloromethylnaphthalene (3 equiv, 350 μL, 2.33 mmol). The protected derivative was obtained in 41% yield (500 mg, white solid). The deprotection was achieved following procedure IV. **15**: 33% yield (100 mg, white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 8.20−7.35 (m, 14H, H-np), 5.61 (d, *J*= 11.4 Hz, 1H, CH_2-np), 5.58 (d, *J*= 11.4 Hz, 1H, CH_2-np), 5.52 (d, *J*= 3.6 Hz, 1H, H-1′), 5.08 (d, *J*= 11.4 Hz, 2H, CH_2-np), 4.05 (dd, *J*= 8.2, 9.6 Hz, 1H, H-3′), 3.98−3.88 (m, 2H, H-5′, H-6′b), 3.87−3.82 (m, 2H, H-5, H-4), 3.72−3.65 (m, 2H, H-6, H-6′), 3.59 (dd, *J*= 8.4 Hz, 1H, H-4′), 3.42 (m, 1H, H-3), 3.39 (dd, *J*= 3.6, 9.7 Hz, 1H, H-2′), 3.26 (m, 1H, H-1), 2.44 (m, *J*= 4.2, 12.5 Hz, 1H, H-2), 1.85 (m, *J*= 12.6 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CD_3OD) δ 135.3−133.1 (6C-np), 129.8−125.2 (14CH-np), 98.5 (C1′), 84.1 (C4), 81.9 (C6), 78.6 (C3′), 77.2 (C5, C5′), 74.3 and 74.2 (2CH_2-np), 71.9 (C4′), 62.0 (C6′), 54.7 (C2′), 50.7 and 50.5 (C1, C3), 30.0 (C2). LRMS (MALDI, DHB) *m/z*: 626 $[M + Na]^+$, 604 $[M + H]^+$. HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd, 626.2842; found, 626.2847.

3′,4′,6-Tri-*O*-1″-naphthylmethylene Paromamine 16. Compound **16** was synthesized following procedure III from tetratritylated paromamine[74] (1.0 g, 0.77 mmol) and 1-chloromethylnaphthalene (468 μL, 3.10 mmol). The tritylated derivative obtained was deprotected following procedure IV. **16**: 11% yield (86 mg, white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (d,1H, H-np), 7.81−7.19 (m, 20H, H-np), 5.61 (d, *J*= 11.2 Hz, 1H, CH_2-np), 5.54 (d, *J*= 3.2 Hz,1H, H-1′), 5.21 (d, *J*= 12.4 Hz, 1H, CH_2-np), 5.13 (d, *J*= 12.4 Hz, 1H, CH_2-np), 5.06 (d, *J*= 11.2 Hz, 1H, CH_2-np), 4.92−4.83 (m, 2H, CH_2-np), 4.16 (m, *J*= 7.6 Hz, 1H, H-3′), 4.06 (m, 1H, H-5′), 3.82 (m, 2H, H-4, H-5), 3.76−3.65 (m, 2H, H-6, H-6), 3.62−3.52 (m, 3H, H-2′, H-4′, H-6′), 3.39 (m, 1H, H-3), 3.25 (m, 1H, H-1), 2.42 (m, 1H, H-2), 1.81 (m, *J* = 12.6 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CD_3OD) δ 135.3−132.2 (9C-np), 130.0−124.4 (21CH-np), 97.0 (C1′), 83.2 (C4), 81.8 (C6), 77.6 (C3′), 77.1 (C5, C5′), 76.9 (C4′), 74.3 and 73.4 and 73.0 (3CH_2-np), 61.1 (C6′), 53.9 (C2′), 50.5 and 50.3 (C1, C3), 29.9 (C2). HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd, 744.3649; found, 744.3616. HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd, 766.3468; found, 766.3468.

3′,6-Di-*O*-1″-naphthylmethylene Neamine 17. Compound **17** was synthesized following procedure I from **22** (1.0 g, 0.77 mmol) and 1-chloromethylnaphthalene (350 μL, 2.3 mmol). The protected derivative was obtained in 41% yield (white solid). The deprotection was achieved following procedure IV. **17**: 57% yield (white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 8.30−7.40 (m, 14H, H-np), 6.11 (d, *J*= 3.6 Hz, 1H, H-1′), 5.58 (dd, *J*= 11 Hz, 2H, CH_2-np), 5.07 (dd, *J*= 11.0 Hz, 2H, CH_2-np), 4.25 (dd, *J*= 8.6, 9.7 Hz, 1H, H-3′), 4.15 (m, 2H, H-4, H-5′), 3.91 (dd, *J*= 9.1 Hz, 1H, H-5), 3,71 (dd, *J*= 9.5 Hz, 1H, H-6), 3.57 (dd, *J*= 8.6 Hz, 1H, H-4′), 3,49−3.23 (m, 4H, H-1, H-3, H-2′, H-6′), 3.16 (dd, *J*= 8.8, 13.6 Hz, 1H, H-6′), 2.47 (m, 1H, H-2), 2.04 (m, *J*= 12.6 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CD_3OD) δ 135.4−133.1 (6C-np), 128.1−125.2 (14CH-np), 96.5 (C1′), 82.5 (C6), 78.8 (C4), 78.3 (C5), 77.7(C3′), 74.6 and 74.5 (2CH_2-np), 73.4 (C4′), 72.5 (C5′), 54.5 (C2′), 50.9 (C1), 50.5 (C3), 41.9 (C6′), 30.0 (C2). LRMS (MALDI, DHB) *m/z*: 641 $[M + K]^+$, 625 $[M + Na]^+$, 603 $[M + H]^+$. HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd, 625.3002; found, 625.3003. HRMS (ESI^+) *m/z*: $[M + K]^+$ calcd, 641.2741; found, 641.2713.

3′,4′,6-Tri-*O*-1″-naphthylmethylene Neamine 18. Compound **18** was synthesized following procedure III from **22** (1.0 g, 0.77 mmol) and 1-chloromethylnaphthalene (468 μL, 3.08 mmol). The protected derivative was obtained in 38% yield (white solid). The deprotection

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was achieved following procedure IV. **18**: 52% yield (white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 8.24−7.11 (m, 21H, H-np), 6.00 (d, J= 3.1 Hz, 1H, H-1′), 5.69 (d, J= 11.2 Hz, 1H, CH_2-np), 5.37 (d, J= 12.5 Hz, 1H, CH_2-np), 5.24 (d, J= 12.4 Hz, 1H, CH_2-np), 5.17 (d, J= 11.2 Hz, 1H, CH_2-np), 4.99 (m, J= 12.4 Hz, 1H, CH_2-np), 4.88 (d, J= 12.3 Hz, 1H, CH_2-np), 4.40 (dd, J= 7.8, 8.0 Hz, 1H, H-3), 4.34 (m, 1H, H-5), 4.18 (dd, J = 9.7 Hz, 1H, H-4), 3.97 (dd, J = 9.1 Hz, 1H, H-5), 3.79 (dd, J = 9.5 Hz, 1H, H-6), 3.64 (dd, J = 3.3, 8.7 Hz, 1H, H-2′), 3.56−3.44 (m, 2H, H-3, H-4′), 3.34 (m, 1H, H-1), 3.10−2.94 (m, 2H, H6′, H6′), 2.54 (m, 1H, H-2), 2.10 (m, J = 12.7 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CD_3OD) δ 135.2−132.3 (9C-np), 130.3−124.2 (21CH-np), 96.2 (C1), 82.3 (C6), 80.0 (C4), 78.6 (C4′), 78.0 (C5), 77.6 (C3′), 74.4 and 73.7 and 73.2 ($3CH_2$-np), 72.1 (C5′), 53.9 (C2′), 50.8 (C1), 50.1 (C3), 41.2 (C6′), 30.0 (C2). LRMS (MALDI, DHB) m/z: 781 $[M + K]^+$, 765 $[M + Na]^+$, 743 $[M + H]^+$. HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 743.3809; found, 743.3835. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 765.3628; found, 765.3620.

2-(3′-Bromopropyl)naphthalene 19. Compound **19** was synthesized following procedure V from 1,3-dibromopropane. Yield: 33% (4.00 g, white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83−7.33 (m, 7H, H-np), 3.43 (t, 2H, J = 6.5 Hz CH_2−Br), 2.95 (t, 2H, J = 7.2 Hz, CH_2-np), 2.26 (q, 2H, J = 7.0 Hz, CH_2). HRMS (ESI/ASAP) m/z: $[M + H]^+$ calcd, 249.0279; found, 249.0276. HRMS (ESI/ASAP) m/z: $[M - Br]^+$ calcd, 169.1017; found, 169.1012.

2-(4′-Bromobutyl)naphthalene 20. Compound **20** was synthesized following procedure V from 1,4-dibromobutane. Yield: 23% (4.38 g, colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83−7.33 (m, 7H, H-np), 3.46 (t, 2H, J = 6.5 Hz CH_2−Br), 2.83 (t, 2H, J = 7.3 Hz, CH_2-np), 1.99−1.85 (m, 4H, CH_2). HRMS (ESI/ASAP) m/z: $[M + H]^+$ calcd, 263.0435; found, 263.0429. HRMS (ESI/ASAP) m/z: $[M - Br]^+$ calcd, 183.1174; found, 183.1174.

2-(6′-Bromohexyl)naphthalene 21. Compound **21** was synthesized following procedure V from 1,6-dibromohexane. Yield: 18% (3.80 g, colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88−7.38 (m, 7H, H-np), 3.44 (t, 2H, J = 6.8 Hz CH_2−Br), 2.83 (t, 2H, J = 7.6 Hz, CH_2-np), 1.89 (t, 2H, J = 7.0 Hz, CH_2−CH_2Br), 1.77 (t, 2H, J = 7.5 Hz, CH_2−CH_2-np), 1.57−1.39 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.1 and 133.7 and 132.0 (3C-np), 127.9−125.1 (7CH-np), 36.0 (CH_2-np), 34.0 (CH_2−Br), 32.8 (CH_2−CH_2Br), 31.2 (CH_2−CH_2np), 28.5 and 28.1 ($2CH_2$). HRMS (ESI/ASAP) m/z: $[M + H]^+$ calcd, 291.0748; found, 291.0744. HRMS (ESI/ASAP) m/z: $[M - Br]^+$ calcd, 211.1487; found, 211.1489.

3′,6-Di-*O*-[3″-(2‴-naphthyl)propyl] Neamine 34 and 3′,4′,6-Tri-*O*-[3″-(2‴-naphthyl)propyl] Neamine 41. Compounds **23** and **30** were prepared following procedure II from **22** (2.58 g, 2.0 mmol) and **19** (2.0 g, 8.0 mmol). The protected derivatives were obtained with 22% (690 mg) and 16% (556 mg) yields, respectively (white solids). **23**: HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 1627.8185; found, 1627.8159. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 1649.8010; found, 1649.8008. **30**: HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 1795.9130; found, 1795.9139. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 1817.8949; found, 1817.8948. The deprotection was achieved following procedure IV. **34**: 99% yield (white solid). ^{1}H NMR (400 MHz, MeOD) δ 7.82−7.38 (m, 14H, H-np), 6.02 (d, J= 3.6 Hz, 1H, H-1′), 4.11−4.02 (m, 4H, H-5′, H-4, CH_2O), 3.93 (dd, J= 8.6, 10.3 Hz, 1H, H-3′), 3.84−3.74 (m, 3H, H-5,CH_2O), 3.50−3.31 (m, MeOH + 5H, H-6′, H-4′, H-2′, H-6, H-3), 3.29−3.26 (m, 1H, H-1), 3.14 (dd, J= 8.7, 12.3 Hz, 1H, H-6′) 2.90−2.84 (m, 4H, CH_2-np), 2.50 (td, J= 4.0, 8.5 Hz, 1H, H-2), 2.16−2.00 (m, 5H, H-2, CH_2CH_2O). ^{13}C NMR (100 MHz, CD_3OD) δ 135.2−133.6 (6C-np), 129.0−126.2 (14CH-np), 96.7 (C1′), 82.4 (C6), 78.6 (C4), 77.7 (C3′), 77.6 (C5), 74.7 and 74.2 ($2CH_2O$), 73.4 (C4′), 71.8 (C5′), 54.6 (C2′), 50.7(C1), 50.2 (C3), 41.8 (C6′), 33.4 ($2CH_2$ np), 32.8 and 32.6 ($2CH_2CH_2O$), 29.8 (C2). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 659.38031; found, 659.3804. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 681.36226; found, 681.3629. **41**: 98% yield (white solid). ^{1}H NMR (400 MHz, MeOD) δ 7.81−7.22 (m, 21H, H-np), 5.96 (d, J= 3.4 Hz, 1H, H-1′), 4.15 (td, J= 2.0, 9.0 Hz, 1H, H-5′), 4.10−4.02 (m, 2H, H-4, CH_2O), 3.96 (dd, J= 8.5, 9.9 Hz, 1H, H-3′), 3.85−3.72 (m, 4H, H-5, CH_2O), 3.67 (td, J= 6.6, 8.9 Hz, 1H, CH_2O), 3.54 (td, J= 6.7, 8.9 Hz, 1H, CH_2O), 3.48−3.25 (m, MeOH + 5H, H-6′, H-2′, H-6, H-3, H-1), 4.21 (t, J = 8.5 Hz, 1H, H-4′), 3.14 (dd, J= 9.5, 13.1 Hz, 1H, H-6′), 2.87 (t^2, J= 7.9 Hz, 2H, CH_2-np), 2.74 (t, J= 7.4 Hz, 2H, CH_2-np), 2.61 (t, J= 7.6 Hz, 2H, CH_2-np), 2.49 (td, J= 3.4, 12.0 Hz, 1H, H-2), 2.17−1.92 (m, 5H, H-2, CH_2−CH_2O), 1.78 (q^2, J= 6.6 Hz, 2H, CH_2−CH_2O). ^{13}C NMR (100 MHz, CD_3OD) δ 135.2−133.6 (9C-np), 129.1−126.3 (21CH-np), 96.6 (C1′), 82.4 (C6), 81.0 (C4′), 79.0 (C4), 77.6 (C3′), 74.6 (C5), 74.0 and 73.6 ($3CH_2$−O), 71.1 (C5′), 54.4 (C2′), 50.7 (C1), 50,0 (C3), 41.5 (C6′), 33.4 and 33.1 ($3CH_2$-np), 32.6 ($3CH_2$−CH_2O), 29.9 (C2). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 827.4742; found, 827.4747. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 849.4562; found, 849.4553.

3′,6-Di-*O*-[4″-(2‴-naphthyl)butyl] Neamine 35 and 3′,4′,6-Tri-*O*-[4″-(2‴-naphthyl)butyl] Neamine 42. Compounds **35** and **42** were prepared following procedure II from **22** (1.5 g, 1.16 mmol) and **20** (1.22 g, 4.65 mmol). The protected derivatives **24** and **31** were obtained in 31% and 14% yields (white solids), respectively. **24**: HRMS (ESI^+) m/z: $[M + K]^+$ calcd, 1693.8057; found, 1693.8027. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 1677.8323; found. 1677.8321. **31**: HRMS (ESI^+) m/z: $[M + K]^+$ calcd, 1875.9158; found, 1875.9187. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 1859.9431; found, 1859.9430. The deprotection was achieved following procedure IV. **35**: 98% yield (white solid). ^{1}H NMR (400 MHz, MeOD) δ 7.76−7.30 (m, 14H, H-np), 5.90 (d, J= 3.5 Hz, 1H, H-1′), 4.03−3.90 (m, 4H, H-5′, H-4′, CH_2O), 3.82 (dd, J= 8.6, 10.2 Hz, 1H, H-3′), 3.73−3.63 (m, 3H, H-5, CH_2O), 3.40−3.21 (m, MeOH + 5H, H-6′, H-4′, H-2′, H-6, H-3), 3.19−3.15 (m, 1H, H-1), 3.06 (dd, J= 8.7, 13.2 Hz, 1H, H-6′), 2.78 (t, J= 6.9 Hz, 4H, CH_2-np), 2.40 (td, J= 3.9, 12.3 Hz, 1H, H-2), 1.95 (q, J= 12.4 Hz, 1H, H-2), 1.78−1.64 (m, 8H, CH_2). ^{13}C NMR (100 MHz, CD_3OD) δ 136.2−134.6 (6C-np), 129.9−127.1 (14CH-np), 97.8 (C1′), 83.3 (C6), 80.0 (C4), 78.6 and 78.5 (C3′, C5), 75.8 and 75.5 ($2CH_2O$), 74.4 (C4′), 72.8 (C5′), 55.5 (C2′), 51.7 (C1), 51.1 (C3), 42.8 (C6′), 37.8, 37.9 ($2CH_2$-np), 31.8 and 31.7 ($2CH_2$), 31.0 (C2), 29.8 ($2CH_2$). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 687.4116; found, 687.4118. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 709.3936; found, 709.3929. **42**: 98% (white solid). ^{1}H NMR (400 MHz, MeOD) δ 7.71−7.15 (m, 21H, H-np), 5.79 (d, J= 3.3 Hz, 1H, H-1′), 4.01 (td, J= 2.2, 9.2 Hz, 1H, H-5′), 3.93−3.88 (m, 2H, H-4, CH_2O), 3.79 (t, J= 9.1 Hz, 1H, H-3′), 3.73−3.68 (m, 1H, CH_2O), 3.63−3.59 (m, 4H, H-5, CH_2O), 3.46 (td, J= 6.6, 8.4 Hz, 1H, CH_2O), 3.31−3.14 (m, MeOH + 4H, H-6′, H-2′, H-6, H-3), 3.12−3.00 (m, 3H, H-6′, H-4′, H-1), 2.72 (t, 2H, J= 6.6 Hz, CH_2-np), 2.62 (t, J= 7.1 Hz, 4H, CH_2-np), 2.34 (td, J= 4.0, 12.4 Hz, 1H, H-2), 1.89 (q, J= 12.5 Hz, 1H, H-2), 1.73−1.43 (m, 12H, CH_2). ^{13}C NMR (100 MHz, CD_3OD) δ 135.2−133.6 (9C-np), 128.9−126.2 (21CH-np), 96.7 (C1′), 82.3 (C6), 80.7 (C4′), 79.5 (C4), 77.6 and 77.5 (C3′, C5), 74.8 and 74.2 ($3CH_2O$), 71.3 (C5′), 54.4 (C2′), 50.7 (C1), 50.0 (C3), 41.5 (C6′), 36.9 and 36.8 ($3CH_2$-np), 30.9 and 30.7 ($3CH_2$), 30.1 (C2), 29.0 and 28.9 ($3CH_2$). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 869.5212; found, 869.5212. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 891.5031; found, 891.5035.

3′,6-Di-*O*-[6″-(2‴-naphthyl)hexyl] Neamine 36. Compound **36** was synthesized following procedure II from **22** (1.5 g, 1.17 mmol) and **21** (1.35 g, 4.65 mmol). The protected derivative 25 was obtained in 30% yield (white solid). The deprotection was achieved following procedure IV. **36**: 97% yield (white solid). ^{1}H NMR (400 MHz, MeOD) δ 7.76−7.28 (m, 14H, H-np), 5.81 (d, J= 3.3 Hz, 1H, H-1′), 4.00 (td, J= 2.5, 5.7 Hz, 1H, H-5′), 3.98−3.86 (m, 2H, H-4, CH_2O), 3.81 (t, J= 4.1 Hz, 1H, CH_2O), 3.72 (t, J= 9.8 Hz, 1H, H-3′), 3.68−3.58 (m, 3H, H-5, CH_2O), 3.37−3.32 (m, 2H, H-4′, H-6), 3.29−3.21 (m, MeOH + 2H, H-6′, H-3), 3.17−3.10 (m, 2H, H-2′, H-1), 3.04 (dd, J= 8.6, 13.1 Hz, 1H, H-6′), 2.74 (t, J= 7.5 Hz, 4H, CH_2-np), 2.33 (td, J= 3.6, 12.4 Hz, 1H, H-2), 1.82 (q, J= 12.3 Hz, 1H, H-2), 1.73−1.59 (m, 8H, CH_2), 1.41−1.32 (m, 8H, CH_2). ^{13}C NMR (100 MHz, CD_3OD) δ 135.2−133.5 (6C-np), 128.8−126.1 (14CH-np), 96.8 (C1′), 82.4 (C6), 79.0 (C4), 77.6 (C3′, C5), 75.1 and 74.7 ($2CH_2O$), 73.4 (C4′), 71.8 (C5′), 54.5 (C2′), 50.7 (C1), 50.1 (C3), 41.8 (C6′), 37.0 ($2CH_2$-np), 32.5 ($2CH_2$), 31.1 and 31.0 ($2CH_2$), 30.3 ($2CH_2$, C2), 27.0 and 26.9 ($2CH_2$). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 743.4742; found, 743.4739. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 765.4562; found, 765.4562.

3′,6-Di-*O*-butyl Neamine 37. Compound **37** was synthesized following procedure I from **22** (1.0 g, 0.77 mmol) and 1-bromobutane (250 μL, 2.31 mmol). The protected derivative **26** was obtained in 37% yield (white solid). The deprotection was achieved following procedure

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IV. **37**: 61% yield (white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 5.9 (d, J= 3.7 Hz, 1H, H-1′), 4.00−3.82 (m, 4H, H-4, H-5′, CH_2O), 3.79 (dd, J= 8.5, 10.3 Hz, 1H, H-3′), 3.66 (m, J= 9.1 Hz, 1H, H-5), 3.64−3.56 (m, 2H, CH_2O), 3.39−3.20 (m, 5H, H-3, H-6, H-2′, H-4′, H-6′), 3.15 (m, 1H, H-1), 3.03 (dd, J= 8.7, 13.3 Hz, 1H, H-6′), 2.39 (m, J= 4.2, 12.5 Hz, 1H, H-2), 1.92 (m, 1H, J= 12.6 Hz, H-2), 1.62−1.50 (m, 4H, CH_2), 1.35−1.24 (m, 4H, CH_2), 0.86 (t, J= 7.4 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, CD_3OD) δ 96.6 (C1′), 82.3 (C6), 78.6 (C4), 77.7 (C5), 77.5 (C3′), 74.7 and 74.4 ($2CH_2O$), 73.4 (C4′), 71.8 (C5′), 54.5 (C2′), 50.7 (C1), 50.2 (C3), 41.8 (C6′), 33.2 and 33.1 ($2CH_2$), 29.8 (C2), 20.1 ($2CH_2$), 14.3 ($2CH_3$). LRMS (MALDI, DHB) m/z: 457 $[M + Na]^+$, 435 $[M + H]^+$. HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 435.3183; found, 435.3180. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 457.3002; found, 457.3007.

3′,6-Di-*O*-nonyl Neamine 39. Compound **39** was synthesized following procedure I from **22** (250 mg, 0.19 mmol) and 1-bromononane (111 μL, 0.58 mmol). The protected derivative **28** was obtained in 52% yield (155 mg, white solid). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 1543.9124; found, 1543.9163. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 1565.8944; found. 1565.8983. The deprotection (134 mg) was achieved following procedure IV. **39**: 60% yield (55 mg, white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 5.98 (d, J= 3.6 Hz, 1H, H-1′), 4.11−3.92 (m, 4H, H-4, H-5′, CH_2O), 3.87 (dd, J= 8.5, 10.3 Hz, 1H, H-3′), 3.79−3.64 (m, 3H, H-5, CH_2O), 3.48−3.37 (m, 3H, H-3, H-4′, H-6′), 3.36−3.21 (m, 3H, H-1, H-6, H-2′), 3.13 (dd, J= 8.7, 13.3 Hz, 1H, H-6′), 2.47 (m, J= 4.0, 12.4 Hz, 1H, H-2), 2.01 (m, J= 12.5 Hz, 1H, H-2), 1.78−1.61 (m, 4H, CH_2CH_2O), 1.32 (br s, 24H, $12CH_2$), 0.93 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ 96.8 (C1′), 82.4 (C6), 79.0 (C4), 77.7 (C5), 77.6 (C3′), 75.1 (CH_2O), 74.7 (CH_2O), 73.4 (C4′), 71.7 (C5′), 54.6 (C2′), 50.7 (C1), 50.2 (C3), 41.8 (C6′), 33.1 ($2CH_2$), 31.2 and 31.0 and 30.7 and 30.5 ($8CH_2$), 30.1 (C2), 27.0 and 26.9 and 23.7 ($5CH_2$), 14.4 ($2CH_3$). HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 597.4562; found, 597.4562. HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 575.4742; found, 575.4737.

3′,6-Di-*O*-octadecyl Neamine 40. Compound **40** was synthesized following procedure II from **22** (0.50 g, 0.34 mmol) and 1-bromooctadecane (0.52 g, 1.56 mmol). The protected derivative **29** was obtained in 25% yield (colorless oil). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 1711.9124; found, 1711.9127. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 1733.8944; found, 1733.8642. The deprotection was achieved following procedure IV. **40**: 62% yield (white solid). ^{1}H NMR (400 MHz, MeOD), ^{1}H NMR (400 MHz, CD_3OD) δ 5.97 (d, J= 3.6 Hz, 1H, H-1′), 4.07−4.03 (m, 2H, H-4, H-5′), 3.97−3.85 (m, 3H, H-3′, CH_2O), 3.75−3.62 (m, 3H, H-5, CH_2O), 3.46−3.19 (m, MeOH + 6H, H-1, H-3, H-6, H-2′, H-4′, H-6′), 3.12 (dd, J= 8.7, 13.3 Hz, 1H, H-6′), 2.45 (td, J= 4.0, 12.4 Hz, 1H, H-2), 2.01 (q, J= 12.5 Hz, 1H, H-2), 1.68−1.61 (m, 4H, $2CH_2$), 1.27 (br s, 60H, $15CH_2$), 0.90−0.87 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ 96.7 (C1′), 82.3 (C6), 78.9 (C4), 77.6 (C5), 77.5 (C3′), 75.1 (CH_2O), 74.7 (CH_2O), 73.3 (C4′), 71.9 (C5′), 54.5 (C2′), 50.7 (C1), 50.2 (C3), MeOH, 41.8 (C6′), 33.1−23.7 ($32CH_2$), 30.0 (C2), 14.5 ($2CH_3$). HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 827.7565; found, 827.7570. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 849.7384; found, 849.7415. HRMS (ESI^+) m/z: $[M + K]^+$ calcd, 865.7124; found, 865.7121.

3′,4′,6-Tri-*O*-butyl Neamine 43. Compound **43** was synthesized following procedure III from **22** (1.0 g, 0.77 mmol) and 1-bromobutane (332 μL, 3.08 mmol). The protected derivative **32** was obtained in 35% yield (white solid). The deprotection was achieved following procedure IV. **43**: 82% yield (white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 5.79 (d, J = 3.5 Hz, 1H, H-1′), 4.01 (m, J= 9.1, 2.4 Hz, 1H, H-5′), 3.90 (dd, J = 9.7 Hz, 1H, H-4), 3.85−3.77 (m, 2H, H-3′, 1H CH_2O), 3.70−3.41 (m, 6H, H-5, 5H CH_2O), 3.30−3.16 (m, 4H, H-3, H-6, H-2′, H-6′b), 3.14−3.01 (m, 3H, H-1, H-4′, H-6′a), 2.33 (m, J = 4.0, 12.4 Hz, 1H, H-2eq), 1.87 (m, J = 12.6 Hz, 1H, H-2ax), 1.56−1.38 (m, 6H, $3CH_2$), 1.30−1.18 (m, 6H, $3CH_2$), 0.84−0.77 (m, 9H, $3CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ 96.5 (C1′), 82.1 (C6), 80.4 (C4′), 78.8 (C4), 77.4 (C5), 77.0 (C3′), 74.6 (CH_2O), 74.2 (CH_2O), 73.8 (CH_2O), 71.3 (C5′), 54.0 (C2′), 50.5 (C1), 49.9 (C3), 41.2 (C6′), 33.2 (CH_2), 33.1 (CH_2), 32.9 (CH_2), 29.6 (C2), 20.1 (CH_2), 20.0 (CH_2), 19.9 (CH_2), 14.2 (CH_3), 14.1 ($2CH_3$). LRMS (MALDI, DHB) m/z: 529 $[M + K]^+$, 513 $[M + Na]^+$, 491 $[M + H]^+$. HRMS (ESI^+) m/z: $[M + H]^+$ calcd, 481.3809; found, 491.3799. HRMS (ESI^+) m/z: $[M + Na]^+$ calcd, 513.3628; found, 513.3625.

Antimicrobial Activity Determination. The minimal inhibitory concentrations (MICs) were determined by a geometric microdilution method according to the recommendations of the CLSI norms for Gram-negative strains (*E. coli* (ATCC 25922, Ec06AB003 (Arm), EcPAZ505H8101, and EcLS8058.1), *P. aeruginosa* (ATCC 27853, PA02, PA03, PAO1, PA21, PA22, PA405, PA406, and Psa.F03), *A. lwoffi* (ATCC 17925 and Al.88-483), *Citrobacter amalonaticus* (Ca06AB0010 (Arm)), *Enterobacter aerogenes* (06AB008 (Arm)) and *S. aureus* strains (ATCC 33592 HA-MRSA and VRSA VRS-2)).[86,87]

The method was slightly modified for *S. aureus* (ATCC 25923), *S. aureus* SA-1199B (harboring resistance to fluoroquinolones through overexpression of the NorA efflux pump), *S.aureus* MsrA (resistant to 14- and 15-membered macrolides, harboring the multicopies plasmid pUL 5054 coding for an efflux pump), *S. aureus* APH2″-AAC6′ (aminoglycoside-6′-*N*-acetyltransferase/2″-*O*-phosphoryltransferase), *S. aureus* APH3′ (aminoglycoside-3′-*O*-phosphoryltransferase), and *S. aureus* ANT4′ (aminoglycoside-4′-*O*-phosphoryltransferase). Briefly, the plates were incubated at 37 °C, and bacterial growth was monitored at 650 nm after 1, 4, 7, and 24 h of growth. Ampicillin (16 mg/L) was used as a positive control, and 2 μL of DMSO was used as a negative control. The extract was considered to be very active if there was no bacterial growth after 24 h incubation, active if bacterial growth was less than 10% of the negative control, and inactive if bacterial growth was more than 10% of the negative control.

Assessment of Eukaryotic Cell Viability. Cell viability and growth capacity were assessed by evaluating their metabolic activity using the MTT assay (reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium in the mitochondria to a purple formazan crystal).[88] Briefly, cells exposed for 24 h to 10 μM of compounds were then incubated for 1 h with 0.2 mg/mL MTT. The reaction was stopped by the addition of dimethyl sulfoxide (DMSO). The OD was measured at 590 and 660 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General information for the synthesis, ^{1}H and ^{13}C NMR spectra, and HPLC profiles and purities of the evaluated amphiphilic derivatives. Structure−activity relationships data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Phone +32 2 764 73 74; Fax: +32 2 764 73 63; E-mail: Marie-Paule.Mingeot@uclouvain.be (M.-P.M.-L.).

*Phone +33 4 76 63 53 17; Fax +33 4 76 63 52 98; E-mail: Jean-Luc.Decout@ujf-grenoble.fr (J.-L.D.).

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AAC, aminoglycoside *N*-acetyltransferase; AG, aminoglycoside; ANT, aminoglycoside *O*-nucleotidyltransferase; APH, amino-

glycoside *O*-phosphoryltransferase; ax, axial; CA, cationic amphiphiles; eq, equatorial; 2NB, 2-naphthylbutyl; 2NH, 2-naphthylhexyl; 2NM, 2-naphthylmethylene; 2NP, 2-naphthylpropyl; np, naphthyl ring (NMR); 2QM, 2-quinolylmethylene; MRSA, methicillin resistant *S. aureus*; VRSA, vancomycin resistant *S. aureus*

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