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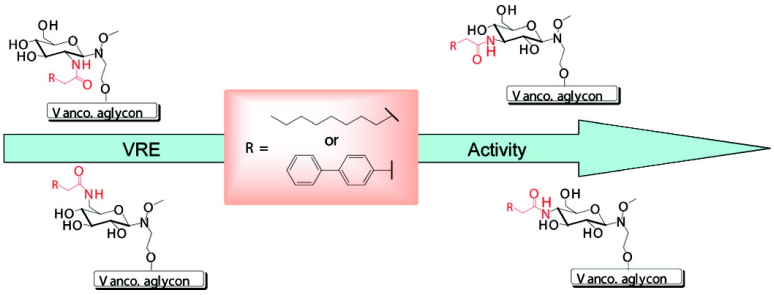


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# Model for Antibiotic Optimization via Neoglycosylation: Synthesis of Liponeoglycopeptides Active against VRE

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***Abstract:*** The neoglycosylation of a methoxyamine-appended vancomycin aglycon with all possible *N*′-decanoylglucopyranose and *N*′-biphenoylglucopyranose regioisomers led to the production of a focused set of liponeoglycopeptide variants in good yields and with excellent stereoselectivity. High-throughput antibacterial assays employing a unique set of vancomycin-resistant *Enterococci faecalis* and *Enterococci faecium* clinical isolates revealed that the nature and regiochemistry of glycosyl lipidation modulated vancomycin-resistent *Enterococci* potency. In contrast to prior work with lipoglycopeptides, this study reveals the glucose C3′ or C4′ as the optimal position for neoglycopeptide lipidation. This purely chemical method for the diversification of the glycolipid portion of lipoglycopeptide antibiotics is simple to perform on a large scale, requires minimal synthetic effort in sugar donor preparation, and provides access to highly active antibiotics that are not easily prepared by other state-of-the-art methods.

## Introduction

The global emergence of vancomycin-resistant *Enterococci* (VRE),[1] coupled with the recent transfer of vancomycin (**1**) resistance to highly pathogenic *Staphylococcus aureus* (*S. aureus*) strains,[2,3] has provided a major impetus for developing new glycopeptide derivatives (Figure 1, **2**−**11**). Although efficient chemoenzymatic strategies for glycopeptide optimization have recently emerged,[4] the chemical modification of glycopeptide sugar substituents remains among the most successful strategies to date.[5] From these extensive studies, two major "pharmacophores" have emerged, the first of which was inspired by the natural lipoglycopeptide teicoplanin (**2**, Figure 1). These analogues (e.g., **3**)[6] carry a signature 2′-*N*-acyl glucosyl moiety, illustrated by teicoplanin (**2**) and dalbavancin (**4**), as a replacement for the natural disaccharide of vancomycin (**1**). The alternative sugar-modified pharmacophore derives from the chlorobiphenyl-based 3′-alkylation of a glycopeptide-attached vancosamine as exemplified by oritavancin (**5**) and chlorobiphenyl vancomycin (**6**).[5a,7] In general, compounds containing the straight-chain 2′-*N*-acyl glucolipid retain activity against VanB-type VRE by avoiding the induction of resistance genes in the targeted bacteria, which allows these antibiotics to bind to the transglycosylase substrate Lipid II and inhibit bacterial cell wall biosynthesis. In contrast, compounds containing the chlorobiphenyl-substituted vancosminylglucosyl disaccharide retain activity against both VanA- and VanB-type VRE by direct inhibition of the transglycosylase, even in the absence of the Lipid II substrate.[5b] Yet, despite advances in understanding the mechanisms of vancomycin resistance and how to overcome them, the systematic study of the lipoglycopeptide structure−activity relationship (SAR) and its application toward antibiotic optimization remains restricted by the lack of efficient, divergent, synthetic strategies for lipoglycopeptide sugar modification.

Neoglycosides are formed by the chemoselective ligation of an unprotected, unactivated reducing sugar with an alkoxyamine-containing aglycon.[8,9] The stereoselectivity of the neoglycosylation reaction is dictated in part by the sugar donor, and in the case of glucose and GlcNAc, the $\beta$-anomer forms exclusively.[8] In the context of natural product glycorandomization, neogly-

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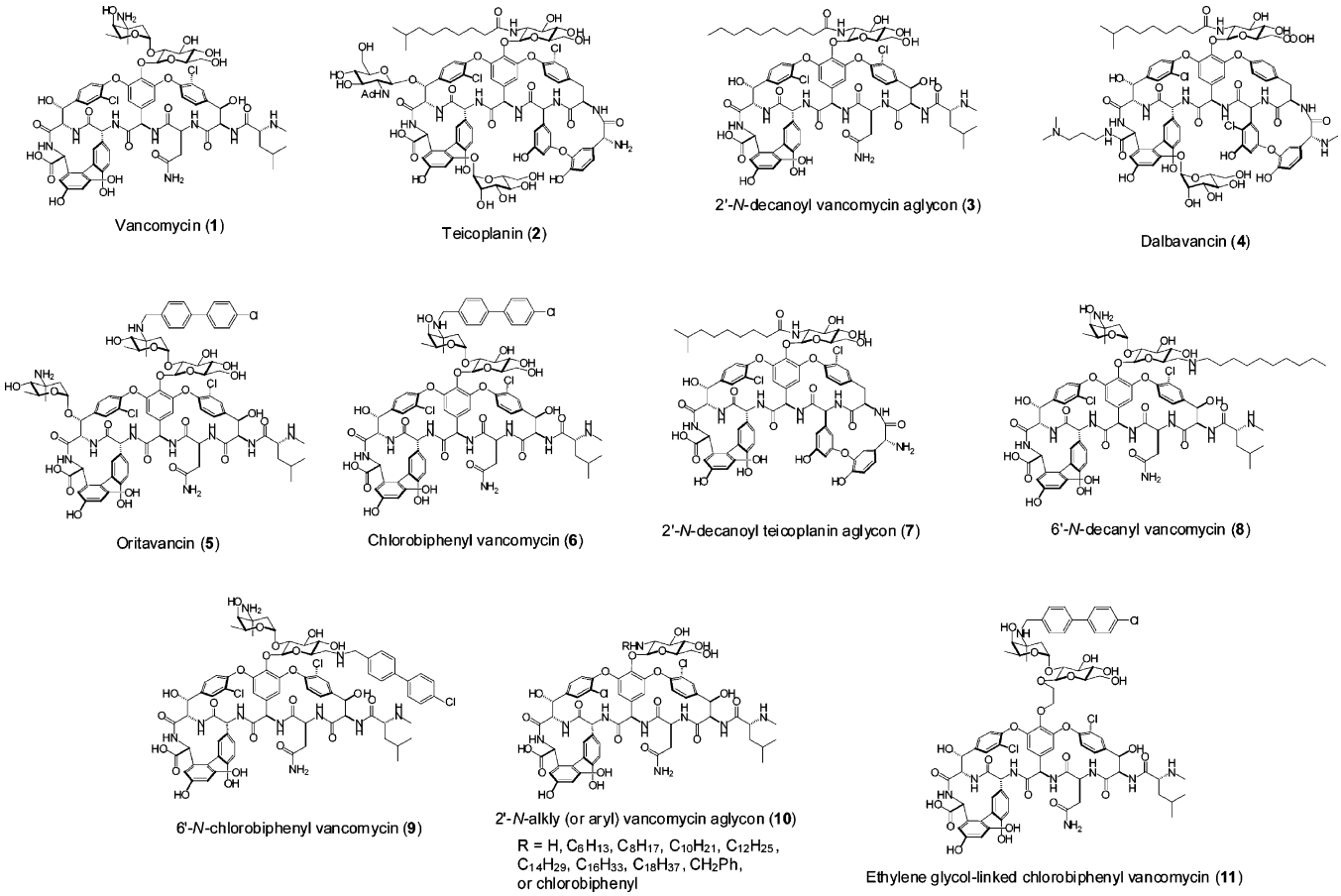


***Figure 1.*** Naturally occurring and synthetically modified (lipo)glycopeptide antibiotics. Vancomycin and teicoplanin are natural products, whereas all the other compounds have been prepared by semisynthetic methods, including chemoenzymatic techniques. Compounds **5**, **6**, **9**, and **11** are VanA-active, whereas **2**−**4**, **7**, **8**, and **10** are VanB-active.

cosylation has allowed for the rapid construction of libraries of biologically active glycosides prohibitively difficult to access by traditional chemical glycosylation.[9] We hypothesized that the application of this chemistry to lipoglycopeptides would provide access to a wider range of chemical diversity than has been achieved thus far in this class of clinically important compounds. Herein we describe the application of chemoselective "neoglycosylation" to expediently evaluate the effect of both "lipid" regiochemistry and the "lipid" composition of lipoglycopeptide variants on their biological activity.

## Results and Discussion

To initiate the application of this unique chemistry toward lipoglycopeptides, the requisite methoxyamine functionality was installed at the A4 position of **1**—the natural position of disaccharide attachment—through an ethylene glycol-type linker (Scheme 1). This strategy was based on a previous report that replacing the natural *O*-glycosidic linkage in chlorobiphenyl vancomycin with an ethylene glycol linker (see Figure 1, **11**) resulted in only a small decrease in activity.[10] Specifically, the Alloc/allyl-protected **1** aglycon **12** was prepared according to a modified literature procedure.[11] To highlight the ease and scalability of the installation of the reactive methoxyamine, beginning with 16 g of **1**, handle **13** was installed selectively at the A4 position in five simple steps to provide neoglycoside aglycon **14** in a 49% overall crude yield. Notably, crude products were advanced throughout this route and, due to the chemoselectivity of neoglycosylation, the crude nature of **14** did not interfere with the culminating neoglycosylation reaction.

For the pilot neoglycosylation, crude aglycon **14** was reacted with a 10-fold excess of 2′-*N*-decanoyl-D-glucose in 2.5% trifluoroacetic acid/dimethyl sulfoxide (TFA/DMSO) at 40 °C, and the ligation reaction was monitored by HPLC (Figure 2a). A significant amount of starting material (20 min) was consumed after 24 h, and a single new peak was observed at 34 min. Within 48 h, the starting material was consumed, and the newly generated material was subsequently isolated and fully characterized by NMR spectroscopy and FT-MALDI-MS. An examination of the 1D $^1$H and 2D TOCSY spectra verified that the isolated product was homogeneous, representing one compound and not a mixture of regio- or stereoisomers. The relevant glucolipid signals were subsequently identified by TOCSY, HSQC, and HMBC experiments (Figure 2b−d). The attachment of the glucolipid regioselectively to the methoxyamine was

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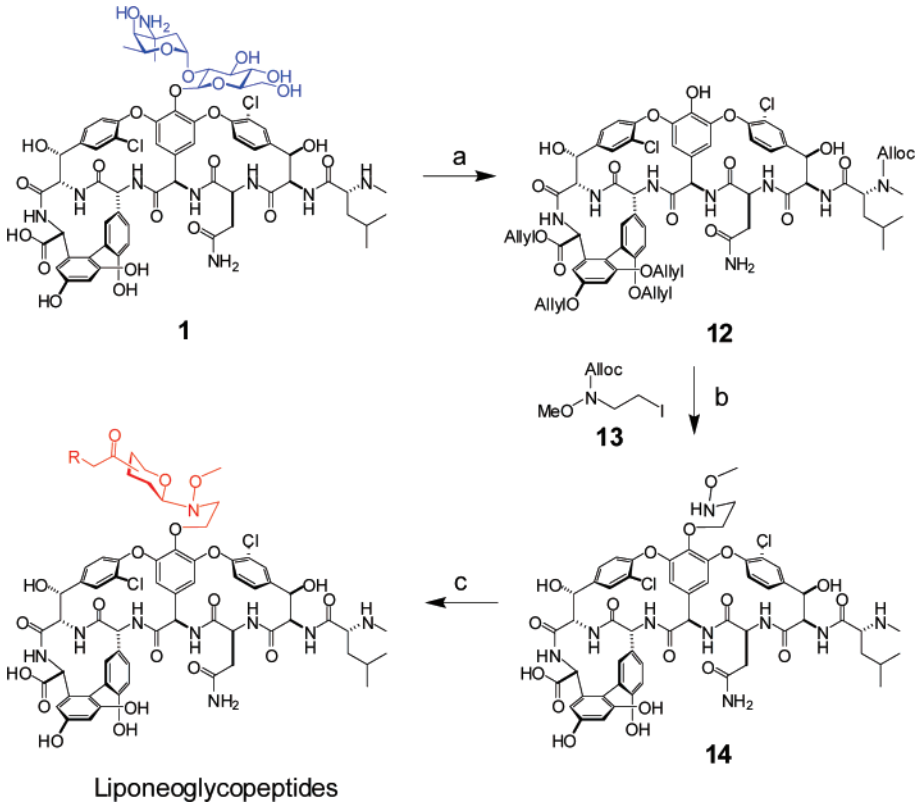
***Scheme 1.*** Synthesis of "Liponeoglycopeptide" Antibiotics[a]




[a] (a) (i) 4 equiv of Alloc-OSu, 3.3 equiv of $NaHCO_3$, DMF, rt, 16 h; (ii) 9 equiv of allyl bromide, 4.5 equiv of $Cs_2CO_3$, DMF, rt, 16 h; (iii) 1% HBr/HOAc, 6 equiv of PhSH, rt, 0.5 h. (b) (i) 2 equiv of **13**, 1.2 equiv of $Cs_2CO_3$, DMF, rt, 48 h; (ii) 0.7 equiv of $Cl_2Pd(PPh_3)_2$, 120 equiv of $Bu_3SnH$, DMF/HOAc (3:1), rt, 0.5 h, 49% crude (80% pure) over 5 steps. (c) 10 equiv of *N*-acyl-D-glucose, 2.5% TFA/DMSO, 40 °C, 24−72 h, 61% conversion (average).

***Table 1.*** Conversions, Yields, and H1 *J* Values for the Neoglycosylation Reactions

| compound | conversion (%)[a] | yield (%)[b] | anomeric $^1$H *J* value[c] |
|---|---|---|---|
| **15** | 70 | 25 | 9.6 |
| **16** | 53 | 14 | 8.8 |
| **17** | 54 | 26 | 8.8 |
| **18** | 44 | 14 | 9.0 |
| **19** | 26 | 15 | 10.0 |
| **20** | 81 | 15 | 8.8 |
| **21** | 80 | 26 | 9.0 |
| **22** | 78 | 30 | 8.8 |

[a] Determined by HPLC. [b] Isolated yield of pure compound. [c] Determined by 1D TOCSY.

verified by a strong HMBC correlation between the anomeric proton and the methylene protons adjacent to the glycosidic nitrogen. A subsequent 1D TOCSY experiment on the glucolipid spin system demonstrated an anomeric coupling constant of 9.6 Hz, consistent with a $\beta$-configured glucosidic linkage in **15**.

Following the pilot reaction conditions, seven additional lipid-variant analogues were prepared in parallel (Figure 3a) using 65 mg of crude aglycon **14** per reaction. The 2-, 3-, and 6-substituted glucolipid donors for these reactions were synthesized directly from the corresponding aminoglucosides, which were either commercially available (2-amino-D-glucopyranose) or prepared according to literature procedures (3- and 6-amino-D-glucopyranose).[12] The 3- and 6-amino-D-glucopyranoses were efficiently prepared on large scale and derivatized with lipid appendages without protecting groups—a marked advantage over classical glycosylation strategies. In contrast, the synthesis of the 4-substituted glucolipids did require 1,2-protection and deprotection, before and after lipid attachment, respectively. To confirm reaction stereoselectivity, each new product was analyzed by NMR as previously described for **15**. TOCSY experiments established that, as in the case of **15**, all of the isolated materials were single isomers. Further 1D TOCSY experiments on the relevant glucolipid spin systems provided anomeric coupling constants of 8.8−10.0 Hz, demonstrating complete $\beta$ stereoselectivity, regardless of the sugar donor.[12] Table 1 summarizes the synthetic results obtained in this study and highlights the capacity of our approach to rapidly create liponeoglycopeptide variants.

In the final neoglycosylation reaction, all eight sugar donors provided the desired products in a single step with conversions ranging from 26 to 81% and isolated yields from 14 to 30% (Table 1). While the yields of this culminating glycosylation reaction are lower than we desired, the general ease and efficiency of the overall synthetic strategy is advantageous over existing alternatives. Although the syntheses for the corresponding *O*-glycosides of compounds **15**−**22** have not been reported, the syntheses of similar analogues (Figure 1, compounds **3** and **6**−**11**) may provide some insight into the prior state-of-the-art in lipoglycopeptide sugar modification. Perhaps the most striking difference between the current strategy and preexisting ones is the overall synthetic design. Previous syntheses of lipoglycopeptides have been largely target-oriented linear routes,[6,10,13,14] leading to the synthesis of desired analogues on a small scale

(12) See the Supporting Information.

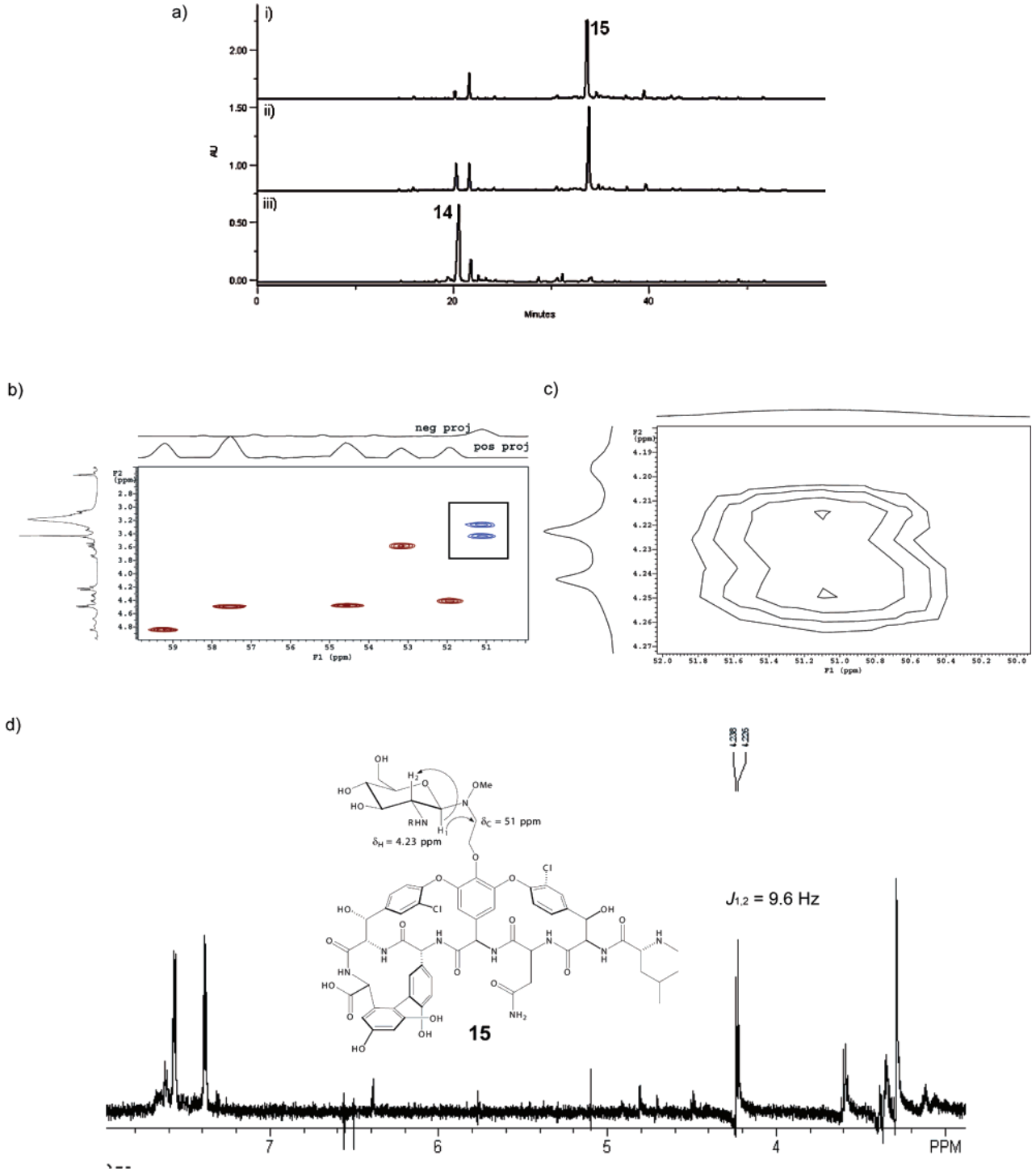



***Figure 2.*** Pilot neoglycosylation: Formation and characterization of compound **15**. (a) HPLC analysis is shown for the pilot glycosylation reaction: (i) 48 h reaction aliquot; (ii) 24 h reaction aliquot; (iii) 0 h reaction aliquot. After 24 h, the starting material (**14**) was partially consumed, and a new peak at 34 min was observed. The starting material was mostly consumed after 48 h, giving a 70% conversion to the product **15**. (b) An HSQC experiment on **15** at 500 MHz and 77 °C shows the methylene α to the glycosidic nitrogen as a negative signal at 51.1 ppm in the inset. (c) An HMBC experiment under the same conditions shows a strong correlation between the anomeric proton (H1) at 4.23 ppm and the methylene carbon identified in (b). (d) A 1D TOCSY experiment at 800 MHz and 45 °C on the glucolipid spin system shows an anomeric coupling constant ($J_{1,2}$) of 9.6 Hz.

and encompassing a relatively small amount of chemical diversity by modifying a single sugar position. In contrast, the use of neoglycosylation is highly divergent from a single intermediate, which can be prepared on a multigram scale in 49% overall yield (80% pure) without any chromatography. Once the neoglycosylation aglycon is in hand, a single step can encompass diversification of the lipid and/or sugar attachment. Furthermore, while chemoenymatic advances in glycosylation and acylation hold promise for exploring the SAR of sugar diversity, applications reported to date remain restricted by reaction scale and enzyme specificity.[4c,4e,15]

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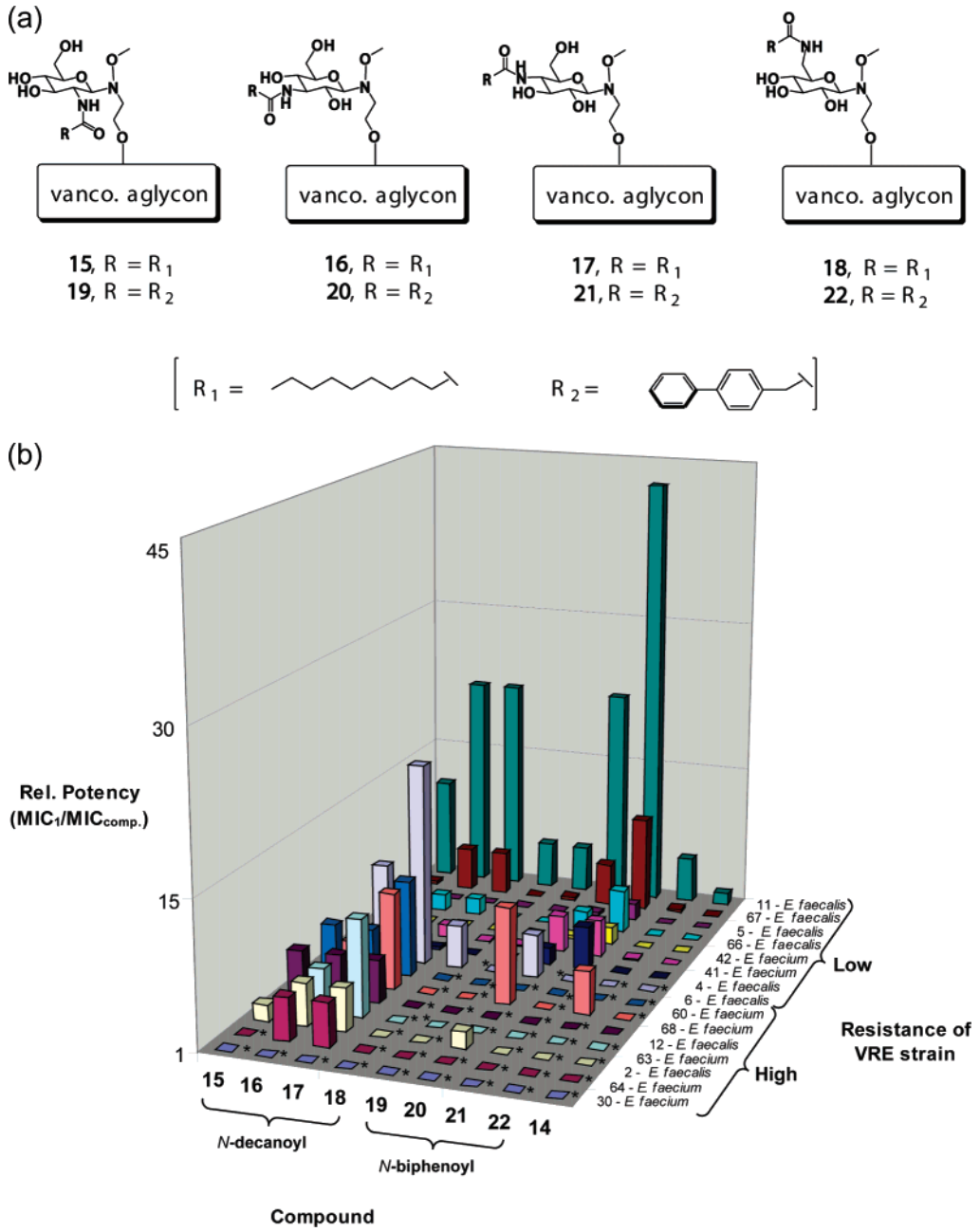


***Figure 3.*** Structure and activity of neolipoglycopeptides. (a) Representation of the **1** substitutions at position A4. (b) Relative potency of liponeoglycopeptides against a VRE panel, as defined by the MIC of **1** ($MIC_1$) divided by the MIC of the compound of interest ($MIC_{comp.}$) for each strain. MIC values were obtained using a standard microdilution assay. The MIC is defined as the lowest concentration at which no growth was visible after incubation at 35 °C for 22 h. VRE strains showed $MIC_1$ from 1 to 1024 $\mu$g/mL and included primary clinical isolates and standard ATCC strains. An asterisk (∗) indicates no bacteriostatic activity was observed at or below the compound's solubility limit (100 $\mu$g/mL) under the assay conditions.

Liponeoglycopeptides **15**−**22** and aglycon **14**, in purified form, were subsequently tested against a panel of 15 different **1**-resistant clinical isolates of *Enterococci*, representing low- and high-level VRE. Figure 3b illustrates the corresponding VRE activity of these liponeoglycopeptides, with the best compound displaying a >40-fold enhancement in potency over **1**. This broad analysis revealed the *N*′-biphenoyl analogues **19**−**22** to display specificity toward the low-level VRE strains and the *N*′-decanoyl derivatives **15**−**18** to display specificity toward the highly resistant strains. Interestingly, while the *N*′-decanoyl series performed better overall, both series of compounds showed a marked preference for substitution at the sugar 3′- or 4′-position. Previous studies with 2′-*N*-decanoyl-**1** and 6′-*N*-decanoyl-**1** (Figure 1, compouunds **3** and **8**, respectively) revealed these modifications to favor activity against low-level VRE.[6,13] Additionally, the insertion of an ethylene glycol linker between the aglycon and sugar (Figure 1, compound **11**)[10] in a previous report did not alter the low-/high-level VRE bias of the parent compound. Conversely, *N*′-decanoyl substitution within liponeoglycopeptides **16** and **17** favors activity against high-level VRE. These perceived differences in VRE specificities among related lipoglycopeptides and liponeoglycopeptides, although preliminary, may implicate mechanism of action distinctions. In addition, although structural differences may not

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allow for a direct comparison between lipoglycopeptides and liponeoglycopeptides, the current study implicates the glucosyl 3′- or 4′-position as a target to consider for future glycopeptide derivatization.

## Conclusions

In summary, we have developed a purely chemical method for the diversification of the glycolipid portion of lipoglycopeptide antibiotics that is simple to perform on a large scale, requires minimal synthetic effort in sugar donor preparation, and provides access to highly active antibiotics that are not easily prepared by other state-of-the-art methods. Our results demonstrate that the natural glycopeptide *O*-glycosidic linkage can be replaced with the neoglycoside *N*-glycosidic linkage while enhancing biological activity. We also find that, with liponeoglycopeptides, VRE activity is favored by the attachment of the lipid at the "unnatural" 3- or 4-position, versus the "natural" 2-position, of the glucosyl moiety. In addition, this new class of "liponeoglycopeptides" may add to the repertoire of novel reagents to aid in the study of transpeptidase/transglycosylase inhibition mechanisms,[16] as well as new strategies to delineate the molecular details of bacterial cell wall biosynthesis.[17]

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**Supporting Information Available:** Synthetic procedures, spectroscopic data, procedures for antibiotic testing, strain identification, and raw biological data. This material is available free of charge via the Internet at http://pubs.acs.org.

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