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Synthesis and Evaluation of Selected Key Methyl Ether Derivatives of Vancomycin Aglycon

Christine M. Crane,[†] Joshua G. Pierce,[†] Siegfried S. F. Leung,[‡,§] Julian Tirado-Rives,[‡] William L. Jorgensen,[‡] and Dale L. Boger*[,†]

[†]*Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and* [‡]*Department of Chemistry, Yale University, 225 Prospect Street, PO Box 208107, New Haven, Connecticut 06520-8107.* [§]*Present address: Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, California 94158*

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A select series of methyl ether derivatives of vancomcyin aglycon were prepared and examined for antimicrobial activity against vancomycin-sensitive *Staphylococcus aureus* and vancomycin-resistant *Enterococci faecalis* as well as their binding affinity for D-Ala-D-Ala and D-Ala-D-Lac. The intent of the study was to elucidate the role selected key methyl groups may play in the improvement of the in vitro antimicrobial profile of the tetra methyl ether derivative of vancomycin aglycon against vancomycin-resistant *Enterococci faecalis* previously reported. In these studies, methodology for selective derivatization of the A-, B-, and D-ring was developed that defines the relative reactivity of the four phenols of vancomycin aglycon, providing a foundation for future efforts for site-directed modification of the vancomycin aglycon core.

Introduction

Vancomycin (**1**) is the antibiotic of last resort used to treat Gram-positive bacterial infections, especially those caused by methicillin-resistant *Staphylococcus aureus* and for patients allergic to β-lactam antibiotics. Vancomycin's biological activity arises from its ability to bind to the terminal L-Lys-D-Ala-D-Ala portion of the bacterial cell wall peptidoglycan precursor, preventing transpeptidase-catalyzed cross-linking and maturation of the bacterial cell wall.[1] An inducible vancomycin-resistance pathway has evolved in *Enterococcoci* bacterial strains (VanA[a] and VanB) that is now beginning to emerge in *Staphylococcus aureus* (*S. aureus*),[1e,f] whereby the *C*-terminal amino acid is modified from D-alanine to D-lactic acid (Figure 1).[1,2] This single atom modification (NH → O) reduces the affinity of **1** for the cell wall peptidoglycan precursor 1000-fold, rendering the antibiotic ineffective, with a corresponding 1000-fold drop in antimicrobial activity.[1d] This loss of affinity and antimicrobial activity can be attributed to both the loss of a key H-bond and the introduction of lone pair repulsive interactions between the residue 4 carbonyl of vancomycin and the ester oxygen of the D-Lac on the *C*-terminus of the ligand.[3,4a] The former contributes no more than 10-fold and the latter at least 100-fold to the experimentally observed 1000-fold loss in affinity for the ligand.[3,4a]

Significant efforts have been directed toward the discovery of next-generation glycopeptide antibiotics that address the emerging vancomycin resistance.[4−11] Numerous modifications have been introduced to the natural product that have

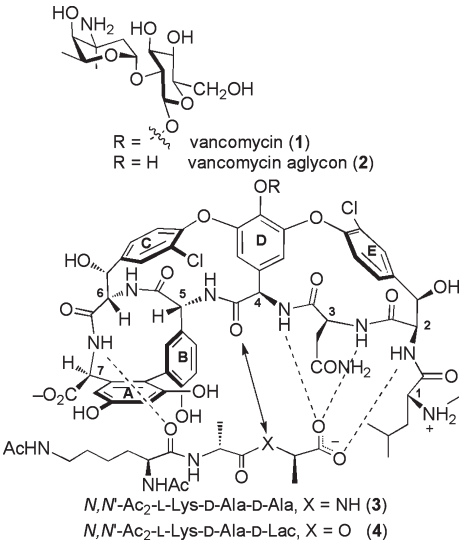

Figure 1. Schematic representation of the interactions between vancomycin (**1**), vancomycin aglycon (**2**), and model ligands *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Ala (**3**) and *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Lac (**4**).

improved the in vitro antimicrobial activity of these vancomycin derivatives against vancomycin-sensitive and resistant strains.[5−8] In our own efforts and complementary to semisynthetic modifications of key residues,[7,8] the offending residue 4 carbonyl of the natural product was removed and provided [Ψ[CH$_2$NH]Tpg^4]vancomycin aglycon (**5**), a compound prepared by total synthesis, which exhibited significantly improved activity against resistant VanA bacteria and dual binding properties for both peptidoglycan analogues *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Ala (**3**) and *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Lac (**4**), (Figure 2).[4a]

In the course of these and related efforts and initially enlisting derivatives of key synthetic intermediates,[4c] we found

*To whom correspondence should be addressed. Phone: 858-784-7522. Fax: 858-784-7550. E-mail: boger@scripps.edu.

[a]Abbreviations: VanA, vancomycin/teicoplanin type-A resistant; VanB, teicoplanin-senstitive and vancomycin type-B resistant; VRE, vancomycin-resistant *Enterococci*; H-bond, hydrogen bond; D-Lac, D-lactic acid; TFA, trifluoroacetic acid; Pd/C, palladium on activated carbon; MIC, minimum inhibitory concentration.

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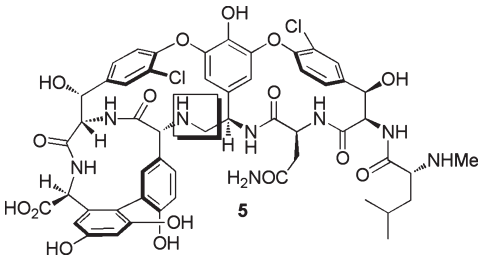
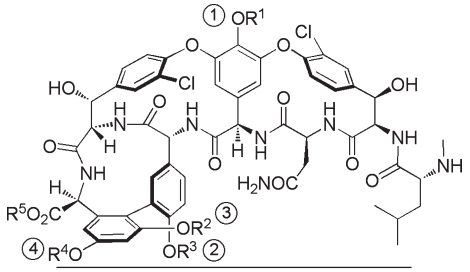


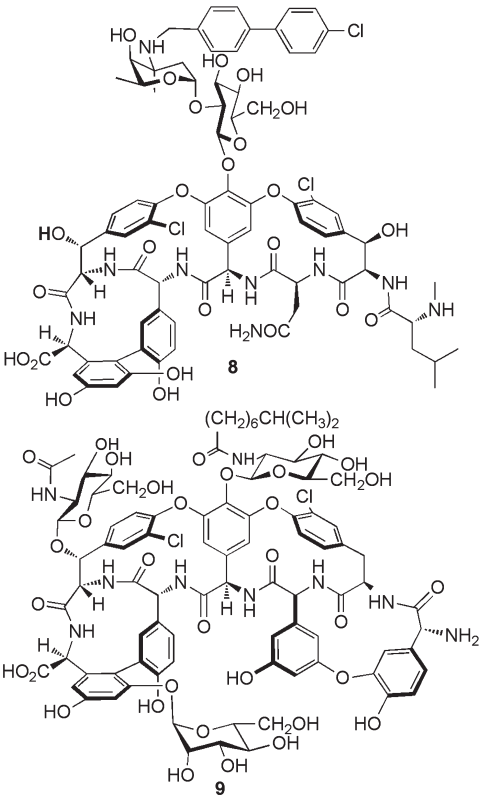
Figure 2. [Ψ[CH_2NH]Tpg^4]Vancomycin aglycon (**5**).



Compound	R^1	R^2	R^3	R^4	R^5
2	H	H	H	H	H
6	Me	Me	Me	Me	H
7	Me	Me	Me	Me	Me
10	H	Me	H	Me	H
11	H	H	Me	Me	H
12	H	Me	Me	Me	H
13	Me	H	H	H	H

Figure 3. Analogues of vancomcyin aglycon.

that derivatives of vancomycin aglycon (**2**), in which each of the four phenols were protected as methyl ethers (Figure 3), exhibited effective antimicrobial activity against both vancomycin sensitive and resistant bacteria (Figure 4). In further exploring the activity, we found that such hydrophobic derivatives of vancomycin aglycon (e.g., **6**−**7**) exhibited equipotent antimicrobial activity against the sensitive and VanB resistant bacteria (Figure 4) and displayed improved activity against VanA bacteria without possessing significantly altered binding affinity for model ligands **3** and **4**.[7,8] This profile of activity is identical, but less potent than that of chlorobiphenyl vancomycin[1a,6] (**8**, and the related analogue oritavancin (LY333328)[5,12]) and teicoplanin (**9**),[7] which bear hydrophobic side chains on the saccharides attached to the phenol of the central residue (Figure 4). Impressively, analogous methyl ether derivatization of the teicoplanin or ristocetin aglycons similarly provided equipotent antimicrobial activity against sensitive and VanB resistant bacteria, establishing the generality of the observations.[7] Notably, derivatives such as **6**−**7** lack a lipid side chain and a disaccharide, suggesting that membrane anchoring[13] and transglycosylation inhibition[14] effects invoked for teicoplanin and **8** may not account for the origin of the effects with **6**. In an extension of this work and in concert with computational studies,[11] we sought to further define the role methyl substitution of the phenols plays in contributing to these properties. Most notable in the computational studies was the generalized trend for a residue 7 C3 methoxy (vs phenol hydroxy, R^2 herein, Figure 3) substituent to improve binding to both D-Ala-D-Ala and D-Ala-D-Lac, whereas analogous changes at C5 (R^4 herein, Figure 3) were benign and those at residue 5 at C4 (R^3 herein, Figure 3) were



MIC, μg/mL	*S. aureus*	*E. faecalis VanB*	*E. faecalis* VanA
vancomycin (**1**)	1	>100	>100
aglycon (**2**)	0.6	>100	>100
6	1.3	2.5	80
7	1.6	1.6	40
8	0.03	0.03	10
teicoplanin (**9**)	0.2	0.2	>100

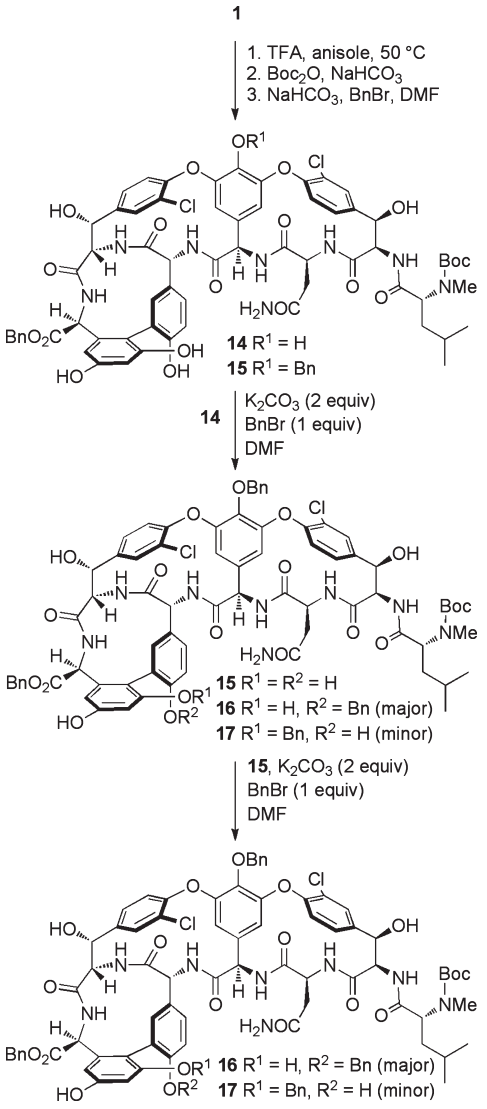
Figure 4. Antimicrobial activity.

counterproductive to D-Ala-D-Lac binding. Especially attractive was the projected binding characteristics of compound **10** (R^1 = R^3 = H, R^2 = R^4 = Me, Figure 3) that was computed to improve affinity for D-Ala-D-Ala by 1.2 kcal/mol and D-Ala-D-Lac by 1.7 kcal/mol. Consequently, compound **10** and its comparison dimethyl ether isomer **11** were of special interest to us to examine. For this purpose, selective derivatization of the phenols was established and provided a key set of mono-, bis-, and tris-methyl ethers **10**−**13** (Figure 3) and both the association constants (K_a) for the ligands **3** and **4** as well as their in vitro antimicrobial activity established. Significantly, a sequential reactivity order (Figure 3) for the four phenols of the vancomycin aglycon was established that will permit additional future semisynthetic modifications.

Chemistry

Vancomycin aglycon (**2**) was prepared according to a modified literature procedure by TFA-mediated deglycosylation of **1** in the presence of excess anisole (Scheme 1).[15] Boc-protection, followed by benzyl ester formation (2 equiv BnBr, $NaHCO_3$, DMF, 25 °C, 18 h, 47%) provided **14**. The use of $NaHCO_3$ (vs Na_2CO_3) allows for selective esterification of the carboxylic acid, whereas stronger inorganic bases led to competitive phenol alkylation. However, the yields of ester **14** were significantly lower if more than two equivalents of

Scheme 1



BnBr was used, as competing monobenzyl ether formation of the D-ring phenol was observed even with $NaHCO_3$ to provide **15** (Scheme 1). Subsequent, deliberate stepwise alkylation of **14** (1 equiv BnBr, 2 equiv K_2CO_3, DMF, 25 °C, 18 h) provided the D-ring monobenzyl ether **15** (42%), and a mixture of bis-benzyl ethers **16** and **17** (25%, Scheme 1) resulting from D-ring benzyl ether formation, followed by A- or B-ring benzyl ether formation. The mixture of bis-benzyl ethers **16** and **17** could also be obtained from deliberate benzylation of **15** (1 equiv BnBr, 2 equiv K_2CO_3, DMF, 25 °C, 18 h) in 56% yield (providing a 2:1 mixture of **16**/**17**), without additional over benzylated products being isolated. The above result defines a stepwise order of alkylation of the phenols of vancomycin aglycon that is depicted in Figure 3, and this was also observed when methyl iodide was used as the alkylating agent (data not shown).

The mixture of **16** and **17** (2:1 regioselectivity, respectively) was separated, and the assignment for each structure was conducted by 2D-ROESY (Figure 5). Compound **16** displayed diagnostic nuclear Overhauser enhancement (NOE) cross-peaks between the unalkylated phenol protons of the A-ring (OH^a and OH^b) to their respective adjacent aromatic protons (H^1 and H^2), one of which they share (H^2), and the

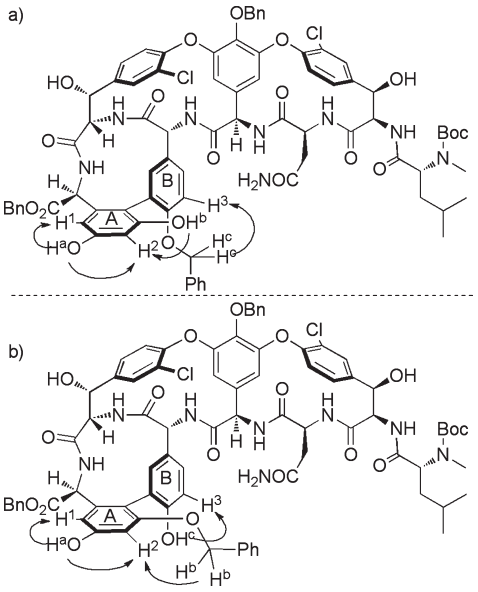
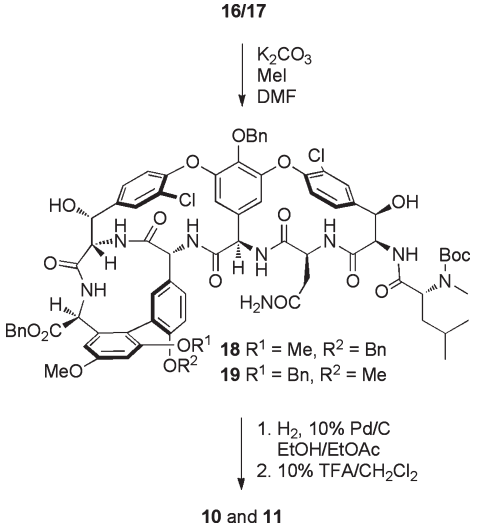


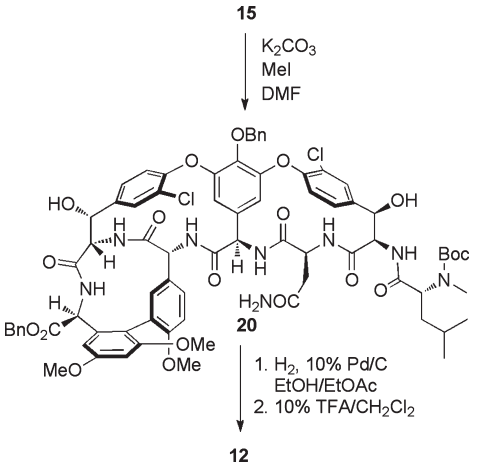
Figure 5. Diagnostic NOE cross-peaks for (a) **16** and (b) **17** in 100% DMSO-d_6.

Scheme 2

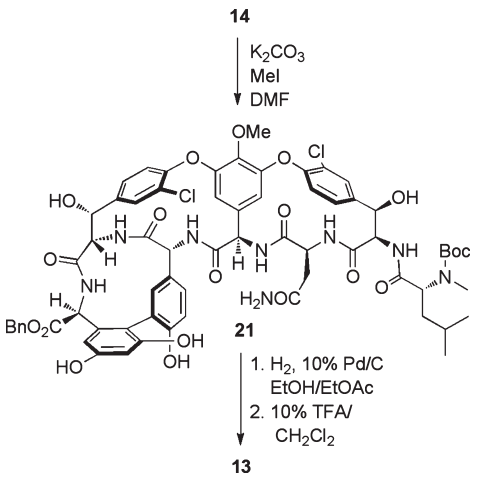


benzylic protons of the B-ring benzyl ether (CH_2^c) exhibited a diagnostic NOE cross-peak with its adjacent aromatic proton H^3 on the B-ring (Figure 5a, for the 2D-ROESY of **16** see the Supporting Information Figure 1SI). Conversely, compound **17** displayed NOE cross-peaks between the unalkylated phenol proton of the A-ring (OH^a) to its respective adjacent aromatic protons (H^1 and H^2), the benzylic protons of the A-ring benzyl ether exhibited a diagnostic NOE cross-peak with its adjacent shared aromatic proton H^2 on the A-ring, and the unalkylated phenol of the B-ring displayed a diagnostic NOE cross-peak to its adjacent buried aromatic H^3 proton (Figure 5b, for 2D-ROESY of **17** see the Supporting Information Figure 2SI). Subsequent global methylation of **16** (40 equiv methyl iodide, 10 equiv K_2CO_3, DMF, 25 °C, 18 h) provided the fully alkylated derivative **18** in 54% yield (Scheme 2). Global methylation of regioisomer **17** proved more challenging and in the end required additional methyl iodide (60 equiv) to provide the desired fully alkylated intermediate **19**. Subsequent hydrogenolysis of the benzyl ethers and benzyl ester of **18**

Scheme 3



Scheme 4



and **19** using H_2 (g) over 10% Pd/C (EtOH/EtOAc, 25 °C, 4−16 h), followed by acidic removal of the Boc-group with TFA in methylene chloride (1:9, 25 °C, 15 min), provided the target molecules **10** and **11** as their trifluoroacetic acid (TFA) salts after RP-HPLC purification, in 81% and 56% yield, respectively (Scheme 2, for 2D-ROESY data see the Supporting Information Figures 3SI−4SI).

Tris-methyl ether derivative **12** was prepared starting from **15** (Scheme 3). Methylation of the remaining phenols (40 equiv methyl iodide, 10 equiv K_2CO_3, DMF, 25 °C, 18 h) provided **20** in 58% yield. Intermediate **20** was debenzylated (H_2, 10% Pd/C, EtOH/EtOAc, 25 °C, 3−16 h) and then treated with TFA/CH_2Cl_2 (1:9, 25 °C, 15 min) to afford **12** as its TFA salt after HPLC purification in 62% yield (for 2D-ROESY data, see the Supporting Information Figure 5SI).

In a similar manner, the D-ring mono methyl ether **21** (Scheme 4) was prepared by monomethylation of the D-ring of **14** (1 equiv methyl iodide, 2 equiv K_2CO_3, DMF, 25 °C, 18 h). Subsequent hydrogenolysis of the benzyl ester (H_2, 10% Pd/C, EtOH/EtOAc, 25 °C, 3−16 h), and removal of the Boc group (TFA/CH_2Cl_2, 25 °C, 15 min) provided the D-ring methyl ether **13** as its TFA salt in 82% yield after HPLC purification (for 2D-ROESY data, see the Supporting Information Figure 6SI).

Results and Discussion

The antimicrobial properties of **10**−**13** were determined against a vancomycin-sensitive *Staphylococcus aureus* (*S. aureus*, strain ATCC 25923), a vanomycin-resistant and teicoplanin-sensitive (VanB) *Enterococcus faecalis* (*E. faecalis*, strain ATCC 51299), and a vancomycin- and teicoplanin-resistant (VanA) *E. faecalis* (strain BM4166) in a microtiter plate-based antimicrobial assay (Table 1).[4,7,8] The VanB strain (ATCC 51299) and the latter VanA strain (BM4166) possess a vancomycin (**1**) inducible resistance pathway that becomes operative upon treatment with glycopeptide antibiotics. VanA and VanB utilize D-Ala-D-Ala peptidoglycan cell wall precursors when left unchallenged but will switch to D-Ala-D-Lac peptidoglycan cell wall precursors upon treatment with a glycopeptide. The association constants (K_a) for ligands **3** and **4** and free energies of binding ($\Delta\Delta G_b$) for compounds **10**−**13** were determined using a well-established UV-difference titration assay.[16] The experimentally determined K_a was then used to calculate $\Delta\Delta G_b$s, which were then compared to those determined computationally (Table 2).[7,8,11,16] For an representative saturation binding curve with **3** and **4** and subsequent Scatchard analysis, see the Supporting Information Figures 7SI−8SI.

Table 1. Antimicrobial Properties

compd	*S. aureus*[a]	MIC (μg/mL) *E. faecalis*[b] (VanA)	*E. faecalis*[c] (VanB)
1	1.25 (1.25)[e]	2000 (2000)[e]	120 (125)[d,e]
2	1.25 (0.625−1.25)[d,e]	640 (640)[d,e]	40 (80)[d]
6	1.25 (0.625)[d]	80 (40)[d]	2.5
7	1.25 (0.625−1.6)[d]	40 (40−50)[d]	1.25 (1.6−10)[d]
10	10	640	120
11	10	640	120
12	5	640	10
13	1.25	240	40

[a]Vancomycin-sensitive *Staphylococcus aureus* (strain ATCC 25923). [b]Vancomycin-resistant *Enterococci faecalis* (VanA, strain BM4166). [c]Vancomycin-resistant *Enterococci faecalis* (VanB, strain ATCC 51299). [d]Taken from refs 7, 8. [e]Taken from refs 4.

The compounds exhibited an interesting range of activity in the antimicrobial assays against the sensitive and the resistant bacteria. Whereas the aglycon **2** containing all four free phenols and the permethylated derivative **6** containing four methyl ethers were equally potent against the sensitive *S. aureus* strain (MIC = 1.25 μg/mL, strain ATCC 25923), partial methylation of the phenols led to significant losses in activity. Only **13**, bearing a single methyl ether on the D-ring phenol, matched the activity of **2** and **6**, whereas the trimethyl ether **12** and the two isomeric dimethyl ethers **10** and **11** experienced 4-fold and 8-fold losses in activity, respectively. Their activity against the resistant VanB strain of *E. faecalis* (strain ATCC 51299) was even more striking and, with the exception of the vancomycin aglycon **2** itself, displayed a similar trend. The mono methyl ether **13** bearing a D-ring methyl ether, like **2** itself, was relatively inactive against this VanB strain (MIC = 40−80 μg/mL), and the two isomeric dimethyl ethers **10** and **11** were even less active (MIC = 120 μg/mL). In contrast, the activity improved substantially, with **12** bearing three methyl ethers (MIC = 10 μg/mL) and the tetramethyl ether **6** exhibited a potency (MIC = 2.5 μg/mL) essentially equivalent to its activity against sensitive *S. aureus*.

Especially interesting was the behavior against a resistant *E. faecalis* VanA strain (BM4166). VanA *E. faecalis*, which is resistant to teicoplanin (MIC > 100 μg/mL) and is also 100-fold resistant to chlorobiphenyl vancomycin **8** (10 μg/mL for VanA vs 0.03 μg/mL for sensitive strain), was resistant to not only vancomycin aglycon (**2**, MIC = 640 μg/mL), but to **10**−**12** as

Table 2. Binding Properties

	association constants (K_a, M^{-1})		$\Delta\Delta G_b^{obs,c}$ ($\Delta\Delta G_b^{cal,d}$ kcal/mol)	
compd	**3**[a] ($\times 10^5$)	**4**[b] ($\times 10^2$)	**3**[a]	**4**[b]
2	1.7(1.7)[e]	1.2 (1.2−2.0)[e,f]	0.0 (0.0)[d]	4.3 (nd)
6	1.8 (1.4)[f]	1.1 (1.6)[f]	0 (1.0 ± 0.7)[d]	4.4 (3.9 ± 0.6)[d]
10	4.3	1.2	−0.5 (-1.2 ± 0.4)[d]	4.4 (2.7 ± 0.4)[d]
11	1.7	1.0	0 (nd)	4.5 (nd)
12	1.6	1.1	0.1 (1.0 ± 0.7)[d]	4.4 (3.6 ± 0.5)[d]
13	1.8	1.0	0 (nd)	4.5 (nd)

[a] *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Ala. [b] *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Lac. [c] The free energy of binding ($\Delta\Delta G_b$) was tabulated from the experimentally determined K_as using the equation $\Delta\Delta G_b = RT \ln K$, where $K = {}^1K_a/{}^xK_a$; ${}^1K_a$ is the association constant for the complex of **2** (taken from ref 4 only) and ligand **3**; ${}^xK_a$ is the association constant for the complex of compound X with ligand of interest, see reference 11. [d] Taken from ref 11. [e] Taken from ref 8. [f] Taken from ref 4. nd = not computationally determined.

well. However, it exhibited sensitivity to **13**, albeit at a modest level (MIC = 240 μg/mL), and exhibited a significantly increased sensitivity with only **6** (MIC = 80 μg/mL). Like **8** and teicoplanin, this activity is observed at concentrations between 50−200-fold what is required for activity against sensitive bacteria.

The dimethyl ether derivative **10** did exhibit an increased binding affinity for D-Ala-D-Ala (ligand **3**, 0.5 kcal/mol) as projected by the computational studies (1.2 kcal/mol),[11] but it did not improve the affinity for D-Ala-D-Lac (ligand **4**) as projected (1.7 kcal/mol).[11] Overall, the binding affinities for either D-Ala-D-Ala or D-Ala-D-Lac were relatively unaffected by the methylation state of the phenols and do not correspond to the relative behavior of the compounds in the antimicrobial assays. The computations were correct in predicting modest effects on binding for most of the methylations, the outcome of which is not obvious due to the substantial changes in hydrogen bonding that accompany conversion of an alcohol to an ether.

Although there are several explanations that could account for this behavior and there are several ways to represent the trends in antimicrobial activity, one feature that stands out is the simple extent, not specific site, of methylation. This seemingly small change in the structures has a pronounced effect on the hydrophobic character of the compounds as judged by the chromatographic behavior (see the Supporting Information). For sensitive *S. aureus*, the marked changes in their activity may indicate a parabolic relationship with hydrophilic/hydrophobic character where the intermediate character (partial phenol methylation) is detrimental to antimicrobial activity, but either hydrophilic or the most hydrophobic derivatives are effective (Figure 6).

Extending this to an interpretation of the resistant VanB *E. faecalis* results only requires that the organism remain sensitive to the hydrophobic vancomycin aglycon derivatives, but lose their sensitivity to the hydrophilic derivatives and those of intermediate hydrophilic character (40−100 fold). Finally, only the most hydrophobic derivative **6** retains activity against resistant VanA *E. faecalis*, albeit at a reduced level, whereas the other derivatives were inactive. The only exception to this latter trend was **13**, which was 200-fold less active against resistant VanA *E. faecalis* than sensitive *S. aureus*. A simple interpretation of this behavior is that such hydrophobic derivatives of vancomycin aglycon fail to effectively trigger the inducible resistance mechanism, resulting in the switch of peptidoglycan precursor from D-Ala-D-Ala to D-Ala-D-Lac. Evidence has been disclosed to indicate that this sensing of the glycopeptide challenge can arise from cell surface receptor recognition of either the antibiotic itself[17] or the build-up of a

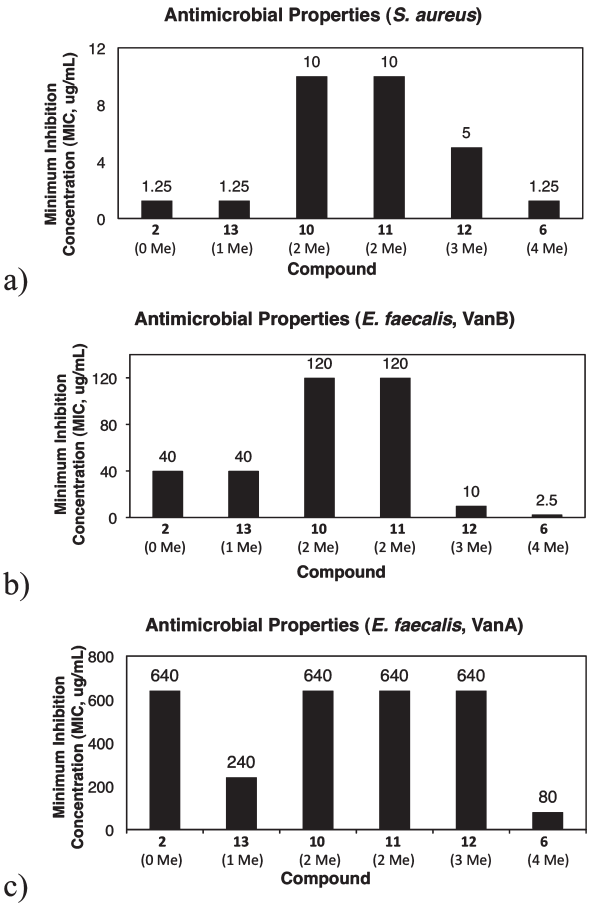


Figure 6. Bar graph representation of antimicrobial properties for compounds tested. (a) Against *S. aureus*. (b) Against *E. faecalis* (VanB). (c) Against *E. faecalis* (VanA).

bacterial cell wall biosynthetic intermediate[18] by a two-component signaling pathway. Independent of the mechanism, the results herein suggest that the hydrophobic permethylated vancomycin aglycon derivatives, and the related teicoplanin and ristocetin aglycon methyl ethers,[7] act by binding to D-Ala-D-Ala, fail to induce the resistance pathway resulting in D-Ala-D-Lac peptidoglycan generation, and serve as a distinct class of glycopeptide antibiotics effective against resistant bacteria.

The derivatives disclosed herein that display consistent activity against both sensitive and resistant VanB and VanA bacteria lack the lipophilic side chain found in teicoplanin (membrane anchoring effect) and the chlorobiphenyl disaccharide of **8** that may contribute to transglycosylase (vs transpeptidase) inhibition. We have speculated that the success of the derivatives is directly related to their hydrophobic

character and their ability to penetrate the bacterial cell membrane sufficiently to avoid induction of the two component signaling pathway responsible for initiating and maintaining the remodeling of the peptidoglycan precursor from D-Ala-D-Ala to D-Ala-D-Lac. We have suggested that **6** and **7** act by the same mechanism as vancomycin (binds D-Ala-D-Ala) but at different physical sites in the bacterial cell wall (intramembrane rather than at the cell surface), potentially avoiding recognition by the two component signaling system or leading to the build up of a different, earlier bacterial cell wall precursor. Regardless of the mechanism, further studies with such derivatives may help define additional mechanisms for avoiding the induction or emergence of bacterial resistance.

Conclusions

We have reported the first stepwise substitution of the four phenols of vancomycin aglycon, defining a rank-order alkylation and indicating each phenol has a distinct reactivity, which was used to prepare a series of key partially methylated derivatives of the vancomycin aglycon. This series was utilized to further define the contribution methyl substitution of the phenols makes in a unique series of glycopeptide antibiotic derivatives that are active against vacomycin sensitive and resistant bacteria.

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Supporting Information Available: Compounds **2** and **6** were prepared according to published procedures.[7,15] Full experimental details, characterization for all new intermediates and final compounds, and 2D-ROESY for all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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