Synthesis and Evaluation of Vancomycin Aglycon Analogues That Bear Modifications in the N-Terminal D-Leucyl Amino Acid

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The synthesis and biological evaluation of a series of vancomycin aglycon analogues bearing alternative residue 1 *N*-methyl-D-amino acids are described. The analogues were prepared to define whether H-bonding D-amino acids could improve the affinity for the model ligands *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Ala (**2**) and *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Lac (**3**) and improve antimicrobial activity against vancomycin-sensitive or vancomycin-resistant bacteria. Additionally, a series of analogues with appended nucleophiles (hydrazines and amines) on the residue 1 D-amino acids are described that were examined for their ability to react with the C-terminal ester of **3**, forming a covalent attachment of L-Lys-D-Ala to the natural product analogues.

Introduction

Vancomycin (**1**) is used as the antibiotic of last resort for the treatment of Gram-positive bacterial infections, especially those caused by methicillin-resistant *Staphylococcus aureus*, and for patients who are allergic to β-lactam antibiotics. Vancomycin binds to the peptide terminal portion of the bacterial cell wall peptidoglycan precursor, specifically to the sequence L-Lys-D-Ala-D-Ala, thereby inhibiting transpeptidase-catalyzed cross-linking and maturation of the bacterial cell wall.[1] However, *Enterococcocci* bacterial strains, VanA and VanB,[a] have developed an inducible vancomycin-resistance pathway, in which the C-terminal amino acid is modified from D-alanine to D-lactic acid (Figure 1).[1,2] This modification (NH → O) reduces the affinity of **1** for the ligand 1000-fold and renders the antibiotic ineffective, with a corresponding 1000-fold drop in antimicrobial activity.[1d] This loss of affinity has been partitioned into the loss of a key H-bond and 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.[3,4] The former contributes 10-fold and the latter 100-fold to the experimentally observed 1000-fold loss in affinity for the ligand.[3,4]

A significant amount of the lost affinity and antimicrobial activity can be restored by simply removing the offending residue 4 carbonyl of the natural product.[4] The resulting methylene derivative Ψ[CH_2NH]Tpg[4]]vancomycin aglycon (**4**) was recently prepared by total synthesis and was shown to exhibit significant activity against resistant VanA bacteria and dual binding properties for both *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Ala (**2**) and *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Lac (**3**) (Figure 2).[4] In continuing efforts to improve on the properties of **4**, we wished to establish whether further modification at the N-terminus of **4** with unnatural amino acids presenting appended H-bond donating groups that H-bond to the residue 2 carbonyl of the ligand might further enhance its affinity for the model ligand **2** or **3**,

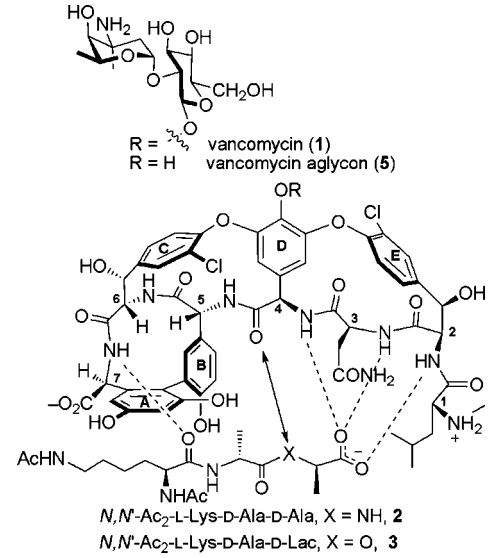

Figure 1. Schematic representation of the interactions between vancomycin (**1**), vancomycin aglycon (**5**), and model ligands *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Ala (**2**) and *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Lac (**3**).

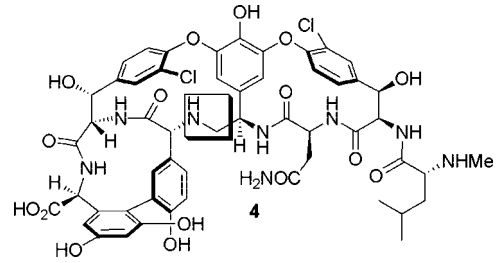

Figure 2. [Ψ[CH_2NH]Tpg[4]]Vancomycin aglycon.

and thereby further improve the antimicrobial properties. However, rather than embark on a total synthesis of such analogues, the premise could be easily investigated first by preparing the analogues on the aglycon of vancomycin **5** (Figure 3), where semisynthetic procedures are well-established.[5,6] Thus, the analogues containing N-terminal D-serine (**6**), *O*-*tert*-butyl-D-serine (**7**), D-threonine (**8**), mono- or bidentate H-bond donating analogues of 3-amino-D-phenylalanine (**9**−**11**), 3-aminomethyl-D-phenylalanine (**12**), and their corresponding hydra-

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[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; DCC, dicyclohexylcarbodiimde; TFA, trifluoroacetic acid; Pd/C, palladium on activated carbon; EtNCO, ethyl isocyanate; Pt/C, platinum on activated carbon; $NaBH_3CN$, sodium cyanoborohydride; MIC, minimum inhibition concentration; Et_3N, triethylamine.

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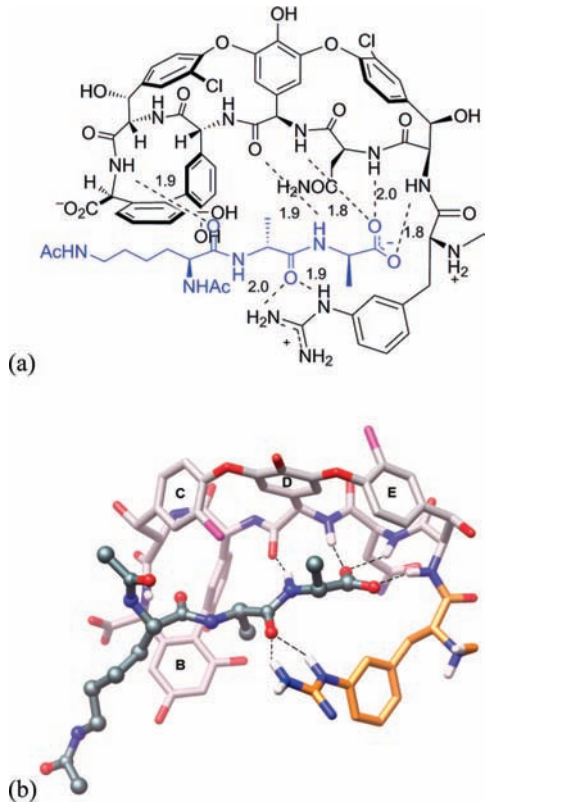


Figure 3. Proposed binding of the N-terminally modified vancomycin analogue **11** with ligand **2** as determined by molecular modeling using the program MOLOC.[7a,b] Molecular modeling was preformed starting with the X-ray crystal structure of **5** cocrystallized with **2** determined to 1.8 Å resolution (PDB code 1FVM):[7c,d] (a) schematic view; (b) ball and stick representation. Black dashed lines represent H-bonds. Distances between H-atom and heteroatom of H-bonding partner are given in angstroms. Color code is as follows: ligand skeleton, dark-gray; vancomycin skeleton, light-gray; N-terminal amino acid skeleton, orange; O, red; N, blue; H, white. Figure was generated with the molecular graphics program Chimera.[8]

zine counterparts **13**−**15** were prepared herein for examination (Figure 4). The modifications at the N-terminus of **5** were expected to alter the binding pocket for the ligand (Figures 1SI and 2SI of Supporting Information), an effect that could be compensated for by the H-bond donating residues.

The effect of N-terminal modification on the antimicrobial activity for a series of chlorobiphenyl vancomycin derivatives has been explored by Kahne and co-workers.[9] Chlorobiphenyl vancomycin and related analogues operate with a different or additional mode of action than vancomycin and are ∼100-fold more active against vancomycin-sensitive bacteria and vancomycin-resistant *Enterococci* (VRE). Moreover, and unlike vancomycin, this activity is maintained when the N-terminal D-leucyl residue is modified or completely removed.[1a,10] Williams and co-workers[5] probed the charge verses nonpolar contributions of the *N*-methylammonium and D-leucyl moieties of **1**, and Hunt and co-workers[11] have examined the antimicrobial properties of *N*-demethylvancomycin (2-fold enhancement). In addition, Preobrazhenskaya and co-workers[12] studied the effect on antimicrobial activity of N-acylation or nitrosylation at the N-terminus of vancomycin. Most recently, Williams and co-workers reported the effect of acylation at the N-terminus *N*-methylamine of **1** on the affinity for **2**.[13]

Chemistry

Desleucylaglucovancomycin (**16**) was prepared in two steps according to literature procedures starting from vancomycin

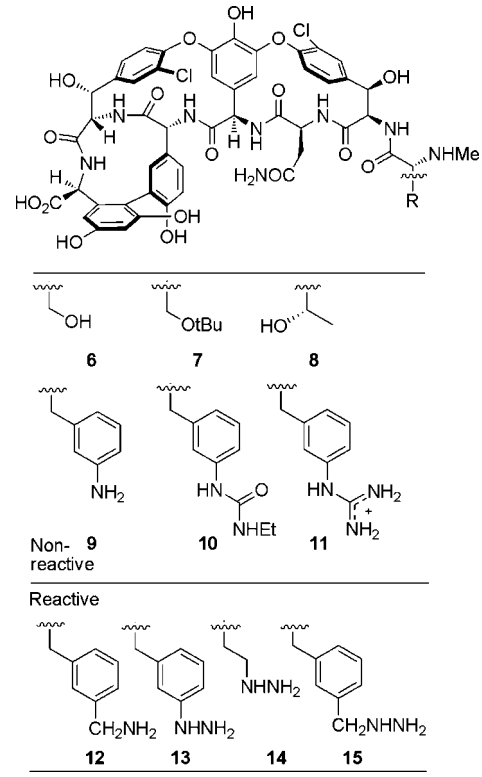
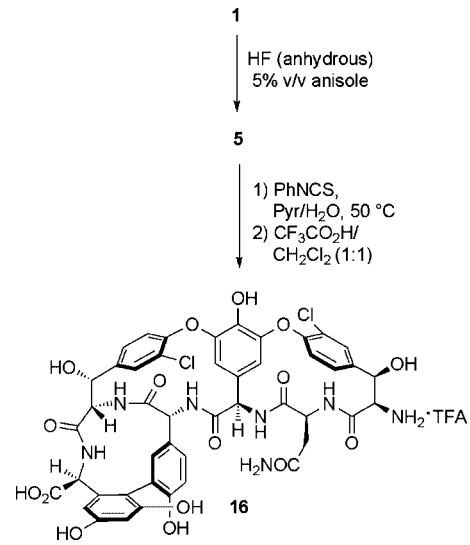


Figure 4. Target molecules.

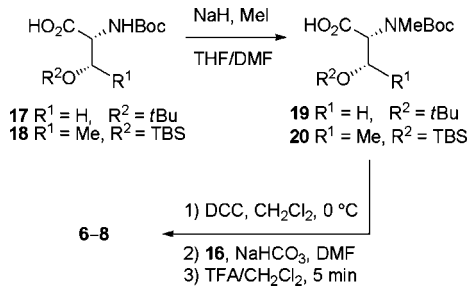
Scheme 1



hydrochloride (**1**), which was deglycosylated to provided the intermediate vancomycin aglycon (**5**).[14] Subsequent removal of the N-terminal D-leucyl residue of **5** by Edman degradation using the sequential action of phenyl isothiocyanate and trifluoroacetic acid provided **16** as reported (Scheme 1).[6]

Target molecules **6** and **7** were prepared from commercially available *N*-Boc-*O*-*tert*-butyl-D-serine (**17**), and **8** was accessed using *N*-Boc-*O*-*tert*-butyldimethylsilyloxy-D-threonine (**18**). Selective N-methylation using the conditions of Cheung and Benoiton[15] provided the corresponding *N*-Boc-*N*-methylamino acids **19** and **20** in 92% and 60% yields, respectively. Dicyclohexylcarbodiimde (DCC) mediated symmetrical anhydride formation of **19** and **20** followed by coupling to the free amine of **16** in the presence of sodium bicarbonate ($NaHCO_3$) provided the protected target molecules. Subsequent treatment of the

Scheme 2



resulting intermediates with trifluoroacetic acid (TFA) in dichloromethane provided the TFA salts of target molecules **6**, **7**, and **8** with yields for the two-step transformation of 24%, 47%, and 53%, respectively, after purification by reverse-phase HPLC (Scheme 2).

Compounds **9**−**11** were obtained from intermediate **20**, which in turn was prepared from commercially available *N*-Boc-3-nitro-D-phenylalanine (**21**, Scheme 3). Selective N-methylation of **21** using the conditions of the Cheung and Benoiton[15] provided **22** in 82% yield. Subsequent esterification with methyl iodide (MeI) and potassium carbonate (K_2CO_3) provided ester **23** in 94% yield, which was reduced to amine **20** (H_2, 10% Pd/C, 98%). Intermediate **20** was hydrolyzed and protected to provide **24** in 75% yield by sequential action of LiOH and di-*tert*-butyl dicarbonate (Boc_2O). DCC-mediated symmetrical anhydride formation of **24** and coupling with **16** in the presence of $NaHCO_3$ provided the target molecule **9** as its bis-TFA salt in 51% yield (two steps), after treatment with 1:1 TFA in dichloromethane and purification by reverse-phase HPLC (Scheme 3).

Intermediate **20** was treated with ethyl isocyanate (EtNCO) to generate the ethylurea **25** in 97% yield. Hydrolysis of ester **25** with LiOH provided the carboxylic acid **26** (88%), which was coupled to **16** in the presence of $NaHCO_3$ following preparation of its symmetrical anhydride using DCC. The target molecule **10** was isolated by reverse-phase HPLC as its TFA salt in a 44% yield (two steps) after treatment of the protected intermediate with TFA in dichloromethane (Scheme 3).

The guanidine derivative **11** was also obtained from intermediate **20** (Scheme 3). Treatment of **20** with commercially available *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine (**27**)[16] provided the bis-protected guanidine derivative **28** in 47% yield. Subsequent hydrolysis of ester **28** with LiOH provided the carboxylic acid **29** (66%) and was followed by treatment of **29** with DCC to prepare the symmetrical anhydride, which was coupled with the free amine of **16** in the presence of $NaHCO_3$. The target molecule **11** was isolated by reverse-phase HPLC as its bis-TFA salt in 27% yield (two steps) after treatment of the protected intermediate with TFA in dichloromethane (Scheme 3).

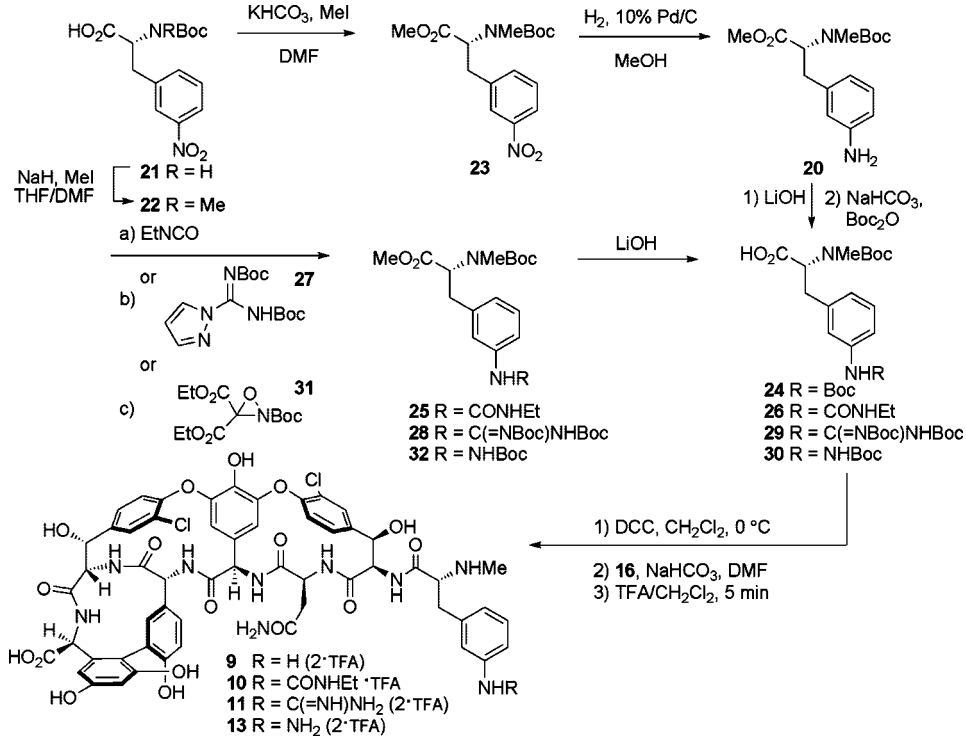
The phenylhydrazine amino acid precursor **30** was obtained in two steps from intermediate **20**. Amination of the aniline of **20** with oxaziridine **31**[17] proceeded in modest, but sufficient yield (23%) with both standard and extended reaction times (24−72 h) as the bis-aminated side product predominated (not shown). Subsequent hydrolysis of the methyl ester **32** with LiOH provided the carboxylic acid **30** in 65% yield. The acid **30** was then transformed to its symmetrical anhydride with DCC and coupled with **16** in the presence of $NaHCO_3$. The protected intermediate was treated with TFA in dichloromethane to provide the desired target molecule **13** (49%, two steps) as its bis-TFA salt after purification by reverse-phase HPLC. Compound **13** was somewhat unstable and was used for all biological and UV-difference titration assays within 1 day after liberation of the hydrazine **13** (Scheme 3). Compounds **14** and **15** were subsequently prepared, as these analogues were envisioned to have improved stability (Figure 4).

Target molecules **12** and **15** were prepared starting from commercially available *N*-Boc-3-cyano-D-phenylalanine (**33**, Scheme 4). Selective N-methylation following the conditions of Cheung and Benoiton[15] provided carboxylic acid **34** in 97% yield, which was esterified with MeI/$KHCO_3$, in DMF to provide ester **35** in 99% yield. Reduction of the nitrile to the corresponding methylamine with hydrogen gas (H_2), Raney Ni, and aqueous 28% ammonium hydroxide (NH_4OH) in methanol provided the amine **36** in 97% yield. Alternative hydrogenation conditions employing H_2 and 10% Pd/C at atmospheric pressure or 50 psi in methanol, tetrahydrofuran, or acetic acid provided only complex mixtures, and reactions performed with Raney Ni in the absence of NH_4OH cleanly provided the bis-alkylamine byproduct. Subsequent protection of the amine **36** with di-*tert*-butyl dicarbonate provided **37** (86%), and hydrolysis of the ester with LiOH in tetrahydrofuran/water provided the desired carboxylic acid **38** in 88%. DCC-mediated anhydride formation of **38** followed by coupling with **16** provided the target molecule **12** as its bis-TFA salt in 47% yield (two-steps) after treatment with 1:9 TFA in dichloromethane and purification by reverse-phase HPLC (Scheme 4).

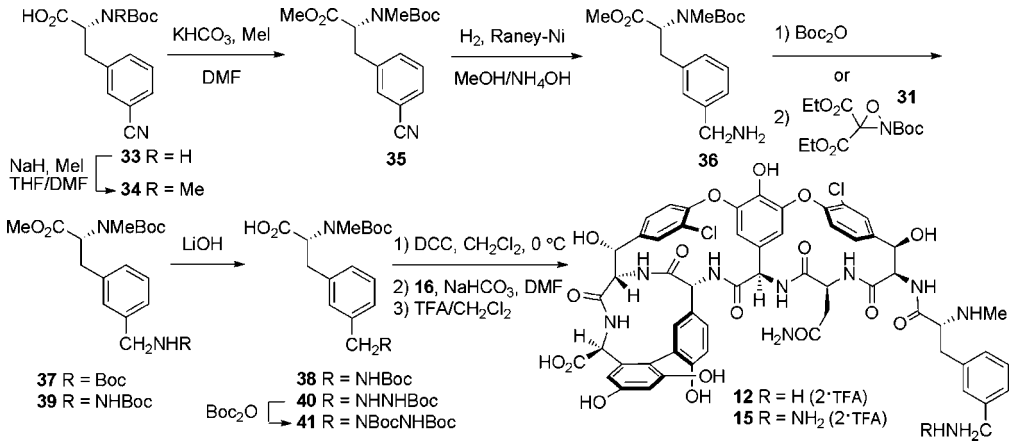
Amination of the amine on **36** with oxaziridine **31**[17] provided the protected hydrazine **39** (58%) in anhydrous toluene (30 min), and the yield was comparable conducting the reaction in dichloromethane (52%) (Scheme 4). Extended reaction times of 24 or 48 h in dichloromethane or toluene provided lower yields of the desired hydrazine (22% and 30−47%, respectively), and the bis-amination byproduct dominated. Hydrolysis of the ester of **39** using LiOH in tetrahydrofuran/water provided the desired carboxylic acid **40** (62%). The hydrazine was then protected with di-*tert*-butyl dicarbonate in the presence of $NaHCO_3$ to provide **41** in 87%. DCC-mediated anhydride formation of carboxylic acid **41** followed by coupling with **16** provided the target molecule **15** as its bis-TFA salt in 52% yield (two steps) after treatment with TFA in dichloromethane (1:9) and purification by reverse-phase HPLC (Scheme 4). As observed with the derivative **13**, the hydrazine function of compound **15** proved unstable, with a lifetime of 3 days when stored as a solid. Therefore, compound **15** was used for all biological and UV-difference titration assays within 1 day after liberation of the free hydrazine. For assay purposes, a crude sample, after deprotection and without purification by reverse-phase HPLC, was used for all assays, as purification caused additional decomposition. However, a purified sample was prepared for characterization purposes.

Compound **14** was prepared from commercially available *N*-Boc-*O*-benzyl-D-homoserine (**42**) initiated by selective N-methylation of **42** as described previously using the conditions of Cheung and Benoiton[15] to provide intermediate **43** (96%, Scheme 5). Subsequent debenzylation under an atmosphere of H_2 gas over 10% Pd/C provided the crude alcohol, which was immediately oxidized to the aldehyde using Dess−Martin periodinane and condensed with *tert*-butyl carbazate to provide the hydrazone **44** (48%, three steps). Reduction of the hydrazone was initially problematic, as bis-alkylhydrazine was the major byproduct (not shown). The hydrazone was cleanly reduced to the hydrazine **45** in 76% yield under an atmosphere of H_2 gas over 5% Pt/C with the additive *tert*-butyl carbazate (2 equiv), which prevented bis-alkyl hydrazine formation. Similar conditions in the absence

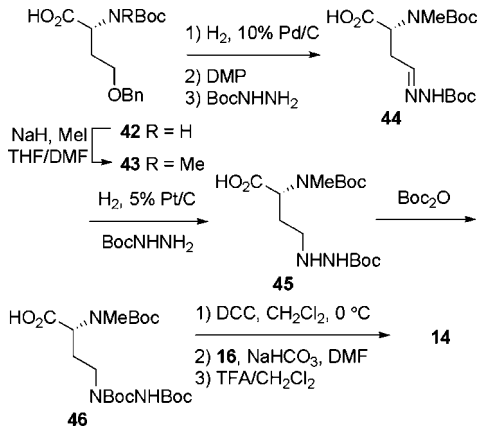
Scheme 3



Scheme 4



Scheme 5



of *tert*-butyl carbazate provided a mixture of unreacted starting material and bis-alkyl hydrazine with only trace amounts of desired product. Other conditions employing sodium cyanoborohydride ($NaBH_3CN$) or hydrogenation over 10% Pd/C in acetic acid, methanol, or tetrahydrofuran at atmospheric pressure or at 50 psi of pressure provided only complex mixtures. Mono-Boc protected hydrazine **45** was then bis-Boc protected with di-*tert*-butyl dicarbonate in a mixture of tetrahydrofuran/water to provide carboxylic acid **46** (49%). DCC-mediated anhydride formation with **46** and coupling with **16** provided the target molecule **14** as its bis-TFA salt in 49% yield (two steps) after treatment with 1:9 TFA in dichloromethane and purification by reverse-phase HPLC (Scheme 5). As observed with hydrazine derivatives **13** and **15**, derivative **14** was also unstable. It was therefore used for all biological and UV-difference titration assays within 1 day after liberation of the hydrazine and stored as a solid. For assay purposes, a crude sample, isolated after deprotection and without purification by reverse-phase HPLC, was used for all assays, as additional purification caused

Table 1. Binding and Antimicrobial Properties

	MIC (μg/mL)		association constants	
compd	*S. aureus*[a]	*E. faecalis*[b]	**2**[c] ($\times 10^5$ M^{-1})	**3**[d] ($\times 10^2$ M^{-1})
1	1.25[e]	2000[e]	2.0[f]	1.8[f]
5	0.625[e]	640[e]	1.7	1.2[f]
6	40	800	0.3	2.1
7	20	320	0.9	2.0
8	40	800	2.6	0.8
9	20	320	0.3	2.1
10	31	640	2.0	4.6
11	10	320	1.1	3.5
12	>10	640	0.8	0.8
13	10	320	0.3	1.9
14	10	>400	1.1	1.5
15	1.25	170	1.7	1.5

[a] Vancomycin-sensitive (strain ATCC 25923). [b] Vancomycin-resistant (VanA, strain BM4166). [c] *N,N′*-Ac$_2$-L-Lys-D-Ala-D-Ala. [d] *N,N′*-Ac$_2$-L-Lys-D-Ala-D-Lac. [e] Taken from ref 18b. [f] Taken from ref 4.

further decomposition. However, a purified sample was prepared for the purposes of characterization.

Results and Discussion

The antimicrobial properties of derivatives **6**−**15** were determined against a vancomycin-sensitive *Staphylococcus aureus* (*S. aureus*, strain ATCC 25923) and a vancomycin-resistant (VanA) *Enterococcus faecalis* (*E. faecalis*, strain BM4166) in a microtiter plate-based antimicrobial assay (Table 1).[4,18] The latter VanA strain maintains an inducible resistance pathway upon treatment with glycopeptide antibiotics, including vancomycin (**1**) and teicoplanin, and is a virulent strain that is difficult to treat compared to a VanB strain. When unchallenged, it utilizes D-Ala-D-Ala peptidoglycan cell wall precursors, but upon treatment with a glycopeptide it will switch to D-Ala-D-Lac peptidoglycan cell wall precursors. The binding properties of **6**−**15** for **2** and **3** were also determined and compared to **1** and **5** using a well-established UV-difference titration assay, which gives association constants (K_a) for the complexes with the model tripeptides **2** and **3** of the peptidoglycan cell wall precursors (Table 1).[18,19] For an exemplary saturation binding curve with **2** or **3** and subsequent Scatchard analysis see the Supporting Information (Figures 3SI and 4SI).

Compounds **6**−**14** all lost a significant amount of antimicrobial activity against a vancomycin-sensitive *S. aureus* (Table 1). Only the vancomycin derivative **15** (MIC = 1.25 μg/mL), which arose as the best of the series, displayed a MIC comparable to vancomycin (MIC = 1.25 μg/mL)[4,18] and the vancomycin aglycon (MIC = 0.625 μg/mL)[4,18] against vancomycin-sensitive *S. aureus* (Table 1). The reduced in vitro antimicrobial properties were paralleled by lower measured affinities (K_a values) of compounds **6**, **7**, **9**, **12**, and **13** for the model ligand **2**, which lost anywhere from 2- to 10-fold in affinity. Interestingly, compounds **8**, **10**, **11**, **14**, and **15** displayed comparable affinities for **2**, and with the exception of **15**, this did not translate to comparable antimicrobial properties (Table 1).

The antimicrobial properties of **6**−**15** were also investigated against a vancomycin-resistant *E. faecalis* (VanA), and the results revealed that **6**−**14** did not display markedly different antimicrobial properties than the natural product. The antimicrobial activities of the N-terminal D-serine and D-threonine analogues **6** and **8** were slightly lower than **5**, even though the former had a similar affinity for the model ligand **3** (Table 1). Analogue **7** bearing the *O-tert*-butyl ether showed improved potency over its hydroxy counterpart **6** and a comparable affinity for **3**; a result that may be attributed to its enhanced hydrophobic character. There was no change in the antimicrobial activities of ethylureido derivative **10** and benzylamine derivative **12**, despite the higher K_a of the former for the ligand **3** and the altered binding pocket (Figure 2SI). Aniline derivative **9**, phenylhydrazine derivative **13**, and alkylhydrazine derivative **14** displayed slightly enhanced antimicrobial properties that paralleled enhanced affinities for **3** (Table 1), again despite the potentially altered binding pocket (Figure 2SI). As observed in the assay against vancomycin-sensitive *S. aureus*, the only analogue that showed enhanced potency against the VanA strain was compound **15** (MIC = 170 μg/mL, Table 1). However, this increase (4-fold) was modest and overall the results did not reveal a unique behavior for the hydrazine series against the vancomycin-sensitive or vancomycin-resistant bacteria. Since the binding affinity of **15** for **3** did not correlate directly with its antimicrobial potency, as **15** had the same affinity as natural product for **3**, it is not clear what factors are contributing to its modestly enhanced potency.

For vancomycin analogues **6**−**8**, any observed loss in affinity may be the result of a change in the overall nonpolar surface area in this region of the natural product (Figure 5S1) as observed for the N-terminal D-alanine and D-glycine analogues of vancomycin reported by Williams and co-workers.[5] These analogues, which in contrast maintained the vancosamine sugar, exhibited a loss in affinity for **2** (20-fold, 0.85×10^5 and 0.77×10^5 M^{-1}, respectively).[5] Further, analysis by molecular modeling[7] of the conformation of the D-serine and D-threonine derivatives **6** and **7** revealed that the hydroxy groups may point away from the carboxylate when the ligand is bound, adopting a more favorable gauche conformation (Figure 5SI). Therefore, it might be expected that the serine derivative **6** would have a lower affinity for **2** and that the analogous gauche conformation for **7** directs the threonine methyl substituent toward the ligand, increasing both the nonpolar surface area in this region of the ligand and its affinity for **2** when compared to **6** (Table 1). However, the reverse trend is observed for the binding results with the ester ligand **3** and the distinctions are modest enough that definitive trends are difficult to discern.

The D-phenylalanine derivatives **9** and **13**, with the H-bond donating groups (aniline or hydrazine) attached directly to the aromatic ring, lost significant affinity for **2** but gained affinity for **3**. Extension of the H-bond donating system by one or two additional atoms from the aromatic ring restores some of the lost affinity for **2** and **3** as demonstrated by compounds **10**, **11** and **14**, **15**, in spite of the altered binding pocket in the N-terminal region of the natural product (Figure 2SI). However, analogue **12** with the single methylene extension still has a slightly lower K_a in comparison to the natural product **5** (Table 1). The most significant positive impact on binding affinity was observed with **10** and **11** and their relative affinity for **3**, which increased 3- to 4-fold potentially reflecting the bidentate H-bond represented in Figure 3.

A series of reaction conditions were explored to investigate the potential of a reaction occurring between the appended hydrazines of analogues **13**−**15** and the C-terminal ester of *N,N′*-Ac$_2$-L-Lys-D-Ala-D-Lac (**3**) (Table 1SI), the hope being that the hydrazine would react with the ester of the bound ligand **3** generating a covalent adduct with the natural product analogue. However, varying the solvent, temperature, concentration, substrate, or pH did not influence the reaction outcome and the desired adduct product was never observed. This result was surprising, as the same intermolecular reaction between **3** and the commercially available hydrazines phenylhydrazine and

2-bromophenylhydrazine with triethylamine (Et_3N) at 70 °C did provide the desired acylated hydrazines (data not shown).

Conclusions

A series of N-terminal derivatives of the vancomycin aglycon were prepared replacing the residue 1 *N*-methyl D-leucyl amino acid with a series of *N*-methyl D-amino acids bearing H-bond donor or reactive nucleophilic substituents. An examination of their antimicrobial activity against vancomycin-sensitive and vancomycin-resistant bacteria and their affinity for model ligands **2** and **3** incorporating D-Ala-D-Ala and D-Ala-D-Lac did not reveal evidence of significant enhancements in activity or affinity, including those capable of formation of covalent adducts derived from their reaction with ligand **3** bearing an ester. Whether this reflects the limitations of our original designs or features of binding not yet recognized is unknown but is a topic of our continuing studies.

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Supporting Information Available: Compounds **5** and **16** were prepared according to published procedures.[6,14] Full experimental details and characterization for all new intermediates and final compounds are provided in the supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

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