Pergamon

Tetrahedron 58 (2002) 6585–6594

TETRAHEDRON

Structural requirements for VanA activity of vancomycin analogues

Zhong Chen, Ulrike S. Eggert, Steven D. Dong, Simon J. Shaw, Binyuan Sun, John V. LaTour and Daniel Kahne*

Department of Chemistry, Princeton University, Princeton, NJ 08544, USA

Received 18 June 2002; accepted 25 June 2002

Dedicated with affection to Professor Yoshito Kishi

Abstract—We have prepared several sets of glycopeptide analogues in order to probe the molecular basis for the activity of derivatives that overcome vanA resistance. The results described in this paper provide compelling evidence that good vanA activity is due to a mechanism of action that does not involve peptide binding. Hypothesizing that this mechanism of action involves an interaction of the disaccharide portion of vancomycin analogues with bacterial transglycosylases, we have prepared a compound in which the vancomycin aglycone is coupled to a known transglycosylase inhibitor that is structurally unrelated to the disaccharides that have been previously investigated. The activity of this compound is excellent. This work provides a clear prescription for the design of better glycopeptide analogues. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Vancomycin is a glycopeptide antibiotic that inhibits peptidoglycan biosynthesis by binding to the D-Ala–D-Ala dipeptide termini of peptidoglycan precursors.[1] It is widely used to treat Gram-positive infections, and has become increasingly important in recent years because it is the only antibiotic capable of curing many multi-drug resistant infections. The emergence of resistance to vancomycin (**1**) in enterococcal strains has aroused considerable concern.[2] The predominant form of vancomycin resistance in enterococcal strains occurs when these bacteria incorporate genes encoding proteins that produce peptidoglycan precursors terminating in D-Ala–D-Lac instead of D-Ala–D-Ala.[3] Efforts to overcome resistance have led to a class of vancomycin derivatives containing hydrophobic substituents such as chlorobiphenyl on the vancosamine nitrogen (e.g. **2**).[4] The activity of these derivatives was initially attributed to a combination of two factors: their ability to anchor to the bacterial membrane near the peptidoglycan precursor; and their ability to dimerize, which was proposed to increase their avidity for peptidoglycan precursors presented in multiple copies on the membrane surface.[5,6]

While hydrophobic substituents do facilitate anchoring of vancomycin derivatives to membranes, and although some of them may promote dimerization, we questioned whether the entropic advantages of membrane anchoring/dimerization would be sufficient to overcome the extremely weak binding of vancomycin to D-Ala–D-Lac.† Further investigation revealed that biological activity against resistant microorganisms was maintained when the vancomycin binding pocket was damaged so that it could not bind even D-Ala–D-Ala, let alone D-Ala–D-Lac. This finding led us to propose that **2** is active against resistant strains because it has a second mechanism of action, one that involves a direct interaction between the substituted disaccharide and some other target important in peptidoglycan synthesis.[8] To test this hypothesis, we have prepared and evaluated the sets of vancomycin analogues described below.

2. Results and discussion

Vancomycin analogues **2**–**8** (Figs. 1 and 2) were synthesized following routes described previously.[9–12] Compound **2** is the vanA-active chlorobiphenyl vancomycin analogue discovered by researchers at Lilly;[4] **2a** is the corresponding desleucyl derivative, which is incapable of peptide binding.[13] Compound **3** resembles compound **2** except that the chlorobiphenyl substituent has been moved from the vancosamine to the C6 position of the penultimate sugar, glucose; **3a** is the corresponding desleucyl derivative.

Keywords: vancomycin; glycopeptide analogues; vanA activity.

* Corresponding author. Tel.: +1-609-258-6368; fax: +1-609-258-2617; e-mail: dkahne@princeton.edu

† It has recently been shown that LY329332, which is similar in structure to chlorobiphenyl vancomycin, does not dimerize or insert into membranes.[7]

0040–4020/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4020(02)00743-3

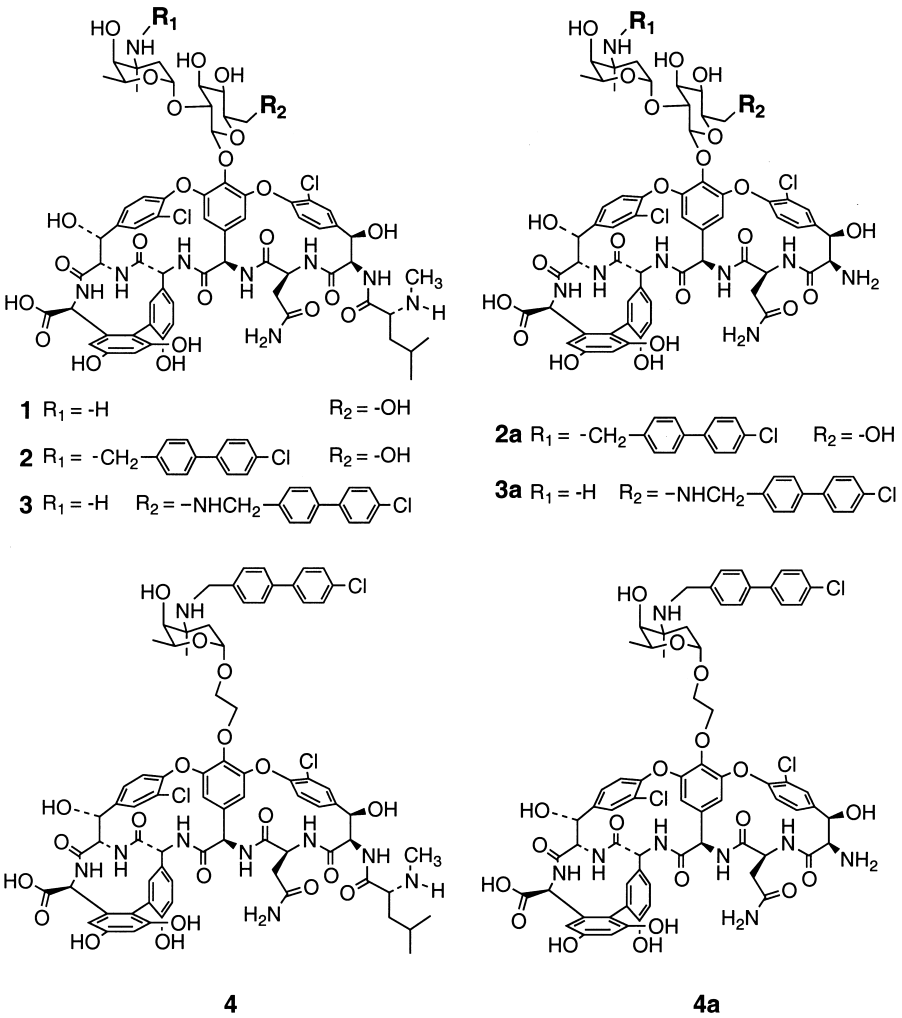


Figure 1. Lipid derivatives of vancomycin and their desleucyl analogues.

Compound **4** also resembles **2** except that the glucose has been replaced by ethylene glycol; **4a** is the corresponding desleucyl derivative. This set of compounds was designed to test whether a particular carbohydrate structure is required for biological activity against resistant bacterial strains. We reasoned that a mechanism involving non-specific membrane interactions would not be sensitive to the position of the lipid substituent on the molecule, but a mechanism involving specific interactions with some other target would be. Minimum inhibitory concentrations for compounds **1**–**4** were measured using a standard microdilution assay and the results are summarized in Table 1.

Table 1. Minimum inhibitory concentrations (MICs, μg/mL) of compounds **1**–**4** against sensitive and VanA vancomycin-resistant strains of *E. faecium*

Compound	*E. faecium*	
	Sensitive	Resistant (VanA)
Vancomycin (**1**)	2	2048
2	<0.025	12.5
3	<0.03	16
4	0.8	63
2a	10	40
3a	64	1024
4a	50	>200

The activity of these compounds indicates that the structure of the substituted disaccharide is important for biological activity. Moving the hydrophobic substituent from the terminal vancosamine sugar to the glucose abolishes peptide-binding-independent activity (compare **2a** and **3a**), consistent with the hypothesis of a second mechanism. The monosaccharide derivatives **4/4a** provide even more compelling evidence that vanA activity involves a specific interaction with some as yet unidentified target rather than a non-specific membrane interaction. In compounds **4/4a** the substituted vancosamino sugar is attached to the aglycone via a flexible linker containing four atoms, thus mimicking the number of atoms between C1 of the vancosamino sugar and the aglycone in compounds **2/2a**. However, even though the sugar in **4/4a** is identical to the terminal sugar in **2/2a** and can be displayed at the same distance from the aglycone, the biological activity of these pairs of compounds is very different.

We then reasoned that if the substituted disaccharide did, in fact, have some biological activity that was distinct from peptide binding, it should be possible to alter the way in which it is attached to the aglycone. We prepared compounds **5** and **6** (Schemes 1 and 2) to evaluate this hypothesis. In compound **5**, the substituted disaccharide is attached via an ethylene glycol linker to the fourth amino

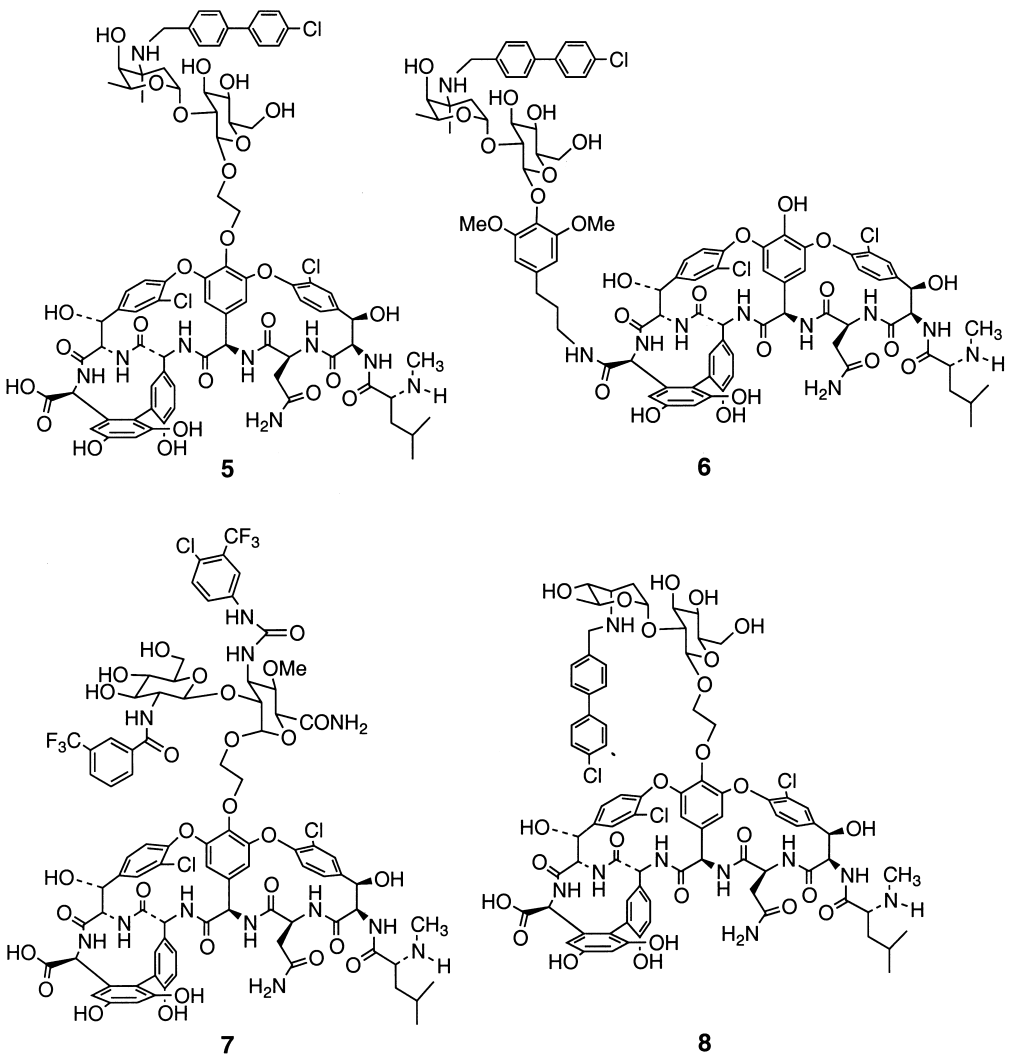


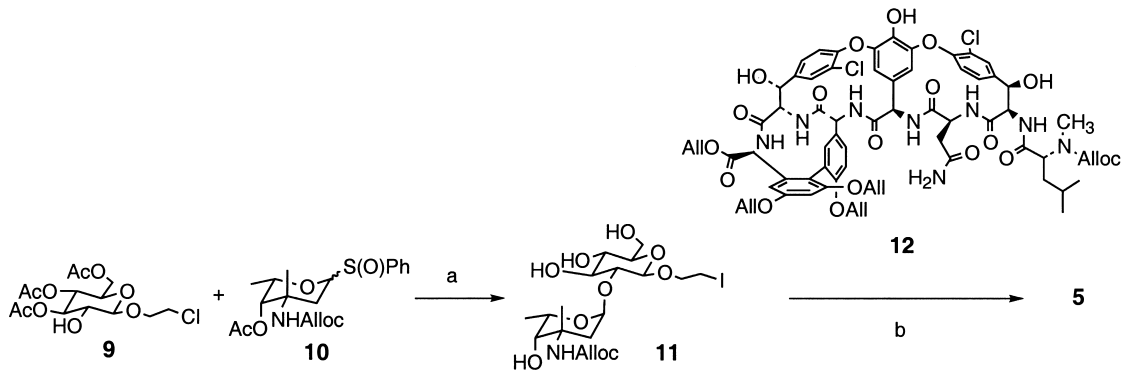
Figure 2. Linked vancomycin derivatives which incorporate both natural and unnatural disaccharides.

acid of the crosslinked heptapeptide aglycone, whereas in compound **6** the same disaccharide is linked via an aryl spacer to the carboxy terminus. As shown in Table 2, both compounds have reasonable activity against vanA-resistant *E. faecium*, with the activity of compound **6** being comparable to that of **2**. Therefore, the orientation of the substituted disaccharide with respect to the aglycone can be altered without destroying activity against resistant strains. This result supports the hypothesis that these vancomycin derivatives are comprised of two separate determinants of biological activity.

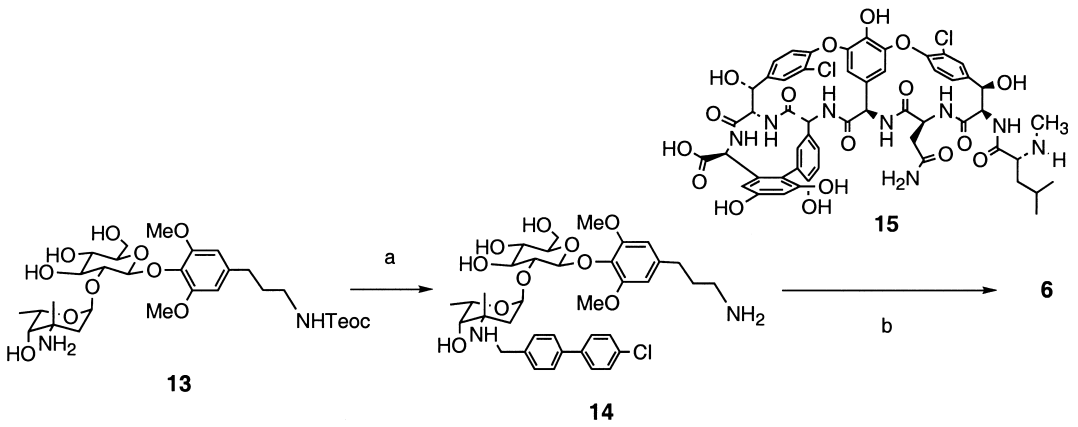
Mechanistic investigations of **2a** in our laboratory have suggested that it inhibits the transglycosylation step of peptidoglycan synthesis even though it cannot bind the peptide portion of the peptidoglycan precursor substrate.[8,14] This result suggested to us that the target of the substituted disaccharide was a component of the transglycosylation complex—perhaps even a bacterial transglycosylase itself. Since the substituted disaccharide on compound **5** was not optimized to block transglycosylation, but merely happened to do so (possibly because it bears a chance resemblance to the carbohydrate substrates of bacterial transglycosylases), we surmised that it should be possible to make a compound with better activity against resistant bacterial strains by attaching a carbohydrate that was designed to block transglycosylation. We prepared compound **7** (Scheme 3) in which a functionalized disaccharide designed to inhibit bacterial transglycosylases (at least when coupled to a phospholipid)[15] was attached to the aglycone at A4 via an ethylene glycol linker, exactly as in the linked chlorobiphenyl derivative **5**.[9] We chose compound **5** as the standard to which to compare **7** because having an ethylene glycol linker rather than a glycosidic linkage to the aglycone facilitates the synthesis. We also prepared compound **8** (Scheme 4), which bears a much closer resemblance to compound **5** than **7** does. The MICs of **7** and **8** were

Table 2. Minimum inhibitory concentrations (MICs, μg/mL) of compounds **5**–**8**

Compound	*E. faecium*	
	Sensitive	Resistant (VanA)
5	<0.01	63
6	0.16	16
7	0.1	16
8	1	>500



Scheme 1. Synthesis of linked vancomycin derivative **5**. (a) (i) Tf_2O, DTBMP, −78 to −20°C, Et_2O/CH_2Cl_2, 84%; (ii) NaI, acetone, 99%; (iii) NaOMe, MeOH, 82%. (b) (i) Cs_2CO_3, DMF, 83%; (ii) $PdCl_2(PPh_3)_2$, Bu_3SnH, DMF/AcOH, 79%; (iii) 4′-chlorobiphenyl-4-carboxaldehyde, DIPEA, $NaBH_3CN$, DMF, 46%.

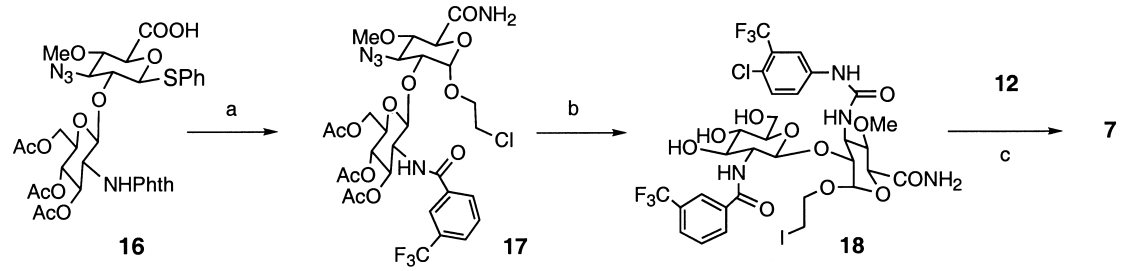


Scheme 2. Synthesis of carboxy-linked derivative **6**. (a) (i) 4′-Chlorobiphenyl-4-carboxaldehyde, DIPEA, $NaBH_3CN$, DMF; (ii) TBAF, 58% over two steps. (b) TBTU, HOBt, DMF, 23%.

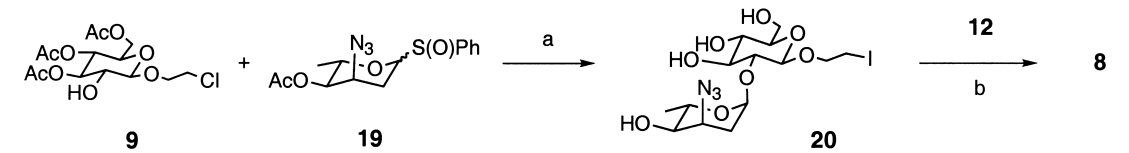
evaluated and compared to that of **5**. Compound **8** has poor activity against vanA strains even though it is structurally related to **5**. Compound **7**, in contrast, shows a four-fold improvement in activity against vanA-resistant bacterial strains compared with compound **5**.

3. Conclusion

While these results do not prove that the vanA activity of the chlorobiphenyl vancomycin disaccharide is due to its ability to inhibit bacterial transglycosylases, they do show that



Scheme 3. Synthesis of linked hybrid **7**. (a) (i) NH_4HCO_3, $(Boc)_2O$, pyridine, CH_3CN, 76%; (ii) NH_2NH_2, THF/CH_3OH, 74%; (iii) 3-(trifluoromethyl)benzoic acid, HATU, DIPEA, DMF, 93%; (iv) Ac_2O, DMAP, pyridine, 92%; (v) $Hg(CF_3CO_2)_2$, $(C_2H_5)_2O{\cdot}BF_3$, 2-chloroethanol, 83%. (b) (i) Me_3P, H_2O/THF/EtOH; (ii) 4-chloro-3-(trifluoromethyl)phenyl isocyanate, CH_2Cl_2/DMF, 72% over two steps; (iii) NaI, acetone; (iv) NaOMe, MeOH, 86% over two steps. (c) (i) Cs_2CO_3, DMF, 84%; (ii) $PdCl_2(PPh_3)_2$, Bu_3SnH, DMF/AcOH, 66%.



Scheme 4. Synthesis of linked vancomycin derivative **8**. (a) (i) Tf_2O, DTBMP, −78 to −20°C, Et_2O/CH_2Cl_2, 91%, 2:1 α/β; (ii) NaI, acetone, 99%; (iii) NaOMe, MeOH, 94%. (b) (i) Cs_2CO_3, DMF, 82%; (ii) PPh_3, THF/H_2O, 81%; (iii) $PdCl_2(PPh_3)_2$, Bu_3SnH, DMF/AcOH, 78%; (iv) 4′-chlorobiphenyl-4-carboxaldehyde, DIPEA, $NaBH_3CN$, DMF, 62%.

the hypothesis has prescriptive value for how to design glycopeptide antibiotics. It should be possible to make still better vancomycin derivatives by attaching even better inhibitors of transglycosylation (and possibly transpeptidation) to the vancomycin aglycone. Whether it will also be possible to make vanA-active peptide-binding antibiotics without necessarily starting from the natural vancomycin aglycone is of considerable interest to us at present and would permit access to an even wider range of structural variants with different properties.

4. Experimental

4.1. General

4.1.1. 2-Iodoethyl 2-*O*-(3-allyloxycarbonylamino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranoside (11). Sulfoxide **10**[16] (156 mg, 0.394 mmol), was azeotroped three times with toluene and dissolved in Et_2O (3 mL). 2-Chloroethyl 3,4,6-tri-*O*-acetyl-β-D-glucopyranoside[12] (**9**, 104 mg, 0.282 mmol) and 2,6-di-*t*-butyl-4-methyl pyridine (174 mg, 0.845 mmol) were azeotroped three times with toluene, dissolved in 1:2 CH_2Cl_2/Et_2O (7.5 mL), and cooled to −78°C. Triflic anhydride (33 μL, 0.197 mmol) was added, then **10** was added over 15 min, and the reaction was stirred at −78°C for 20 min, warmed to −20°C over 1.5 h, stirred for another 30 min, and then quenched with saturated $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3×10 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (27% EtOAc/CH_2Cl_2) to give 151 mg (84%) of 2-chloroethyl 2-*O*-(3-allyloxycarbonylamino-4-*O*-acetyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside as a colorless solid: R_f=0.39 (30% EtOAc/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.89 (m, 1H), 5.28 (br dd, *J*=17.5, 1.5 Hz, 1H), 5.23 (apt, *J*=9.5 Hz, 1H), 5.20 (br d, *J*=9.7 Hz, 1H), 5.08 (d, *J*=4.5 Hz, 1H), 4.99 (apt, *J*=9.5 Hz, 1H), 4.91 (s, 1H), 4.79 (br s, 1H), 4.54–4.43 (m, 4H), 4.27 (dd, *J*=12.5, 5.0 Hz, 1H), 4.17 (dt, *J*=11.0, 5.0 Hz, 1H), 4.13 (dd, *J*=12.5, 2.4 Hz, 1H), 3.82 (dd, *J*=9.5, 8.0 Hz, 1H), 3.78 (dt, *J*=11.0, 4.5 Hz, 1H), 3.72–3.62 (m. 3H), 2.17 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.17–1.95 (m, 2H), 1.67 (s, 3H), 1.12 (d, *J*=6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 172.0, 171.3, 170.5, 170.4, 155.2, 133.6, 118.4, 102.3, 97.9, 76.2, 74.8, 74.8, 72.5, 70.5, 69.4, 65.9, 64.1, 62.7, 53.7, 43.3, 36.0, 24.7, 21.5, 21.4, 21.4, 21.3, 17.8; HRMS (FAB) calcd for $C_{27}H_{40}ClNNaO_{14}$ $[M+Na]^+$ 660.2035, found 660.2028.

A solution of 2-chloroethyl 2-*O*-(3-allyloxycarbonylamino-4-*O*-acetyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (60 mg, 0.094 mmol) and NaI (300 mg, 2 mmol) in acetone (0.8 mL) was refluxed for 60 h, cooled to room temperature, concentrated, and then the residue was dissolved in CH_2Cl_2 and filtered. The filtrate was concentrated and purified by flash chromatography (25% EtOAc/CH_2Cl_2) to yield 68 mg (99%) of 2-iodoethyl 2-*O*-(3-allyloxycarbonylamino-4-*O*-acetyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside as a colorless solid: R_f=0.36 (25% EtOAc/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.89 (m, 1H), 5.28 (dq, *J*=17.5, 1.5 Hz, 1H), 5.22 (m, 2H), 5.08 (d, *J*=4.5 Hz, 1H), 4.98 (apt, *J*=9.5 Hz, 1H), 4.94 (s, 1H), 4.78 (br s, 1H), 4.54–4.43 (m, 4H), 4.27 (dd, *J*=12.5, 5.0 Hz, 1H), 4.17–4.10 (m, 2H), 3.87 (ddd, *J*=11.0, 8.0, 6.5 Hz, 1H), 3.80 (dd, *J*=9.5, 8.0 Hz, 1H), 3.70 (ddd, *J*=9.5, 5.0, 2.3 Hz, 1H), 3.31 (ddd, *J*=10.0, 8.0, 5.5 Hz, 1H), 3.24 (ddd, *J*=10.0, 8.0, 7.0 Hz, 1H), 2.17 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.17–1.95 (m, 2H), 1.68 (s, 3H), 1.13 (d, *J*=6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 171.9, 171.3, 170.6, 170.4, 133.6, 118.4, 102.2, 97.9, 76.1, 75.0, 74.6, 72.5, 71.6, 69.4, 65.9, 64.2, 62.7, 53.6, 36.0, 24.8, 21.5, 21.5, 21.4, 21.3, 17.9, 1.9; HRMS (FAB) calcd for $C_{27}H_{40}INNaO_{14}$ $[M+Na]^+$ 752.1391, found 752.1384.

2-Iodoethyl 2-*O*-(3-allyloxycarbonylamino-4-*O*-acetyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside in 1 mg/mL NaOMe/MeOH (2 mL) was stirred at room temperature for 60 min, quenched with NH_4OAc (20 mg), concentrated and purified by flash chromatography (10% MeOH/CH_2Cl_2) to give 19 mg (82%) of **11**: R_f=0.32 (12% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.95 (m, 1H), 5.41 (d, *J*=4.5 Hz, 1H), 5.31 (dq, *J*=17.5, 1.5 Hz, 1H), 5.19 (dq, *J*=10.5, 1.5 Hz, 1H), 4.56–4.50 (br m, 3H), 4.43 (d, *J*=7.5 Hz, 1H), 4.19 (ddd, *J*=11.5, 7.0, 6.0 Hz, 1H), 3.90–3.84 (m, 2H), 3.67 (m, 1H), 3.52–3.44 (m, 3H), 3.39–3.33 (m, 3H), 3.28 (m, 2H), 2.09 (br d, *J*=13.8 Hz, 1H), 1.87 (dd, *J*=13.8, 4.5 Hz, 1H), 1.66 (s, 3H), 1.23 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 500 MHz) δ 134.7, 117.5, 103.0, 98.5, 79.8, 78.1, 77.5, 73.5, 71.9, 71.8, 66.0, 65.2, 62.9, 54.8, 36.0, 24.3, 18.0, 2.7; HRMS (FAB) calcd for $C_{19}H_{32}INNaO_{10}$ $[M+Na]^+$ 584.0969, found 584.0961.

4.1.2. Compound 5. Disaccharide **11** (13 mg, 0.023 mmol) and the protected vancomycin aglycone **12** (45 mg, 0.032 mmol), synthesized as described,[16] were combined and azeotroped with toluene three times. Cs_2CO_3 (9.1 mg, 0.028 mmol) was added, the mixture was azeotroped twice with toluene, DMF (0.2 mL) was added, and the reaction was stirred at room temperature for 12 h and then quenched with AcOH (one drop). The reaction mixture was precipitated with water (10 mL), centrifuged, and the precipitate purified by flash chromatography (8–14% MeOH/CH_2Cl_2) to give 35 mg (83%) of a white solid: R_f=0.29 (15% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.61 (s, 1H), 7.58 (d, *J*=8.2 Hz, 1H), 7.30 (d, *J*=8.2 Hz, 1H), 7.09 (s, 1H), 6.97 (d, *J*=8.9 Hz, 1H), 6.89 (d, *J*=2.1 Hz, 1H), 6.42 (d, *J*=2.1 Hz, 1H), 5.80–6.14 (m, 6H), 5.72 (s, 1H), 5.06–5.46 (m, 15H), 4.40–4.80 (m, 17H), 4.34–4.42 (m, 1H), 4.17 (s, 1H), 4.08–4.18 (m, 1H), 3.88 (d, *J*=11.3 Hz, 1H), 3.64–3.70 (m, 1H), 3.48–3.54 (m, 2H), 3.40 (s, 1H), 2.93 (s, 3H), 2.36–2.46 (m, 1H), 2.12 (d, *J*=13.7 Hz, 1H), 1.80–1.90 (m, 2H), 1.60 (s, 3H), 1.50–1.60 (m, 1H), 1.20 (d, *J*=6.1 Hz, 3H), 0.97 (d, *J*=4.8 Hz, 3H), 0.93 (d, *J*=5.2 Hz, 3H); HRMS (FAB) calcd for $C_{88}H_{103}Cl_2N_9NaO_{29}$ $[M+Na]^+$ 1842.6136, found 1842.6154.

To this material (30 mg, 0.016 mmol) in 1:1 DMF/AcOH (1.5 mL) was added $PdCl_2(PPh_3)_2$ (8 mg, 0.011 mmol). After the solution was degassed, Bu_3SnH in 50 μL portions was added every 5 min until the reaction was complete. The reaction mixture was precipitated with acetone (40 mL) and

the precipitate was suspended in water (5 mL) and kept at 4°C overnight, after which the suspension was filtered. The filtrate was concentrated and purified by reverse phase HPLC (0–30% CH_3CN/H_2O with 0.1% TFA) to give 21 mg (79%) of the deprotected product as its TFA salt: R_f=0.41 (6:6:1:2 $CHCl_3$/MeOH/H_2O/saturated NH_4OH); ^1H NMR (DMSO-d_6, 500 MHz) δ 9.04 (s, 1H), 8.77 (s, 1H), 8.55 (d, J=5.2 Hz, 1H), 7.84 (s, 1H), 7.46–7.71 (m, 5H), 7.28–7.34 (m, 2H), 7.14 (s, 1H), 6.70–6.79 (m, 3H), 6.39 (s, 1H), 6.26 (s, 1H), 5.94–6.00 (m, 1H), 5.77 (d, J=7.9 Hz, 1H), 5.62 (s, 1H), 5.31 (d, J=4.0 Hz, 1H), 5.10–5.23 (m, 3H), 4.89 (s, 1H), 4.40–4.49 (m, 5H), 4.17–4.24 (m, 4H), 4.01 (s, 1H), 3.86–3.94 (m, 1H), 3.65 (d, J=10.4 Hz, 1H), 3.43–3.46 (m, 1H), 3.07–3.16 (m, 3H), 2.60 (s, 3H), 2.11–2.17 (m, 1H), 1.84–1.87 (m, 1H), 1.53–1.67 (m, 4H), 1.44 (s, 3H), 1.06 (d, J=6.2 Hz, 3H), 0.93 (d, J=6.1 Hz, 3H), 0.88 (d, J=6.1 Hz, 3H); HRMS (FAB) calcd for $C_{68}H_{80}Cl_2N_9O_{25}$ $[M+H]^+$ 1492.4642, found 1492.4648.

To the aforementioned TFA salt (10 mg, 0.006 mmol) in DMF (0.35 mL) was added DIPEA (5.4 μL, 0.031 mmol) followed by 4′-chlorobiphenyl-4-carboxaldehyde (62 μL of a 0.1 M solution in DMF). The reaction was stirred at 60°C for 30 min, $NaBH_3CN$ (19 μL of a 1.0 M solution in THF) was added, the reaction was stirred at 65°C for 4.5 h, cooled to room temperature, and poured into Et_2O (12 mL). The resulting precipitate was purified by reverse phase HPLC (10–60% CH_3CN/H_2O with 0.1% AcOH) to give 5 mg (46%) of the AcOH salt of **5** as a white solid: R_f=0.66 (6:6:1:2 $CHCl_3$/MeOH/H_2O/(saturated NH_4OH)); ^1H NMR (DMSO-d_6, 500 MHz) δ 8.69 (s, 1H), 8.50 (s, 1H), 7.86 (s, 1H), 7.70 (s, 1H), 7.68 (s, 1H), 7.62 (m, 2H), 7.30–7.53 (m, 10H), 7.16 (s, 1H), 6.70–6.80 (m, 2H), 6.39 (s, 1H), 6.26 (s, 1H), 5.77 (d, J=7.9 Hz, 1H), 5.58 (s, 1H), 5.30 (s, 1H), 5.24 (s, 1H), 5.09–5.13 (m, 2H), 4.82 (s, 1H), 4.17–4.42 (m, 10H), 3.89–3.93 (m, 1H), 3.67 (d, J=10.7 Hz, 1H), 3.45 (m, 1H), 3.02–3.13 (m, 4H), 2.29 (s, 3H), 3.12–3.18 (m, 1H), 1.91 (s, 3H), 1.62–1.78 (m, 3H), 1.37–1.51 (m, 4H), 1.09 (d, J=6.4 Hz, 3H), 0.89 (d, J=6.4 Hz, 3H), 0.85 (d, J=6.4 Hz, 3H); HRMS (FAB) calcd for $C_{81}H_{89}Cl_3N_9O_{25}$ $[M+H]^+$ 1692.5035, found 1692.4990.

4.1.3. 4-(3-Aminopropyl)-2,6-dimethoxyphenyl 2-(3-*N*-[4-(4-chlorophenyl)-benzylamino]-2,3,6,trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranoside (14). To a solution of disaccharide **13** (90 mg, 0.14 mmol)[11] in DMF (5 mL) was added 4′-chlorobiphenyl-4-carboxaldehyde (38 mg, 0.18 mmol) and DIPEA (122 μL, 0.7 mmol). The mixture was stirred at 55°C for 30 min, $NaBH_3CN$ (0.7 mL of a 1 M solution in THF) was added and the mixture was stirred at 55°C for 3 h. AcOH (0.4 mL) was then added and the product was partially purified by reverse-phase HPLC, eluting with a linear gradient (15–80% CH_3CN/H_2O with 0.1% AcOH). The lyophilized powder was dissolved in DMF (1 mL) and TBAF (1 mL of a 1 M solution in THF) was added. The mixture was heated at 55°C overnight. The product was purified by reverse-phase HPLC, eluting with a linear gradient (10–60% CH_3CN/H_2O with 0.1% AcOH) to yield 58 mg (58%) of **14**: ^1H NMR (500 MHz, D_2O) δ 7.72 (d, J=8.4 Hz, 2H), 7.67 (d, J=8.4 Hz, 2H), 7.44 (d, J=8.1 Hz, 2H), 7.38 (d, J=8.4 Hz, 2H), 6.76 (s, 2H), 5.32 (s, 1H), 5.23 (d, J=7.3 Hz, 1H), 4.45–4.44 (m, 1H), 3.93–3.88 (m, 7H), 3.79–3.61 (m, 5H), 3.47 (t, J=9.1 Hz, 1H), 3.36 (s, 1H), 3.31–3.27 (m, 1H), 3.03 (t, J=7.7 Hz, 2H), 2.75 (t, J=7.5 Hz, 2H), 2.08–2.00 (m, 4H), 1.77 (s, 3H), 0.93 (d, J=6.6 Hz, 3H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 153.9, 141.3, 139.5, 137.9, 134.2, 1328, 131.1, 129.4, 128.8, 127.8, 106.6, 104.6, 101.2, 97.8, 78.7, 78.4, 77.4, 70.9, 69.7, 64.2, 61.8, 60.2, 58.8, 56.2, 43.6, 39.6, 34.2, 33.1, 29.8, 26.2, 24.1, 22.9, 19.6, 16.4, 13.3; HRMS (FAB) calcd for $C_{37}H_{50}ClN_2O_{10}$ 717.3154 $[M+H]^+$: 717.3151.

4.1.4. Compound 6. To a solution of the vancomycin aglycone (**15**)[17] (16 mg, 0.014 mmol) and disaccharide **14** (5 mg, 0.007 mmol) in DMF (1 mL) was added HOBt (3 mg, 0.018 mmol) and TBTU (6 mg, 0.018 mmol) followed by *N*-methyl morpholine (4 μL, 0.035 mmol). The reaction was stirred overnight at room temperature. The solution was purified by reverse-phase HPLC, eluting with a linear gradient (10–60% CH_3CN/H_2O with 0.1%TFA) to give 3 mg (23%) of the desired product **6**: ^1H NMR (CD_3OD, 500 MHz) δ 8.96 (br d, J=5.5 Hz, 1H), 8.65 (br s, 1H), 8.22 (br t, 5.5 Hz, 1H), 7.74 (d, J=8.3 Hz, 2H), 7.70 (s, 2H), 7.66 (d, J=8.6 Hz, 2H), 7.61 (d, J=8.6 Hz, 3H), 7.49 (d, J=8.3 Hz, 2H), 7.24 (d, J=8.9 Hz, 1H), 7.10 (s, 1H), 6.83, (br s, 1H), 6.80–6.77 (m, 1H), 6.61 (s, 2H), 6.45 (d, J=5.7 Hz, 2H), 5.46 (d, J=4.4 Hz, 1H), 5.42 (s, 1H), 5.35 (s, 1H), 5.31 (s, 1H), 5.17 (d, J=8.0 Hz, 1H), 4.73 (br s, 1H), 4.67 (d, J=5.5 Hz, 1H), 4.58 (q, J=6.5 Hz, 1H), 4.33 (d, J=9.6 Hz, 1H), 4.23 (s, 1H), 4.21 (s, 2H), 4.04 (t, 6.5 Hz, 1H), 3.86 (s, 6H, $-OCH_3$), 3.75 (br d, J=13.0 Hz, 2H), 3.71 (d, J=8.4 Hz, 1H), 3.66 (dd, J=12.3, 5.4 Hz, 1H), 3.60 (s, 1H), 3.55 (t, J=8.8 Hz, 2H), 3.45 (t, J=8.8 Hz, 2H), 3.20–3.14, (m, 1H), 2.95 (d, J=15.6 Hz, 1H), 2.78 (s, 3H), 2.66 (t, J=8.2 Hz, 2H), 2.19 (dd, J=13.8, 4.4 Hz, 1H), 2.04 (d, J=13.1 Hz, 1H), 1.96 (s, 1H), 1.95–1.89 (m, 3H), 1.87 (s, 3H), 1.71–1.63 (m, 2H), 1.16 (d, J=6.4 Hz, 3H), 0.97 (d, J=4.3 Hz, 3H), 0.93 (d, J=4.3 Hz, 3H); HRMS (MALDI) calcd for $C_{90}H_{99}Cl_3N_{10}NaO_{26}$ 1863.5690 $[M+Na]^+$: 1863.5744.

4.1.5. 2-Chloroethyl 2-*O*-(2-deoxy-3,4,6-tri-*O*-acetyl-2-[3-trifluoromethyl-benzamido]-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-β-D-glucopyranosiduronamide (17). To a solution of phenyl 2-*O*-(2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronic acid (**16**, 2.50 g, 3.37 mmol) in 20 mL CH_3CN were added pyridine (0.175 mL), di-*tert*-butyl-dicarbonate (0.96 g, 4.38 mmol) and NH_4HCO_3 (0.35 g, 4.38 mmol), the reaction was stirred at room temperature for 10 h, quenched with methanol (1 mL), diluted with CH_2Cl_2 (30 mL), washed with saturated $NaHCO_3$ (50 mL×2), brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (90% EtOAc/petroleum ether) to afford 1.90 g (76%) of phenyl 2-*O*-(2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide: ^1H NMR ($CDCl_3$, 500 MHz) δ 7.7–7.9 (m, 4H), 7.4–7.5 (m, 2H), 7.25–7.35 (m, 3H), 6.13 (brd, J=2.6 Hz, 1H), 5.85 (dd, J=9.2, 10.6 Hz, 1H), 5.73 (d, J=8.4 Hz, 1H), 5.60 (brd, J=2.9 Hz, 1H), 5.24 (dd, J=9.2, 9.9 Hz, 1H), 4.65 (d, J=9.2 Hz, 1H), 4.43 (dd, J=8.4, 10.6 Hz, 1H), 4.30 (dd, J=4.8, 12.5 Hz, 1H), 4.21 (dd, J=2.6, 12.5 Hz, 1H), 3.92 (m, 1H), 3.63 (d, J=9.5 Hz, 1H), 3.43 (s, 3H), 3.35–3.45

(m, 2H), 3.16 (t, J=9.5 Hz, 1H), 2.08 (s, 3H), 2.04 (s, 3H), 1.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9, 170.3, 170.2, 169.7, 168.3, 134.6, 133.0, 132.2, 131.6, 129.3, 128.5, 123.8, 97.8, 85.7, 81.7, 72.1, 70.8, 69.1, 68.4, 62.2, 60.6, 54.9, 21.0, 20.8, 20.6; HRMS (FAB) calcd for $C_{33}H_{35}N_5NaO_{13}S$ $[M+Na]^+$ 764.1850, found 764.1872.

To a solution of this disaccharide (1.90 g, 2.56 mmol) in a 5:1 mixture of CH_3OH/CH_2Cl_2 (30 mL) was added hydrazine (3.9 mL, 125 mmol). The reaction mixture was stirred 20 h at room temperature, concentrated, and purified by reverse-phase HPLC, eluting with a linear gradient (0–80% CH_3CN/H_2O). The fractions containing the product were combined and concentrated to give 0.90 g (74%) of phenyl 2-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.50–7.55 (m, 2H), 7.25–7.35 (m, 3H), 4.86 (d, J=9.9 Hz, 1H), 4.72 (d, J=8.1 Hz, 1H), 3.90 (dd, J=1.8, 12.1 Hz, 1H), 3.75–3.85 (m, 2H), 3.73 (dd, J=5.1, 11.7 Hz, 1H), 3.69 (t, J=9.5 Hz, 1H), 3.52 (s, 3H), 3.40 (t, J=9.5 Hz, 1H), 3.25–3.35 (m, 3H), 2.64 (t, J=8.1 Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.1, 134.9, 133.3, 130.1, 128.9, 103.7, 87.6, 82.8, 79.7, 78.4, 78.0, 76.4, 72.0, 70.4, 63.1, 60.7, 58.9; HRMS (FAB) calcd for $C_{19}H_{27}N_5NaO_8S$ $[M+Na]^+$ 508.1478, found 508.1481.

To a solution of phenyl 2-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide (0.90 g, 1.85 mmol) and α,α,α-trifluoro-*m*-toluic acid (0.42 g, 2.22 mmol) in DMF (60 mL) were added HATU (0.84 g, 2.22 mmol) and DIPEA (0.39 mL, 2.22 mmol). The reaction was stirred 12 h at room temperature, quenched with CH_3OH (10 mL), and purified by flash chromatography (10% CH_3OH/CH_2Cl_2) to give 1.13 g (93%) of phenyl 2-*O*-(2-deoxy-2-[3-trifluoromethyl-benzamido]-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.26 (s, 1H), 8.18 (d, J=8.1 Hz, 1H), 7.82 (d, J=7.7 Hz, 1H), 7.66 (t, J=7.7 Hz, 1H), 7.4–7.5 (m, 2H), 7.22–7.32 (m, 3H), 5.00 (d, J=8.4 Hz, 1H), 4.87 (d, J=8.8 Hz, 1H), 3.98 (dd, J=8.4, 10.3 Hz, 1H), 3.94 (dd, J=2.6, 11.7 Hz, 1H), 3.7–3.8 (m, 3H), 3.54–3.64 (m, 2H), 3.4–3.5 (m, 4H), 3.3–3.4 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.0, 169.1, 137.1, 135.0, 133.2, 132.3, 131.9, 130.6, 130.1, 129.2, 128.7, 126.6, 125.7, 125.6, 124.5, 102.2, 87.4, 82.6, 79.5, 78.1, 77.1, 75.5, 72.5, 70.6, 63.2, 60.6, 58.6; HRMS (FAB) calcd for $C_{27}H_{30}F_3N_5NaO_9S$ $[M+Na]^+$ 680.1614, found 680.1590.

To a solution of phenyl 2-*O*-(2-deoxy-2-[3-trifluoromethyl-benzamido]-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide (1.13 g, 1.72 mmol) and DMAP (0.42 g, 3.44 mmol) in pyridine (40 mL) was added Ac_2O (8.11 mL, 86.0 mmol). After 2 h at room temperature, the reaction was quenched with CH_3OH (10 mL) and concentrated. The crude solid was washed with H_2O and CH_2Cl_2 to give 1.23 g (92%) of phenyl 2-*O*-(2-deoxy-3,4,6-tri-*O*-acetyl-2-[3-trifluoromethyl-benzamido]-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide: ^{1}H NMR (DMF-d_7, 500 MHz) δ 9.04 (d, J=8.8 Hz, 1H), 8.27 (s, 1H), 8.24 (d, J=7.7 Hz, 1H), 7.95 (d, J=7.7 Hz, 1H), 7.7–7.8 (m, 2H), 7.4–7.5 (m, 3H), 7.25–7.35 (m, 3H), 5.57 (dd, J=9.5, 10.3 Hz, 1H), 5.45 (d, J=8.4 Hz, 1H), 5.17 (d, J=9.9 Hz, 1H), 5.08 (apt, J=10.3 Hz, 1H), 4.2–4.4 (m, 3H), 4.0–4.1 (m, 2H), 3.92 (apt, J=9.9 Hz, 1H), 3.5–3.6 (m, 4H), 3.44 (apt, J=9.5 Hz, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (DMF-d_7, 125 MHz) δ 171.3, 171.0, 170.7, 170.2, 166.5, 136.9, 134.9, 132.4, 131.6, 131.0, 130.8, 130.0, 129.1, 128.0, 126.5, 125.1, 125.1, 124.3, 100.6, 85.2, 81.9, 79.5, 77.0, 74.0, 72.4, 70.3, 69.5, 63.3, 60.3, 56.1, 21.2, 21.1, 20.9; HRMS (FAB) calcd for $C_{33}H_{36}F_3N_5NaO_{12}S$ $[M+Na]^+$ 806.1931, found 806.1968.

To a solution of phenyl 2-*O*-(2-deoxy-3,4,6-tri-*O*-acetyl-2-[3-trifluoromethyl-benzamido]-β-D-glucopyranosyl)-3-azido-3-deoxy-4-*O*-methyl-1-thio-β-D-glucopyranosiduronamide (100 mg, 0.128 mmol) in 2-chloroethanol (5 mL), $BF_3{\cdot}Et_2O$ (0.16 mL, 1.28 mmol) and $Hg(TFA)_2$ (61 mg, 0.192 mmol) were added. After stirring for 4.5 h, the reaction mixture was concentrated and purified by flash chromatography (5–10% CH_3OH/CH_2Cl_2) to afford 80 mg (83%) of compound **17**: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.13 (s, 1H), 8.06 (d, J=8.1 Hz, 1H), 7.83 (d, J=7.7 Hz, 1H), 7.66 (t, J=7.7 Hz, 1H), 5.42 (dd, J=9.5, 10.6 Hz, 1H), 5.12 (d, J=3.3 Hz, 1H), 5.06 (apt, J=9.5 Hz, 1H), 4.97 (d, J=8.4 Hz, 1H), 4.26 (d, J=3.7 Hz, 2H), 4.15 (dd, J=8.4, 10.6 Hz, 1H), 4.12 (d, J=9.9 Hz, 1H), 3.8–4.0 (m, 3H), 3.7–3.8 (m, 2H), 3.64 (apt, J=9.5 Hz, 1H), 3.46 (dd, J=3.7, 10.6 Hz, 1H), 3.44 (s, 3H), 3.19 (apt, J=9.9 Hz, 1H), 2.08 (s, 3H), 2.03 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.9, 172.4, 172.1, 171.4, 168.9, 136.7, 132.2, 132.0, 130.7, 129.4, 125.5, 124.4, 103.8, 99.6, 82.4, 81.0, 74.1, 73.3, 71.8, 70.7, 70.1, 66.0, 63.2, 60.9, 56.4, 43.4, 20.9, 20.7, 20.7; HRMS (FAB) calcd for $C_{29}H_{35}ClF_3N_5NaO_{13}$ $[M+Na]^+$ 776.1770, found 776.1809.

4.1.6. 2-Iodoethyl 2-*O*-(2-deoxy-2-[3-trifluoromethyl-benzamido]-β-D-glucopyranosyl)-3-(4-chloro-3-trifluoromethyl-phenyl)-ureido-3-deoxy-4-*O*-methyl-β-D-glucopyranosiduronamide (18). To a solution of **17** (80 mg, 0.106 mmol) in a mixture of 1:1 EtOH/THF (6 mL), $P(CH_3)_3$ (0.212 mL of a 1.0 M solution in THF) was added. The reaction was stirred for 1.5 h, H_2O (0.212 mL) was added, and stirring continued for another 12 h. The crude amine was concentrated, azeotroped with toluene three times and dissolved in a mixture of 7:1.5 CH_2Cl_2/DMF (8.5 mL). 4-Chloro-3-(trifluoromethyl)-phenyl isocyanate (28 mg, 0.127 mmol) was added, the solution was stirred for 3.5 h, quenched with CH_3OH (2 mL), concentrated, and purified by flash chromatography (10% CH_3OH/CH_2Cl_2) to afford 72 mg of 2-chloroethyl 2-*O*-(2-deoxy-2-[3-trifluoromethyl-benzamido]-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-3-(4-chloro-3-trifluoromethyl-phenyl)-ureido-3-deoxy-4-*O*-methyl-β-D-glucopyranosiduronamide (72% over two steps): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.91 (s, 1H), 7.80 (d, J=8.1 Hz, 1H), 7.35–7.45 (m, 2H), 7.1–7.2 (m, 3H), 5.63 (br s, 1H), 5.25 (br s, 1H), 5.20 (d, J=2.9 Hz, 1H), 5.00 (apt, J=9.9, 9.9 Hz, 1H), 4.2–4.3 (m, 2H), 4.16 (d, J=9.5 Hz, 1H), 3.7–4.0 (m, 8H), 3.3–3.5 (m, 4H), 2.08 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.4, 172.4, 171.9, 171.5, 168.4, 157.0, 140.2, 135.9, 132.7, 131.8, 131.6, 130.3, 129.0, 128.9, 126.3, 125.3, 124.7, 124.1, 123.4, 123.3, 117.9, 103.0,

100.2, 81.4, 79.9, 73.2, 72.4, 70.5, 70.4, 63.3, 60.8, 57.6, 54.1, 43.5, 20.9, 20.7, 20.6; HRMS (MALDI) calcd for $C_{37}H_{40}Cl_2F_6N_4NaO_{14}$ $[M+Na]^+$ 971.1715, found 971.1758.

To 2-chloroethyl 2-*O*-(2-deoxy-2-[3-trifluoromethyl-benzamido]-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-3-(4-chloro-3-trifluoromethyl-phenyl)-ureido-3-deoxy-4-*O*-methyl-β-D-glucopyranosiduronamide (72 mg, 0.076 mmol) in acetone (0.7 mL) was added NaI (171 mg, 1.14 mmol). The reaction was refluxed for 24 h, concentrated, and the crude iodide was dissolved in 1 mg/mL NaOMe/MeOH and stirred for 2 h before quenching with NH_4OAc (40 mg). The solution was concentrated and purified by flash chromatography (15% CH_3OH/CH_2Cl_2) to give 66 mg of compound **18** (86% over two steps): ^1H NMR (CD_3OD, 500 MHz) δ 8.03 (s, 1H), 7.90 (d, J=7.9 Hz, 1H), 7.48 (s, 1H), 7.41 (d, J=7.9 Hz, 1H), 7.1–7.3 (m, 3H), 5.29 (d, J=3.4 Hz, 1H), 4.20 (d, J=9.8 Hz, 1H), 4.01 (apt, J=10.5 Hz, 1H), 3.85–3.97 (m, 4H), 3.80 (d, J=9.5 Hz, 1H), 3.68 (dd, J=6.6, 11.7 Hz, 1H), 3.25–3.45 (m, 10H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.6, 168.5, 157.2, 140.3, 136.5, 132.7, 132.0, 131.7, 131.5, 130.1, 128.9, 128.6, 126.4, 125.5, 124.6, 124.3, 123.4, 118.0, 103.2, 100.0, 81.9, 78.8, 78.3, 74.6, 72.6, 71.3, 63.2, 60.8, 59.5, 54.2, 2.7; HRMS (MALDI) calcd for $C_{31}H_{34}ClF_6IN_4NaO_{11}$ $[M+Na]^+$ 937.0754, found 937.0709.

4.1.7. Compound 7. Disaccharide **18** (22 mg, 0.024 mmol) and protected vancomycin aglycone **12**[16] (48 mg, 0.035 mmol) were combined and azeotroped with toluene three times. Cs_2CO_3 (10 mg, 0.031 mmol) was added followed by DMF (1 mL) and the reaction was stirred at room temperature for 12 h, quenched with a drop of AcOH, concentrated, and purified by flash chromatography (15% CH_3OH/CH_2Cl_2) to afford 44 mg (84%) of the coupled product: ^1H NMR (CD_3OD, 500 MHz) δ 7.99 (s, 1H), 7.91 (d, J=7.7 Hz, 1H), 7.62 (d, J=3.7 Hz, 1H), 7.42 (s, 2H), 7.37 (d, J=7.7 Hz, 1H), 7.28 (d, J=8.4 Hz, 1H), 7.1–7.2 (m, 3H), 7.07 (s, 1H), 7.04 (d, J=8.4 Hz, 1H), 6.92 (d, J=8.8 Hz, 1H), 6.66 (d, J=2.2 Hz, 1H), 6.40 (d, J=1.8 Hz, 1H), 5.7–6.2 (m, 6H), 5.66 (s, 1H), 5.1–5.5 (m, 11H), 5.0–5.1 (m, 2H), 4.27 (m, 1H), 4.16 (s, 1H), 3.94 (d, J=11.0 Hz, 1H), 2.90 (s, 3H), 1.81 (t, J=11.0 Hz, 1H), 0.95 (d, J=6.2 Hz, 3H), 0.90 (d, J=7.3 Hz, 3H); HRMS (MALDI) calcd for $C_{100}H_{105}Cl_3F_6N_{12}NaO_{30}$ $[M+Na]^+$ 2195.5922, found 2195.5891.

To a solution of this compound (22 mg, 0.010 mmol) in 1:1 DMF/AcOH (2 mL) was added $PdCl_2(PPh_3)_2$ (7 mg, 0.010 mmol) followed by Bu_3SnH in 50 μL portions every 5 min until the reaction was complete. The mixture was pipetted into acetone (40 mL), the resulting precipitate was dissolved in 1:1 H_2O/CH_3OH (1.5 mL) and purified by reverse-phase HPLC, eluting with a linear gradient (10–70% CH_3CN/H_2O with 0.1% AcOH). The fractions containing the product were combined and concentrated to give 12 mg of compound **7** (66%): ^1H NMR (DMF-d_7, 500 MHz) δ 8.74 (s, 1H), 8.67 (m, 1H), 8.30 (m, 1H), 7.71 (s, 1H), 7.67 (d, J=6.6 Hz, 2H), 7.49 (d, J=8.1 Hz, 2H), 7.45 (d, J=8.8 Hz, 1H), 7.3–7.4 (m, 3H), 7.28 (t, J=7.3 Hz, 1H), 7.21 (s, 2H), 6.88 (dd, J=2.2, 8.4 Hz, 1H), 6.81 (d, J=8.1 Hz, 1H), 6.71 (d, J=11.7 Hz, 1H), 6.59 (s, 1H), 6.51 (m, 3H), 5.96 (d, J=7.7 Hz, 1H), 5.86 (s, 1H), 5.46 (s, 1H), 5.40 (s, 1H), 5.31 (d, J=11.4 Hz, 2H), 5.2–5.3 (m, 2H), 4.96 (d, J=8.4 Hz, 1H), 4.87 (m, 1H), 4.7–4.8 (m, 1H), 4.77 (d, J=5.1 Hz, 1H), 4.68 (d, J=5.1 Hz, 1H), 4.46–4.64 (m, 3H), 4.0–4.3 (m, 4H), 3.32 (s, 3H), 3.0–3.1 (m, 1H), 1.8–1.9 (m, 2H), 1.4–1.6 (m, 2H), 0.93 (d, J=6.6 Hz, 3H), 0.89 (d, J=6.6 Hz, 3H); HRMS (MALDI) calcd for $C_{84}H_{85}Cl_3F_6N_{12}Na\ O_{28}$ $[M+Na]^+$ 1951.4458, found 1951.4357.

4.1.8. 2-Iodoethyl 2-*O*-(3-azido-2,3,6-trideoxy-α-L-*ribo*-hexopyranosyl)-β-D-glucopyranoside (20). Sulfoxide **19** (72 mg, 0.221 mmol) was azeotroped with toluene three times and dissolved in Et_2O (3 mL). Nucleophile **9** (58 mg, 0.158 mmol) and 2,6-di-*t*-butyl-4-methyl pyridine (97 mg, 0.474 mmol) were azeotroped with toluene three times, dissolved in 1:2 CH_2Cl_2/Et_2O (6 mL), and the solution was cooled to −78°C. Triflic anhydride (18.6 μL, 0.111 mmol) was added followed by the sulfoxide solution (added dropwise over 20 min), and the reaction was stirred at −78°C for 20 min and then slowly warmed to −20°C over 1.5 h. After stirring for another 30 min, the reaction was quenched with saturated $NaHCO_3$ (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (3×8 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (15% EtOAc/CH_2Cl_2) to give 81 mg (91%) of a 2:1 mixture of α and β 2-chloroethyl 2-*O*-(3-azido-4-*O*-acetyl-2,3,6-trideoxy-L-*ribo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside as a colorless solid: R_f=0.45–0.39 (20% EtOAc/CH_2Cl_2); LRMS (ESI) calcd for $C_{22}H_{32}ClN_3NaO_{12}$ $[M+Na]^+$ 588, found 588.

The α/β mixture of 2-chloroethyl 2-*O*-(3-azido-4-*O*-acetyl-2,3,6-trideoxy-L-*ribo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (81 mg, 0.143 mmol) and NaI (300 mg, 2 mmol) was dissolved in acetone (0.8 mL) and heated at reflux for 72 h after which the reaction mixture was cooled to room temperature and the solvent was evaporated. CH_2Cl_2 was added to the residue and the mixture was filtered. The filtrate was concentrated and purified by flash chromatography (12% EtOAc/CH_2Cl_2) to give 61 mg of 2-chloroethyl 2-*O*-(3-azido-4-*O*-acetyl-2,3,6-trideoxy-α-L-*ribo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside as a colorless solid: R_f=0.34 (15% EtOAc/CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.25 (apt, J=9.5 Hz, 1H), 4.99 (apt, J=9.5 Hz, 1H), 4.93 (br d, J=3.7 Hz, 1H), 4.61 (dd, J=9.8, 3.5 Hz, 1H), 4.55 (d, J=8.0 Hz, 1H), 4.46 (dq, J=9.8, 6.2 Hz, 1H), 4.27 (dd, J=12.5, 5.0 Hz, 1H), 4.14–4.08 (m, 3H), 3.95 (ddd, J=11.0, 9.0, 6.0 Hz, 1H), 3.76 (dd, J=9.5, 8.0 Hz, 1H), 3.71 (ddd, J=9.5, 5.0, 2.3 Hz, 1H), 3.32 (m, 2H), 2.14 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.16–1.97 (m, 2H), 1.16 (d, J=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 171.3, 170.9, 170.5, 170.4, 102.3, 96.3, 76.1, 75.8, 74.5, 72.4, 72.0, 69.3, 62.8, 62.7, 55.9, 33.4, 21.5, 21.5, 21.4, 21.3, 17.9, 1.8; LRMS (ESI) calcd for $C_{22}H_{32}IN_3NaO_{12}$ $[M+Na]^+$ 680, found 680.

A solution of 2-chloroethyl 2-*O*-(3-azido-4-*O*-acetyl-2,3,6-trideoxy-α-L-*ribo*-hexopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (40 mg, 0.061 mmol) in 1 mg/mL NaOMe/MeOH (3 mL) was stirred at room temperature for 3.5 h, quenched with NH_4OAc (20 mg), concentrated, and purified by flash chromatography (gradient 7–10% MeOH/CH_2Cl_2) to give 28 mg (94%) of **20** as a colorless solid: R_f=0.35 (12% MeOH/CH_2Cl_2); ^1H NMR (CD_3OD, 500 MHz) δ 5.25

(d, J=4.0 Hz, 1H), 4.46 (d, J=8.0 Hz, 1H), 4.32 (dq, J=9.5, 6.2 Hz, 1H), 4.10 (m, 1H), 3.98–3.86 (m, 3H), 3.68 (m, 1H), 3.52 (m, 1H), 3.42–3.26 (m, 6H), 2.24 (br dd, J=15.1, 2.5 Hz, 1H), 2.01 (ddd, J=15.1, 4.0, 4.0 Hz, 1H), 1.21 (d, J=6.2 Hz, 3H); ^{13}C NMR (CD_3OD, 500 MHz) δ 103.2, 97.2, 79.4, 78.6, 78.0, 74.0, 72.3, 71.8, 65.4, 62.9, 60.2, 33.7, 18.2, 2.3; HRMS (FAB) calcd for $C_{14}H_{24}IN_3O_8Na$ $[M+Na]^+$ 512.0506, found 512.0498.

4.1.9. Compound 8. Disaccharide **20** (12 mg, 0.024 mmol) and the protected aglycone **12** (48 mg, 0.034 mmol) were combined and azeotroped with toluene three times, whereupon Cs_2CO_3 (9.6 mg, 0.029 mmol) was added and the mixture was azeotroped with toluene twice. DMF (0.2 mL) was added, the reaction was stirred at room temperature for 12 h, quenched with a drop of AcOH, and purified by flash chromatography (gradient 7–14% MeOH/CH_2Cl_2) to give 35 mg (82%) of the linked product as a white solid: R_f=0.28 (12% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.66 (d, J=8.2 Hz, 1H), 7.59 (s, 1H), 7.56 (d, J=8.5 Hz, 1H), 7.42 (s, 1H), 7.33 (d, J=8.5 Hz, 1H), 7.07 (s, 1H), 6.94 (d, J=8.5 Hz, 1H), 6.66 (d, J=2.1 Hz, 1H), 6.39 (d, J=2.1 Hz, 1H), 5.76–6.12 (m, 5H), 5.72 (s, 1H), 5.00–5.44 (m, 14H), 4.40–4.74 (m, 14H), 4.28–4.40 (m, 2H), 4.14 (s, 1H), 3.81 (d, J=10.1 Hz, 1H), 3.64 (m, 1H), 3.49 (t, J=4.6 Hz, 1H), 3.39 (t, J=8.9 Hz, 1H), 2.90 (s, 3H), 2.20 (dd, J=14.7, 2.2 Hz, 1H), 1.95 (s, 1H), 1.80–1.88 (m, 1H), 1.44–1.56 (m, 2H), 1.15 (d, J=6.1 Hz, 3H), 0.95 (d, J=6.1 Hz, 3H), 0.90 (d, J=5.5 Hz, 3H); HRMS (FAB) calcd for $C_{83}H_{95}Cl_2N_{11}NaO_{27}$ $[M+Na]^+$ 1770.5674, found 1770.5713.

The coupled product from above (20 mg, 0.011 mmol) and PPh_3 (15 mg, 0.057 mmol) were heated in 6:1 THF/H_2O (0.35 mL) at 55°C for 10 h, and cooled to room temperature. Following precipitation with Et_2O (10 mL), the reaction mixture was purified by reverse phase HPLC (gradient 10–100% CH_3CN/H_2O with 0.1% AcOH) to give 16 mg (81%) of the amine as its AcOH salt: R_f=0.28 (16:4:1:1 $CHCl_3$/MeOH/H_2O/saturated NH_4OH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.68 (d, J=7.9 Hz, 1H), 7.60 (s, 1H), 7.57 (d, J=8.2 Hz, 1H), 7.43 (s, 1H), 7.32 (d, J=8.2 Hz, 1H), 7.05 (s, 1H), 6.94 (d, J=8.9 Hz, 1H), 6.66 (d, J=2.1 Hz, 1H), 6.38 (d, J=2.1 Hz, 1H), 5.76–6.10 (m, 6H), 5.71 (s, 1H), 5.55 (s, 1H), 5.02–5.44 (m, 16H), 4.30–4.76 (m, 19H), 4.10–4.18 (m, 3H), 3.84 (d, J=10.7 Hz, 1H), 3.65 (m, 1H), 3.40–3.60 (m, 3H), 3.40 (dd, J=10.1, 4.3 Hz, 1H), 2.89 (s, 3H), 2.30–2.40 (m, 1H), 1.95–2.05 (m, 1H), 1.45–1.55 (m, 2H), 1.23 (d, J=6.1 Hz, 3H), 0.94 (d, J=5.5 Hz, 3H), 0.89 (d, J=5.5 Hz, 3H); HRMS (FAB) calcd for $C_{83}H_{98}Cl_2N_9O_{27}$ $[M+H]^+$ 1722.5949, found 1722.5934.

To a solution of the protected heptapeptide linked to the amino disaccharide (14 mg, 0.008 mmol) in 1:1 DMF/AcOH (1.5 mL) was added $PdCl_2(PPh_3)_2$ (5.7 mg, 0.08 mmol). The solution was degassed, Bu_3SnH in 50 μL portions was added every 5 min until the reaction was complete, the product was precipitated with acetone (30 mL), redissolved in water (5 mL) and filtered. The filtrate was concentrated and purified by reverse-phase HPLC (gradient 0–30% CH_3CN/H_2O with 0.1% TFA) to give 10 mg (78%) of the deprotected product as the TFA salt: R_f=0.41 (6:6:1:2 $CHCl_3$/MeOH/H_2O/saturated NH_4OH); ^{1}H NMR (DMSO 500 MHz) δ 8.77 (s, 1H), 8.57 (d, J=4.9 Hz, 1H), 7.86 (s, 1H), 7.50–7.70 (m, 4H), 7.47 (d, J=9.8 Hz, 1H), 7.20–7.40 (m, 2H), 7.15 (s, 2H), 6.70–6.80 (m, 3H), 6.40 (s, 1H), 6.26 (s, 1H), 5.99 (t, J=5.5 Hz, 1H), 5.79 (d, J=7.9 Hz, 1H), 5.00–5.30 (m, 5H), 4.88 (s, 1H), 4.58 (t, J=6.1 Hz, 1H), 4.53 (d, J=7.6 Hz, 1H), 4.30–4.50 (m, 4H), 3.90–4.10 (m, 3H), 3.67 (d, J=4.6 Hz, 1H), 3.40–3.50 (m, 2H), 3.00–3.20 (m, 2H), 2.10–2.20 (1H, m), 1.90–2.10 (m, 2H), 1.60–1.70 (m, 2H), 1.50–1.60 (m, 2H), 1.13 (d, J=6.1 Hz, 3H), 0.92 (d, J=6.1 Hz, 3H), 0.88 (d, J=6.1 Hz, 3H); HRMS (ESI) calcd for $C_{67}H_{78}Cl_2N_9NaO_{25}$ $[M+Na]^+$ 1500.4291, found 1500.4250.

To the axial amine (TFA salt, 5 mg, 0.003 mmol) in DMF (0.3 mL) was added DIPEA (2.7 μL, 0.016 mmol) followed by 4′-chlorobiphenyl-4-carboxaldehyde (31 μL of a 0.1 M solution in DMF). The reaction mixture was stirred at 60°C for 30 min, $NaBH_3CN$ (9.4 μL of a 1 M solution in THF) was added, stirring continued for another 70 min, and then the solution was cooled to room temperature and poured into Et_2O (12 mL). The precipitate was purified by reverse phase HPLC (gradient 10–60% CH_3CN/H_2O with 0.1% AcOH) to give 3.4 mg (62%) of **8** as its AcOH salt: R_f=0.65 (6:6:1:2 $CHCl_3$/MeOH/H_2O/saturated NH_4OH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.68 (s, 1H), 8.51 (s, 1H), 7.83 (s, 1H), 7.40–7.60 (m, 13H), 7.16 (s, 1H), 6.94 (s, 1H), 6.70–6.80 (m, 2H), 6.39 (s, 1H), 6.26 (s, 1H), 5.76 (t, J=7.9 Hz, 1H), 5.56 (s, 1H), 5.37 (s, 1H), 5.23 (s, 1H), 5.15–5.25 (m, 2H), 4.82 (s, 1H), 3.90–4.50 (m, 9H), 2.29 (s, 3H), 1.70–1.80 (m, 1H), 1.40–1.50 (m, 2H), 1.06 (d, J=6.1 Hz, 3H), 0.90 (d, J=6.7 Hz, 3H), 0.86 (d, J=6.7 Hz, 3H); LRMS (ESI) calcd for $C_{80}H_{87}Cl_3N_9O_{25}$ $[M+H]^+$ 1677, found 1677.

4.1.10. Determination of minimum inhibitory concentrations.[18] The strains used for all compounds except **3**, **3a** were *Enterococcus faecium* 49624, *E. faecium* CL 4931 (VanA). The strains used for **3**, **3a** were *E. faecium* RLA1, *E. faecium* CL 5242 (VanA). Test compounds were dissolved in DMSO. Minimum inhibitory concentration was determined by a standard broth microdilution assay using Brain Heart Infusion medium. Viable cells were stained blue by adding 50 μL 1 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to each well. The minimum inhibitory concentration is defined as the lowest concentration of compound that prevented visible growth.

Acknowledgments

This work was supported by NIH grant R01GM66174.

References

1. Barna, J. C. J.; Williams, D. H. *Ann. Rev. Microbiol.* **1984**, *38*, 339–357.
2. Murray, B. E. *N. Engl. J. Med.* **2000**, *342*, 710–721.
3. Bugg, T. D.; Dutka-Malen, S.; Arthur, M.; Courvalin, P.; Walsh, C. T. *Biochemistry* **1991**, *30*, 2017–2021.

4. Nagarajan, R. *J. Antibiot.* **1993**, *46*, 118–195.
5. Cooper, M. A.; Williams, D. H. *Chem. Biol.* **1999**, *6*, 891–898.
6. Allen, N. E.; LeTourneau, D. L.; Hobbs, J. N. J. *Antimicrob. Agents Chemother.* **1997**, *41*, 66–71.
7. Kim, S. J.; Cegelski, L.; Studelska, D. R.; O'Connor, R. D.; Mehta, A. K.; Schaefer, J. *Biochemistry* **2002**, *41*, 6967–6977.
8. Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. *Science* **1999**, *284*, 507–511.
9. Sun, B.; Chen, Z.; Eggert, U. S.; Shaw, S. J.; LaTour, J. V.; Kahne, D. *J. Am. Chem. Soc.* **2001**, *123*, 12722–12723.
10. Kerns, R.; Dong, S. D.; Fukuzawa, S.; Carbeck, J.; Kohler, J.; Silver, L.; Kahne, D. *J. Am. Chem. Soc.* **2000**, *122*, 12608–12609.
11. Eggert, U.S. PhD Thesis, Princeton University 2001.
12. Chen, Z. PhD Thesis, Princeton University 2001.
13. Allen, N. E.; LeTourneau, D. L.; Hobbs, J. N. *J. Antibiot.* **1997**, *50*, 677–684.
14. Eggert, U. S.; Ruiz, N.; Falcone, B. V.; Branstrom, A. A.; Goldman, R. C.; Silhavy, T. J.; Kahne, D. *Science* **2001**, *294*, 361–364.
15. Sofia, M. J.; Allanson, N.; Hatzenbuhler, N. T.; Jain, R.; Kakarla, R.; Kogan, N.; Liang, R.; Liu, D. S.; Silva, D. J.; Wang, H. M.; Gange, D.; Anderson, J.; Chen, A.; Chi, F.; Dulina, R.; Huang, B. W.; Kamau, M.; Wang, C. W.; Baizman, E.; Branstrom, A.; Bristol, N.; Goldman, R.; Han, K. H.; Longley, C.; Midha, S.; Axelrod, H. R. *J. Med. Chem.* **1999**, *42*, 3193–3198.
16. Thompson, C.; Ge, M.; Kahne, D. *J. Am. Chem. Soc.* **1999**, *121*, 1237–1244.
17. Nagarajan, R.; Schabel, A. A. *J. Chem. Soc. Chem. Comm.* **1988**, *19*, 1306–1307.
18. NCCLS, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (Approved Standard, NCCLS Document M7-A4)*. 4th ed. National Committee for Clinical Laboratory Standards: Wayne, PA, 1997.