## Hybrid Glycopeptide Antibiotics

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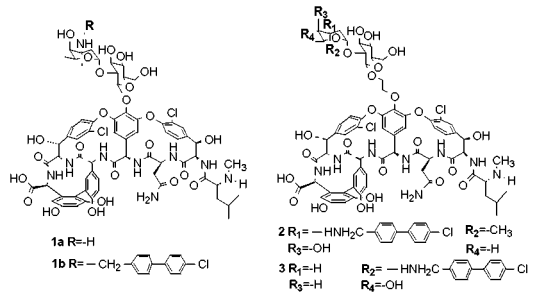
The emergence of resistance to vancomycin (**1a**, Table 1) in enterococcal strains has aroused considerable concern.[1] Efforts to overcome resistance have led to a new class of vancomycin derivatives containing hydrophobic substituents on the vancosamine sugar (e.g., **1b**, Table 1).[2] These glycolipid derivatives are more active than vancomycin against both sensitive and resistant enterococcal strains (**1b** vs **1a**, Table 1). Based on several lines of evidence, we have previously proposed that these glycolipid derivatives of vancomycin are bifunctional molecules, consisting of two biologically active components that interact with different cellular targets.[3,4] The aglycone binds to the D-Ala-D-Ala dipeptide terminus of peptidoglycan precursors; the functionalized disaccharide interacts with proteins involved in the transglycosylation step of peptidoglycan synthesis. We have suggested that this latter mechanism explains how the compounds overcome resistance.[5] If this bifunctional model is correct, then it should be possible to improve the activity of vancomycin derivatives by optimizing the glycolipid moiety for inhibition of transglycosylation.

Substituent changes to the disaccharide of vancomycin have been extensively explored, but there have been only limited efforts to change the sugars attached to the vancomycin aglycone. Glycosylation of the vancomycin aglycone is not a trival operation.[6] It would be useful to have an efficient, general strategy to attach a wide variety of different sugars to the vancomycin aglycone. We reasoned that *if* glycolipid derivatives of vancomycin are bifunctional, then the glycosidic linkage to the phenol might not be critical. If it is not, then we can substitute a simpler linker, which would enable us to explore a wide range of different carbohydrate moieties rapidly.

To evaluate the importance of the glycosidic linkage in the activity of glycolipid derivatives of vancomycin, we prepared compound **2** by the route shown in Scheme 1. The compound was found to have excellent activity against sensitive strains (Table 1). However, compared with compound **1b**, which contains the natural glycosidic linkage, **2** shows a modest decrease in activity (2−5-fold) against resistant strains. Therefore, while linker structure influences activity, it is possible to dispense with the glycosidic linkage itself.[7]

The next issue to address was how to improve on the activity of the linked compound **2**. We have developed solid-phase methods to make substituted disaccharide libraries containing hundreds to thousands of members, but the effort involved is not insignificant.[8] Prior to undertaking the synthesis of a carbohydrate library, we wanted to be sure that changing the carbohydrate structure could lead to significant improvements in activity. We explored a few conservative changes to the natural disaccharide, but all of them led to a decrease in activity. For example,

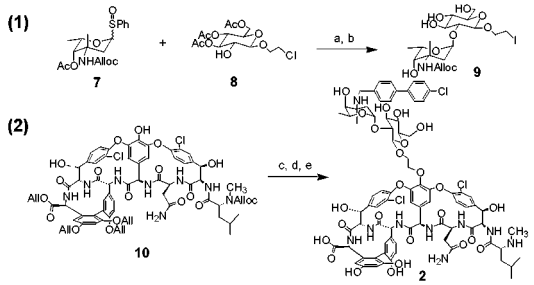
**Table 1.** MICs of Vancomycin Derivatives[a]




| compd | *E. faecium* sensitive[b] | *E. faecium* resistant[c] (VanA) | *E. faecalis* sensitive[d] | *E. faecalis* resistant[e] (VanB) | *S. aureus*[f] |
|---|---|---|---|---|---|
| **1a** | 2 | 2048 | 16 | 2048 | 4 |
| **1b** | <0.025 | 12.5 | 0.1 | 12.5 | <0.025 |
| **2** | <0.01 | 63 | 0.05 | 32 | 0.2 |
| **3** | <0.1 | 125 | 0.25 | 250 | 2 |

[a] MIC values ($\mu$g/mL) were obtained by using a standard microdilution assay. The MIC is defined as the lowest antibiotics concentration that resulted in no visible growth after incubation at 35 °C for 22 h. [b] Bacterial strain 49624. [c] Bacterial strain CL4931. [d] Bacterial strain 29212. [e] Bacterial strain CL4877. [f] Bacterial strain 29213.

**Scheme 1**[a]




[a] Conditions: (a) $Tf_2O$, DTBMP, −78 to −20 °C, $Et_2O/CH_2Cl_2$, 84%. (b) i: NaI, acetone, 99%; ii: NaOMe, MeOH, 82%. (c) **9**, $Cs_2CO_3$, DMF, 83%. (d) $PdCl_2(PPh_3)_2$, $Bu_3SnH$, DMF/AcOH, 79%. (e) 4,4′-Chlorobiphenyl aldehyde, DIEA, $NaBH_3CN$, DMF, 46%.

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(6) Both chemical and enzymatic methods have been used to make a limited number of derivatives. see: (a) Ge, M.; Thompson, D.; Kahne, D. *J. Am. Chem. Soc.* **1998**, *120*, 11014. (b) Thompson, C.; Ge, M.; Kahne, D. *J. Am. Chem. Soc.* **1999**, *121*, 1237. (c) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Winssinger, N.; Hughes, R.; Bando, T. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 240. (d) Solenberg, P. J.; Matsushima, P.; Stack, D. R.; Wilkie, S. C.; Thompson, R. C.; Baltz, R. H. *Chem. Biol.* **1997**, *4*, 195. (e) Losey, H. C.; Peczuh, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; Pelczer, I.; Kahne, D.; Walsh, C. T. *Biochemistry* **2001**, *40*, 4745.
(7) As a control, we have also coupled the natural, unsubstituted disaccharide to the vancomycin aglycone through an ethylene glycol linker. Like vancomycin itself, this compound (**2a**) is active against sensitive strains but not active against resistant strains. We have also attached a chlorobiphenyl-substituted disaccharide to the carboxy terminus of the vancomycin aglycone. The activity of this compound (**2b**) against resistant strains is comparable to the activity of **1b**. Compound **2b** supports the conclusion that the natural linkage is not essential. See Supporting Information for the structures and MIC values of compound **2a** and **2b**.
(8) Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520.

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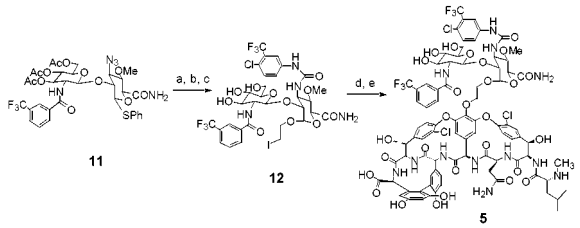
**Scheme 2**[a]



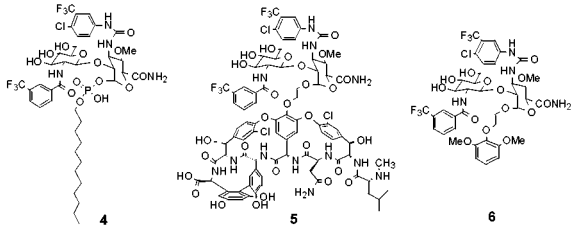

[a] Conditions: (a) $(CF_3CO_2)_2Hg$, $(C_2H_5)_2O{\cdot}BF_3$, 2-chloroethanol, 83%. (b) i: $(Me)_3P$, $H_2O$/THF/EtOH; ii: 4-chloro-3-(trifluoromethyl)phenyl isocyanate, $CH_2Cl_2$/DMF, 72% for two steps. (c) i: NaI, acetone; ii: NaOMe, MeOH, 86% for two steps. (d) **10**, $Cs_2CO_3$, DMF, 84%. (e) $PdCl_2(PPh_3)_2$, $Bu_3SnH$, DMF/AcOH, 66%.

compound **3** (Table 1), an isomer of compound **2**, is less active against both sensitive *and* resistant strains than **1b** or **2**. Although the activity of compound **3** did change, it was not for the better. We needed a more rational approach for compound selection.

We have suggested that the chlorobiphenyl disaccharide moiety on vancomycin analogues **1b** and **2** overcomes resistance by interacting with proteins involved in the transglycosylation step of cell wall biosynthesis.[3,9] If this hypothesis explains the activity of **1b** and **2** against resistant bacterial strains, then one might predict that replacing the disaccharide on **2** with a known transglycosylase inhibitor would produce a still more active compound. The best transglycosylase inhibitor known,[10] moenomycin, is a glycophospholipid containing five hexoses. Sofia and co-workers recently made a solid-phase library of disaccharides based on a fragment of moenomycin and identified compound **4** as having both good antibacterial activity and an ability to inhibit transglycosylation.[11] This disaccharide seemed like a much better starting point than the vancomycin disaccharide because it has better transglycosylase inhibitory activity. Therefore, we prepared the disaccharide *without* the anomeric phospholipid (Scheme 2) and linked it to the vancomycin aglycone to make **5**. The activity of compound **5** is shown in Table 2 along with the activities of **4** and a related analogue **6** that lacks the phospholipid (Table 2). **5** is significantly more active than compound **2** against resistant strains. In fact, it is comparable to or better against these strains than compound **1b** (Table 1), the glycosidically linked prototype, while maintaining excellent activity against sensitive strains. **5** is also more active than **4** even though it lacks the phospholipid anchor, which was previously suggested to be critical for biological activity (**6**, which does not contain either a phospholipid anchor *or* the vancomycin aglycone, has minimal activity).[11a]

The preceding results support the hypothesis that better vancomycin analogues can be made by attaching carbohydrates with good transglycosylase inhibitory activity to the vancomycin aglycone. The functionalized carbohydrate in **5** is based on a disaccharide analogue (**4**) of moenomycin, a known transglycosylase inhibitor.[12,13] The phospholipid anchor in **4** was replaced with the vancomycin aglycone to produce a hybrid compound with activity that far exceeds the activity of the individual components (compare **5** to the mixture of **6** with the vancomycin aglycone, Table 2). The synthesis of a large collection of vancomycin analogues will be greatly facilitated by the replacement of the glycosidic linkage with a simple ethylene glycol linker. Vancomycin has inspired the design of many synthetic peptide binders.[14] Attaching transglycosylase inhibitors to those compounds could be a fruitful approach to the design of new antibiotics. Hence, this work suggests significant opportunities for the design of a large class of new antibiotics comprised of hosts that bind to cell surface peptides attached to specific inhibitors for peptidoglycan-processing enzymes.

**Table 2.** MICs of Moenomycin Disaccharide Derivatives[a]




| | *E. faecium* | | *E. faecalis* | | |
|---|---|---|---|---|---|
| compd | sensitive | resistant (VanA) | sensitive | resistant (VanB) | *S. aureus* |
| **4** | 4 | 4 | 4 | 1 | 2 |
| **5** | 0.1 | 16 | <0.1 | 1 | 0.2 |
| **6** | 250 | 250 | 125 | 250 | 125 |
| **VA**[b] | 2 | >500 | 8 | >500 | 2 |
| **6** + **VA**[c] | 1 | 250 | 4 | 250 | 2 |

[a] Same bacterial strains were used as in Table 1. [b] Vancomycin aglycone. [c] Add mixture of **6** and vancomycin aglycone.

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**Supporting Information Available:** Experimental details, including spectral information, for the synthesis of compounds **11**, **12**, and **5**, as well as MIC data of related compounds **2a** and **2b** (see footnote 7) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) We have confirmed that **5** inhibits transglycosylation in a permeabilized *E. coli* model, like **4**.[3a,11b] In addition, mutant bacterial strains described in ref 13, which are resistant to transglycosylase inhibitors, are also resistant to compounds **4** and **5**.

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