The Role of Hydrophobic Substituents in the Biological Activityof Glycopeptide Antibiotics

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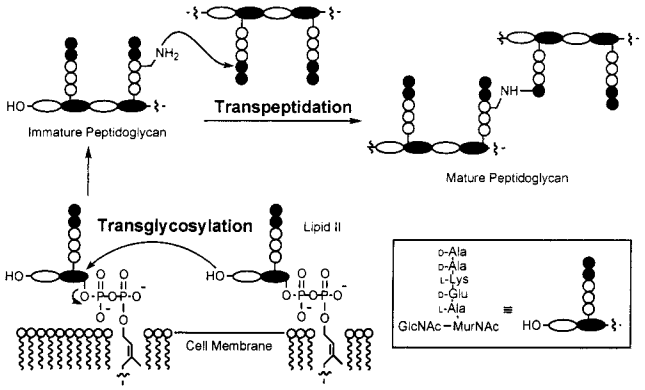
Vancomycin and teicoplanin (Figure 1, **1a** and **2a**) are the two glycopeptide antibiotics that are used clinically.[1] These drugs function against gram positive bacteria by binding to the terminal D-Ala-D-Ala dipeptide of peptidoglycan precursors, preventing maturation of the bacterial cell wall (Scheme 1).[2] Bacteria become resistant to vancomycin and teicoplanin by producing cell wall precursors terminating in D-Ala-D-Lac, a depsipeptide ligand that interacts only weakly with the peptide binding pockets of the drugs.[3] The emergence of such antibiotic resistance poses a serious threat to human health.[4] However, it is possible to overcome resistance by attaching a hydrophobic substituent to the vancosamine nitrogen of vancomycin (Figure 1, **3a**).[5] Although teicoplanin (**2a**) contains a naturally occurring hydrophobic substituent, it is not as active as these vancomycin derivatives (**3a**) against VanA-resistant strains producing D-Ala-D-Lac peptide termini (Table 1).

We have been probing the role of the hydrophobic substituent in the biological activity of vancomycin derivatives by varying its position. In this paper, we report the synthesis and evaluation of a new class of vancomycin derivatives containing hydrophobic substituents on the glucose C6 position (Figure 1, **4a** and **5a**). Like teicoplanin, this class of compounds has the hydrophobic substituent on the sugar directly attached to the aglycone. Below we show that **4a** and **5a** behave more like teicoplanin than like the vancosamine-substituted derivative **3a**, indicating that the position of the hydrophobic substituent influences the mechanism of action.

Although numerous vancosamine-substituted derivatives of vancomycin have been reported, the glucose directly attached to the aglycon has not been modified previously. Because the glucose C6 hydroxyl is the only primary hydroxyl in the molecule, we thought it would be possible to modify this position selectively. Following protection of the amino and carboxylic acid groups, the C6 primary hydroxyl was converted to a mesitylene sulfonyl ester (Scheme 2, **6**). Displacement with azide followed by reduction provided amine derivative **7**, which was converted to the C6 glucosamine derivatives **4a** and **5a** as shown. This synthetic route is straightforward and can be adapted to make a wide range of C6-substituted glucose derivatives of vancomycin.

We compared the minimum inhibitory concentrations (MIC) of teicoplanin (**2a**) and the vancosaminyl-substituted derivative **3a** with the C6 glucosamine derivatives **4a** and **5a**. As shown in Table 1, all the glycopeptides containing a lipid substituent are

Scheme 1. Peptidoglycan Biosynthesis



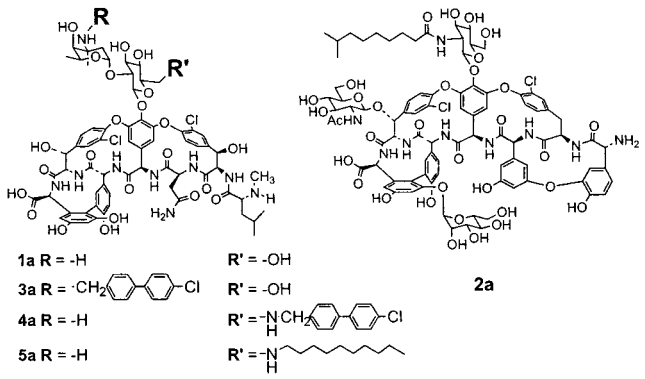


Figure 1. Glycopeptide Antibiotics.

Table 1. MICs against *E. faecium*[a]

intact glycopeptide	sensitive[b]	resistant[c] (VanA)	damaged glycopeptide	sensitive[b]	resistant[c] (VanA)
1a	2	512	**1b**	no activity	no activity
2a	1	128	**2b**	16	2048
3a	<0.03	2	**3b**	4	16
4a	<0.03	16	**4b**	64	1024
5a	0.06	32	**5b**	32	512

[a] MIC values (μg/mL) were obtained using a standard microdilution assay. The MIC is defined as the lowest antibiotic concentration that resulted in visible growth after incubation at 35 °C for 22 h. [b] Bacterial strain used: RLA1. [c] Bacterial strain used: CL5242.

significantly more active than vancomycin itself against vancomycin-sensitive bacterial strains. Moreover, they have good activity against resistant strains as well. In an assay that reveals the step at which peptidoglycan synthesis is inhibited, compounds **2a**−**5a** were found to block transglycosylation, while vancomycin was found to block transpeptidation.[6] Assuming that all compounds bind to D-Ala-D-Ala, this difference in the site of inhibition suggests that glycopeptides containing lipid substituents bind primarily to Lipid II, whereas vancomycin binds primarily to immature (un-cross-linked) peptidoglycan (Scheme 1). Preferential binding to Lipid II would be achieved if the hydrophobic substituents anchor the glycopeptides in bacterial membranes.[7]

Membrane anchoring can explain both the lower MICs against sensitive bacterial strains and the switch in the site of inhibition

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(2) Barna, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* **1984**, *38*, 339.
(3) Bugg, T. D. H.; Wright, G. D.; Dutka-Malen, S.; Courvalin, P.; Walsh, C. T. *Biochemistry* **1991**, *30*, 10408.
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(5) Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L.; Felty-Duckworth, A. M. *J. Antibiot.* **1989**, *42*, 63.
(6) Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. *Science* **1999**, *284*, 507.
(7) Williams has proposed that the lipid substituent on teicoplanin functions as a membrane anchor, and our results are consistent with this hypothesis. See: (a) Mackay, J. P.; Gerhard, U.; Beauregard, D. A.; Westwell, M. S.; Searle, M. S.; Williams, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4581. (b) Cooper, M. A.; Williams, D. H. *Chem. Biol.* **1999**, *6*, 891.

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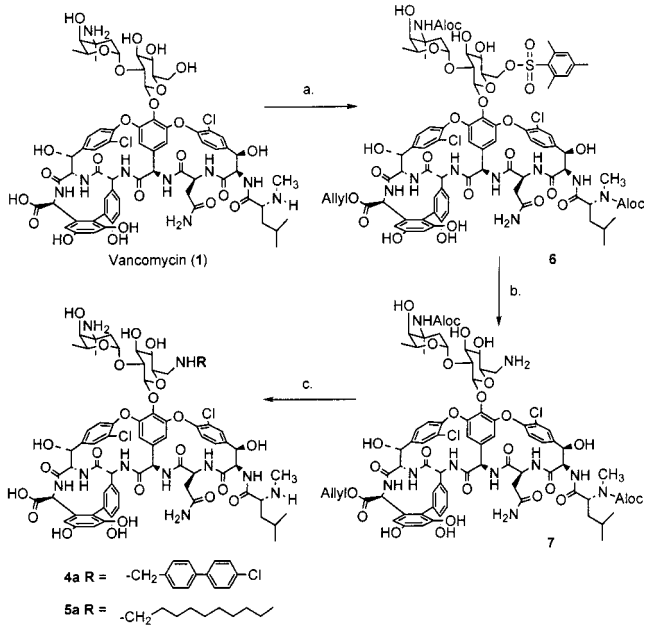
Scheme 2[a]



[a] (a) i. Aloc-NHS, $NaHCO_3$, H_2O/acetone; ii. allyl bromide, $NaHCO_3$, DMSO, 85% from **1**; iii. mesitylenesulfonyl chloride, pyridine, 4 °C, 55%. (b) i. NaN_3, DMF, 85 °C, 60%; ii. PPh_3, THF/H_2O, 60 °C, 82%. (c) i. RCHO, $NaCNBH_3$, DMF, rt, 20−60%; ii. Bu_3SnH, AcOH/DMF, $(Ph_3P)_2PdCl_2$, 88%.

relative to vancomycin. However, it is not obvious how lipid-substituted glycopeptides kill resistant bacteria, which do not contain substrates presenting D-Ala-D-Ala.[8] It is possible that the proximity to Lipid II, which is enforced by membrane anchoring, helps overcome the reduced binding affinity to D-Ala-D-Lac. To determine whether peptide binding plays a significant role in the activity of these lipid-substituted compounds against resistant bacteria, we prepared a set of compounds in which the peptide binding pockets were damaged.

Compounds **2b**−**5b**, which have reduced affinity for D-Ala-D-Ala and D-Ala-D-Lac, were prepared by Edman degradation or reductive hydrolysis (Figure 2).[9] In MIC assays, damaged teicoplanin **2b** and the damaged C6-substituted vancomycin derivatives **4b** and **5b** no longer display activity against vancomycin resistant strains (Table 1). Therefore, the intact parent compounds must kill resistant bacteria by a mechanism that requires peptide binding. In contrast, the damaged chlorobiphenyl vancomycin derivative **3b** has comparable activity against both sensitive and resistant strains. Furthermore, its activity against resistant strains is comparable to that of the parent compound **3a**.

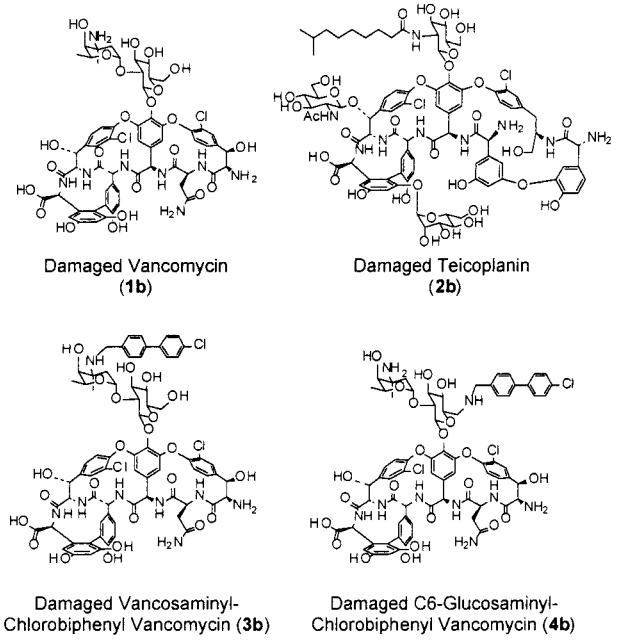


Figure 2. Damaged Glycopeptide Antibiotics.

The damaged compounds **3b** and **4b** are structurally similar. Both of them can anchor in membranes as assessed by their ability to block transglycosylation rather than transpeptidation in the site of inhibition assay. Furthermore, both of them have substantially greater activity than the parent compound, vancomycin, against sensitive bacterial strains. However, the two compounds have significantly different activities against resistant enterococci (Table 1), with **3b** having a MIC of 16 μg/mL and **4b** having a MIC of 1024 μg/mL. We conclude that compounds in the **3** series have a second mechanism of action that is only revealed when peptide binding is abolished.[10,11]

In conclusion, we have uncovered differences in the mechanism of action of hydrophobically substituted glycopeptide derivatives by comparing the biological activities of pairs of compounds containing intact and damaged peptide binding pockets. Like teicoplanin, vancomycin derivatives containing hydrophobic substituents on the sugar have excellent activity against both sensitive and many resistant bacterial strains. For most of the compounds, this activity depends on having an intact peptide binding pocket. The requirement for an intact peptide binding pocket suggests that the primary function of the hydrophobic substituent is to anchor the glycopeptide to membranes, which increases proximity—and thus binding—to Lipid II, the substrate for the transglycosylases. For derivatives substituted on the vancosaminyl sugar (**3a**), however, considerable activity is retained even when the peptide binding pocket is damaged. These compounds have an additional biological activity that cannot simply be due to membrane localization. Identifying the source of this activity should lead to a more rational approach to the design of vancomycin derivatives that overcome resistance.

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(8) It has been suggested that many glycopeptide antibiotics overcome resistance through the formation of dimers as described by: Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1172. The ability of **4a** and **5a** to form dimers was evaluated by affinity capillary electrophoresis (ACE) (for a description of ACE, see: Colton, I. J.; Carbeck, J. D.; Rao, J.; Whitesides, G. M. *Electrophoresis* **1998**, *19*, 367). Separations were performed on a P/ACE MDQ (Beckman Instruments) using a 27 cm silica column (20 cm to detector) capillary at 25 °C with an applied voltage of 10 kV. The electrophoresis buffer was 20 mM sodium phosphate (pH 7.0), and histamine was used as a positively charged marker of electroosmotic flow. Detection was by direct UV absorbance at 200 nm. At concentrations up to 100 μM, the compounds did not display mobility changes consistent with simple dimer formation. Teicoplanin, which does not dimerize, showed similar behavior as demonstrated by: LeTourneau, D. L.; Allen, N. E. *Anal. Biochem.* **1997**, *246*, 62.

(9) (a)Des-leucyl C6 vancomycin derivatives **4b** and **5b** were prepared by first subjecting vancomycin to Edman degradation utilizing a procedure described by Booth, P. M.; Stone, D. J. M.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* **1987**, 1694. (i. PhNCS, pyridine, 40 °C, 1.5 h; ii. TFA, CH_2Cl_2, rt, 3 min, 100%.) The resulting des-leucyl vancomycin was converted to the C6 analogues **4b** and **5b** following the route for vancomcyin in Scheme 2. (b) Damaged teicoplanin was prepared by reductive hydrolysis according to the procedure of: Malabarba, A.; Ciabatti, R.; Kettenring, J.; Ferrari, P.; Vékey, K.; Bellasio, E.; Denaro, M. *J. Org. Chem.* **1996**, *61*, 2137. ($NaBH_4$, EtOH/H_2O, rt, 48h, 20%.)

(10) The possibility that the significant activity differences between **3b** and **4b** can be explained by subtle changes in membrane presentation seems unlikely. No striking differences in activity are observed between **3a** and **4a** when the peptide-binding-dependent mechanism dominates.

(11) This finding has been confirmed using an alternative experimental approach as described by: Goldman, R. C.; Baizman, E. R.; Longley, C. B.; Branstrom, A. A. *FEMS Microbiol. Lett.* 2000, *183*, 209.