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The Structural Basis for Induction of VanB Resistance

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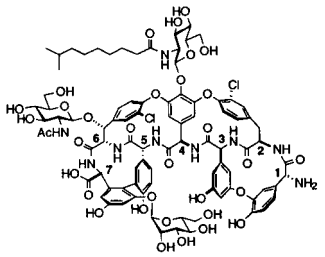
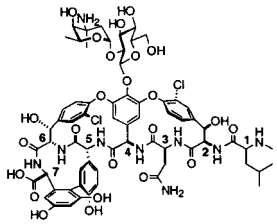
Teicoplanin (**1**) and vancomycin (**2**) are glycopeptide antibiotics used for the treatment of methicillin-resistant Gram-positive bacterial infections.[1] These glycopeptides act by binding to the terminal D-Ala-D-Ala dipeptide of peptidoglycan precursors, preventing maturation of the bacterial cell wall.[2] Because they are the last line of defense for many bacterial infections, the emergence of resistance to glycopeptide antibiotics in enterococci and streptococci has aroused concern.[3] The most common form of resistance to vancomycin occurs in enterococci when the bacteria acquire genes encoding enzymes that remodel the dipeptide terminus of peptidoglycan precursors from D-Ala-D-Ala to D-Ala-D-lactate so that vancomycin cannot bind to them.[4] Strains displaying this type of resistance generally have either a VanA phenotype, which means they are resistant to both vancomycin and teicoplanin, or a VanB phenotype, which means they are resistant only to vancomycin.[5] The genes directly involved in remodeling the peptidoglycan precursors are highly homologous in VanA and VanB strains. Therefore, it is not clear why teicoplanin and vancomycin, which are structurally and mechanistically similar, affect VanB strains differently.



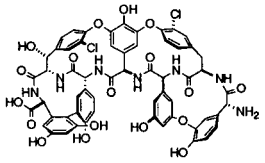
Teicoplanin (**1**)



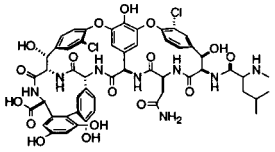
Vancomycin (**2**)

Transcription of the genes involved in remodeling peptidoglycan precursors is under the control of a two-component regulatory system.[6] In VanB strains, this two component system contains a histidine kinase sensor protein, $VanS_b$, that controls the phosphorylation state of a response regulator called $VanR_b$.[7] In the absence of vancomycin, $VanS_b$ functions primarily as a $VanR_b$ phosphatase and prevents promiscuous cellular kinases from inappropriately activating transcription by phosphorylating $VanR_b$. In the presence of vancomycin, $VanS_b$ phosphatase activity is switched off, $VanR_b$ is phosphorylated, and transcription of the genes, VanHBX, that remodel peptidoglycan precursors is initiated.[6] It is not clear how the $VanS_b$ sensor kinase is activated. It has been proposed that differential regulation of the remodeling genes upon exposure to vancomycin and teicoplanin could be explained if the former but not the latter binds specifically to $VanS_b$.[8,9]

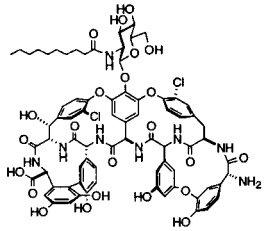
Vancomycin and teicoplanin have similar structures and bind *N*-Acyl-D-Ala-D-Ala with comparable affinity. Nevertheless, there are three distinct structural differences. First, amino acids 1 and 3 of the aglycons are different; second, teicoplanin contains a neutral, lipid-containing monosaccharide on the fourth amino acid of the heptapeptide, while vancomycin contains a positively charged disaccharide at the same position; and third, teicoplanin contains additional sugars on the aglycon.



Teicoplanin aglycone (**3**)



Vancomycin aglycone (**4**)



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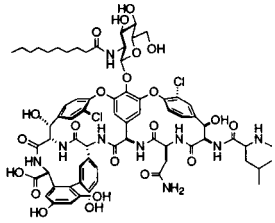

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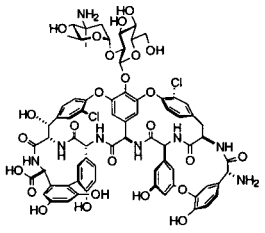

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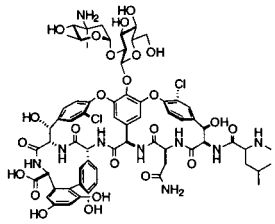

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To determine which structural differences account for differential regulation of the remodeling genes, we prepared a complementary set of vancomycin and teicoplanin analogues and evaluated their activities against VanB-resistant enterococci. Compounds **3** and **4** are the teicoplanin and vancomycin aglycons, respectively. Compound **5** is a teicoplanin analogue containing a C-2 *N*-decanoyl glucosamino sugar but no other sugars; **6** is the corresponding vancomycin analogue; compound **7** is a teicoplanin analogue containing a positively charged disaccharide but no other sugars; **8** is the corresponding vancomycin analogue. Compounds **5** and **6** were prepared from the aglycons **3** and **4** using a chemoenzymatic approach as shown in Scheme 1. For compounds **7** and **8**, glucose and epivancosamine were attached enzymatically to aglycons **3** and

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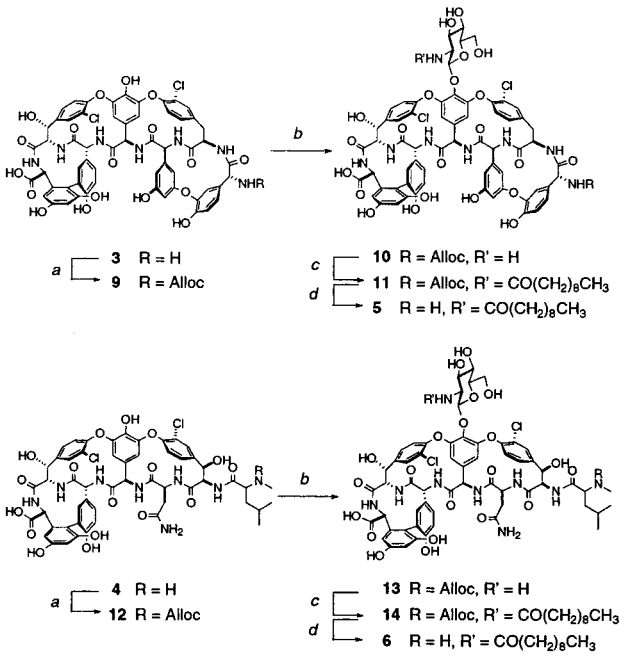
Scheme 1 [a]



[a] Conditions: (a) alloc succinimide (2 equiv), Et_3N, DMF, 3 h, 69% (**9**), 73% (**12**). (b) 2.8 mM UDP 2-NH_2-Glc (4 equiv and 3 equiv relative to **9** and **12**, respectively), 5 μM GtfE, pH 9.0 buffer containing 75 mM Tricine, 2.5 mM tris(2-carboxyethyl)phosphine, 1 mg/mL bovine serum albumin, 37 °C, 40 h, 57% (**10**), 69% (**13**). (c) decanoyl succinimide (3 equiv), Et_3N, DMF, 16 h, 57% (**11**), 55% (**14**). (d) $Me_2NH{\cdot}BH_3$ (6 equiv), $Pd(PPh_3)_4$ (0.4 equiv), DMF, 30 min, 66% (**5**), 69% (**6**).

Table 1. MIC Values against *E. faecalis*[a]

cmpd	sensitive[b]	resistant[c] (VanB)	cmpd	sensitive[b]	resistant[c] (VanB)
1	0.5	0.5	**5**	0.2	4
2	16	>500	**6**	0.2	8
3	2	>500	**7**	8	>130
4	16	>500	**8**	8	>500

[a] MIC values (μg/mL) were obtained using a standard microdilution assay. The MIC is defined as the lowest antibiotic concentration that resulted in no visible growth after incubation at 35 °C for 22 h. [b] Bacterial strain CL4931. [c] Bacterial strain 29212.

4 using glycosyl transferases from the vancomycin and chloroeremomycin biosynthetic clusters.[10]

The minimum inhibitory concentrations of teicoplanin (**1**), and vancomycin (**2**), and compounds **3**−**8** against VanB-sensitive and -resistant bacterial strains are shown in Table 1. Both aglycons have activity against sensitive strains but lose all activity against VanB strains, indicating that they induce resistance. Analogues **5** and **6**, which have different aglycons but the same lipidated monosaccharide, retain activity against VanB strains. When the lipidated monosaccharide is replaced by a positively charged disaccharide, as in **7** and **8**, activity against VanB strains is lost.

These data show that the vancomycin and teicoplanin aglycons are the minimal structural features required to induce resistance; however, their ability to do so can be blocked by the addition of a lipid-substituted carbohydrate. It is unlikely that the lipid-substituted sugars overcome resistance simply by hindering binding to the sensor kinase. If this were the case, one would expect the disaccharide in compounds **7** and **8** to interfere with binding as well.

The preceding results raise the question of how resistance is induced. If resistance is induced by binding of glycopeptides directly to the sensor kinase, then it is necessary to explain why the addition of some carbohydrate moieties at A4 interferes with binding while the addition of other carbohydrate moieties does not. One possibility is that lipid-containing carbohydrate substituents circumvent resistance because they localize near the bacterial membrane where they are not as accessible to the sensor kinase as the other glycopeptides.[11] The other possibility is that the membrane-anchored glycopeptides cause a different set of cell wall intermediates to accumulate because they block the transglycosylation step of peptidoglycan synthesis rather than the transpeptidation step.[12,13] If resistance is induced (or circumvented) by an accumulated intermediate or breakdown product, then differences in the step that is inhibited could have a profound effect on biological activity even if binding to D-Ala-D-Ala is the proximate cause of inhibition.[14,15]

We are continuing to investigate the nature of the signaling mechanism responsible for induction of VanB resistance. The lipoglycopeptides analyzed here, in conjunction with glycopeptide analogues we have previously reported,[12,13,15] may also enable us to differentiate the VanA and VanB signaling pathways. In the meantime, we note that the preceding results provide a clear prescription for how to circumvent VanB resistance in designing better glycopeptide antibiotics.

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Supporting Information Available: Experimental procedures for the syntheses and spectral data of new compounds **5** and **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Malabarba, A.; Nicas, T. I.; Thompson, R. C. *Med. Res. Rev.* **1997**, *17*, 69.
(2) Barna, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* **1984**, *38*, 339.
(3) Murray, B. E. *N. Engl. J. Med.* **2000**, *342*, 710.
(4) Bugg, T. D.; Dutka-Malen, S.; Arthur, M.; Courvalin, P.; Walsh, C. T. *Biochemistry* **1991**, *30*, 2017.
(5) Quintiliani, R. J.; Evers, S.; Courvalin, P. *J. Infect. Dis.* **1993**, *167*, 1220.
(6) Silva, J. C.; Haldimann, A.; Prahalad, M. K.; Walsh, C. T.; Wanner, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11951.
(7) Arthur, M.; Quintilliani, R. J. *Antimicrob. Agents Chemother.* **2001**, *45*, 375.
(8) Baptista, M.; Depardieu, F.; Courvalin, P.; Arthur, M. *Antimicrob. Agents Chemother.* **1996**, *40*, 2291.
(9) Arthur, M.; Depardieu, F.; Courvalin, P. *Microbiology* **1999**, *145*, 1849.
(10) 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.
(11) Evers, S.; Courvalin, P. *J. Bacteriol.* **1996**, *178*, 1302.
(12) It has long been known that glycopeptides such as vancomycin block transglycosylation when assayed in particulate bacterial membranes: Anderson, J. S.; Matsuhashi, M.; Haskin, M. A.; Strominger, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 881. More recently, it has been shown that in cell-based assays, lipid-substituted glycopeptides block transglycosylation, whereas vancomycin blocks transpeptidation: Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. *Science* **1999**, *284*, 507.
(13) Kerns, R.; Dong, S. D.; Fukuzawa, S.; Carbeck, J.; Kohler, J.; Silver, L.; Kahne, D. *J. Am. Chem. Soc.* **2000**, *122*, 12608.
(14) Baptista, M.; Rodrigues, P.; Depardieu, F.; Courvalin, P.; Arthur, M. *Mol. Microbiol.* **1999**, *32*, 17.
(15) It is possible that transglycosylation inhibitors circumvent resistance because they trigger another signal that causes rapid cell death. Hence, induction of resistance cannot be observed. See: Eggert, U. S.; Ruiz, N.; Falcone, B. V.; Branstrom, A. A.; Goldman, R. C.; Silhavy, T. J.; Kahne, D. *Science* **2001**, *294*, 361.

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