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A Redesigned Vancomycin Engineered for Dual D-Ala-D-Ala and D-Ala-D-Lac Binding Exhibits Potent Antimicrobial Activity Against Vancomycin-Resistant Bacteria

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Supporting Information

ABSTRACT: The emergence of bacteria resistant to vancomycin, often the antibiotic of last resort, poses a major health problem. Vancomycin-resistant bacteria sense a glycopeptide antibiotic challenge and remodel their cell wall precursor peptidoglycan terminus from D-Ala-D-Ala to D-Ala-D-Lac, reducing the binding of vancomycin to its target 1000-fold and accounting for the loss in antimicrobial activity. Here, we report [Ψ[C(=NH)NH]Tpg^4]vancomycin aglycon designed to exhibit the dual binding to D-Ala-D-Ala and D-Ala-D-Lac needed to reinstate activity against vancomycin-resistant bacteria. Its binding to a model D-Ala-D-Ala ligand was found to be only 2-fold less than vancomycin aglycon and this affinity was maintained with a model D-Ala-D-Lac ligand, representing a 600-fold increase relative to vancomycin aglycon. Accurately reflecting these binding characteristics, it exhibits potent antimicrobial activity against vancomycin-resistant bacteria (MIC = 0.31 μg/mL, VanA VRE). Thus, a complementary single atom exchange in the vancomycin core structure (O → NH) to counter the single atom exchange in the cell wall precursors of resistant bacteria (NH → O) reinstates potent antimicrobial activity and charts a rational path forward for the development of antibiotics for the treatment of vancomycin-resistant bacterial infections.

Vancomycin (**1**) is the most widely recognized member of an important family of glycopeptide antibiotics.[1] Clinical uses of vancomycin include its use in the treatment of patients on dialysis, and patients allergic to β-lactam antibiotics.[2] However, its most important use is in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, for which vancomycin is the drug of last resort.[3,4] The prevalence of MRSA in U.S. intensive care units (ICU, 60% of SA infections are MRSA, 2003)[5] and the movement of MRSA from a hospital-acquired to a community-acquired infection have intensified the need to combat such resistant bacterial infections. Concurrent with the emergence of community-acquired MRSA, vancomycin-resistant strains of other bacteria are also on the rise with U.S. ICU isolates of vancomycin-resistant *Enterococcus faecalis* (VRE) approaching 30% (2003),[5] albeit in strains remaining sensitive to other antibiotics. Most feared is the emergence of MRSA strains now insensitive or resistant to vancomycin (VISA and VRSA) even in developed countries.[6,7] This poses a major health problem and has stimulated efforts to develop vancomycin analogues[8,9] or alternative antibiotics for the treatment of such vancomycin-resistant bacterial infections.[7,10]

Vancomycin inhibits bacterial cell wall synthesis by binding to the peptidoglycan peptide terminus D-Ala-D-Ala found in cell wall precursors,[11] sequestering the substrate from transpeptidase and inhibiting cell wall cross-linking. The D-Ala-D-Ala complex with the antibiotic is stabilized by an array of hydrophobic van der Waals contacts and five hydrogen bonds (H-bonds) lining the vancomycin binding pocket (Figure 1).[12] Vancomycin-resistant bacteria (VanA and VanB) sense the antibiotic challenge[13] and subsequently remodel their precursor peptidoglycan terminus from D-Ala-D-Ala to D-Ala-D-Lac.[14,15] Normal D-Ala-D-Ala production continues despite the presence of vancomycin, but a late-stage remodeling to D-Ala-D-Lac ensues to avoid the action of the antibiotic. The substitution of a linking ester for the amide with the exchange of a single atom (NH → O) reduces the binding to vancomycin 1000-fold and accounts for the 1000-fold higher MICs seen in VRE clinical isolates.[14] One key, but subtle insight to emerge from this characterization of vancomycin-resistant bacteria is that efforts to redesign vancomycin for their treatment should target compounds that not only bind D-Ala-D-Lac, but that also maintain binding to D-Ala-D-Ala.

The complex of vancomycin with D-Ala-D-Lac lacks the central H-bond of the D-Ala-D-Ala complex and suffers a repulsive lone pair interaction between the vancomycin residue 4 carbonyl and D-Ala-D-Lac ester oxygens (Figure 1). We provided an experimental estimation of the magnitude of these two effects by examining the model ligands **2**−**4**, revealing that it is the repulsive lone pair interactions (100-fold), not the H-bond loss (10-fold), that is responsible for the largest share of the reduced binding affinity (1000-fold).[16] These observations had important ramifications on our redesign of vancomycin to bind D-Ala-D-Lac, suggesting that efforts could focus principally on removing the destabilizing lone pair interaction rather than reintroduction of a H-bond and that this may be sufficient to compensate for the majority of the binding affinity lost with D-Ala-D-Lac.

In conjunction with studies on the total synthesis of the glycopeptide antibiotics[17−22] and concurrent with efforts probing systematic modifications to vancomycin itself,[23] we initiated efforts on the redesign of vancomycin and its aglycon **5** to bind D-Ala-D-Lac.[24] We focused our attention on [Ψ[C(=NH)NH]Tpg^4]-vancomycin aglycon (**6**), replacing the residue 4 amide with the corresponding amidine (Figure 2). The key question addressed

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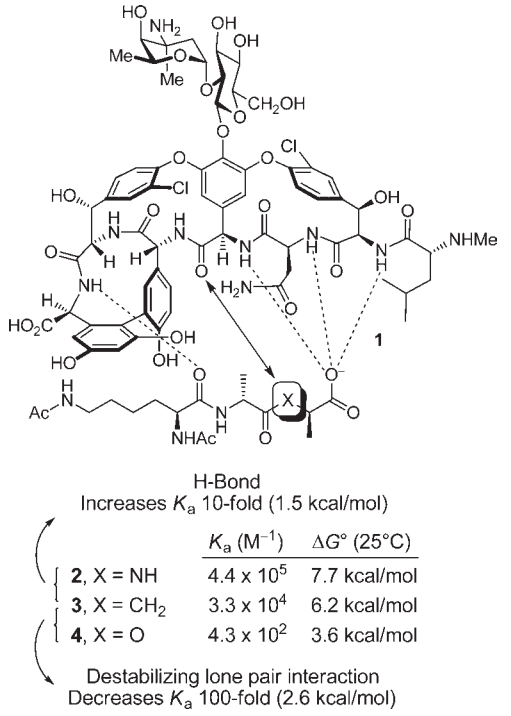

Figure 1. Structure of vancomycin (**1**), representation of its interaction with model ligands **2**−**4**, and measured binding data.[16]

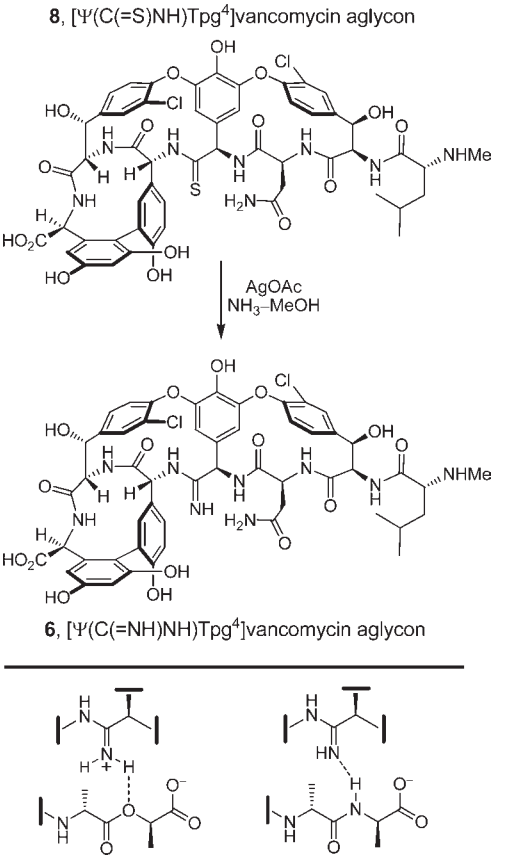

Figure 2. Structure of [Ψ[C(═NH)NH]Tpg^4]vancomycin aglycon (**6**), its preparation from [Ψ[C(═S)NH]Tpg^4]vancomycin aglycon (**8**), and potential dual binding behavior of the amidine in **6** toward D-Ala-D-Lac and D-Ala-D-Ala.

with **6** is whether the incorporation of the residue 4 amidine could accommodate D-Ala-D-Lac binding by removing the destabilizing electrostatic interaction and perhaps serving as a H-bond donor, while simultaneously maintaining affinity for D-Ala-D-Ala by virtue of serving as a H-bond acceptor (Figure 2). Such binding characteristics of **6** were not easy to anticipate as it is not clear whether the ester oxygen of D-Ala-D-Lac could serve as a H-bond acceptor,[25] or whether an amidine, which is likely protonated, might remain a good H-bond acceptor for D-Ala-D-Ala. Since the utility of an amidine as an amide isostere in peptides has been essentially unexplored,[26−28] the projected binding properties of **6** were even more unclear. Key to the preparation of **6** herein is the use of [Ψ[C(═S)NH]Tpg^4]vancomycin aglycon (**8**), bearing a residue 4 thioamide, for single-step, site-specific amidine introduction. Among its many attributes, this strategy not only permits access to **6**, but it also allows late stage synthetic access to related analogues including the interesting thioamide **8** itself, and alternative access to our prior methylene derivative **7**[24] from a common late stage intermediate.

Treatment of the fully deprotected vancomycin aglycon thioamide **8**, prepared by a total synthesis[29] modeled on our preceding work,[19−21,24] with silver acetate (AgOAc, 10 equiv) in methanol saturated with ammonia (NH_3−MeOH) at 25 °C (12 h) directly provided the amidine **6** cleanly as a colorless solid that is stable to extensive handling (Figure 2). It is considerably more polar than **5** and **8**, likely reflecting amidine protonation. It is readily soluble in water (H_2O) or H_2O−MeOH, but insoluble in acetonitrile (MeCN), and it required addition of trifluoroacetic acid (TFA) to the sample before reverse-phase high-performance liquid chromatography (HPLC) purification. The simplicity of this transformation does not do justice to the efforts that went into its development. A number of instructive alternative approaches were examined, establishing the experience needed to conduct this reaction within the chemical and structural framework of a fully functionalized and fully deprotected vancomycin aglycon.

The results of the examination of **6** are summarized in Figure 3 alongside those of vancomycin aglycon (**5**) and the synthetic methylene derivative **7**,[24] lacking the amide carbonyl. Both the C═N bond length of an amidine (1.30 vs 1.23 Å) and the van der Waals radii of nitrogen (1.55 vs 1.52 Å) closely approximate those of an amide carbonyl and oxygen atom, suggesting that an amidine may serve geometrically and sterically as an effective amide isostere. The binding affinity[30] of **6** with the model D-Ala-D-Ala ligand **2** was found to be only approximately 2-fold less than the vancomycin aglycon itself and 15-fold greater than the methylene derivative **7**, suggesting that the amidine functions well as a H-bond acceptor for the amide NH in the model ligand. Moreover, this binding affinity of **6** was maintained with the model D-Ala-D-Lac ligand **4**, representing a nearly 600-fold increase in affinity relative to the vancomycin aglycon (**5**) and a more than 10-fold increase relative to the methylene derivative **7**. Importantly, **6** displays effective, balanced binding affinity for both model ligands (K_a **2**/**4** = 1.05) at a level that is within 2- to 3-fold that exhibited by vancomycin aglycon (**5**) for D-Ala-D-Ala. Accurately reflecting these binding properties, **6** exhibited potent antimicrobial activity (MIC = 0.31 μg/mL) against VanA resistant *E. faecalis* (VanA VRE, BM4166), the most stringent of vancomycin-resistant bacteria, being equipotent to the activity that vancomycin (**1**) and vancomycin aglycon (**5**) display against sensitive bacterial strains (MIC = 0.3−2 μg/mL).

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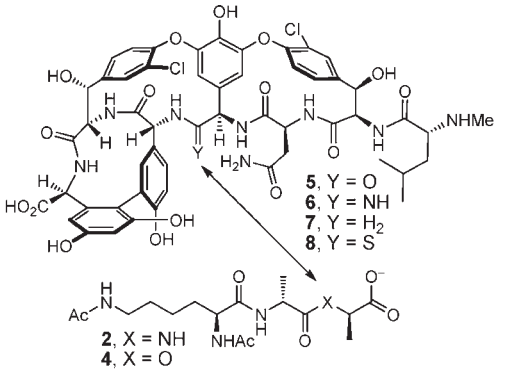

	ligand, K_a (M^{-1}) ($\Delta G°$, kcal/mol)			MIC (μg/mL)[a]
compound	**2**, X = NH	**4**, X = O	K_a(**2**)/K_a(**4**)	*E. faecalis* (VanA)
5, Y = O	1.7×10^5 (7.1)	1.2×10^2 (2.8)	1400	640
6, Y = NH	7.3×10^4 (6.6)	6.9×10^4 (6.6)	1.05	0.31
7, Y = H_2	4.8×10^3 (5.0)	5.2×10^3 (5.1)	0.9	31[b]
8, Y = S	1.7×10^2 (3.0)	1.1×10^1 (1.4)	–	>640

[a]MIC = minimum inhibitory concentration required for complete growth inhibition. *E. faecalis* (BM4166, VanA VRE). [b]Taken from ref. 24.

Figure 3. Assessment of **6** and comparison with key residue 4 amide modifications.

Although the behavior of **6** toward the D-Ala-D-Ala ligand **2** may not be too surprising, requiring the unprotonated amidine to function effectively as a H-bond acceptor for the ligand amide NH, its binding to the D-Ala-D-Lac ligand **4** is remarkable. There is no precedent on which to suggest that the residue 4 amidine could function as a H-bond donor to the ester oxygen of the D-Ala-D-Lac ligand sufficient to achieve this level of increased affinity. Rather, we suggest that this is additionally and largely the result of a now stabilizing electrostatic interaction between the protonated amidine and the ester oxygen lone pairs (Figure 2). Thus, removal of the vancomycin carbonyl oxygen atom and its destabilizing electrostatic interaction with the D-Ala-D-Lac ester oxygen atom (lone pair/lone pair repulsion) and its replacement with a protonated amidine nitrogen and its complementary stabilizing electrostatic interaction reinstates essentially full binding affinity to the altered ligand. Beautifully, this represents a complementary single atom exchange in the antibiotic (O → NH) to counter the single atom exchange in the cell wall precursors of resistant bacteria (NH → O).

Although [Ψ[C(=S)NH]Tpg^4]vancomycin aglycon (**8**) was prepared as the immediate precursor to **6**, it also proved especially interesting to examine. Since a thioamide is regarded as a weaker H-bond acceptor than an amide, the affinity of **8** for the D-Ala-D-Ala ligand **2** was anticipated to be reduced relative to the vancomycin aglycon, whereas its binding with the D-Ala-D-Lac ligand **4** was not as easily predicted. However, its behavior proved equally stunning, failing to bind either the model D-Ala-D-Ala or D-Ala-D-Lac ligand to any appreciable extent and being inactive as an antimicrobial agent. Most remarkable of these observations is the 1000-fold loss in affinity for the D-Ala-D-Ala ligand **2** relative to the vancomycin aglycon, indicating that this seemingly benign change in a single atom (O → S) in going from the amide to thioamide is sufficient to completely disrupt binding. Although the weaker H-bonding ability of a thioamide is likely contributing to this lowered affinity, the magnitude of the loss indicates something more fundamental is responsible. We suggest that both the increased bond length of the thiocarbonyl (1.66 vs 1.23 Å) and the increased van der Waals radii of sulfur (1.80 vs 1.52 Å) are sufficient to sterically displace and completely disrupt the intricate binding of D-Ala-D-Ala. These contrasting observations further underscore the remarkable behavior of the amidine **6**.

The clinical impact of such redesigned glycopeptide antibiotics is likely to be important, charting a rational approach forward in the development of antibiotics for the treatment of vancomycin-resistant bacterial infections. Since the single atom exchange described here is a deep-seated change that entails the selective transformation of one of seven amides in the vancomycin core structure, this was accomplished initially by total synthesis. In addition to semisynthetic approaches to **6** and **8** that now may be explored with the benefit of authentic samples in hand, a provocative ramification of the observations is the possibility that Nature also may have discovered this solution to the redesign of vancomycin for dual D-Ala-D-Ala and D-Ala-D-Lac binding in the form of related natural products yet to be isolated or characterized. In this respect, such residue 4 amidine derivatives possess the same nominal molecular weight as the corresponding amides, but are more polar, and it is possible they have been overlooked in screening efforts to date. Finally, we note that beyond the impact of unraveling the subtle details of the interaction of vancomycin with its biological target and their ramifications, the studies provide fundamental new insights into molecular recognition events, replacing a lost H-bond not with a reengineered reverse H-bond, but by replacing the resulting destabilizing electrostatic interaction with a stabilizing electrostatic interaction.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details and complete ref 6. This material is available free of charge via the Internet at http://pubs.acs.org.

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