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Investigation into the functional impact of the vancomycin C-ring aryl chloride

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ABSTRACT

A vancomycin aglycon analogue that possesses a reduced C-ring and an intact E-ring chloride was prepared and its antimicrobial activity towards *Staphylococcus aureus* and binding affinity to model cell wall ligands were established. Comparison of the derivative with a series of vancomycin aglycon analogues that possess and lack the chloro substituents on the aryl C- and E-rings defines the impact and further refines the role the C-ring chloride plays in promoting both target binding affinity and binding selectivity for D-Ala-D-Ala and its impact on antimicrobial activity.

Vancomycin (**1**) and structurally related glycopeptide natural products have proven to be invaluable antibiotics over the past fifty years.[1] Most recently, vancomycin is prescribed as the drug of last resort for the treatment resistant Gram-positive bacterial infections, including methicillin-resistant *Staphylococcus aureus* (MRSA).[2] Vancomycin disrupts bacterial cell wall biosynthesis by binding to the peptide terminus D-Ala-D-Ala found in peptidoglycan precursors through a network of five hydrogen bonds, thereby inhibiting cell wall cross-linking.[2,3] Alarmingly, vancomycin-resistant bacteria have emerged which are able to overcome this mode of action. In the more prevalent resistant bacterial strains, VanA and VanB, the D-Ala-D-Ala terminus is remodeled to D-Ala-D-Lac resulting in a 1000-fold decrease in antibiotic binding affinity and an accompanying 1000-fold loss of antimicrobial activity.[2–5] Additionally, several resistant strains (VanC, VanE, and VanG) have been found to possess an altered D-Ala-D-Ser peptide terminus.[6–8] Although not as severe, these bacteria display reduced sensitivity to vancomycin antimicrobial activity, resulting from a reduced binding affinity for D-Ala-D-Ser.[5,9]

Our efforts to overcome vancomycin bacterial resistance have enlisted both total and semi-synthesis studies and have provided several vancomycin analogues that are effective against resistant bacteria.[10–12] As a result of these studies, we have a strong interest in understanding the intricacies of the binding properties of vancomycin.[13] In a recent report, we disclosed synthetic efforts capable of selective Pd(0)-catalyzed conversion of the E-ring aryl chloride in vancomycin aglycon derivatives to a boronic acid, permitting the synthesis of alternatively substituted E-ring analogues to probe the impact of this characteristic feature of the glycopeptides antibiotics.[14] Many E-ring aryl chloride substitution analogues were well tolerated and it was the presence, but not the nature, of a substituent on the E-ring that was important for maintaining binding affinity for a terminal D-Ala-D-Ala model peptide and antimicrobial activity. Herein, we disclose an initial study on the impact and role of the C-ring aryl chloride through the synthesis and evaluation of the reduced C-ring vancomycin aglycon possessing the E-ring aryl chloride (**3**) and its comparison with a series of vancomycin aglycon analogues (**2**–**5**) that posses and lack the chloro substituents on the C and E-aryl rings (Fig. 1, Table 1).

Eremomycin, a naturally occurring glycopeptide also possesses a vancomycin heptapeptide scaffold with a reduced C-ring and intact E-ring aryl chloride.[1,15] However due to the presence of alternative amino-sugars on the periphery of the molecule, comparisons to vancomycin do not provide a clear indication as to the role of the C-ring chloride in binding and antimicrobial activity. Our studies on the selective borylation of the *N*-Boc vancomycin aglycon (**6**) revealed that a major byproduct from the reaction was the reduced C-ring aryl chloride aglycon possessing an E-ring aryl boronic acid (**7**, Fig. 2).[14] With this intermediate in hand, copper(II)-mediated chlorination and *N*-Boc deprotection provided the desired vancomycin aglycon analogue **3**, capable of direct assessment of only the impact of the C-ring aryl chloride.

For comparison, a complete series of vancomycin aglycon analogues that possess and lack the aryl chloride substituents was assembled. Compounds **4** and **5** which lacked the E-ring chloride and both C- and E-ring aryl chlorides, respectively, were prepared as previously described by Harris through hydrogenation of vancomycin aglycon.[16]

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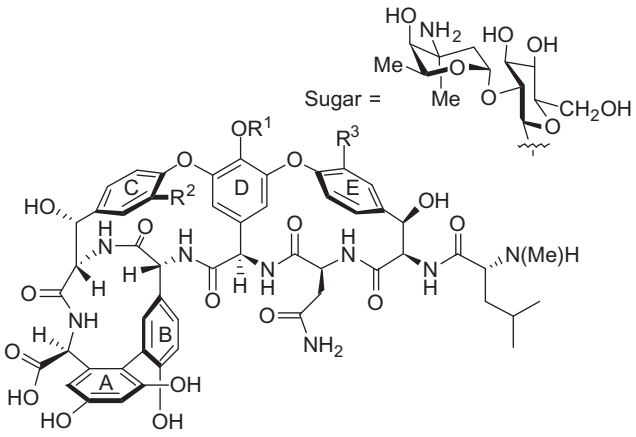

Figure 1. Vancomycin and vancomycin aglycon analogues.

Table 1
Vancomycin analogues

Compound	R^1	R^2	R^3
1	Sugar	Cl	Cl
2	H	Cl	Cl
3	H	H	Cl
4	H	Cl	H
5	H	H	H

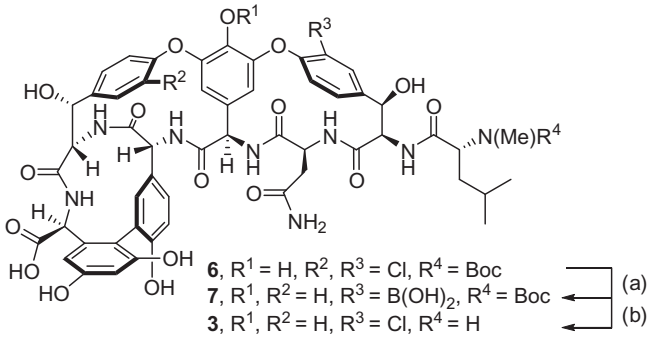

Figure 2. (a) $(HO)_4B_2$ (3 equiv), X-Phos-Pd-G2 (0.3 equiv), KOAc (3 equiv), X-Phos (0.6 equiv), EtOH, 60 °C, 6 h, 19% **7**; (b) $CuCl_2$, $MeOH/H_2O$ (60 °C, 2 h) then TFA/CH_2Cl_2, (25 °C, 20 min), 52% (two steps) $R^1 = R^2 = H$, $R^3 = Cl$, $R^4 = H$.

The antimicrobial activity of vancomycin and the vancomycin aglycon analogues **2**–**5** against sensitive *S. aureus* were obtained according to a standard microtiter plate-based antimicrobial assay (Table 2).[11,10h] Ligand binding studies were performed according to the procedure of Nieto and Perkins with the ligands *N,N′*-Ac_2-L-Lys-D-Ala-X (X = D-Ala, D-Ser, Gly, D-Phe) and the results are summarized in Table 2.[17] These ligands represent known (X = Gly) and additional possible vancomycin binding sites in the bacterial cell wall of resistant bacteria (X = D-Ser).

Removal of just the C-ring aryl chloride with **3** led to a >eight-fold drop in activity against sensitive *S. aureus* compared to the vancomycin aglycon. This loss in activity was more substantial than the decrease in activity seen upon the selective removal of the E-ring aryl chloride in **4**. The difference in antimicrobial activity can be explained through ligand binding studies with *N,N′*-Ac_2-L-Lys-D-Ala-D-Ala, which revealed that selective removal of the C-ring chloride with **3** led to an approximate four-fold loss in binding, whereas the selective E-ring chloride removal with **4** led to a smaller two-fold decrease in binding affinity. Interestingly, the loss of binding for **3** and **4** for D-Ala-D-Ala are additive and explain the poorer binding observed for the analogue **5**, lacking both the C- and E-ring chlorides.

For the model D-Ala-D-Ser ligand, analogue **3** was slightly more effective at binding than the aglycon. Nevertheless, the binding affinity was reduced ca. 30-fold for both analogues. The lower binding with D-Ala-D-Ser accounts for the decreased antimicrobial activity of vancomycin on VanC, VanE, and VanG bacterial strains. For D-Ala-Gly, analogue **3** was two-fold less effective than that of the vancomycin aglycon which is a slightly smaller decrease compared to that seen with D-Ala-D-Ala (ca. three-fold). For the larger D-Ala-D-Phe ligand, it was possible that the removal of the C-ring aryl chloride would enable better binding due to decreased steric constraints. However, both **2** and **3** showed equally reduced binding towards the model D-Ala-D-Phe ligand.

These observations further refine the role of the C-ring aryl chloride in target binding selectivity and binding affinity. Its presence helps define the vancomycin binding pocket and increase binding affinity for terminal D-Ala-D-Ala ligands over alternative bacterial cell wall binding sites and its impact is larger than that of the E-ring chloride. Additionally, the comparative binding studies of **2** and **3** with model cell wall ligands provide a valuable insight into and starting point for evaluating C-ring analogues replacing the chloride with alternative substituents.

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Supplementary data

Supplementary data (full experimental details and characterization for all final compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.080.

Table 2
Binding and antimicrobial properties

Compd	MIC (μg/mL)	Association constant for *N,N′*-Ac_2-L-Lys-D-Ala-**X**				
	S. aureus[a]	K_a, M^{-1} **D-Ala** ($\times 10^5$)	$\Delta\Delta G_b^{obs,b}$	K_a, M^{-1} **D-Ser** ($\times 10^3$)	K_a, M^{-1} **Gly** ($\times 10^4$)	K_a, M^{-1} **D-Phe** ($\times 10^2$)
1	1.25	1.8				
2	1.25	1.4	—	3.3	1.0	9.1
3	10	0.38	0.77	4	0.49	9
4	5	0.68	0.42			
5	20	0.18	1.21			

[a] Vancomycin-sensitive *Staphylococcus aureus* (ATCC 25923).
[b] The free energy of binding ($\Delta\Delta G_b$) was tabulated from the experimentally determined K_a using the equation $\Delta\Delta G_b = RT\ln K$, where $K = {}^1K_a/{}^xK_a$; ${}^1K_a$ is the association constant for the complex of **2** with *N,N′*-Ac_2-L-Lys-D-Ala-D-Ala; ${}^xK_a$ is the association constant for the complex of compound **X** with *N,N′*-Ac_2-L-Lys-D-Ala-D-Ala.

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