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Probing the Role of the Vancomycin E-Ring Aryl Chloride: Selective Divergent Synthesis and Evaluation of Alternatively Substituted E-Ring Analogues

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Abstract

The selective functionalization of vancomycin aglycon derivatives through conversion of the E-ring aryl chloride to a reactive boronic acid, and its use in the synthesis of a systematic series of vancomycin E-ring analogues are described. The series was used to examine the E-ring chloride impact in binding D-Ala-D-Ala and on antimicrobial activity. In contrast to the reduced activity of the unsubstituted E-ring derivatives, hydrophobic and relatively non-polar substituents approach or match the chloro substituted vancomycin and was insensitive to the electronic character of the substituent (e.g. Cl vs CN/OMe), whereas highly polar substituents fail to provide the enhancements. Moreover, the active permethylated vancomycin aglycon derivatives exhibit VanB VRE antimicrobial activity at levels that approach (typically within 2-fold) their activity against sensitive bacteria. The robust borylation reaction also enabled the functionalization of a minimally protected vancomycin aglycon (*N*-Boc-vancomycin aglycon), and provides a direct method for the preparation of previously inaccessible analogues.

Introduction

Glycopeptide natural products have had a rich history as efficacious antibiotics. Since its introduction into the clinic in 1958, vancomycin has been used continuously for over 50 years, most recently as a drug of last resort for the treatment of resistant Gram-positive bacterial infections, including methicillin-resistant *Staphylococcus aureus* (MRSA).^{1,2} Vancomycin selectively disrupts bacterial cell wall biosynthesis by binding the peptide terminus D-Ala-D-Ala of the peptidoglycan precursor through a network of five hydrogen bonds thereby inhibiting transpeptidase-catalyzed cell wall cross-linking and causing cell lysis (Figure 1).^{2,3} For the first three decades of clinical use, there were no reports of bacterial resistance to vancomycin. However, vancomycin-resistant enterococci (VRE) and *Staphylococcus aureus* (VRSA) strains have emerged in the past 25 years.^{2–4} The most prevalent resistant bacterial strains, VanA and VanB VRE, sense the presence of vancomycin, and reprogram the D-Ala-D-Ala precursor terminus to D-Ala-D-Lac through a two component signaling cascade,⁵ resulting in a 1000-fold decrease in antibiotic binding affinity and a corresponding 1000-fold drop in antimicrobial activity.^{2–4} This decrease in binding affinity for D-Ala-D-Lac is the result of the loss of a key central hydrogen bond and

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Supporting Information Available

Compounds **5**, **6**, and **10** were prepared according to published procedures.²⁰ Full experimental details and characterization for all final compounds are provided in the supporting information. This material is available free of charge via the internet at <http://pubs.acs.org>.

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introduction of repulsive lone pair interactions between the vancomycin residue 4 amide carbonyl and the D-Ala-D-Lac ester oxygen (Figure 1).⁶

Efforts to overcome vancomycin bacterial resistance have focused largely on two fronts: (1) the redesign of the vancomycin core through the use of total synthesis,⁷ and (2) modification on the periphery of the molecule through semi-synthesis.^{8–10} In recent reports, we have disclosed synthetic efforts focused on the core modification of residue 4.⁷ Removal of the residue 4 amide oxygen provided [Ψ [CH₂NH]Tpg⁴]vancomycin aglycon,^{7b} which eliminated the destabilizing lone pair interactions and led to improved D-Ala-D-Lac binding and antimicrobial activity against VanA VRE. More recently, this work led to the total synthesis of a key residue 4 thioamide precursor that upon silver(I)-promoted amidine formation¹¹ enabled the synthesis of [Ψ [C(=NH)NH]Tpg⁴]vancomycin aglycon, which exhibited fully effective dual D-Ala-D-Ala and D-Ala-D-Lac binding and fully reinstated antimicrobial activity against VanA VRE.^{7c,7d}

Complementary to such efforts, semisynthetic modifications to vancomycin have provided the largest share of analogues for examination^{8–10} and have been performed on the majority of the available reactive peripheral sites on the vancomycin skeleton. However, two key sites on vancomycin that have been only briefly explored are the C- and E-ring aryl chlorides.^{12–14} The first study on the role of the aryl chlorides by Harris revealed that the removal of one (E-ring) or both aryl chlorides through hydrogenolysis diminished antimicrobial activity. Similar results have since been reported for related glycopeptides containing the characteristic aryl chlorides, but have been largely restricted to comparisons between structures that possess and lack the chloro substituent.^{1,15} Despite being a prominent structural feature, the magnitude of their impact on the antimicrobial properties and their functional role remain unclear. Moreover and despite their structural similarity, observations to date suggest the C- and E-ring aryl chlorides may serve different functional roles. We have anticipated that the C-ring chloride affects both target binding affinity and steically impacts ligand binding selectivity, defining the top of a small hydrophobic binding pocket selective for D-Ala-D-Ala and smaller ligands, whereas the more removed E-ring chloride contributes to binding affinity.¹² Additionally, Williams has proposed that the E-ring chloride stabilizes *in situ* dimerization in vancomycin-type antibiotics that in turn enhances antimicrobial activity.¹⁶

Although chemically equivalent, the E-ring chloride is located outside the binding pocket and is more accessible for selective modification. In an ongoing effort to understand the intricacies of the binding properties of vancomycin, we set out to define the impact of E-ring aryl substituents on its binding affinity to D-Ala-D-Ala and its relationship to antimicrobial activity. Herein, we report studies that provide the systematic modification of the E-ring aryl chloride through the selective palladium(0)-catalyzed borylation of *N*-Boc vancomycin aglycon **5** and the permethylated vancomycin aglycon derivative **6** (Figure 2). The synthesis of a reactive aryl boronic acid intermediate enabled the divergent¹⁷ synthesis and biological evaluation of 16 vancomycin aglycon analogues containing systematic changes to the E-ring substituent.

The permethylated vancomycin and teicoplanin derivatives exhibit ligand binding properties and *in vitro* antimicrobial activity against sensitive bacterial strains at levels indistinguishable from the unprotected vancomycin aglycon, which in turn are essentially equivalent to those of vancomycin itself.^{8a,8b} Significantly, and unlike the free aglycons, the permethylated derivatives exhibit pronounced improvements in antimicrobial activity against VanB, but not VanA vancomycin-resistant enterococci, exhibiting a potency matching or approaching their activity against sensitive bacteria (Figure 3).^{8b} This latter feature proved general for other glycopeptide antibiotics in the family including teicoplanin,

which was used to define the VanB phenotype.¹⁸ Because they lack the carbohydrate domain potentially involved in transglycosylase inhibition¹⁹ as well as lipid acyl chains potentially involved in membrane anchoring,^{2,3} we have been especially interested in further exploring the behavior of the permethylated aglycon derivatives in order to provide insights into their mechanism of overcoming VanB resistance. Thus, the impact of the E-ring aryl chloride on the binding and antimicrobial activity was examined systematically with the permethylated vancomycin aglycon derivatives and with a smaller number of free aglycon derivatives.

Results and Discussion

Chemistry

Several synthetic and structural challenges arise in the synthesis of such vancomycin analogues. Reactions involving high temperatures (>100 °C) are known to cause rearrangements, isomerizations, and retro-aldol side reactions. Strong bases can further lead to additional degradation, and the solubility properties of vancomycin can limit reaction scope. To minimize possible side reactions and to improve substrate solubility, the permethylated vancomycin aglycon derivatives **6** and **10** available from vancomycin in 4 and 5 steps, respectively, were examined as initial substrates (eq 1).²⁰

Central to a successful approach for modification of the E-ring chloride was its transformation to an intermediate more amenable to systematic modification than direct substitution from the chloride, which we found has limited reaction scope (Scheme 1). Initial efforts focused on the formation of the corresponding aniline through a Suzuki–Miyaura coupling²¹ with an alkenyl boronic acid followed by alkene oxidative cleavage and Curtius rearrangement of the resulting carboxylic acid or a direct Pd(0)-catalyzed aryl amination.^{22,23} Diazonium salt formation then provides a more reactive species, which could be utilized for functionalization of the E-ring.^{7,24} However, and in part because of the sea of coordinating functionality and the inherent complexity of the substrate, the direct Pd(0)-catalyzed aminations examined on either **6** or **10** and conducted intermittently over the course of a decade using a variety of catalysts, amines, and ammonia equivalents did not result in formation of the desired aniline derivative. The alternative and already lengthy Curtius rearrangement route suffered from epimerization of the C-terminus methyl ester under Suzuki–Miyaura coupling conditions with (*E*)-styrylboronic acid.

As an alternative, we examined the corresponding boronic acid and related derivatives,²⁵ using recent palladium catalyst developments enabling the borylation of aryl chlorides under mild conditions.²⁶ Initial investigation for the coupling of bis(pinacolato)diboron conducted with **10** found that the aryl chlorides underwent oxidative addition as evidenced by the generation of reduction products, but only trace amounts of a mono-borylated product could be detected. Attempts to increase the reaction temperature and times only led to over reduction of both the C- and E-ring aryl chlorides. Adoption of borylation conditions^{26e} using tetrahydroxydiboron (X-Phos, B₂(OH)₄, EtOH, 80 °C, 3 h) with **6** provided a 1:1 ratio of a single mono-borylated product **12** with reduction of the second aryl chloride, and the bis-borylated product **13** (Figure 4). By decreasing the reaction time, the reduction of the second chloride was diminished leading to the observance of a single mono-borylated product with the second aryl chloride intact. The preferential reaction of one of the two aryl chlorides led us conclude that the E-ring chloride reacted first as previously shown by Harris and later by Arimoto.^{12,13} Competitive reaction of the C-ring chloride could be further minimized by reducing the reaction temperature from 80 °C to 35 °C and allowed for careful monitoring of the reaction.

With further optimization of the reaction conditions (Figure 4), borylation of **6** occurred in 55% overall yield, providing the desired product **11** in 45% as a 3:1 mixture with the reduced C-ring product **12** and 10% of the bis-borylated product **13** (Figure 4). Although this 3:1 mixture of **11** and **12** could be separated and each product characterized, we found it more convenient to use **11** as this 3:1 mixture in the subsequent functionalization reactions and to purify (HPLC) the resulting products following the aryl substituent introduction.

The extension of these observations to *N*-Boc vancomycin aglycon (**5**) bearing a single protecting group and containing four free phenols, an unprotected carboxylic acid, two free alcohols, a primary carboxamide and six amides, provided the analogous mono-borylation product **15** (Figure 5). Initially, we found that the yield of the desired borylation product **15** was consistently low (< 10%), when using the conditions optimized for **6**. However, with the use of the second generation X-Phos precatalyst, the reaction was found to proceed more smoothly and provided improved yields of **15**. Due to the ease of purification and handling and because of our interest in the permethylated vancomycin aglycon derivatives, the methyl ether borylation products were used to examine the derivatization substitution reactions.

Copper(II)-mediated bromination (CuBr₂) of **11** occurred smoothly to provide the desired E-ring bromide **14a** in 90% yield (Scheme 2).²⁷ Following *N*-Boc deprotection and reversed-phase HPLC purification, 2D-NMR spectroscopic analysis of the product (**14b**) unambiguously established that the borylation occurred on the E-ring. Treatment of **14a** with AlBr₃-EtSH cleanly effected global deprotection,²⁴ removing the four aryl methyl ethers, the methyl ester and the *N*-Boc group in a single step to afford the fully deprotected E-ring bromo analogue **16**. This fully deprotected analogue was also accessed through bromination of the boronic acid derivative **15** (CuBr₂, 50 °C, 2 h), followed by simple acid-catalyzed *N*-Boc deprotection (80% over 2 steps).

Subsequent efforts to prepare the E-ring aniline as an alternative functionalization substrate was achieved through a copper-catalyzed coupling of the boronic acid **11** with aqueous ammonia (Scheme 3).²⁸ Although stable as the protected *N*-Boc derivative (**17a**), isolation of the desired aniline proved difficult following *N*-Boc deprotection, as decomposition of the sensitive aniline product was experienced upon final purification. To circumvent the stability issue, the aniline was methylated (MeI, K₂CO₃, DMF) to provide the more stable dimethylaniline derivative in 42% yield. As evident in these studies, the more stable boronic acid was a preferred intermediate over the aniline **17a** for the late stage divergent synthesis of vancomycin analogues. Subsequently, a series of compounds **19a–28a** containing a

systematic series of aryl substituents were synthesized from the boronic acid **11** through copper- and palladium-mediated reactions (Scheme 3).²⁹ It is noteworthy that these metal-mediated boronic acid substitution reactions are conducted on a substrate that contains countless Lewis basic sites and sensitive functionality (e.g., Asp primary carboxamide, β -hydroxyamides) within a macrocycle noted for sequestering metals, yet proceed effectively. In addition and as a validation of an alternative strategy enlisting a more reactive E-ring halogen derivative, the bromo derivative **14a** was found to undergo selective Stille coupling³⁰ with SnMe_4 to provide **27a** in 45% yield without evidence of competitive C-ring chloro substitution. This same analogue **27a** was also accessed from the boronic acid **11** through Suzuki coupling with MeI .³¹

Following formation of each analogue, *N*-Boc deprotection and HPLC purification provided the corresponding permethylated vancomycin aglycon analogue for biological testing (**11b**, **14b**, **18b–28b**). In addition and complementary to the examination of **26a**, compound **26a** was subjected to global deprotection to provide **29** in order to further establish that the ligand binding data of the methyl ether analogues mirrored that of the free aglycon (Figure 6). In addition, compound **16** (prepared as previously noted through bromination of **14a**), and compound **30** (prepared through selective hydrogenation of **2**), were prepared and examined as additional free aglycon analogues.

Antimicrobial and Ligand Binding Activity

The antimicrobial activity of analogues **11b**, **14b**, and **18b–30** presented in Table 2 were obtained in a microtiter plate-based antimicrobial assay against vancomycin-sensitive *S. aureus* (strain ATCC 25923) and vancomycin-resistant and teicoplanin-sensitive *E. faecalis* (VanB, strain ATCC 51299).^{7b,7d,8,24b} Ligand binding studies were performed according to the procedure of Nieto and Perkins with the tripeptide *N,N'*- $\text{Ac}_2\text{-L-Lys-D-Ala-D-Ala}$ (**3**) and the results are summarized in Table 2.³²

As anticipated from prior studies, removal of the E-ring chloride led to a four-fold reduction in antimicrobial activity against sensitive *S. aureus* (**2** vs **30** and **7** vs **25b**) and a two-fold reduction in binding affinity for the model $\text{Ac}_2\text{-L-Lys-D-Ala-D-Ala}$ ligand. No distinction in either property was observed between the fully deprotected vancomycin aglycon derivatives or the corresponding permethylated derivatives, where they were found to exhibit indistinguishable relative and absolute values. All derivatives bearing hydrophobic or relatively nonpolar E-ring substituents approached or matched the properties of the vancomycin aglycon or permethylated aglycon and exceeded the potency and affinity of **30** and **25b** lacking the E-ring substituent. Most significant is the observation that this was seen with both strong electron-withdrawing (e.g., $\text{R} = \text{CN}$) and strong electron-donating substituents (e.g., $\text{R} = \text{OMe}$), indicating that the electronic properties of the E-ring substituent are not contributing to the binding affinity for D-Ala-D-Ala and the resulting antimicrobial activity against sensitive organisms. The exceptions to this enhancement displayed by an E-ring substituent were those bearing a strong polar substituent (OH , NO_2 , NMe_2 , B(OH)_2), which either matched or were found to be even less effective than the E-ring unsubstituted vancomycin aglycon derivative. Here again the effects appear to be independent of the electronic properties of the substituent (e.g., NO_2 and NMe_2) and are seen in both the ligand binding affinities and antimicrobial activity. The most surprising of the ineffective substituents was NO_2 , where the extent of the diminished properties exceeded initial expectations, and there may be additional features limiting its behavior that we do not yet appreciate.

Significantly, the antimicrobial data obtained for the permethylated vancomycin aglycon series reinforces our previous findings that permethylation of the vancomycin aglycon

provides enhanced activity towards VanB vancomycin-resistant enterococci (VanB VRE).⁸ With the exception of the nitro derivative and although they display slightly decreased activity relative to **7**, each displayed VanB VRE antimicrobial activity only 1–8 fold lower (typical 2-fold) than its corresponding activity against sensitive *S. aureus* and each is 10–50 times more potent than vancomycin and typically 20 times more potent than the vancomycin aglycon. Like previous binding studies, the permethylated vancomycin analogues do not show increased or substantially altered binding for *N,N'*-Ac₂-L-Lys-D-Ala-D-Lac or D-Ala-D-Ala, they lack the carbohydrate domain thought to be responsible for alternative or additional transglycosylase (vs transpeptidase) inhibition, and they do not contain appended lipid side chains found in tecioplanin for membrane anchoring. Thus, they constitute a unique series of vancomycin derivatives capable of addressing VanB vancomycin resistance. Although the origin of this increased activity has not been established, it is likely derived from the bacteria inability to sense the antibiotic challenge and initiate the peptidoglycan remodeling.

Finally, it is worth highlighting that the E-ring azide (**19b**) is among the most effective chloride replacements, suggesting an especially effective site at which to introduce and conduct photoaffinity cross-linking reactions, and it is also among the highest yielding of the substituents to be introduced (90%), suggesting a powerful way forward for further functionalizations via alkyne cycloaddition reactions.

Conclusions

Complementary to efforts to functionalize natural products through introduction of diversifying reactive functionality, the work herein constitutes an example of utilizing unreactive latent functionality inherent in the natural product to provide such a late stage diversification. The selective Pd(0)-catalyzed conversion of the E-ring aryl chloride in vancomycin aglycon derivatives to a boronic acid while leaving the C-ring aryl chloride intact permitted the synthesis and evaluation of a systematic series of substituents used to probe the impact of this characteristic feature of the glycopeptides antibiotics. This catalytic Pd(0)-mediated selective boronic acid functionalization could be conducted not only on a protected vancomycin aglycon (**6**), but also the minimally protected *N*-Boc vancomycin aglycon (**5**) possessing four phenols, two secondary alcohols, six amides, and the C-terminus carboxylic acid as well as the potentially competitive C-ring aryl chloride.

In contrast to the reduced effectiveness of the unsubstituted E-ring derivatives and regardless of the electronic character of the substituent, hydrophobic and relatively non polar E-ring substituents approached or matched the antimicrobial and binding properties of the chloro substituted vancomycin derivative, whereas highly polar substituents (OH, NMe₂, NO₂, B(OH)₂) failed to provide the enhancements. Most significant, the antimicrobial activity against sensitive bacteria paralleled the binding affinity for a model D-Ala-D-Ala ligand and was insensitive to the electronic character of the substituent where both strong electron-withdrawing (e.g., R = CN) and strong electron-donating (e.g., R = OMe) substituents provide the enhancement observed with the E-ring chloride substituent. Additionally and unlike vancomycin and the vancomycin aglycon derivatives that exhibit a 40- to 100-fold loss in antimicrobial activity against VanB VRE, the permethylated vancomycin aglycon derivatives exhibit VanB VRE antimicrobial activity at levels that approach (typically within 2-fold) their activity against sensitive bacteria, confirming the generality of earlier observations with the much more limited series (E-ring Cl). Finally, in addition to finding that most small E-ring substituents provide a modest improvement in the antimicrobial and binding properties of the glycopeptide aglycons independent of their electronic properties, the studies lay the groundwork and provide the methodology for more

substantial functionalization studies involving larger perturbations to the structure of vancomycin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

Ala	alanine
Lac	lactic acid
Boc	t-butyloxycarbonyl
dba	dibenzylideneacetone
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
o-tol	ortho-tolyl
TBS	<i>tert</i> -butyldimethylsilyl
VRE	vancomycin-resistant enterococci
VRSA	vancomycin-resistant <i>Staphylococcus aureus</i>
X-Phos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl
X-Phos-Pd-G2	Chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl) [2-(2'-amino-1,1'-biphenyl)]palladium(II)
K_a	association constant

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Figure 1. Schematic representation of the interactions between vancomycin (1), vancomycin aglycon (2), and model ligands *N,N'*-Ac₂-L-Lys-D-Ala-D-Ala (3) and *N,N'*-Ac₂-L-Lys-D-Ala-D-Lac (4).

Figure 2.
Structures of **5** and **6**

Compound	<i>S. aureus</i> ^a	<i>E. faecalis</i> ^b	<i>E. faecalis</i> ^c
		VanA	VanB
vancomycin (1)	1	>100	>100
vancomycin aglycon (2)	0.8	>100	>100
7	1.25	50	1.25
teicoplanin	0.2	>100	0.2
teicoplanin aglycon (8)	1.6	>100	25
9	3.2	>100	3.2

^a*Staphylococcus aureus* (ATCC 25923).

^b*Enterococcus faecalis* (VanA, BM4166).

^c*Enterococcus faecalis* (VanB, ATCC 51299).

Figure 3.
Comparison MIC ($\mu\text{g/mL}$) values for key permethylated vancomycin and teicoplanin aglycons illustrating their unique activity against VanB VRE.

$\text{Pd}(\text{OAc})_2$	X-Phos	Time	T ($^\circ\text{C}$)	Product Ratio
(equiv)	(equiv)			11 : 12 : 13
0.25	0.75	3 h	80	0 : 1 : 1
0.1	0.3	3 h	80	0 : 1 : 1
0.1	0.3	0.5 h	80	1 : 2 : 2
0.1	0.3	0.5 h	60	2 : 1 : 1
0.1	0.3	6.5 h	35	3 : 1 : 1

Figure 4.
 Selective Borylation of **6** and representative optimization studies.

Figure 5.
Borylation of *N*-Boc vancomycin aglycon.

Figure 6.
N-Boc and global deprotection of the permethylated vancomycin aglycon analogues.

Scheme 1.

Scheme 2.

Scheme 3.

Table 2

Antimicrobial and Binding Properties

Compound	MIC ($\mu\text{g/mL}$)		Association constant for 3	
	<i>S. aureus</i> ^a	<i>E. faecalis</i> ^b (VanB)	K_a , M^{-1} ($\times 10^5$) ^c	$\Delta \Delta G_b$ ^d
Vancomycin (1)	1.25	120	1.8 (2.3–3.9) ^e	
Vancomycin Aglycon (2)	1.25	40	1.4	-
16 (-Br)	2.5	40	1.1	0.14
29 (-OH)	5	40	0.53	0.57
30 (-H)	5	80	0.68	0.42
Permethyl Aglycon Derivatives				
7 (-Cl)	1.25	2.5	1.3 (2.0)	-
11b (-B(OH) ₂)	5	10	0.23	1.03
14b (-Br)	2.5	5	1.1	0.1
18b (-NMe ₂)	5	10	0.34	0.79
19b (-N ₃)	1.25	5	1.2	0.05
20b (-NO ₂)	>40	>40	0.097	1.56
21b (-CO ₂ CH ₃)	2.5	10	1.0	0.16
22b (-I)	2.5	10	1.3	0
23b (-OMe)	2.5	5	1.2	0.05
24b (-CN)	2.5	5	1.1	0.1
25b (-H)	5	5	0.71	0.36
26b (-OH)	5	5	0.88	0.23
27b (-CH ₃)	2.5	5	1.2	0.05
28b (-CF ₃)	2.5	20	1.2	0.05

^aVancomycin-sensitive *Staphylococcus aureus* (ATCC 25923).

^bVancomycin-resistant *Enterococcus faecalis* (VanB, ATCC 51299).

^cBinding to *N,N'*-Ac₂-L-Lys-D-Ala-D-Ala (**3**).

^dThe 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** or **8** with **3**; XK_a is the association constant for the complex of compound **X** with **3**.

^eTaken from ref 8a.