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A stepwise dechlorination/cross-coupling strategy to diversify the vancomycin 'in-chloride'

Tyler J. Wadzinski, Katherine D. Gea, Scott J. Miller *

Department of Chemistry, Yale University, PO Box 208107, New Haven, CT 06520-8107, United States

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ABSTRACT

In an effort to rapidly access vancomycin analogues bearing diverse functionality at the 6_c-Cl (the 'in-chloride') position, a two-step dechlorination/cross-coupling protocol was developed. Conditions for efficient cross-coupling of the relatively unreactive 6_c-Cl group were found that ensure high conversion with minimal product decomposition. A set of 2_c-dechloro-6_c-functionalized vancomycin derivatives was prepared, and antibiotic activities of the compounds were evaluated against a panel of vancomycin-resistant and vancomycin-susceptible strains. Results from biological testing further underscore the steric sensitivity of vancomycin's binding pocket.

Glycopeptide natural product antibiotics, vancomycin (**1**) in particular, have seen widespread use and extensive study over the past half century.[1,2] The glycopeptide antibiotics inhibit cell wall biosynthesis by coordinating the D-Ala-D-Ala peptide termini of peptidoglycan precursors, disrupting peptidoglycan cross-linking.[3,4] Strong binding to D-Ala-D-Ala results from a network of five hydrogen bonds along with hydrophobic interactions within the glycopeptide binding pocket (Fig. 1).[5–10] For a period after its discovery, vancomycin was considered the 'antibiotic of last resort;' however, clinical resistance was first observed in the 1980s and has become increasingly prevalent since then.[11–14] In an effort to combat resistant infections and better understand resistance mechanisms, synthetic chemists have generated a diverse range of vancomycin analogues through both semisynthesis[15] and total synthesis.[16,17]

The 6_c-Cl group (the 'in-chloride'), located in vancomycin's binding pocket, has inspired revealing studies over the past three decades. Harris and co-workers reported a method to prepare dechlorinated vancomycin derivatives via palladium-catalyzed hydrogenation and found 2_c-Cl, oriented toward the convex face of the molecule, to be significantly more reactive than the in-chloride group.[15a] The authors prepared 2_c-dechlorovancomycin (**2**) with 2_c,6_c-didechlorovancomycin (**3**) observed as the product of overreaction. More recently, Arimoto and co-workers utilized Suzuki–Miyaura (SM) coupling to access a library of 2_c-functionalized and 2_c,6_c-difunctionalized vancomycin analogues, again observing substantially higher reactivity at 2_c-Cl relative to 6_c-Cl.[15k] Boger also reported an elegant 2_c-borylation protocol that allowed unique access to organometallic substitution reactions at the 2_c-position.[15l] Moreover, although a synthesis of 6_c-dechlorovancomycin has not yet been reported, Boger and co-worker recently prepared the aglycone of 6_c-dechlorovancomycin, enabling binding studies and antibiotic measurements.[15m]

In recent years, our group has become interested in discovering approaches for the site-selective modification of vancomycin, and a single-step and site-selective synthesis of 6_c-functionalized vancomycin has emerged as a goal.[18] Toward this goal, we sought to better understand the reactivity of vancomycin's 6_c-Cl position absent the competing, more favorable, reaction at 2_c-Cl under SM coupling conditions. We identified **2** as a suitable substrate for such studies, and a modified method based on that of Harris et al. was found to furnish **2** with minimal overreaction to **3** (Scheme 1).[19] Furthermore, we anticipated that products resulting from such reactivity studies could possess increased biological activity and inspire future research efforts toward 6_c-Cl modification. Herein, we report a stepwise dechlorination/cross-coupling strategy to access 2_c-dechloro-6_c-functionalized vancomycin derivatives and antibiotic evaluation of novel reaction products featuring diverse functionality at the in-chloride position.

Due to the substrate's unique combination of reactivity, stability, and solubility properties, unusual challenges in the SM cross-coupling of **2** were encountered during reaction optimization. We found that aqueous conditions were required for substrate solubility, but that rapid decomposition of boronic acid coupling partners in water limited conversion.[20–23] Additionally, product decomposition was unavoidable as a function of long reaction times; excess

* Corresponding author. Tel.: +1 203 432 9885; fax: +1 204 436 4900.
E-mail address: scott.miller@yale.edu (S.J. Miller).

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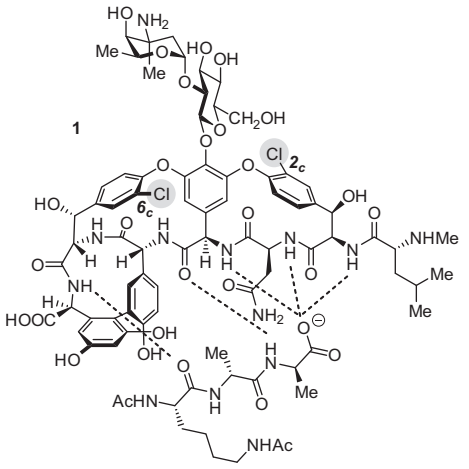
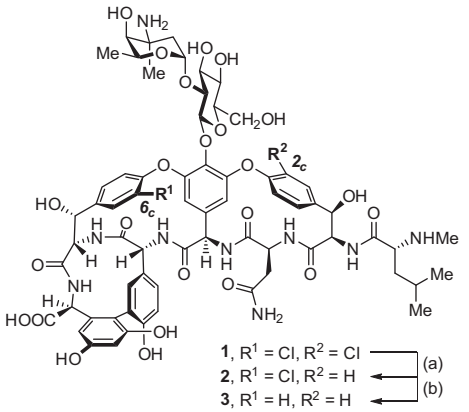


Figure 1. Schematic representation of model ligand *N,N′*-Ac_2-L-Lys-D-Ala-D-Ala bound to vancomycin.



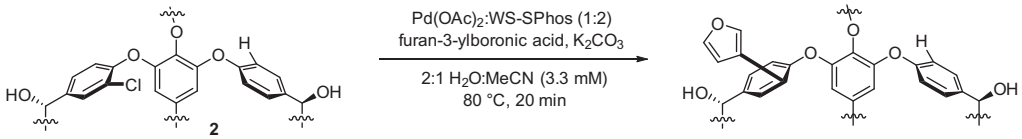
Scheme 1. Stepwise dechlorination of vancomycin (**1**). Reagents and conditions: (a) 10% Pd/C, H_2 (1 atm), H_2O, rt, 3 h, 23%; (b) 10% Pd/C, H_2 (4 atm), H_2O, rt, 24 h, 75%.

base (relative to boronic acid) was also found to accelerate this decomposition under the reaction conditions.[24] The byproduct resulting from product decomposition was not identified; however, LCMS analysis revealed an identical isotope pattern to that of the product (see Supporting information for more detail). Minimizing both types of decomposition (decomposition of boronic acid and decomposition of product) was found to be critical for efficient SM coupling of **2**.

We began reaction optimization by investigating conditions utilizing high catalyst loading along with dropwise addition of a boronic acid/K_2CO_3 solution over a short reaction time (Table 1, entry 1). Longer reaction times led to increased byproduct formation and lower product yield (entry 2); yet, fewer equivalents of boronic acid and base led to decreased byproduct formation and higher product yield (entry 3). We expected that dropwise addition of a boronic acid/K_2CO_3 solution would help avoid boronic acid decomposition and enable higher conversion; however, we observed nearly identical conversion upon switching to a 'dump-and-stir' protocol (entry 4; longer dropwise additions were not examined due to significant byproduct formation over longer reaction times). Next, we investigated conditions employing an excess of boronic acid relative to K_2CO_3 to further suppress base-promoted product decomposition, and a slight increase in product yield was, indeed, observed (entry 5). Lower catalyst loading was also examined but afforded decreased yield due to low conversion, presumably because the slower coupling was unable to outcompete rapid boronic acid decomposition (entries 6 and 7). Ultimately, the conditions in entry 5 were best able to minimize decomposition of both boronic acid and product, leading to the highest product yield.

Once optimized SM reaction conditions were identified, a small library of 2_c-dechloro-6_c-functionalized vancomycin derivatives was prepared (Table 2). The antibiotic activities of vancomycin (**1**) and vancomycin derivatives **2**–**10** were then measured against vancomycin-sensitive *Staphylococcus aureus* and *Enterococcus faecalis* strains and vancomycin-resistant strains of *E. faecalis* (VanA and VanB) using a standard microtiter plate-based antimicrobial assay (Table 3).[25] In every case, **2** exhibited an approximately four-fold reduced activity compared to **1** (entries 1 and 2), and the activity of **3** was reduced approximately eight-fold relative to **1** (entries 1 and 3). These findings are consistent with those of Boger and co-worker, who measured similar reductions in activity against a vancomycin-sensitive strain of *S. aureus* for dechlorinated vancomycin aglycone derivatives relative to vancomycin aglycone.[15l,m] In these prior studies, comparative binding of model cell wall ligands to **1**–**3**[15a] as well as dechlorovancomycin aglycone derivatives[15l,m] suggested that the reduced antibiotic activity results from reduced binding efficiency between dechlorovancomycin analogues and cell wall precursors. The studies also indicate that decreases in binding affinity arising from dechlorination at 2_c-Cl and 6_c-Cl are additive in the case of 2_c,6_c-didechlorovancomycin analogues. Accordingly, the antibiotic activity of **2** is expected to be the most reliable

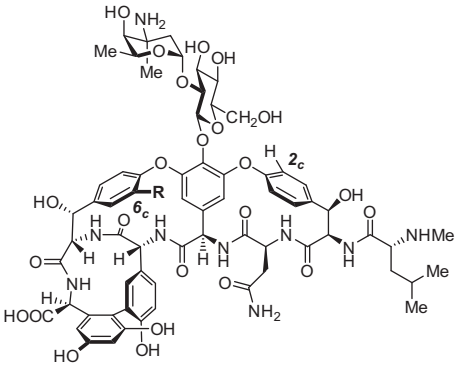
Table 1
Representative optimization studies for the SM coupling of **2**[a]

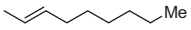
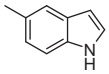
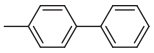
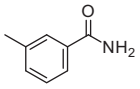
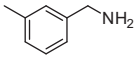


Entry	Catalyst (%)	Boronic acid (equiv)	K_2CO_3 (equiv)	Boronic acid/K_2CO_3 addition time (min)	Starting material (%)	Product (%)	Byproduct (%)
1	50	10	10	20	13	77	10
2[b]	50	10	10	20	9	57	34
3	50	5	5	20	15	81	4
4	50	5	5	0	14	81	5
5	**50**	**5**	**4**	**0**	**14**	**83**	**3**
6	25	5	5	20	31	64	4
7[b]	25	5	5	20	27	51	22

[a] Yields represented by uncorrected HPLC integrations at 280 nm.
[b] Reaction time = 60 min.

Table 2
Structures of 2_cdechloro-6_c-functionalized vancomycin analogues[a]



Compd (% isolated[b])	R	Compd (% isolated[b])	R
4 (21%)		**8** (7%)	
5 (11%)			
6 (52%)		**9** (22%)	
7 (29%)		**10** (16%)	

[a] Optimized reaction conditions were employed for all compounds except **4** and **7**, which required more forcing conditions. See Supporting information for additional details.
[b] Isolated yields refer to HPLC purified material that is chromatographically homogeneous for biological testing.

Table 3
Antibiotic activity data (MIC, μg/mL) for 2_c-dechloro-6_c-functionalized vancomycin analogues against vancomycin-susceptible and vancomycin-resistant bacteria[a]

Entry	Compound	*S. aureus* (MSSA)	*S. aureus* (MRSA)	*E. faecalis* (VSE)	*E. faecalis* (VRE; VanB)	*E. faecalis* (VRE; VanA)
1	**1**	1	1	2	8	>64
2	**2**	4	4	8	64	>64
3	**3**	8	8	32	>64	>64
4	**4**	>64	>64	>64	>64	>64
5	**5**	>64	>64	>64	>64	>64
6	**6**	64	64	>64	>64	>64
7	**7**	>64	>64	>64	>64	>64
8	**8**	>64	>64	>64	>64	>64
9	**9**	>64	>64	>64	>64	>64
10	**10**	>64	>64	>64	>64	>64
11	Teicoplanin	0.5	0.5	0.25	0.25	64
12	Linezolid	2	1	2	1	1

[a] MSSA = methicillin-susceptible *S. aureus*, ATCC 29213; MRSA = methicillin-resistant *S. aureus*, MMX 2002; VSE = vancomycin-susceptible *E. faecalis*, MMX 101; VRE = vancomycin-resistant *E. faecalis*, VanB = MMX 202, VanA = MMX 486. See Supporting information for additional details.

baseline for comparison when considering the activities of novel 2_c-dechloro-6_c-functionalized vancomycin analogues **4**–**10**.

We observed that 6_c-*trans*-1-octen-1-yl (**4**) and 6_c-(4-biphenyl) substitution (**5**) reduced activity beyond the threshold of the assay (>64 μg/mL) for all strains tested, including vancomycin-sensitive strains (Table 3, entries 4 and 5). These observations are consistent with MIC measurements of 2_c,6_c-difunctionalized vancomycin derivatives against vancomycin-sensitive and vancomycin-resistant strains of *S. aureus*, *Enterococcus faecium*, and *E. faecalis* reported by Arimoto and co-workers.[15k] The authors found that larger hydrocarbon-based substituents (*trans*-1-octenyl and *trans*-(5-phenyl)-1-pentenyl) reduced activity beyond the detection threshold of 64 μg/mL while the relatively smaller substituent *trans*-1-propenyl displayed an activity similar to that of vancomycin. As observed in the present study, no compounds exhibited measurably increased activity against vancomycin-resistant strains. Taken with MIC data for dechlorovancomycin derivatives, our data re-assert that vancomycin's antibiotic activity is highly sensitive to the binding pocket's steric environment.

Nevertheless, we hypothesized that hydrogen-bonding, nucleophilic, or charged functionality at the 6_c-position might increase biological activity through conformational changes or through noncovalent or covalent interactions with cell wall precursors. Compounds **6**–**10** were prepared and tested for their potential effects. While 6_c-(furan-3-yl)-substituted analogue **6** inhibits vancomycin-sensitive *S. aureus* strains at high concentration (64 μg/mL), inhibition was not detected for any *E. faecalis* strains examined (>64 μg/mL; Table 3, entry 6). Interestingly, compounds bearing primary amino (**7**), indolyl (**8**), amido (**9**), and fluorophenyl (**10**) functionality all failed to inhibit any strains examined in this study (>64 μg/mL; Table 3, entries 7–10). As observed with dechloro and hydrocarbon functionality at the 6_c-position, steric constraints play the predominant role in determining the antibiotic activity of compounds **6**–**10** despite their diverse functionality. Indeed, the only example of these 2_c-dechloro-6_c-functionalized vancomycin analogues that possesses any measurable activity is furanyl derivative **6**, which bears the smallest substituent at the 6_c-position.

In summary, we have developed a generalizable, stepwise approach to access 2_c-dechloro-6_c-functionalized vancomycin derivatives, and MIC measurements of novel compounds have further advanced understanding of the binding pocket's steric sensitivity. This stepwise approach will enable the preparation

and evaluation of vancomycin derivatives beyond the scope of the present study. Selective chemical modification of the binding pocket of vancomycin continues to provide opportunities for creative analogue design.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.12.027.

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24. As summarized in Ref. 15l: '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.'
25. Minimum inhibitory concentrations (MICs) were determined by Micromyx, LLC (Kalamazoo, MI) in accordance with CLSI guidelines. See Supporting information for further details.