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Synthesis and Evaluation of Vancomycin and Vancomycin Aglycon Analogues that Bear Modifications in the Residue 3 Asparagine

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Abstract—The synthesis and biological evaluation of a set of residue 3 analogues of vancomycin and its aglycon are described. These investigations follow from the promising biological activity of a protected and synthetically modified vancomycin aglycon analogue in which the asparagine side chain was modified to possess a nitrile, rather than a carboxamide. Although this modification typically was detrimental to antimicrobial activity, hydrophobic vancomycin aglycon analogues that lack a lipid anchor as well as the disaccharide are detailed that exhibit unusual potency against VanB, but not VanA, resistant bacteria.

Vancomycin is the prototypical member of a class of clinically important glycopeptide antibiotics[1–3] enlisted as the drugs of last resort for the treatment of resistant bacterial infections or for patients allergic to β-lactam antibiotics.[4] Its structural complexity, the interest in defining its structural features responsible for cell wall biosynthesis inhibition in sensitive bacteria,[5] the emergence of clinical resistance,[6] and the determination of its molecular origin have generated considerable interest in vancomycin and related agents.[7–9] Herein, we describe the synthesis and evaluation of a series of analogues of vancomycin that bear modifications to the residue 3 asparagine (Fig. 1, Table 1).

To date, the only disclosed modifications in the vancomycin residue 3 are limited to two naturally occurring compounds. The first of these is M43G, which contains a residue 3 glutamine containing an additional methylene in the side chain. The second is M43F, which contains a residue 3 aspartic acid where the side chain carboxamide has been hydrolyzed to the corresponding carboxylic acid. M43G and M43F were found to be 4- and 8-fold less active than **1** in antimicrobial assays, respectively.[10] In the course of efforts on the total synthesis of the vancomycin aglycon,[9] we observed that intermediates containing an L-β-cyanoalanine in place of the residue 3 L-Asp exhibited surprisingly effective activity against both vancomycin sensitive and resistant bacteria. This result was especially interesting since these compounds contain a synthetic modification in the asparagine side chain central to the D-Ala-D-Ala binding pocket where the side chain carboxamide has been converted to a nitrile. Based on these observations, and with a knowledge that the importance of residue 3 has not been previously explored, a series of vancomycin aglycon and vancomycin analogues bearing this residue 3 nitrile versus carboxamide modification has been prepared and evaluated.

Chemistry

Compounds **2**–**4** were prepared as described previously.[9] Compound **5** was obtained through simultaneous deprotection of the four methyl ethers, methyl ester, and Boc group of the *N*-Boc derivative of **4** ($AlBr_3$, EtSH, 5 h). Compounds **6** and **7** were prepared by selective alkylative methyl ester derivatization of *N*-Boc vancomycin aglycon under conditions that do not promote phenol *O*-alkylation as described in Scheme 1.

Initial attempts to hydrolyze the methyl ester of **4** and related compounds to obtain the free acid were unsuccessful due to competing nitrile hydrolysis. Thus,

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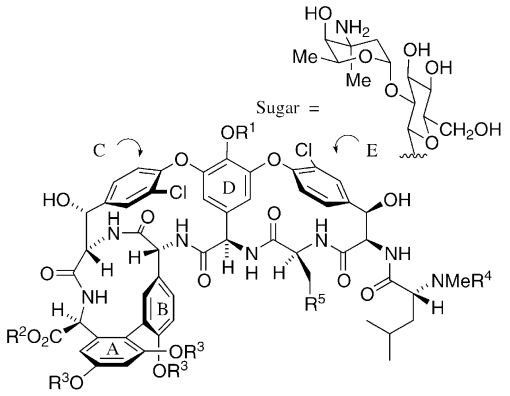
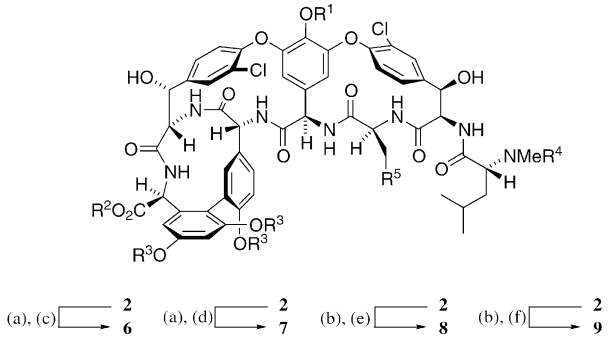


Figure 1. Analogues of vancomycin and vancomycin aglycon.

Table 1. Vancomycin analogues

Compd	R^1	R^2	R^3	R^4	R^5
1	Sugar	H	H	H	$CONH_2$
2	H	H	H	H	$CONH_2$
3	Me	Me	Me	H	$CONH_2$
4	Me	Me	Me	H	CN
5	H	H	H	H	CN
6	H	Me	H	H	$CONH_2$
7	H	Me	H	H	CN
8	Me	H	Me	H	$CONH_2$
9	Me	H	Me	H	CN
13	Sugar	Me	H	H	CN
14	Sugar	H	H	H	CN
15	Sugar	Me	H	H	$CONH_2$

compounds **8** and **9** were accessed by way of the corresponding benzyl ester as described in Scheme 1. The nitrile and carboxamide analogues of vancomycin methyl ester, compounds **13** and **15**, respectively, were obtained from a silylated vancomycin intermediate **10**.[8] The residue 3 carboxamide of **10** was selectively dehydrated to give the nitrile **11** (4.0 equiv TFAA, 6.0 equiv pyridine, CH_2Cl_2, 0 °C, 15 min, 87%), which was desilylated (Bu_4NF–HOAc 1:1, 60 equiv, THF, 8 days, 48%) to give **12**. Subsequent *N*-deprotection (H_2, 10% Pd/C, CH_3OH, 4 h, 60%) provided the key vancomycin methyl ester analogue **13** containing the residue 3 nitrile modified asparagine. All attempts to hydrolyze the methyl ester of **13** to provide **14** were unsuccessful. Under basic conditions, hydration of the nitrile to the carboxamide was found to occur faster than methyl ester hydrolysis consistent with observations first made in the course of our vancomycin aglycon synthesis.[9] Consequently, we enlisted the corresponding benzyl ester analogue **10a** so that the C-terminus carboxylic acid protecting group could be cleaved by hydrogenolysis in order to yield **14** (Scheme 2).

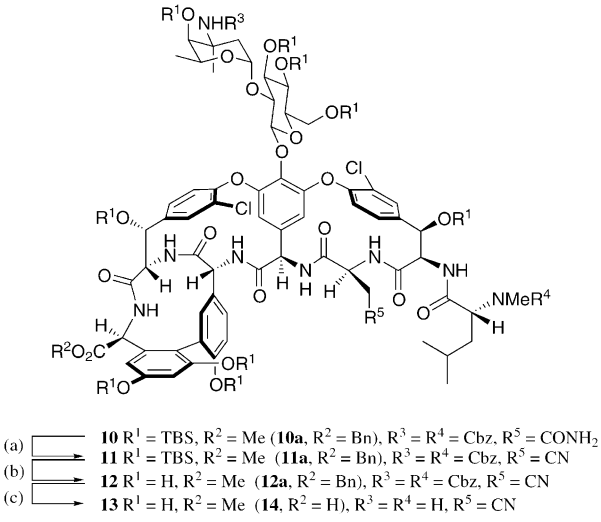


Scheme 1. (a) (1) $Boc_2O/NaHCO_3$, dioxane/H_2O, 5 h; (2) MeI, $NaHCO_3$, DMF, 4 h, 70% (two steps) R^1 = H, R^2 = Me, R^3 = H, R^4 = Boc; (b) (1) $Boc_2O/NaHCO_3$, dioxane/H_2O, 5 h; (2) BnBr, $NaHCO_3$, DMF, 16 h, 43% (two steps); (3) MeI, K_2CO_3, DMF, 16 h, 44%, R^1 = Me, R^2 = Bn, R^3 = Me, R^4 = Boc; (c) HCl/dioxane, 0 °C, 20 min, 68%; (d) (1) TFAA/pyridine, 0 °C, 10 min, THF, 52%; (2) HCl/dioxane, 0 °C, 20 min, 52%; (e) (1) HCl/dioxane, 0 °C, 20 min, 73%; (2) H_2/10% Pd–C, CH_3OH, 12 h, 25%; (f) (1) TFAA/pyridine, 0 °C, 10 min, THF, 51%; (2) HCl/dioxane, 0 °C, 20 min, 38%; (3) H_2/10% Pd–C, CH_3OH, 18 h, 60%.

Antimicrobial Activity

Antimicrobial assays were performed according to a standard procedure[11] and the results are summarized in Table 2.

Within the series of analogues examined, six pairs constitute direct comparisons between a residue 3



Scheme 2. (a) TFAA, pyridine (87%); (b) Bu_4NF–HOAc 1:1 (48%); (c) H_2, 10% Pd–C, CH_3OH (60%).

Table 2. Minimal inhibitory concentrations (μg/mL)

Compd	*Staph. aureus*[a]	*Enterococcus faecium*[b]	*Enterococcus faecium*[c]	*Enterococcus faecalis*[d]	*Enterococcus faecalis*[e]
1	1.25	2.5	500	2000	125
2	0.625	2.5	160	640	80
3	0.625	1.25	40	40	10
4	0.625	1.25	40	40	2.5
5	10	20	640	640	320
6	5	10	320	640	320
7	1.25	5	320	320	320
8	0.625	2.5	40	40	20
9	0.31	2.5	160	160	20
13	2.5	10	320	640	160
14	20	80	320	320	320
15	2.5	2.5	640	640	80

[a]*Staphylococcus aureus* (ATCC 25923).
[b]*Enterococcus faecium* (ATCC 35667).
[c]*Enterococcus faecium* (Vancomycin resistant).
[d]*Enterococcus faecalis* (VanA, BM4166).
[e]*Enterococcus faecalis* (VanB, ATCC 51299).

carboxamide versus nitrile (**1** vs **14**, **2** vs **5**, **3** vs **4**, **6** vs **7**, **8** vs **9**, and **15** vs **13**), and the comparisons are summarized in Table 3.

With the notable exception of nitrile **4**, which matched or exceeded the potency of the corresponding carboxamide **3** in both the sensitive and resistant bacteria examined, the nitrile derivatives were typically less effective than the corresponding carboxamides. Notably, the residue 3 nitrile derivatives of vancomycin (**14**) and the vancomycin aglycon (**5**) were $\geq 10\times$ less potent than the natural carboxamides **1** and **2** against vancomycin-sensitive bacteria although there was little potency distinction with the resistant bacteria. By contrast, the nitrile derivatives of the aglycon methyl ethers (**4** > **9**) proved to be more potent than vancomycin or its aglycon and **4** was especially potent against VanB *Enterococcus faecalis*. However, this potency enhancement appears to be related to the aryl methyl ether functionalization since the corresponding carboxamides also exhibit comparable activity (**3** vs **4** > **8** vs **9**) with **3** also exhibiting good activity against VanB *E. faecalis*.

Peptide Binding Studies

The binding constant for the formation of a 1:1 antibiotic–peptide complex was determined according to the procedure of Nieto and Perkins[12] for the vancomycin analogues with the tripeptides *N*,*N*′-diacetyl-L-Lys-D-Ala-D-Ala and *N*,*N*′-diacetyl-L-Lys-D-Ala-D-Lac and the results are summarized in Table 4. A comparison of the carboxamide versus nitrile derivatives ability to bind the D-Ala-D-Ala or D-Ala-D-Lac ligands revealed that the carboxamide was typically equally effective or more effective than the corresponding nitrile. The notable exception rests with the comparison of **3** with **4** where the nitrile **4** was significantly and reproducibly better at binding D-Ala-D-Ala than **3**. In fact, **4** was found to be uniquely more effective than any derivative examined. Interestingly, **3** and **4** were indistinguishable in binding D-Ala-D-Lac, and both were the best derivatives in doing so. However, the binding assays did not correlate directly with the antimicrobial potencies nor did they reveal a unique behavior for the aryl methyl ethers or nitriles suggesting that other distinguishable features are contributing to the in vitro antimicrobial potencies.

The most obvious distinction is the much more hydrophobic character of **3** and **4**, and to the lesser extent that of **8** and **9**, which track with the activity against VanB. It is possible that these changes in structure convey properties to the analogues in a manner like the lipid chains found in teicoplanin[15] or the hydrophobic substituents of vancosamine derivatized vancomycin analogues.[15,16] Like the impact on teicoplanin but not that of the latter, these hydrophobic changes do not impact VanA activity, but only increase activity against inducible VanB.[15] This observation and the relatively unaffected peptide binding affinities suggest that it may be the membrane localization or cellular compartmental site of action (intracellular vs extracellular), and not the mechanism of action, which is affected by the structural changes. As such, derivatives **3** and **4** may be considered new leads for further exploration, especially for treatment of inducible VanB bacterial infections, prototypical examples of a unique approach to overcoming VanB resistance, and valuable tools for understanding the mechanism(s) of action of the glycopeptide antibiotics.[17]

Table 3. Comparison of MIC for residue 3 $CONH_2$/CN pairs (μg/mL)

Compd	*S. aureus*[a]	*E. faecium*[b]	*E. faecium*[c]	*E. faecalis*[d]	*E. faecalis*[e]
1/14	1.25/20	2.5/80	500/320	2000/320	125/320
2/5	0.625/10	2.5/20	160/640	640/640	80/320
3/4	0.625/0.625	1.25/1.25	40/40	40/40	10/2.5
6/7	5/1.25	10/5	320/320	640/320	320/320
8/9	0.625/0.31	2.5/2.5	40/160	40/160	20/20
15/13	2.5/2.5	2.5/10	640/320	640/640	80/160

[a]*Staphylococcus aureus* (ATCC 25923).
[b]*Enterococcus faecium* (ATCC 35667).
[c]*Enterococcus faecium* (Vancomycin resistant).
[d]*Enterococcus faecalis* (VanA, BM4166).
[e]*Enterococcus faecalis* (VanB, ATCC 51299).

Table 4. Comparison of peptide binding constants (K_a) for residue 3 $CONH_2$/CN pairs

Compd	Lys-Ala-Ala (K_a)$\times 10^{-5}$	Lys-Ala-Lac (K_a)$\times 10^{-2}$
1/14	2.3–3.9[12,13]/2.5	3.3[14]/2.2
2/5	5.8/1.5	2.1/1.5
3/4	2.0/9.9	3.5/3.3
6/7	5.7/1.2	1.8/1.4
8/9	1.4/1.2	1.6/2.1
15/13	1.2/1.8	1.6/2.8

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11. A 50 μL volume of 0.5 M McFarland solution was diluted with 12.5 mL of inoculum solution. 49 μL was inoculated into a well of a V-shaped 96-well microtiter plate. A 1 μL aliquot of an antibiotic solution (DMSO) was added to the well. After up to 24 h of incubation at 37 °C, minimal inhibitory concentrations were determined by monitoring the turbidity in the wells. The lowest agent concentration capable of eliminating turbidity is reported as the minimal inhibitory concentration (MIC).
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17. Full experimental details and characterization of all synthetic intermediates and final products (**3**–**15**) can be provided upon request.