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Synthesis and Evaluation of Methyl Ether Derivatives of the Vancomycin, Teicoplanin, and Ristocetin Aglycon Methyl Esters

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Abstract—A series of methyl ether derivatives of the vancomycin, teicoplanin, and ristocetin aglycon methyl esters was synthesized and their antimicrobial activity was established. These derivatives exhibit increased activity against VanB resistant strains of bacteria equipotent with that observed with sensitive bacteria.

Vancomycin is the leading member of the clinically important glycopeptide antibiotics enlisted to treat resistant bacterial infections and for patients allergic to β-lactam antibiotics.[1–3] Although vancomycin is immensely useful, the emergence of vancomycin clinical resistance has underscored the importance of developing new drugs with improved antibacterial activity.[4] The most common forms of resistance are found in enterococci where the peptidoglycan precursor is induced to shift from a D-Ala-D-Ala to D-Ala-D-Lac peptide terminus to which vancomycin binds 1000-fold less effectively.[5] Strains resistant to both vancomycin and teicoplanin (VanA) or sensitive to teicoplanin, but resistant to vancomycin (VanB) have been identified that bear this same basis of resistance.[5] Although the origin of this selective VanB sensitivity to teicoplanin is not presently well understood, it does serve as the basis for the VanA/VanB classification.

In the course of several studies addressing the synthesis of glycopeptide antibiotics that act by inhibiting bacterial cell wall biosynthesis, we have developed methodology to access a variety of semisynthetic derivatives.[6,7] In a previous study in which analogues of vancomycin possessing modifications to the residue 3 asparagine were evaluated, we observed that analogues bearing a C-terminus methyl ester and with the phenols protected as methyl ethers showed promising activity.[8] These hydrophobic derivatives of vancomycin showed improved activity against inducible VanB suggesting that they possess properties analogous to teicoplanin which have been attributed to the lipid sidechain.[9] Herein, we disclose the synthesis and evaluation of a similar series of analogues of teicoplanin and ristocetin in which the phenols have been transformed to the corresponding methyl ethers.

Preparation of vancomycin aglycon (**2**) was accomplished by HF mediated cleavage of the disaccharide.[10] Synthesis of derivatives **3** and **4** (Fig. 1 and Table 1) was carried out according to our previously disclosed sequence.[11] The aglycon was converted to **3** by protection of the amine terminus as the *N*-Boc carbamate followed by *O*-methylation of the phenolic hydroxyls and carboxylic acid with $TMSCHN_2$. Removal of the *N*-Boc with 4N HCl in dioxane generated **3**. Compound **4** was synthesized from *N*-Boc protected vancomycin aglycon by selective dehydration of the residue 3 carboxamide to

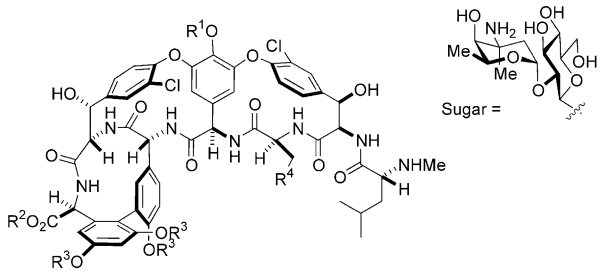

Figure 1. Analogues of vancomycin and vancomycin aglycon.

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Table 1. Vancomycin analogues

Compd	R^1	R^2	R^3	R^4
1	Sugar	H	H	$CONH_2$
2	H	H	H	$CONH_2$
3	Me	Me	Me	$CONH_2$
4	Me	Me	Me	CN

give the corresponding nitrile.[8,11] The intermediate nitrile was then subjected to methylation with $TMSCHN_2$ followed by cleavage of the *N*-Boc with 4 N HCl to provide **4**.

Teicoplanin (**5**) is 2–8 times more potent,[12] possesses a lower toxicity,[13] exhibits a longer half-life,[14] and is easier to administer and monitor than vancomycin.[15] Teicoplanin (**5**), but not the teicoplanin aglycon (**6**), shows activity against inducible VanB but not against VanA. It has been suggested that the aliphatic sidechain present on the sugars of teicoplanin may serve as a membrane anchor and help to localize the antibiotic at the bacterial cell surface.[9] This membrane localization may account for the improved activity of teicoplanin and has been suggested to be responsible for the VanB activity.[9,16] Various explanations have been advanced to account for these observations. In addition to increased effectiveness of D-Ala-D-Lac binding due to the fixed proximity,[9] the lipid substitution or membrane localization could prevent binding to a sensor kinase that signals VanB induction or alter the cell wall biosynthesis step inhibited by the antibiotic (i.e., transglycosylase versus transpeptidase).[16] Our observation of VanB activity with the methyl ether derivatives of the vancomycin aglycon methyl ester suggested that it might be the hydrophobic character of the antibiotic, not the unique nature of the lipid sidechain of teicoplanin, that is responsible for this activity. Thus, we explored the properties of the methyl ether derivatives of two teicoplanin aglycon esters **7** and **8** (Fig. 2 and Table 2). Teicoplanin aglycon (**6**) was prepared from teicoplanin (**5**) by sulfuric acid mediated hydrolysis of the sugars.[17]

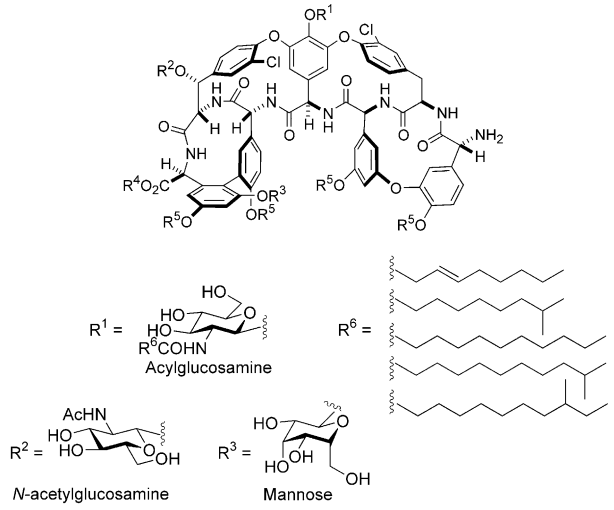

Figure 2. Analogues of teicoplanin and teicoplanin aglycon.

Derivative **7** was accessed by protection of **6** as the *N*-Boc carbamate with Boc_2O followed by esterification with $NaHCO_3$ and methyl iodide. Subsequent *O*-methylation of the phenolic hydroxyls with $TMSCHN_2$ followed by *N*-Boc cleavage by treatment with 4 N HCl in dioxane generated **7**. Analogue **8** was prepared in an analogous manner by benzyl esterification of the *N*-Boc protected aglycon with $NaHCO_3$ and benzyl bromide. *O*-methylation of the phenolic hydroxyls with $TMSCHN_2$ and *N*-Boc cleavage upon reaction with 4 N HCl in dioxane generated **8**.

Ristocetin A (**9**) is a member of the glycopeptide family of antibiotics related to vancomycin and it was originally isolated from *Nocardia lurida* (later reclassified as *Amycolatopsis orientalis*).[18] Although it was clinically employed to treat bacterial infections in the late 1950s, undesirable platelet aggregation led to its discontinuation. However, the aglycon of ristocetin A (**10**) has been found to be slightly more active than its parent and, more importantly, is free of the undesirable side effects.[19] Ristocetin (**9**) and the ristocetin aglycon (**10**), like vancomycin and its aglycon, do not exhibit potent activity against VanB bacteria. Consequently, it was of interest to establish whether methyl ether derivatives of the ristocetin aglycon that possess a C-terminus methyl ester might exhibit VanB activity (Fig. 3 and Table 3). Ristocetin aglycon was prepared from ristocetin A (**9**)

Table 2. Teicoplanin analogues

Compd	R^1	R^2	R^3	R^4	R^5
5	Sugar	Sugar	Sugar	H	H
6	H	H	H	H	H
7	Me	H	Me	Me	Me
8	Me	H	Me	Bn	Me

Table 3. Ristocetin analogues

Compd	R^1	R^2	R^3	R^4
9	Sugar	Sugar	Sugar	H
10	H	H	H	H
11	Me	Me	H	Me

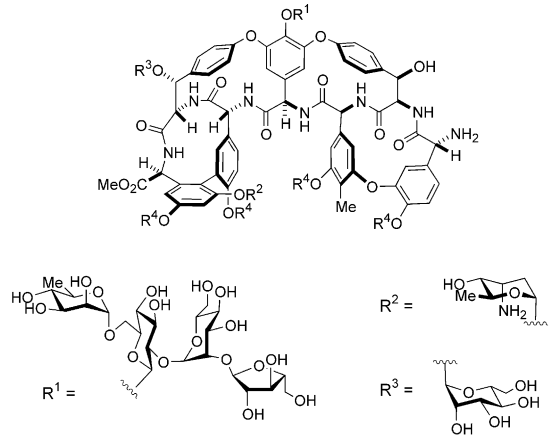

Figure 3. Analogues of ristocetin and ristocetin aglycon.

Table 4. Minimal inhibitor concentration (μg/mL)

Compd	*S. aureus*[a]	*E. faecalis* (VanA)[b]	*E. faecalis* (VanB)[c]
1	0.8	>100	25.0
2	0.8	>100	25.0
3	1.6	50	1.6
4	1.6	50	1.6
5	0.2	>100	0.2
6	1.6	>100	25.0
7	3.2	>100	3.2
8	25.0	>100	3.2
9	1.6	>100	12.5
10	0.8	>100	25.0
11	3.2	>100	3.2

[a]*Staphylococcus aureus* (ATCC 25923).
[b]*Enterococcus faecalis* (VanA, BM4166).
[c]*Enterococcus faecalis* (VanB, ATCC 51299).

using our recently reported anhydrous HF deglycosidation protocol. Aglycon **10** was converted to **11** by a three-step sequence using conditions developed during efforts on a total synthesis of the ristocetin aglycon. Aglycon **10** was protected as the *N*-Boc carbamate with Boc_2O and $NaHCO_3$ in aqueous dioxane to provide the *N*-Boc aglycon in 85% yield. Methylation of the phenolic hydroxyls using $TMSCHN_2$ in benzene/methanol gave the fully methylated intermediate in 40% yield. Removal of the *N*-Boc carbamate by treatment with HCl in dioxane provided **11** in 84% yield.

Antimicrobial assays were performed according to a standard procedure[20] and the minimum inhibitory concentrations of compounds **1**–**11** against sensitive *S. aureus*, and VanA and VanB *Enterococcus faecalis* are summarized in Table 4.

In agreement with previous observations, vancomycin (**1**) and ristocetin (**9**) show a >10-fold drop in activity against VanB *E. faecalis* when compared to sensitive *S. aureus*, while teicoplanin (**5**) was equally effective against both strains. The aglycons of vancomycin (**2**), teicoplanin (**6**), and ristocetin (**10**) exhibit comparable activity against sensitive *S. aureus* but lose activity against the VanB strain. In sharp contrast, the methyl ether derivatives of the aglycon methyl esters **3**, **4**, **7**, and **11** were equipotent against both sensitive *S. aureus* and VanB *E. faecalis*. Even more intriguing, the benzyl ester analogue of teicoplanin aglycon **8** was not active against *S. aureus* although it showed good activity against VanB *E. faecalis*. The series of new compounds tested showed no activity against VanA *E. faecalis* although the weak activity of **3** and **4** previously observed[8] was also observed herein.

Thus, in three different series of glycopeptide antibiotics, the conversion to methyl ether derivatives of the aglycon methyl esters provides derivatives that are equally effective against sensitive and inducible VanB bacteria. Also clear from the examinations is that the introduction of these hydrophobic changes do not impact VanA resistance. This behavior is analogous to that of teicoplanin and suggests that the methyl ether/methyl ester derivatization may confer properties related to the lipid sidechain of teicoplanin or the hydrophobic substituents of vancosamine derivatized vancomycin analogues.[16] If so, it may not be the unique structure of the teicoplanin lipid sidechain or its membrane anchoring properties that convey VanB activity, but rather that these hydrophobic modifications may affect the membrane localization or cellular compartmental site of action (intracellular versus extracellular) without altering the mechanism of action (D-Ala-D-Ala binding). Notably, the derivatives lack the glycopeptide carbohydrates making it unlikely that they are direct transglycosylase inhibitors[16,21] although indirect inhibition by D-Ala-D-Ala binding cannot be excluded. Similarly, membrane anchoring in its classical sense is not viable for these derivatives which lack a lipid sidechain.

Complementary to prior examples of hydrophobic substitution of the sugars, these studies define a new class of semisynthetic derivatives of the glycopeptide aglycons that shows promise for the treatment of both sensitive and inducible VanB bacterial infections. In addition, this unique class of derivatives provides a valuable new tool for exploring the mechanism of action of the glycopeptide antibiotics and the origin of inducible VanB resistance.[22]

Acknowledgements

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20. Fresh cultures of *S. aureus*, *E. Faecalis* VanA and VanB strains were prepared one day before the experiments. The bacterial suspensions were diluted with the culture medium (Muller–Hinton broth) to achieve the turbidity equivalent to 1:100 dilution of 0.5 M Macfarland solution. The diluted bacterial suspensions (0.5 mL) were supplemented with 2.5 μL of serially diluted antibiotic in DMSO, and incubated for 24 h at 37 °C on the rotating wheel. Bacterial growth was determined by measuring turbidity of the culture at 600 nm.
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22. For **7-Boc**: $[\alpha]_D^{25}$ +95 (*c* 0.18, CH_3OH); ^1H NMR (CD_3OD, 313 K, 500 MHz) δ 7.60 (s, 1H), 7.55 (d, $J=8.8$ Hz, 1H), 7.39 (s, 1H), 7.37 (d, $J=8.4$ Hz, 1H), 7.22 (dd, $J=1.9$, 8.5 Hz, 1H), 7.16 (d, $J=1.9$ Hz, 1H), 7.13–7.12 (m, 2H), 7.08 (d, $J=8.5$ Hz, 1H), 7.01–6.99 (m, 3H), 6.72 (d, $J=2.0$ Hz, 1H), 6.50 (br s, 1H), 6.35 (d, $J=2$ Hz, 1H), 6.24 (s, 1H), 6.20 (br s, 1H), 5.90 (br s, 1H), 5.62 (br s, 1H), 5.43 (br s, 1H), 5.30 (s, 1H), 5.13 (br s, 2H), 4.87 (m, 1H), 4.79 (s, 1H), 4.58 (s, 1H), 4.15 (s, 3H), 4.10 (br s, 1H), 3.87 (s, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 3.27 (m, 1H), 3.02 (s, 3H), 2.97 (br s, 1H), 1.47 (s, 9H); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.6, 172.4, 172.3, 172.0, 163.0, 162.1, 160.6, 159.1, 157.6, 155.8, 155.2, 155.1, 154.7, 154.3, 152.5, 151.3, 145.9, 142.9, 142.6, 142.3, 140.2, 136.9, 134.3, 132.3, 132.2, 132.1, 131.3, 131.2, 129.9, 128.9, 128.8, 128.7, 128.4, 128.2, 125.8, 125.2, 125.0, 122.4, 115.3, 114.8, 108.1, 106.3, 101.2, 100.7, 99.3, 98.1, 81.2, 73.0, 69.3, 67.5, 64.6, 62.1, 59.4, 58.3, 57.1, 56.6., 56.4, 56.2, 56.1, 55.9, 53.2, 53.1, 51.7, 28.9; FABHRMS (NBA-CsI) *m/z* 1528.2978 ($M^- + Cs$, $C_{70}H_{67}Cl_2N_7O_{20}$ requires 1528.2872).

For **8-Boc**: $[\alpha]_D^{25}$ +71 (*c* 0.36, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.60 (d, $J=1.8$ Hz, 1H), 7.56 (dd, $J=1.8$, 8.0 Hz, 1H), 7.44–7.34 (m, 7H), 7.23 (dd, $J=1.8$, 8.1 Hz), 7.17 s, 1H), 7.13–7.08 (m, 3H), 6.98–7.03 (m, 3H), 6.66 (d, $J=2.4$ Hz, 1H), 6.49 (s, 1H), 6.21 (br s, 2H), 6.18 (d, $J=2.4$ Hz, 1H), 5.94 (s, 1H), 5.22 (d, $J=12.0$ Hz, 1H), 5.11 (s, 2H), 4.89–4.87 (m, 1H), 4.85 (s, 1H), 4.56 (s, 1H), 4.14 (s, 3H), 4.11 (br s, 1H), 3.78 (s, 3H), 3.73 (s, 3H), 3.60–3.58 (m, 6H), 3.30–3.29 (m, 1H), 2.97 (s, 3H), 2.80 (br s, 1H), 1.47 (s, 9H); ^{13}C NMR (DMF-d_7, 150 MHz) δ 173.0, 172.4, 172.1, 171.1, 170.3, 170.0, 169.3, 162.2, 161.4, 160.2, 158.2, 156.5, 153.8, 153.6, 152.1, 151.4, 150.3,145.5, 143.8, 141.5, 137.6, 137.3, 137.2, 136.3, 135.5, 132.6, 131. 8, 131.5, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 128.9, 127.8, 127.3, 125.3, 125.1, 124.7, 124.3, 122.3, 121.6, 116.0, 113.6, 108.0, 107.7, 107.1, 106.7, 105.7, 105.3, 105.0, 99.5, 79.6, 73.2, 67.6, 63.8, 61.9, 59.9, 58.7, 57.9, 57.8, 57.2, 56.7, 56.6, 56.2, 55.9, 55.8, 55.7, 36.5, 28.9; FABHRMS (NBA-CsI) *m/z* 1606.3114 ($M^+ + Cs$, $C_{76}H_{71}Cl_2N_7O_{20}$ requires 1606.3193).

For **11-Boc**: $[\alpha]_D^{25}$ −13 (*c* 0.28, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 7.79 (d, $J=8.0$ Hz, 1H), 7.57 (d, $J=8.0$ Hz, 1H), 7.39 (d, $J=8.3$ Hz, 1H), 7.29–7.25 (m, 1H), 7.21 (d, $J=8.6$ Hz, 1H), 7.20–7.16 (m, 2H), 7.07 (d, $J=8.4$ Hz, 1H), 7.05–7.03 (m, 1H), 7.01–6.99 (m, 2H), 6.97–6.94 (m, 2H), 6.85–6.82 (m, 1H), 6.71 (d, $J=2.2$ Hz, 1H), 6.34 (d, $J=2.2$ Hz, 1H), 6.22 (s, 1H), 6.19 (s, 1H), 5.78 (br s, 1H), 5.66 (s, 1H), 5.57 (s, 1H), 5.33 (s, 1H), 5.30 (s, 1H), 5.18–5.14 (m, 2H), 5.05 (d, $J=4.8$ Hz, 1H), 4.78 (s, 1H), 4.60 (s, 1H), 4.10 (br s, 4H), 3.86 (s, 3H), 3.82 (s, 3H), 3.77–3.75 (m, 6H), 3.74 (s, 3H), 2.96 (s, 3H), 2.06 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 172.3, 170.7, 169.6, 168.8, 161.4, 160.1, 159.7, 158.3, 157.4, 156.7, 156.1, 155.8, 155.4, 151.9, 140.2, 138.8, 136.8, 136.2, 134.4, 132.1, 130.3, 130.1, 129.7, 129.4, 129.2, 127.8, 127.7, 124.3, 123.3, 122.6, 122.2, 120.5, 116.2, 116.0, 115.4, 114.5, 114.2, 113.6, 108.6, 107.2, 106.9, 105.8, 105.3, 99.1, 79.6, 79.3, 64.1, 61.7, 61.5, 59.7, 58.1, 57.7, 57.1, 56.5, 56.3, 56.2, 55.9, 55.7, 55.4, 55.3, 52.6, 28.7; MALDIFTMS (DHB) *m/z* 1380.4545 ($M^+ + Na$, $C_{71}H_{71}N_7O_{21}$ requires 1380.4595).