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Design and Synthesis of New Vancomycin Derivatives

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Abstract—A set of vancomycin derivatives with lipid chain attached *via* a glyceric acid linker was designed and synthesized. A concise synthesis towards these derivatives was developed and the IC50s of these new lipoglycopeptides were tested. Some of them showed very potent activity against both vancomycin sensitive and resistant strains.

The glycopeptides antibiotics are the most important drugs in current use for the treatment of Gram-positive bacterial infections. They function through binding to the bacterial cell wall substrate D-Ala-D-Ala and thus inhibiting the transpeptidation step. In 1990s, vancomycin resistant enterococci (VREs) and vancomycin-resistant staphylococcus aureus (VRSA) emerged which posed a very serious public health problem. This led to new interests in the development of antibiotics of different class as well as new derivatives of the glycopeptides.

Many different types of vancomycin derivatives were prepared and studied. The most successful finding is the discovery by Nagarajan *et al* that attaching lipid chain to the vancomycin carbohydrate portion led to significant potency boost towards both vancomycin sensitive and resistant strains.[1] These modifications eventually led to the development of a new glycopeptide antibiotic called oritavancin which is now close to FDA filing (Figure 1). Studies suggest that these new vancomycin derivatives with lipid chain attached (lipoglycopeptides) have dual mechanisms by inhibiting both transpeptidation and the transglycosylation steps of the cell wall biosynthesis.[2] The alycone portion binds the cell wall substrate and the lipid carbohydrate portion inhibits the transglycosylases at the same time. Interestingly, we notice that another transglycosylase inhibitor – moenomycin (Figure 1) - also has a similar structure feature where a long lipid chain is attached to its carbohydrate portion via a glyceric acid linker. Kahne group has extensively studied the functions of the C55 lipid chain as well as the carbohydrate portion.[3] However, the function of this glyceric acid phosphate linker remains unknown.

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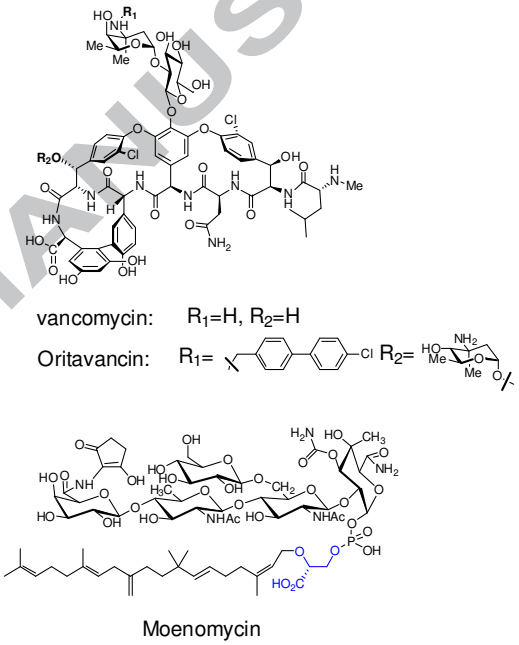


Figure 1. Chemical structures of vancomycin, oritavancin and moenomycin.

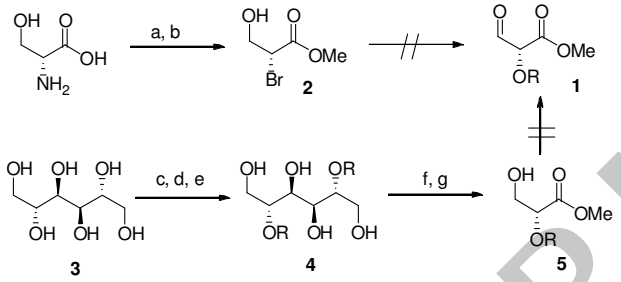
During the development of the lipoglycopeptides, it was found that these derivatives have poorer solubility in water compared to vancomycin. In addition, the greasy chain caused unwanted ion-channel side effect. How to improve aqueous solubility while improving the potency is a challenging problem in this area.

We have noticed the similarity between the lipoglycopeptides and moenomycin. It was interesting to understand the role of the glyceric acid-phosphate portion of moenomycin is. Here we designed a set of vancomycin derivatives with lipid chains attached to the carbohydrate portion *via* a similar glyceric acid linker. Our assumption is that an important structural feature "borrowed" from a transglycosylase inhibitor should

also be helpful to the transglycosylase inhibiting effect of the vancomycin carbohydrate portion. In addition, the added polarity of this acid linker should also help to lower the LogP, improve the solubility and reduce the unwanted ion channel side effect. On the other hand, studies on these analogs may also give us some hints of the role of this glyceric acid linker in moenomycin.

A straightforward way for making these new derivatives is through the reductive-amination between ester **1** and vancomycin (Scheme 1). We tried a few methods to make this unique ester. At first, we converted serine into methyl 2-bromo-3-hydroxypropanoate **2** through diazonium reaction and esterification. However, all attempts to displace the *alfa*-bromide with alkoxide only led to the decomposition of **2**. We then tried the Kahne procedure and prepared the ester **5**.[4] However, the final oxidation of the hydroxyl group failed completely with every oxidation reagent we tried, presumably due to the instability of the final product.

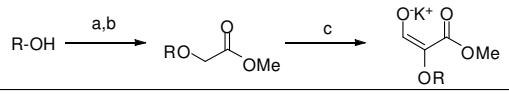
Scheme 1[a]. Attempted syntheses of compound 1.



[a]Reagents and conditions: (a) $NaNO_2$, HBr; (b) MeOH, HCl; (c) PMB dimethoxy ketal, TsOH; (d) RBr, NaH; (e) AcOH, H_2O; (f) $NaIO_4$, THF, H_2O; (g) $NaClO_2$, NaH_2PO_4, H_2O, 2-methyl-2-butene; then MeOH, HCl.

After lots of literature searching and experimentation, we found that the condensation of methyl 2-benzoxy-acetate with methyl formate can provided the sodium salt of desire product in one single step (entry 1, table 1).[5] The product is quite stable as potassium salt. This method can also be applied to other 2-alkoxy-acetates to give a series of 3-oxy propionates. Proton NMR indicated that these compounds are in their enolate form which makes them much more stable.

Table 1[a] Synthesis of various methyl 2-aloxy-3-oxo-propanoates**.**

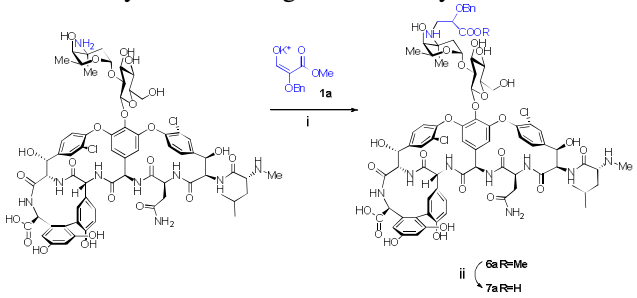


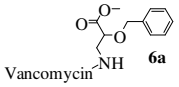
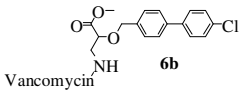
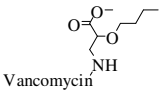
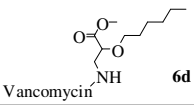
Entry	R=	Product	Yield[%]
1	benzyl	**1a**	63
2	chlorobiphenyl methyl	**1b**	79
3	*n*-butyl	**1c**	66
4	*n*-hexyl	**1d**	55

[a]Reagents and conditions: (a) chloroacetic acid, NaH, THF; (b) $SOCl_2$, MeOH; (c) KO^{t}Bu, methyl formate, ether.

Next we studied the reductive-amination reaction between compounds **1a**-**1d** and vancomycin. Following the known condition (DMF, $NaBH_3CN$, 80℃)[1], we only obtained the enamine intermediate. Attempts to reduce this intermediate with various reducing agents all failed (entry 1, 2, 3, table 2). During the isolation of this intermediate, we noticed that it was less stable under acidic conditions. With this in mind, we adjusted the acidity of the reaction media. A 1:1 mixture of DMF and acetic acid turned out to be the best reaction solvent and high temperature (80 ℃) was also needed. Complete reduction of the enamine intermediate was achieved to give **6a** (entry 4, table 2) [6]. Compounds **6b**-**6d** were synthesized in similar fashion (entry 5-8, table 2). Thus, a concise synthesis of these vancomycin derivatives with lipid-glyceric linker was developed. Further hydrolysis of the methyl esters was achieved using LiOH to give the final products **7a**-**7d**.[7]

Table 2[a] Synthesis of targeted vancomycin derivatives**.**

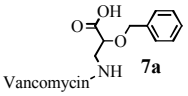
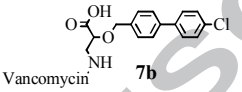
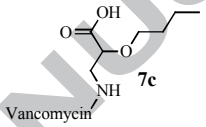
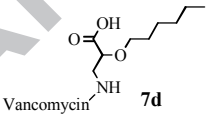


Entriy	Substrates	Reaction conditions	Products	Yield[%]
1	**1a**	$NaBH(OAc)_3$/ DMF	 **6a**	0
2	**1a**	$NaBH(OAc)_3$/ THF	**6a**	0
3	**1a**	$NaBH_3CN$/ DMF	**6a**	0
4	**1a**	$NaBH_3CN$/ DMF/AcOH	**6a**	62
5	**1b**	$NaBH_3CN$/ DMF/AcOH	 **6b**	54
6	**1c**	$NaBH_3CN$/ DMF/AcOH	 **6c**	80
7	**1d**	$NaBH_3CN$/ DMF/AcOH	 **6d**	68

[a]Reagents and conditions: (i) $NaBH_3CN$, DMF/AcOH=1/1, 80℃; (b) $LiOH•H_2O$, THF/H_2O.

The anti-bacterial activities of these derivatives were tested against vancomycin sensitive and medium resistant strains.[8] Their IC50s were listed in table 3. From the IC50 data, it showed that the new analogs with longer lipid chain were generally more potent (entry 8 *vs.* 9, table 3). The acid analogs **7a**-**7d** have similar activities as the esters **6a**-**6d**. The most potent compound, **7b**, is as active as the known chlorobiphenyl vancomycin.

Table 3. Sixteen-hour MICs (μg/mL) of vancomycin, chlorobiphenyl vancomycin and compounds **6a**-**6d**, **7a**-**7d** for selected bacterial strains. Vancomycin sensitive strains: *Staphylococcus aureus* Newman; vancomycin intermediate resistance strain: *Staphylococcus aureus* Mu 50.

Entry	Compound	MICs	
		Newman	Mu50
1	vancomycin	2	8
2	chlorobiphenyl vancomycin	<0.125	2
3	**6a**	8	32
4	**6b**	<0.125	2
5	**6c**	16	64
6	**6d**	2	4
7	 **7a**	16	64
8	 **7b**	<0.125	2
9	 **7c**	64	128
10	 **7d**	4	8

Thus, we have developed a concise synthetic route towards a set of vancomycin lipid analogs with a glyceric acid linker. Some of these analogs showed good activities towards both vancomycin sensitive and resistant strains. We are actively testing their solubility as well as their ion-channel binding activities.

Acknowlegement

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5. Synthesis of **1a**: To a solution of methyl 2-(benzyloxy)acetate (1.0 g, 5.2 mmol, 1 eq) in 6 mL of anhydrous ether was added potassium *tert*-butoxide (0.63 g, 5.2 mmol, 1.1 eq) and methyl formate (0.53 g,. 8.8 mmol, 1.7 eq). The mixture was stirred at room temperature for 15 hours. The precipitated solid was filtered and washed with ether (15 mL) to give **1a** (734mg, 63%) as a white solid. ^{1}H NMR (300 MHz，DMSO, δ) : 8.55 (dd, J=12.4Hz, 1H), 7.30 (m, 5H), 4.52 (s, 2H), 3.40 (s, 3H) ppm. LRMS (ESI): calcd for $C_{11}H_{12}KO_4$ 246.0, found 206.9[M-39].

6. Synthesis of **1b**: The synthesis of compound **1b** is similar to the synthesis of compound **1a**. ^{1}H NMR (300 MHz，DMSO, δ): 8.57 (s, 1H), 7.48-7.71 (m, 8H), 4.61 (s, 2H), 3.40 (s, 3H) ppm. ^{13}C NMR (75 MHz，DMSO, δ): 166.98, 166.07, 140.23, 139.09, 137.14, 132.08, 128.90, 128.36, 128.24, 126.03, 123.64, 72.65, 48.60 ppm. LRMS (ESI): calcd for $C_{17}H_{14}KClO_4$ 356.0, found 317.0[M-39].

7. Synthesis of **6a**: To a solution of vancomycin (0.74 g, 0.5 mmol, 1 eq) in 40 mL of anhydrous DMF was added **1a** (150 mg, 0.6 mmol, 1.2 eq). The mixture was stirred at 80℃ for 2 hours until HPLC indicated the disappearance of vancomycin. To the mixture was added AcOH (8mL) and $NaBH_3CN$ (63 mg, 1.0mmol, 2 eq). The reaction was stirred for another 2 hours. The reaction mixture was directly subjected to preparative reverse phase HPLC purification to give product **6a** (508 mg, 62% yield) as a white solid. HRMS (ESI): calcd for $C_{77}H_{87}Cl_2N_9O_{27}$ 1639.5088, found 1640.5133[M+H].

Synthesis of **7a**: To a solution of LiOH•H_2O (7.2 mg, 0.17 mmol, 10 eq) in 0.57 mL of THF and 0.57 ml of water was added compound **6a** (30 mg, 0.018 mmol, 1 eq). The mixture was stirred at room temperature for 25 minutes. The reaction was quenched with AcOH (0.1 mL) and then concentrated under reduced pressure. The residue was purified by preparative reverse phase HPLC to give product **7a** (19 mg, 64% yield). LRMS (ESI): calcd for $C_{76}H_{85}Cl_2N_9O_{27}$ 1625.4932, found 1626.4990 [M+H] and 1648.4827 [M+Na].

Synthesis of **6b**: Compound **6b** was synthesized similar to compound **6a**. ^{1}H NMR (300 MHz, DMSO, δ): 9.12 (s, 1H), 8.64 (d, *J*=2.8 Hz, 1H), 8.48 (d, *J*=5.6 Hz, 1H), 8.25 (s, 1H), 8.17 (m, 2H), 7.93 (s, 1H), 7.86 (s, 1H), 7.53-7.63 (m, 4H), 7.47-7.52 (m, 2H), 7.34-7.42 (m, 4H), 7.18-7.26 (m, 2H), 6.92 (s, 2H), 6.77 (m, 1H), 6.72 (d, *J*=6.0 Hz, 1H), 6.67 (s, 1H), 6.62 (s, 1H), 6.42 (d, *J*=2.1 Hz, 1H), 6.26 (d, *J*=2.1 Hz, 1H), 5.74 (m, 1H), 5.69 (d, *J*=5.7 Hz, 1H), 5.55 (s, 1H), 5.43 (s, 1H), 5.24-5.38 m, 3H), 5.11-5.19 (m, 2H), 4.88 (m, 1H), 4.61 (q, *J*=11.7 Hz, 1H), 4.51 (s, 2H), 4.43 (d, *J*=2.8 Hz, 1H), 4.42 (d, *J*=5.6 Hz, 1H), 4.34 (q, *J*=7.2 Hz, 1H), 4.19 (d, *J*=7.5 Hz, 1H), 4.09 (m, 1H), 3.71 (m, 2H), 3.67 (s, 3H), 3.59 (m, 1H), 3.50 (m, 1H), 3.31 (m, 3H), 3.27 (s, 1H), 3.17 (m, 2H), 2.96 (m, 1H),2.78 (m,1H), 2.35 (s, 3H), 2.14 (m, 2H), 1.49-1.59 (m, 4H), 1.10 (s, 3H), 1.04 (s, 3H), 0.89 (d, *J*=6.3 Hz, 3H), 0.84 (d, *J*=6.4 Hz, 3H) ppm. HRMS (ESI): calcd for $C_{83}H_{90}Cl_3N_9O_{27}$ 1749.5012, found 1750.5076 [M+H].

Synthesis of **7b**: Compound **7b** was synthesized similar to compound **7a**. ^{1}H NMR (300 MHz, DMSO, δ) : 9.44 (s,1H), 9.12 (s, 1H), 8.64 (d, *J*=2.8 Hz, 1H), 8.48 (d, *J*=5.6 Hz, 1H), 8.25 (s, 1H), 8.17 (m, 2H), 7.93 (s, 1H), 7.86 (s, 1H), 7.53-7.63 (m, 4H), 7.47-7.52 (m, 2H), 7.34-7.42 (m, 4H), 7.18-7.26 (m, 2H), 6.92 (s, 2H), 6.77 (m, 1H), 6.72 (d, *J*=6.0 Hz, 1H), 6.67 (s, 1H), 6.62 (s, 1H), 6.42 (d, *J*=2.1 Hz, 1H), 6.26 (d, *J*=2.1 Hz, 1H), 5.74 (m, 1H), 5.69 (d, *J*=5.7 Hz, 1H), 5.55 (s, 1H), 5.43 (s, 1H), 5.24-5.38 m, 3H), 5.11-5.19 (m, 2H), 4.88 (m, 1H), 4.61 (q, *J*=11.7 Hz, 1H), 4.51 (s, 2H), 4.43 (d, *J*=2.8 Hz, 1H), 4.42 (d, *J*=5.6 Hz, 1H), 4.34 (q, *J*=7.2 Hz, 1H), 4.19 (d, *J*=7.5 Hz, 1H), 4.09 (m, 1H), 3.71 (m, 2H), 3.59 (m, 1H), 3.50 (m, 1H), 3.31 (m, 3H), 3.27 (s, 1H), 3.17 (m, 2H), 2.96 (m, 1H),2.78 (m,1H), 2.35 (s, 3H), 2.14 (m, 2H), 1.49-1.59 (m, 4H), 1.10 (s, 3H), 1.04 (s, 3H), 0.89 (d, *J*=6.3 Hz, 3H), 0.84 (d, *J*=6.4 Hz, 3H) ppm. HRMS (ESI): calcd for $C_{82}H_{88}Cl_3N_9O_{27}$ 1735.4855, found 1736.4874 [M+H].

8. **IC50 measurement**: Media and bacterial strains: Tryptic Soy Broth (TSB) medium was used to cultivate *Staphylococcus aureus* Newman strain (vancomycin sensitive) and *Staphylococcus aureus* Mu 50 strain (vancomycin intermediate resistance).

MIC (Minimum Inhibitory Concentration) values were tested for all compounds. Compounds were dissolved with DMSO to 1.28 mg/mL as stock solution. All samples were diluted with culture broth to 128 μg/mL as the initial concentration. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 64 μg/mL to 0.125 μg/mL. 100 μL of each dilution was distributed in 96-well plates, as well as sterile controls, growth controls (containing culture broth plus DMSO, without compounds) and positive controls (containing culture broth plus vancomycin hydrochloride). Each test and growth control well was inoculated with 5 μL of a bacterial suspension (about 10^5 CFU/well). The 96-well plates were incubated at 37 ℃ for 16 h. MIC values of these compounds against *Staphylococcus aureus* Newman strain and *Staphylococcus aureus* Mu 50 strain, was defined as the lowest concentration to inhibit the bacterial growth completely.

Graphical abstract