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Novel Semi-Synthetic Glycopeptide Antibiotics Active Against Methicillin-Resistant S*taphylococcus aureus* (MRSA) and Vancomycin-Resistant Enterococci (VRE): Doubly-Modified Water-Soluble Derivatives of Chloroorienticin B

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Abstract—A series of *N*-alkylated and aminomethylated derivatives of chloroorienticin B, a vancomycin-related glycopeptide antibiotic, were synthesized. Doubly-modified derivatives having both hydrophobic and hydrophilic substituents exhibited potent antibacterial activity against MRSA and VRE along with considerable water-solubility.

Introduction

Vancomycin, one of the typical glycopeptide antibiotics, has been widely used for treatment of gram-positive bacteria infections especially those caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, the emergence and spread of vancomycin-resistant enterococci (VRE) is a growing clinical problem worldwide.[1] In the last few years, chemical modifications of vancomycin-related glycopeptide antibiotics have been extensively explored and the introduction of a hydrophobic side chain on the nitrogen of the amino sugar moiety was found to be effective for potent activity against VRE.[2] Among the derivatives with hydrophobic substituents on the amino sugar moiety, LY333328 has shown promise and is currently under clinical study.[3] Hydrophobic substituents on the amino sugar moiety are considered to have unfavorable effects on water-solubility, therefore another substituent is required to increase the water-solubility of the derivatives. However most of the derivatives reported thus far have only one hydrophobic substituent on the amino sugar moiety and there are few reports with respect to the derivatives with two or more substituents.[2e,2i,4] Among them, doubly-modified glycopeptide derivatives (derivatives of teicoplanin aglycon) that have both hydrophobic and hydrophilic substituents have exhibited considerable activity against gram-positive bacteria including VRE.[4] This report prompted us to prepare the novel semi-synthetic glycopeptide antibiotics.

We report herein the chemical modification of chloroorienticin B,[5] a vancomycin-related glycopeptide antibiotic, and show that its derivatives are active against MRSA and VRE and possess considerable water solubility (see Fig. 1).

Results and Discussion

We first prepared the derivatives having hydrophobic substituents on the amino sugar nitrogen to enhance the antibacterial activity especially against VRE. The synthesis of *N*-alkylated derivatives of chloroorienticin B is shown in Scheme 1. The secondary amino group of the N-terminus was first protected by a PMZ (*p*-methoxybenzyloxy carbonyl) group[6] and the resulting *N*-protected intermediate (**1**) was subjected to reductive *N*-alkylation.[2g] Deprotection of the PMZ group by treatment with CF_3CO_2H afforded *N*-modified derivatives of chloroorienticin B.

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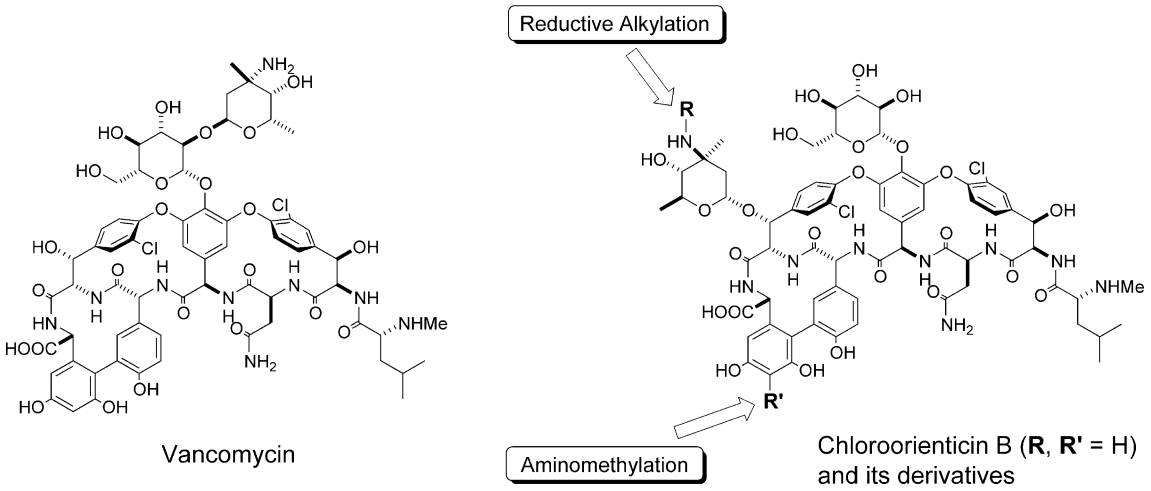
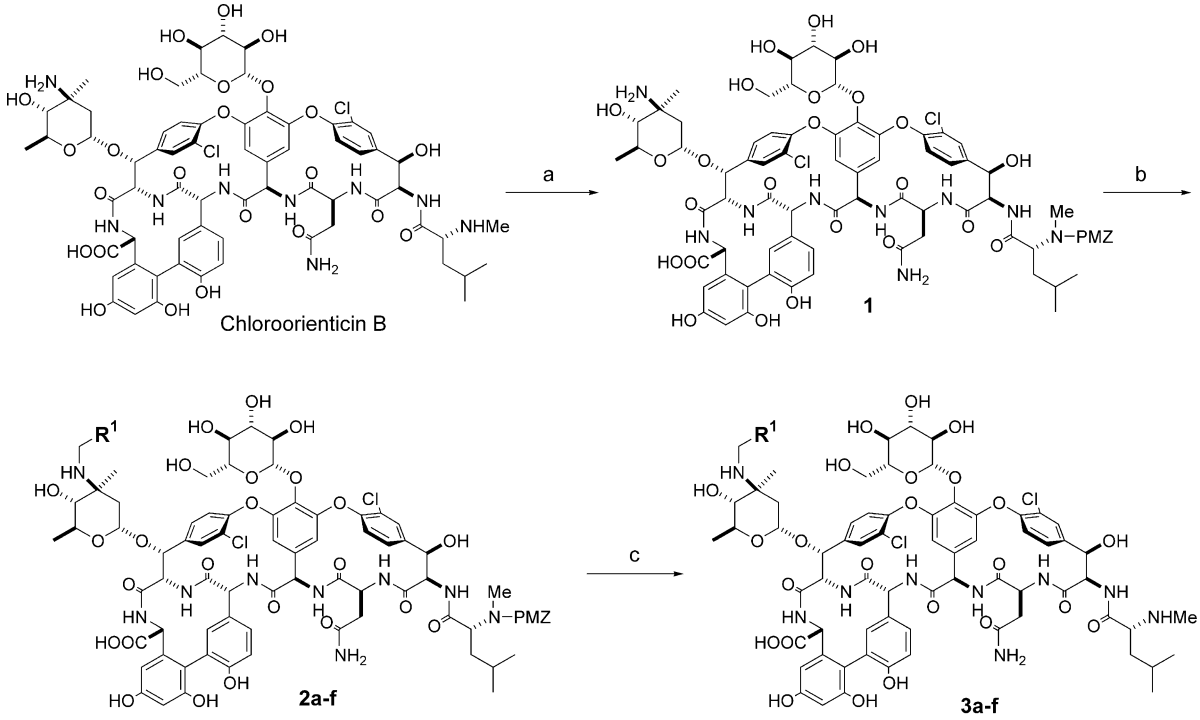


Figure 1. Chemical structure of vancomycin and chloroorienticin B.

All of the final products were obtained as hydrochlorides after purification by reverse-phase (ODS) column chromatography eluted with dil.HCl aq-CH_3CN followed by lyophilization. The chemical structures of the compounds obtained were confirmed by ^1H NMR and mass spectra and their purity was demonstrated by HPLC and elemental analysis.

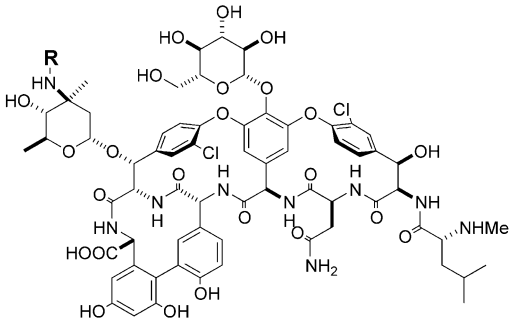
The in vitro antibacterial activities of the compounds prepared as described above are shown in Table 1 with values of MIC[7] (μg/mL) against MRSA and VRE (Van A enterococci). Compounds with the *p*-substituted benzyl group (**3b**–**3f**) were found to have potent activity against MRSA (4–16 times more potent than vancomycin) and VRE (MIC: 3.13–12.5 μg/mL). The derivative having a long aliphatic chain (**3a**) also showed potent antibacterial activity. Among them, compound **3d** having a 4-[(*E*)-2-(4-chlorophenyl)-vinyl]benzyl moiety was found to have the most well-balanced antibacterial activity against MRSA and VRE.

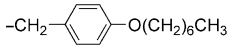
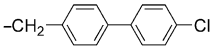
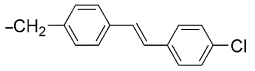
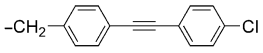
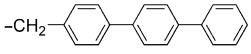
All the compounds having hydrophobic substituents on the amino sugar nitrogen shown in Table 1 exhibited potent antibacterial activity, but they were sparingly soluble in water (less than 1%), which precluded further pharmacological evaluation. We next focused our efforts on preparing more water-soluble derivatives by further modification of the compounds that have potent activity against MRSA and VRE. We chose compound **3d**

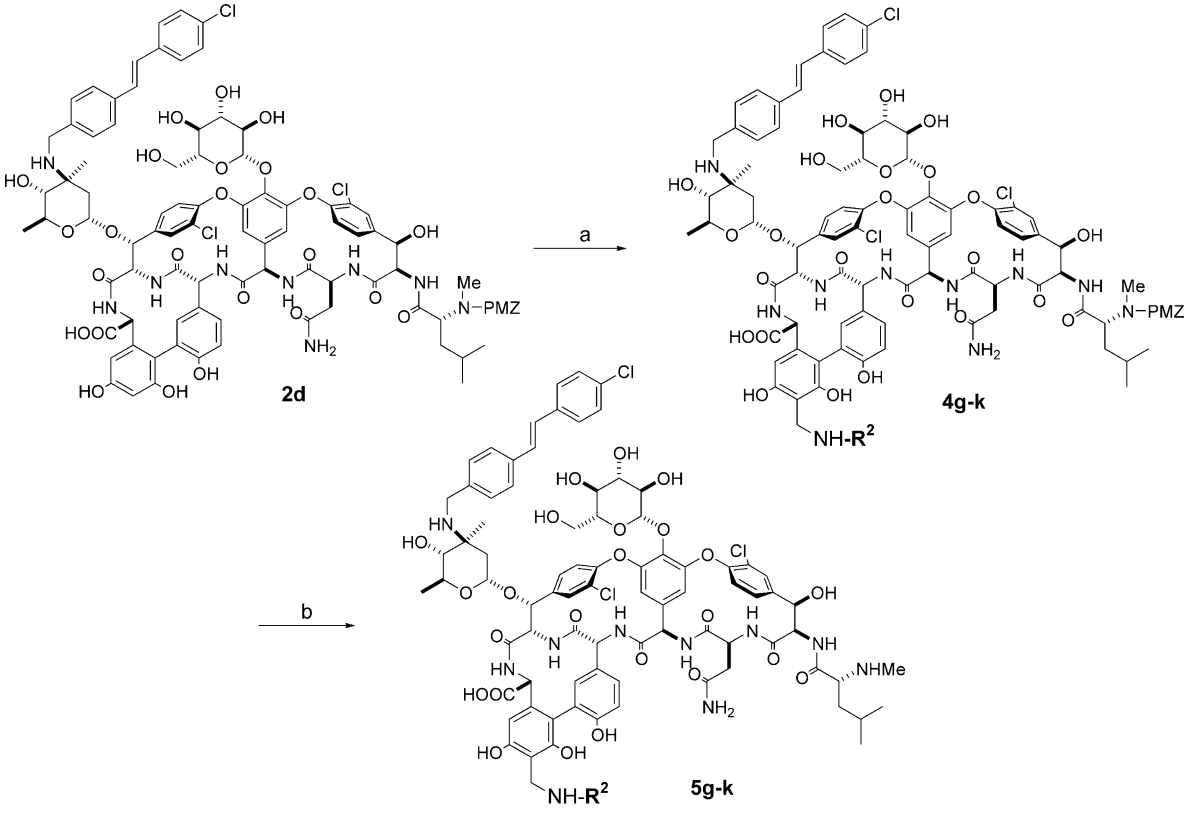


Scheme 1. Reagents and conditions: (a) S-*p*-methoxybenzyloxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine (MZ-SDP), Et_3N, DMSO-DMF; (b) R^1-CHO, $NaBH_3CN$, MeOH–DMF; (c) CF_3CO_2H–H_2O.

Table 1. In vitro antibacterial activity against MRSA and VRE

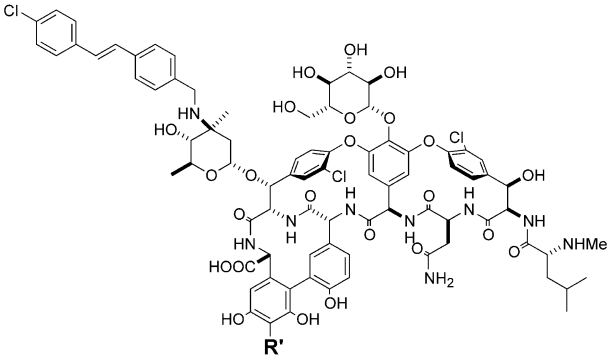


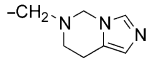
Compd	R	MIC (μg/mL)		
		Staphylococcus aureus SR3637 (MRSA)	*Enterococcus faecalis* SR7914 (VRE)	*Enterococcus faecium* SR7917 (VRE)
3a	$-(CH_2)_{12}CH_3$	0.2	12.5	6.25
3b		0.39	25	12.5
3c		0.1	12.5	6.25
3d		0.2	6.25	6.25
3e		0.2	12.5	6.25
3f		0.39	12.5	3.13
Chloroorientation B	–H	0.78	> 100	> 100
Vancomycin		1.56	> 100	> 100



Scheme 2. Reagents and conditions: (a) R^2-NH_2, 37%-HCHO aq, NaOH aq, CH_3CN–H_2O; (b) CF_3CO_2H–H_2O.

Table 2. In vitro antibacterial activity of the aminomethylated derivatives of **3d**



Compd	R′	MIC (μg/mL)			Solubility in water[a] (%)
		Staphylococcus aureus SR3637 (MRSA)	*Enterococcus faecalis* SR7914 (VRE)	*Enterococcus faecium* SR7917 (VRE)	
3d	H	0.2	6.25	6.25	< 1
5g	$-CH_2NH$ ⌒ NMe_2	0.39	12.5	6.25	10
5h	$-CH_2NH$ ⌒ $^+NMe_3 \cdot Cl^-$	0.39	12.5	6.25	10
5i	$-CH_2NH$ ⌒ NMe_2	0.78	12.5	6.25	10
5j	$-CH_2NH$ ⌒ NH_2 (CO$_2$H)	0.39	12.5	12.5	8
5k	$-CH_2-$ 	0.78	6.25	3.13	10
	Chloroorienticin B	0.78	> 100	> 100	> 10
	Vancomycin	1.56	> 100	> 100	> 10

[a]Solubility as hydrochlorides.

as a parent compound for further chemical modification leading to a derivative with better water-solubility.

Preparation of more water-soluble derivatives of **3d** was achieved by aminomethylation[8] (Mannich reaction) of the resorcinol moiety as shown in Scheme 2. Intermediate **2d** was subjected to the Mannich reaction affording the aminomethylated products **4g–k**, followed by removal of the PMZ group to give aminomethylated derivatives **5g–k**.

As shown in Table 2, the water-solubility of the aminomethylated derivatives prepared was much greater than that of the parent compound **3d**. As for the antibacterial activity, compounds having tertiary and quaternary amino groups (**5g–i**) and the amino acid moiety (**5j**) have somewhat decreased antibacterial activity against MRSA and VRE (especially against *Enterococcus faecalis*). However, the compound with the 5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine-6-methyl moiety (**5k**)[9] was found to be as active as its parent **3d** against *E. faecalis* and more active against *Enterococcus faecium* along with improved water-solubility. The activity of **5k** against MRSA was less potent than **3d**, but comparable to chloroorienticin B and vancomycin.

The introduction of hydrophobic side chains is considered to be essential for potent antibacterial activity especially against VRE, but such hydrophobic substituents have the negative effect on water-solubility of the resultant compounds. In the previous reports concerning doubly-modified teicoplanin derivatives that have both hydrophobic and hydrophilic substituents,[4] the most active compounds had the activity comparable to **5k**. However these compounds were derivatives of teicoplanin aglycon, which are structurally different from chloroorienticin B derivatives, and are devoid of the sugar moiety which plays an important role in water-solubility. The doubly-modified compound **5k** presented herein with a hydrophobic substituent on an amino sugar moiety and a hydrophilic one on a resorcinol moiety is a novel derivative of vancomycin-related glycopeptide antibiotics that has potent antibacterial activity against MRSA and VRE with considerable water-solubility (10%).

In conclusion, the results presented in this paper demonstrate the possibility that more water-soluble semi-synthetic glycopeptide antibiotics with potent activity against MRSA and VRE can be prepared by modification of the parent glycopeptide by introducing both hydrophobic and hydrophilic substituents. This methodology is considered to be useful for discovering novel semi-synthetic glycopeptide antibiotics.

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6. Preliminary experiments revealed that the N-terminus secondary amino group of the peptide backbone was more reactive than the amino sugar nitrogen, therefore protection of N-terminus amino group was found to be necessary prior to reductive *N*-alkylation.
7. Minimum Inhibitory Concentrations (MICs) were determined by National Committee for Clinical Laboratory Standards (NCCLS)-recommended microbroth dilution methods using cation-adjusted Mueller Hinton broth (Difco Laboratories, Detroit, MI, USA).
8. Pavlov, A. Y.; Lazhko, E. I.; Preobrazhenskaya, M. N. *J. Antibiot.* **1997**, *50*, 509.
9. Histamine dihydrochloride was used for aminomethylation of **2d**.