Bioorganic & Medicinal Chemistry Letters 21 (2011) 6732–6738

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antibacterial activity of N^4-mono alkyl derivatives of novel glycopeptide LYV07ww01

Chang Shao [a], Weicheng Zhou [a,*], Shunli Zhang [a], Wei Wei [b], Lingao Ruan [b], Yiping Jin [c], Xinqiang Sun [c], Yanqin Huang [d]

[a] State Key Lab of New Drug & Pharmaceutical Process, Shanghai Key Lab of Anti-Infectives, Shanghai Institute of Pharmaceutical Industry, State Institute of Pharmaceutical Industry, Shanghai 200437, China
[b] Shanghai Health Creation Center for Biopharmaceutical R&D, Shanghai 200240, China
[c] Xinchang Pharmaceutical Factory, Zhejiang Medicine Co., Ltd, Xinchang 312500, Zhejiang, China
[d] MicuRx (Shanghai) Pharmaceuticals, Inc., Shanghai 201203, China

ARTICLE INFO

Article history:
Received 18 August 2011
Revised 13 September 2011
Accepted 14 September 2011
Available online 18 September 2011

Keywords:
Synthesis
Antibacterial
Glycopeptide
LYV07ww01

ABSTRACT

Thirty-one N^4-mono alkyl derivatives of novel glycopeptide LYV07ww01 were synthesized by the reductive alkylation and their in vitro antibacterial activity was tested. The benzyl derivatives showed potent activity, especially against vancomycin-resistant enterococci and penicillin-resistant *Streptococcus pneumoniae*.

Vancomycin (**1**, see Fig. 1) and the related compounds are called glycopeptide antibiotics. **1** is produced by *Amycolatopsis orientalis* and was first isolated by Eli Lilly and Company in 1956. It became available for clinical use upon its FDA approval in 1958. It has been one of the few antibiotics effective against nosocomial infections caused by Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus* (**MRSA**).[1] Its antibacterial activity appears to be based on inhibition of peptidoglycan transglycosylation and/or transpeptidation by binding to –D-Ala-D-Ala, which is the terminus of cell-wall precursor peptides.[2] However, the appearance of vancomycin-resistant enterococci (**VRE**) and the possibility of its spread to other pathogens become a growing clinical problem worldwide recently.[3] In the case of **VRE**, the terminus of the precursor peptides varies from –D-Ala-D-Ala to –D-Ala-D-Lac, the affinity of vancomycin for depsipeptide ligands is decreased by about 1/1000; therefore, antibacterial activity also decreases.[4] The concern of increasingly frequent vancomycin resistance has highlighted the need for modification of glycopeptide derivatives. The biphenylmethyl derivative of A82846B, known as oritavancin (Fig. 2), has been found to be more active against both the susceptible and vancomycin-resistant pathogens than vancomycin.[5] The recent studies have shown that the introduction of a hydrophobic side chain on the nitrogen of the amino sugar moiety in glycopeptides was found to be effective for potent activity against **VRE**.[6] Telavancin and dalbavancin are also included as the successful examples (see Fig. 2), the former has been approved onto the market by FDA in 2009 and the latter is in clinical trial. A common structural characteristic in these semi-synthetic glycopeptides is a hydrophobic side-chain substituent at the amino-sugar, which is attached to the phenolic-hydroxyl group of amino acid residue 4 of the aglycon structure.[7]

As recently described in the Chinese patent, LYV07ww01 (**2**, see Fig. 1) can be obtained through genetic manipulation of native vancomycin producer *A. orientalis* HCCB10007.[8] **2** is a novel three-sugar glycopeptide with the same scaffold as vancomycin, but another vancosamine appended to amino acid residue 6. It is very similar to A82846B (LY264826, Chloroorienticin A, see Fig. 1) which has 4-*epi*-vancosamine units both in amino acid residue 4 and in amino acid residue 6.[9] So far, the structural modification on **2** has not been reported, herein we report the synthesis of a series of **2** derivatives with N^4-alkylation of vancosamine in amino acid residue 4. Their in vitro antibacterial activities were evaluated against a panel of pathogens, including Gram-positive bacteria resistant to current therapies.

The target compounds were prepared by reductive alkylation.[10] This reaction was accomplished by treating **2** with a slight excess of the desired aliphatic or aromatic aldehydes, heating to 65 °C in

* Corresponding author. Tel./fax: +86 21 35052484.
E-mail address: profzhouwc@yahoo.com.cn (W. Zhou).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.09.045

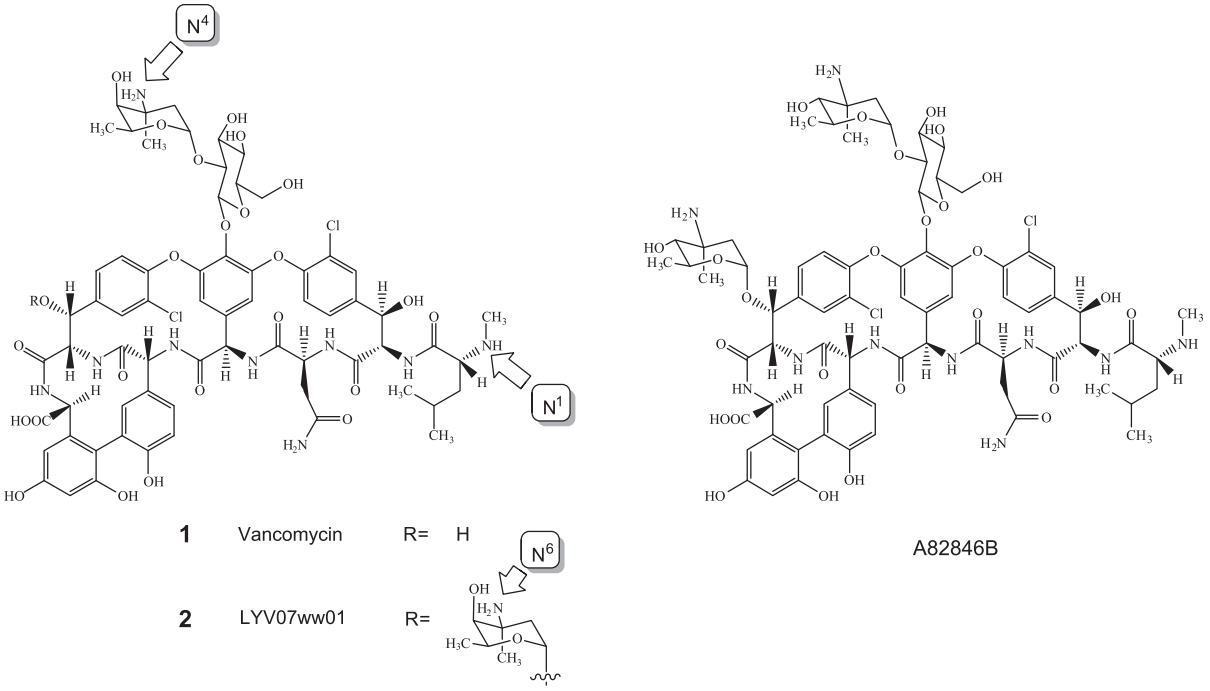


Figure 1. Structures of **1**, **2** and A82846B.

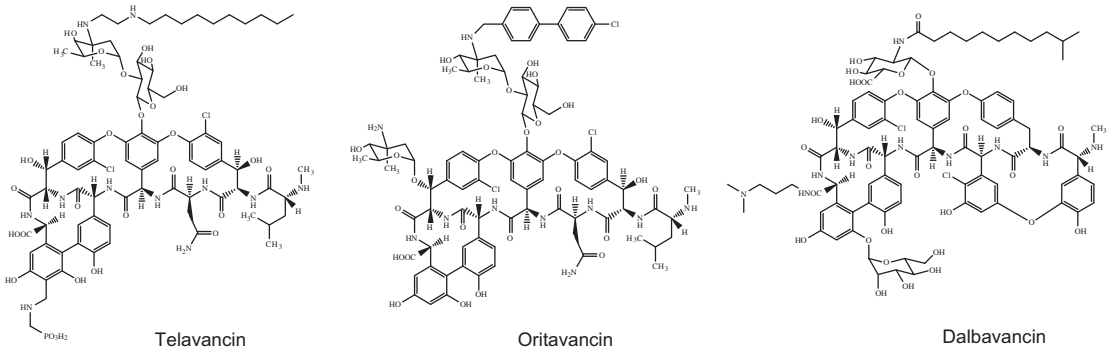
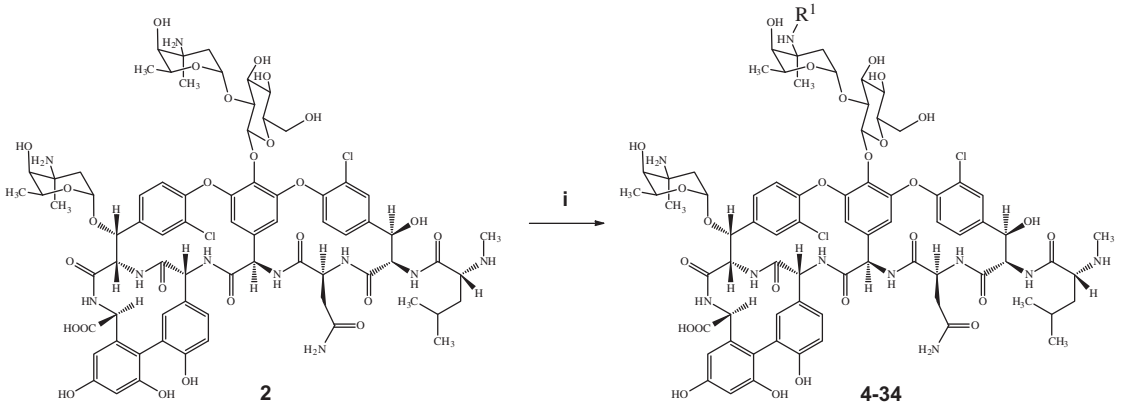
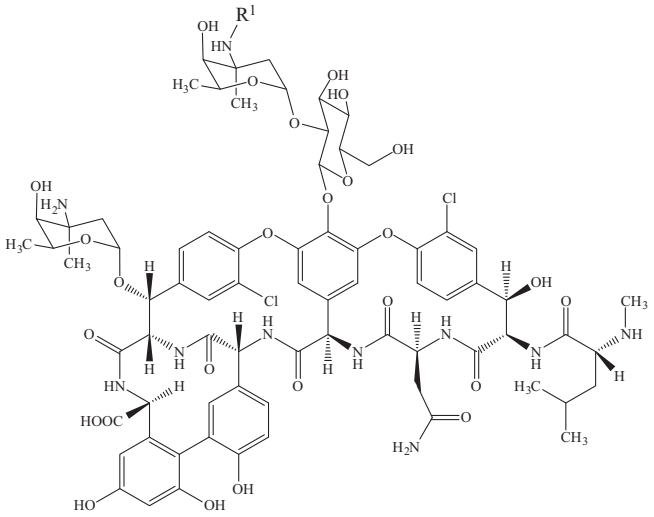


Fig. 2. Structures of telavancin, oritavancin and dalbavancin.



Scheme 1. Reagents and conditions: (i) (1) R'CHO, DMF/MeOH (1:1), 65 °C 2 h; (2) $NaBH_3CN$, rt.

Table 1
N^4-mono alkyl derivatives of novel glycopeptide LYV07ww01



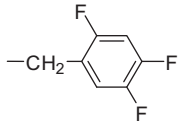
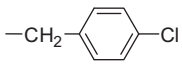
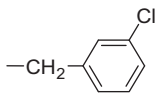
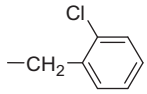
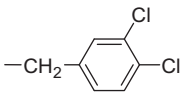
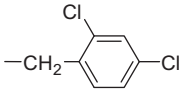
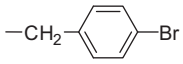
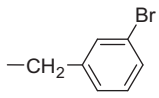
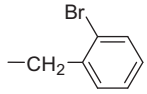
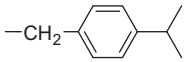
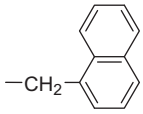
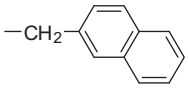
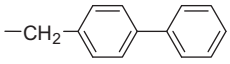
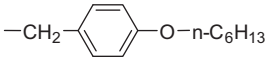
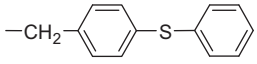
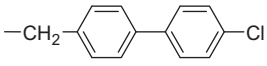
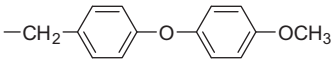
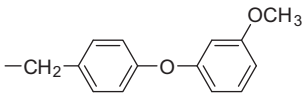
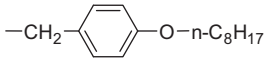
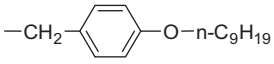
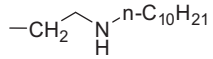
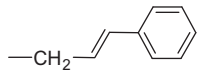
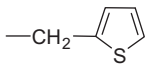
Compd	R^1	Yield (%)	$[\alpha]_D^{25}$	ESI-MS (m/z)	MIC (μg/mL)[d]					
					S. aur	*S. pne*	*S. alb*	*E. fae*	*S. gam*	*S. epi*
1	Vancomycin hydrochloride		−25[a]	1448.33 (M+H)	0.78	0.39	3.13	6.25	6.25	>25
2	H		−80[b]	1613.60 (M+Na)	0.39	0.098	0.78	3.13	0.78	>25
4		21.2	−71[b]	1735.25 (M+H)	1.56	1.56	3.13	1.56	12.5	12.5
5		13.6	−52[c]	1737.49 (M+Na)	3.13	1.56	3.13	3.13	1.56	12.5
6		22.5	−56[c]	1715.66 (M+H)	1.56	0.78	1.56	1.56	0.78	>25
7		20.5	−62[c]	1751.26 (M+Na)	1.56	0.78	3.13	1.56	1.56	6.25
8		36.1	−76[b]	1749.41 (M+H)	3.13	0.78	3.13	6.25	0.78	>25
9		17.2	−46[c]	1749.60 (M+H)	3.13	3.13	3.13	1.56	1.56	25
10		20.9	−60[c]	1781.41 (M+Na)	1.56	1.56	1.56	1.56	6.25	6.25
11		15.3	-66[c]	1781.44 (M+Na)	0.78	0.78	0.78	0.78	3.13	>25

Table 1 (*continued*)

Compd	R^1	Yield (%)	$[\alpha]_D^{25}$	ESI-MS (m/z)	MIC (μg/mL)[d]					
					S. aur	*S. pne*	*S. alb*	*E. fae*	*S. gam*	*S. epi*
12		17.8	−49[c]	1759.53 (M+H)	1.56	0.195	1.56	1.56	0.39	>25
13		15.6	−16[c]	1711.50 (M+H)	0.78	0.78	1.56	1.56	1.56	3.13
14		27.1	−60[b]	1723.36 (M+H)	1.56	0.78	1.56	1.56	1.56	6.25
15		17.3	−59[c]	1731.35 (M+H)	1.56	0.78	1.56	1.56	6.25	>25
16		32.8	−63[c]	1731.70 (M+H)	1.56	1.56	3.13	3.13	0.78	>25
17		30.4	−35[c]	1779.69 (M+Na)	3.13	1.56	1.56	1.56	0.78	6.25
18		15.0	−59[c]	1803.50 (M+Na)	6.25	3.13	6.25	6.25	3.13	>25
19		20.5	+143[c]	1889.66 (M+H)	3.13	3.13	3.13	3.13	3.13	>25
20		24.2	−34[c]	1791.19 (M+H)	6.25	3.13	3.13	6.25	3.13	6.25
21		18.5	−13[c]	1804.56 (M+2)	1.56	1.56	3.13	1.56	1.56	>25
22		18.5	−42[c]	1804.62 (M+2)	3.13	3.13	3.13	3.13	0.78	>25
23		25.8	−63[c]	1810.79 (M+2)	12.5	6.25	12.5	12.5	6.25	>25
24		23.8	−59[c]	1823.75 (M+H)	12.5	12.5	25	12.5	12.5	>25
25	$-CH_2$-*n*-C_3H_7	18.6	−106[b]	1647.65 (M+H)	0.78	0.39	1.56	>25	1.56	>25
26	$-CH_2$-*n*-C_4H_9	26.8	−99[b]	1661.65 (M+H)	1.56	0.39	3.13	>25	0.78	>25
27	$-CH_2$-*n*-C_5H_{11}	26.7	−104[b]	1675.65 (M+H)	1.56	0.78	3.13	>25	1.56	>25
28	$-CH_2$-*n*-C_6H_{13}	18.0	−95[b]	1689.70 (M+H)	1.56	0.78	1.56	>25	1.56	>25
29	$-CH_2$-*n*-C_7H_{15}	13.1	−60[b]	1703.50 (M+H)	3.13	0.39	3.13	1.56	1.56	>25
30	$-CH_2$-*n*-C_8H_{17}	13.6	−100[b]	1717.60 (M+H)	3.13	1.56	6.25	>25	1.56	>25
31	$-CH_2$-*n*-C_9H_{19}	28.9	−59[b]	1731.36 (M+H)	6.25	6.25	6.25	>25	6.25	>25

(*continued on next page*)

Table 1 (*continued*)

Compd	R^1	Yield (%)	$[\alpha]_D^{25}$	ESI-MS (m/z)	MIC (μg/mL)[d]					
					S. aur	*S. pne*	*S. alb*	*E. fae*	*S. gam*	*S. epi*
32		15.1	−57[c]	1774.46 (M+H)	6.25	3.13	6.25	12.5	3.13	>25
33		11.7	−47[c]	1707.72 (M+H)	3.13	1.56	3.13	3.13	1.56	>25
34		5.9	−52[c]	1711.61 (M+Na+2)	25	6.25	>25	12.5	25	>25

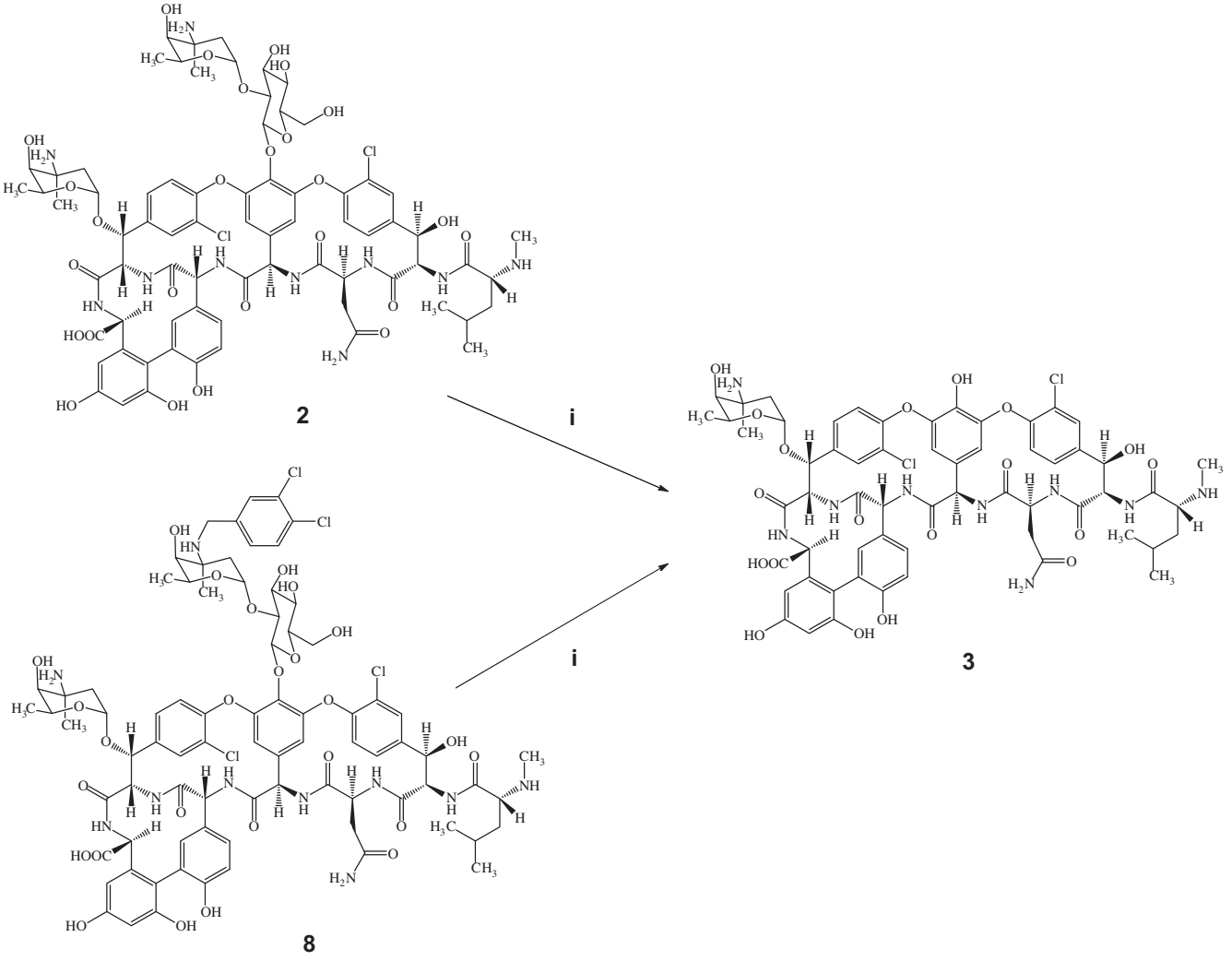
S. aur: *Staphylococcus aureus* 26003, *S. pne*: *Streptococcus pneumoniae* 31002, *S. alb*: *Staphylococcus albus* 26101, *E. fae*: *Enterococcus faecium* 32220, *S. gam*: *Streptococcus gamma* 32206, *S. epi*: *Streptococcus epidermidis* 26069.

[a] *c* 0.1, H_2O.

[b] *c* 0.1, CH_3OH.

[c] *c* 0.1, DMSO.

[d] MIC was tested by the CLSI two-fold dilution method.



Scheme 2. Reagents and conditions: (i) TFA, rt.

Table 2
Antibacterial activity (MIC μg/mL) against vancomycin-resistant bacteria

	MIC (μg/mL)[d]							
	S. aureus		*E. faecalis*		*E. faecium*		*S. pneumoniae*	
	MSSA[a] ATCC 25923	MRSA ATCC 33591	VSE ATCC 29212	VRE (Van B) ATCC 51299	VSE ATCC 35667	VRE MEFA 0039	PISP[b] ATCC 49619	PRSP[c] MSPN 0003
1	0.5	0.5	1	8	0.25	>32	0.125	0.25
2	0.25	0.125	0.25	0.5	0.125	8	0.063	0.125
7	1	0.5	0.5	0.5	0.25	8	⩽0.031	0.125
10	1	0.5	0.5	0.5	⩽0.031	2	⩽0.031	⩽0.031
11	1	0.5	0.25	0.5	⩽0.031	2	⩽0.031	⩽0.031
12	1	0.5	0.25	0.5	0.125	4	⩽0.031	0.063
13	1	0.5	0.5	0.5	0.125	4	0.063	0.125
14	1	0.5	0.5	0.5	⩽0.031	2	⩽0.031	⩽0.031
25	0.5	0.5	0.5	1	0.25	32	0.063	0.125

[a] MSSA: methicillin-susceptible *Staphylococcus aureus*.
[b] PISP: penicillin-intermediate *S. pneumoniae*.
[c] PRSP: penicillin-resistant *S. pneumoniae*.
[d] MIC was tested by the CLSI broth microdilution method.

DMF/MeOH (1:1) as a solvent, followed by adding $NaBH_3CN$ as a reducing agent and stirring at room temperature (see Scheme 1). After work-up, the crude products were purified by reverse-phase HPLC with dil. HCOOH aq-CH_3OH as the eluant and the desired fractions were concentrated to a small volume and neutralized with saturated sodium bicarbonate to remove the formic acid, then the aqueous solution was extracted with *n*-butanol. The organic layer was separated and washed with water, then evaporated in vacuo to dryness. The solid was collected by filtration and washed with acetone and dried in vacuo.[13] In the case of compound **32**, after the reaction of **2** with *N*-Fmoc-*N*-decylaminoacetaldehyde as above, the additional de-protection was carried with 10% diethylamine in DMF for 30 min at room temperature to remove the *N*-Fmoc-protective group. The data from both ^1H NMR and mass spectra (Electrospray Ionization, ESI) indicated that the products were the mono-alkyl derivatives (Table 1).

The structure of the products was confirmed by chemical degradation as described in the case of A82846B.[11] The selective cleavage of compound **2** and **8** was accomplished by the reaction with TFA at room temperature respectively, and the de-disaccharide compound **3** was found as the major product in both of reactions by HPLC (see Scheme 2). The result indicated that the site of alkylation was taken place on the disaccharide amino group (N^4), neither on the *N*-methylleucine (N^1) nor the mono-saccharide (N^6).

The in vitro antibacterial activity of the compounds against Gram-positive bacteria was tested with vancomycin as the positive control. Minimum inhibitory concentration (MIC) values were determined using agar two-fold dilution method according to CLSI and the data was collected in Table 1.

The result showed that both the benzyl and the aliphatic derivatives maintained good or moderate antibacterial activities. Compounds **4**, **6**, **7**, **9**–**15**, **17, 21** had potent activity against *Enterococcus faecium* 32220 (4–8 times more active than **1,** 2–4 times more active than **2**), while the derivatives having aliphatic chains (**25**–**32**) had decreased antibacterial activity against **VSE** (Table 1).

The methoxybenzyl derivative (**13**) was the most active compound against all 5 Gram-positive pathogens in the alkyloxybenzyl derivatives, as the side chain was extended, the activity decreased (**18, 23, 24**). A similar trend was observed for a series of aliphatic derivatives, as the carbon length was increased from C_4 to C_{10} (**25**–**31**), the MIC against *S. aureus* 26003 decreased from 0.78 to 6.25 μg/mL.

The polyaromatic derivatives including biphenyl and fused aromatic analogs (**15**–**17, 20**) exhibited an improvement in activity against *E. faecium* 32220, as well as the biphenyl ether (and the sulfur bioisostere) derivatives **21**, **22**, **19**. However, replacement of the aromatic ring with heterocycle such as the thiophene group (**34**) provided no advantage over the benzyl analog.

As shown in Table 1, compounds **7**, **10–14**, **25** had the activities comparative to **1**. Encouraged by the above exciting results, these compounds were undergone the in vitro test against the Gram-positive resistant bacteria and the results were summarized in Table 2. Although all seven modified derivatives did not give significant improvement against *S. aureus* (MSSA or MRSA) compared to **1** or **2**, the compounds **10**, **11**, **14** were 4 times more active than **2** against *E. faecium* (VSE and VRE), especially, the six benzyl derivatives (**7**, **10–14**) gave the 4–16 times more activity than **1** against **VRE** including *E. faecalis* and *E. faecium*. The derivatives with halogens (**7**, **10**–**12**) or isopropyl (**14**) showed better activity against *Streptococcus pneumoniae,* both PISP and PRSP.

Arhin mentioned that the in vitro activity against S. aureus of oritavancin was increased when polysorbate 80 was added, but the latter did not affect the activity of vancomycin.[12] The antibacterial test in this paper was undergone without polysorbate 80, the effect on our compounds' MIC of polysorbate 80 will be worthy for investigation.

In summary, a series of **2** derivatives with N^4 alkylation of vancosamine in amino acid residue 4 were synthesized and their in vitro antibacterial activities were evaluated. The benzyl derivatives showed potent activity, especially against vancomycin-resistant enterococci and penicillin-resistant *S. pneumoniae* and compounds **10**, **11** and **14** are worthy to the further study.

Acknowledgment

The work was supported by "973 project (Grant No. 2010CB735601)" from Ministry of Science and Technology of China.

References and notes

1. Kahne, D.; Leimkuhler, C.; Lu, W.; Walsh, C. *Chem. Rev.* **2005**, *105*, 425.
2. Nitanai, Y.; Kikuchi, T.; Kakoi, K.; Hanamaki, S.; Fujisawa, I.; Aoki, K. *J. Mol. Biol.* **2009**, *385*, 1422.
3. (a) Walsh, C. T.; Fisher, S. L.; Park, I. S.; Prahalad, M.; Wu, Z. *Chem. Biol.* **1996**, *3*, 21; (b) Shlaes, D. M.; Etter, L.; Gutmann, L. *Antimicrob. Agents Chemother.* **1991**, *35*, 776.
4. (a) Walsh, C. *Nature* **2000**, *406*, 775; (b) Leung, S. S. F.; Tirado-Rives, J.; Jorgensen, W. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1236.
5. Malabarba, A.; Nicas, T. I.; Thompson, R. C. *Med. Res. Rev.* **1997**, *17*, 69.
6. Nicas, T. I.; Mullen, D. L.; Flokowitsch, J. E.; Preston, D. A.; Synder, N. J.; Zweifel, M. J.; Wilkie, S. C.; Rodriguez, M. J.; Thompson, R. C.; Cooper, R. D. G. *Antimicrob. Agents Chemother.* **1996**, *40*, 2194.
7. Allen, N. E.; Letourneau, D. L.; Hobbs, J. N., Jr. *J. Antibiot.* **1997**, *50*, 677.

8. Ge, M.; Huang, H.; Wei, W.; Ruan, L. G.; Yang, S.; Zhu, L.; Jiang, W. H.; Chen, D. J.; Luo, M. Y.; Yang, Z. J.; Xia, X.; Li, Q. S.; Wang, T. J.; Yin, Y.; Jin, W. X.; Yang, T. CN 101928331A.
9. Naoki, T.; Toshiyuki, K.; Masaaki, K.; Yoshihiro, T. *J. Antibiot.* **1988**, *41*, 1506.
10. Cooper, R. D. G.; Snyder, N. J.; Zweifel, M. J.; Staszak, M. A.; Wilkie, S. C.; Nicas, T. I.; Mullen, D. L.; Futler, T. F.; Rodriguez, M. J. *J. Antibiot.* **1996**, *49*, 575.
11. (a) Nagarajan, R.; Berry, D. M.; Schabel, A. A. *J. Antibiot.* **1989**, *42*, 1438; (b) Wanner, J.; Tang, D.; McComas, C. C.; Crowley, B. M.; Jiang, W.; Moss, J.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1169.
12. Arhin, F. F.; Sarmiento, I.; Belley, A.; McKay, G. A.; Draghi, D. C.; Grover, P.; Sahm, D. F.; Parr, T. R., Jr.; Moeck, G. *Antimicrob. Agents Chemother.* **2008**, *52*, 1597.
13. *Representative experiment for the reductive alkylation*: Compound **2** (500 mg, 0.3 mmol, 1.0 equiv) and 4-bromobenzaldehyde (72 mg, 0.4 mmol, 1.3 equiv) was dissolved in DMF/MeOH (1:1, 10 mL). The reaction mixture was stirred for 2 h in 65 °C, then cooled to room temperature and $NaBH_3CN$ (38 mg, 0.6 mmol, 2.0 equiv) was added. The reaction mixture was stirred for additional 2 h at room temperature. Methanol was removed in vacuo, and then acetone was added. The precipitate was filtered, washed by acetone and dried in vacuo to give the crude product. HPLC purification [Waters 2489, column: Sepax BR-C18 21.2 × 100 mm, gradient eluant: CH_3OH–water 5–30% (0.1% HCOOH)] provided the desired fractions. The eluent was concentrated to a volume of 20 mL and neutralized with saturated sodium bicarbonate to remove the formic acid, and then was extracted with n-butanol (20 mL × 3). The organic layer was separated and washed with water, then evaporated in vacuo to dryness. The solid was collected by filtration and washed with acetone and dried in vacuo to give pure compound **10** (110 mg, 20.9%) as an off white solid.