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Design, Synthesis, and Antibacterial Activity of Demethylvancomycin Analogues against Drug-Resistant Bacteria

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Five novel N-substituted demethylvancomycin derivatives were rationally designed and synthesized by using a structure-based approach. The in vitro antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA), gentamicin-resistant *Enterococcus faecalis* (GRE), methicillin-resistant *Streptococcus pneumoniae* (MRS), and vancomycin-resistant *Enterococcus faecalis* (VRE) were evaluated. One of the compounds, *N*-(6-phenylheptyl)demethylvancomycin (**12a**), was found to exhibit more potent antibacterial activity than vancomycin and demethylvancomycin. Compound **12a** was also found to be ~18-fold more efficacious than vancomycin against MRSA; however, the two compounds were found to have similar efficacy against MRS. Furthermore, compound **12a** exhibited a favorable pharmacokinetic profile with a half-life of 5.11 ± 0.52 h, which is longer than that of vancomycin (4.3 ± 1.9 h). These results suggest that **12a** is a promising antibacterial drug candidate for further preclinical evaluation.

Introduction

Antimicrobial resistance is an emerging threat to global health. Infections caused by drug-resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcus faecalis* (VRE), are the most serious.[1,2] There is therefore an urgent demand for new antibiotics against drug-resistant bacteria.

The glycopeptide antibiotic vancomycin (Figure 1) has a unique history.[3] Regarded by some as the agent of last resort for refractory Gram-positive bacterial infections; this first and only commercially available glycopeptide antibiotic has become the drug of choice for empiric therapy in much of the world and is more widely used as a generic medication.[4] Its antibacterial activity appears to result from the inhibition of enzymatic peptidoglycan transglycosylation and/or transpeptidation reactions by binding to D-Ala-D-Ala, the terminus of bacterial cell wall precursor peptides.[5] However, in the case of VRE, the terminus of the precursor peptides is D-Ala-D-Lac instead; the affinity of vancomycin for this terminus is only about 1/1000 of that for the normal terminus (D-Ala-D-Ala), and the resulting antibacterial activity of vancomycin is also much weaker toward VRE than toward more sensitive bacteria.[2,6] According to the proposed resistance mechanisms, strategies for targeting drug-resistant bacteria could include: 1) screening for compounds with new chemical structures and with novel mechanism(s) of action, 2) modifying the structures of existing antibiotics and antibacterial drugs, which could be aided by studying mechanism(s) of action and structure–activity relationship (SAR) data for the existing drugs, or 3) finding a substance that can boost the host defense mechanism and/or attenuate the microbial pathogenicity.

Recent studies have shown that introducing a hydrophobic side chain on the nitrogen of the amino sugar moiety in glycopeptide antibiotics was able to confer potent activity against VRE while maintaining potency against MRSA. Telavancin, oritavancin, and dalbavancin are successful examples of this.[7,8] Among these, telavancin was approved by FDA in 2009 for the treatment of complicated skin and skin structure infections (cSSSIs) caused by Gram-positive bacteria, including MRSA. Both dalbavancin and oritavancin are in the late stages of clinical trials for the treatment of cSSSIs. Telavancin, with a long decylaminoethyl group as the side chain, has a strong tendency to form dimers and become anchored to the membrane. As a result, telavancin not only binds tightly to the D-Ala-D-Ala terminus of the peptidoglycan in vancomycin-sensitive bacterial strains but is also able to bind to the D-Ala-D-Lac terminus of the peptidoglycan in vancomycin-resistant bacterial strains.[9–11] On the other hand, oritavancin, with an *N*-chlorobiphenyl group as the side chain, has been proposed to have two cell wall binding sites: the well-known peptidoglycan D-Ala-D-Ala terminus and the pentaglycyl bridging segment.[12,13]

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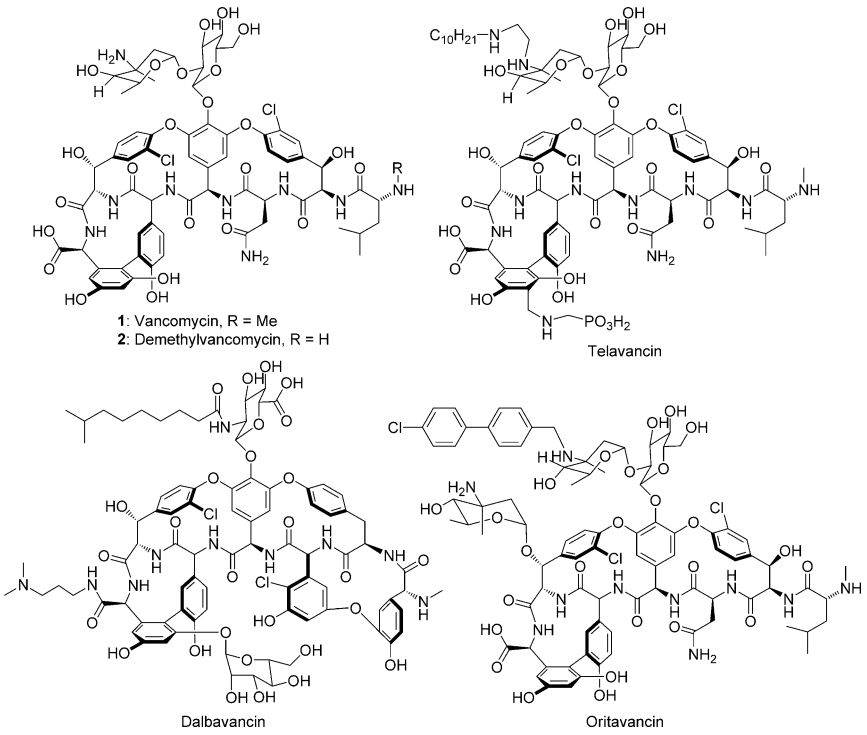


Figure 1. Chemical structures of the antibacterial glycopeptides.

We recently reported the synthesis of seventeen novel N-substituted (*N*-arylmethylene or -aliphatic substituents) demethylvancomycin derivatives and their antibacterial activities against *Clostridium difficile*.[14] It was found that several compounds with *N*-arylmethylene substituents, structurally similar to oritavancin, showed more potent antibacterial activity against *C. difficile* than vancomycin (**1**) or demethylvancomycin (**2**). More interestingly, preliminary data showed that one analogue (i.e., *N*-undecyl-demethylvancomycin) exhibited potent antibacterial activity against vancomycin-resistant *Enterococcus faecium* (ATCC 700802). This observation suggested that further modifications to this analogue, with a long N-alkyl group, could potentially provide promising drug candidates against drug-resistant bacteria.

As a part of our ongoing work to extend the SAR study of vancomycin and demethylvancomycin, and to elucidate the corresponding mechanisms of action, we decided to prepare a set of analogues with long alkyl side chain substitutions at the nitrogen of the amino sugar moiety of demethylvancomycin. Thermodynamically, orienting the long flexible alkyl chains might lead to a significant entropic penalty, due to the loss of both rotational and translational degrees of freedom. However, if optimal hydrophobic or aromatic fragments are introduced to gain favorable binding enthalpy, the overall binding strength can be improved by enthalpy–entropy compensation. Considering the biphenyl substituted structure of oritavancin, the reported binding mode of glycopeptide with peptidoglycan,[12] and the crystal structure of vancomycin,[15] it was reasoned that introducing an aromatic ring at the end of an aliphatic side chain may enhance the binding affinity of the resulting glycopeptide with the peptidoglycan amino acid residues and bestow structural features for activity against the drug-resistant bacteria. Thus, we designed and synthesized a series of *N*-arylalkyl demethylvancomycin derivatives. These new compounds (**12a**–**e**), along with our previously reported analogues (**12f**–**v**), were evaluated in the current study for biological activity against the drug-resistant bacteria.

Results and Discussion

Design

The X-ray crystal structure of vancomycin bound to the peptidoglycan of VRE provided the starting point for the rational design of new analogues. Molecular docking showed a large space between the amino sugar moiety and the peptidoglycan in the original vancomycin–peptidoglycan complex. By retaining multiple hydrogen bonds with D-Lac and L-Lys,[2] for a seven-carbon side chain, the distance between the center of the phenyl group (such as in **12a**) and the peptidoglycan Gly4 would be 3.2 Å, potentially creating a NH–π interaction (Figure 2). Although this NH–π interaction is not as strong as a hydrogen bonding or ionic interaction, it may still make a binding energy contribution and could potentially increase the binding affinity.[16] Therefore, we designed a series of demethylvancomycin derivatives by connecting an aromatic group to the amino sugar moiety of demethylvancomycin through a seven-carbon linker. We hoped that the NH–π

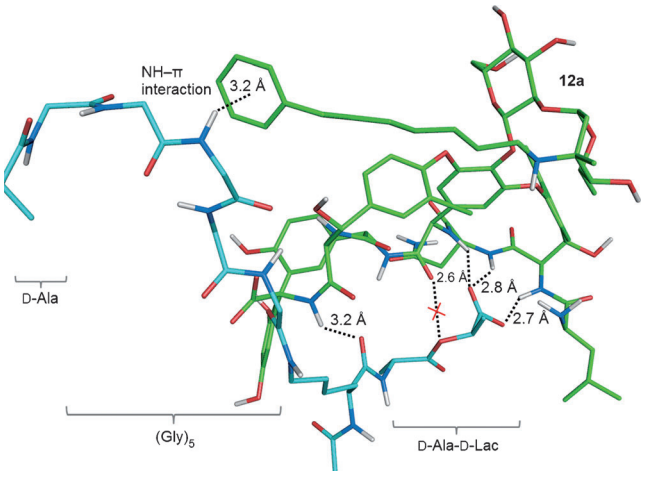
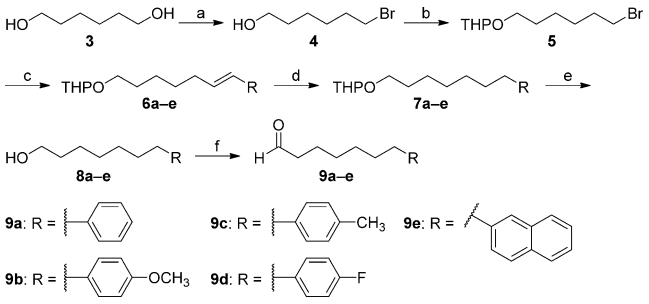


Figure 2. Proposed binding mode of **12a** with the peptidoglycan of VRE. Vancomycin derivative **12a** (green) and peptidoglycan (cyan) are shown as stick models.

interaction could enhance the binding affinity of demethylvancomycin derivatives for the peptidoglycan on the cell wall of VRE.

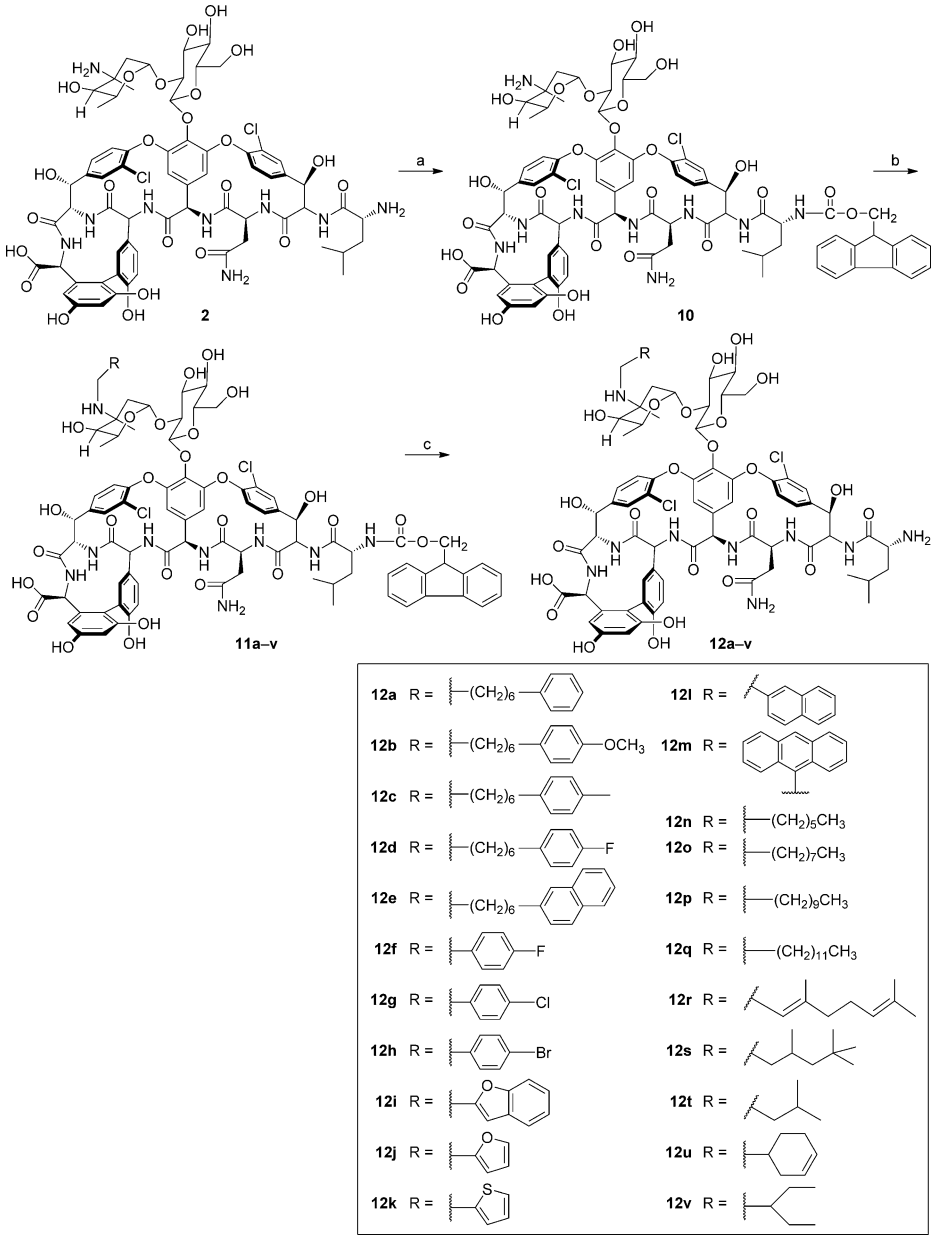
Synthesis

The synthesis of aldehydes **9a–e** started from commercially available 1,6-hexanediol (**3**), as illustrated in Scheme 1. One of the hydroxy groups of **3** was converted into a bromide by reacting with aqueous hydrobromide in toluene to afford 6-bromo-hexanol (**4**) in 98% yield. The remaining hydroxy group of 6-bromo-hexanol (**4**) was then protected with tetrahydropyranyl (THP) in the presence of pyridinium *para*-toluenesulfonate (PPTS) in dichloromethane to furnish **5** in 98% yield. Intermediate **5** was heated with triphenylphosphine at 120 °C under neat conditions to form the corresponding phosphonium salt, which was then reacted with the respective aldehydes via a Wittig reaction (lithium hexamethyldisilazide as a base) to form **6a–e** in 20–30% yield. It should be noted that there was no desired phosphonium salt formation if toluene or ethyl acetate was used as the solvent, even at reflux. The resulting compounds, **6a–e**, were subsequently reduced to **7a–e** in 80–98% yield using Pd/C catalytic hydrogenation at room temperature. After removing the THP group of **7a–e** with *para*-toluenesulfonic acid (*p*-TsOH), **8a–e** were obtained in 85–98% yield. Intermediates **8a–e** were then oxidized with pyridinium chlorochromate and silicon dioxide to afford the corresponding aldehydes **9a–e** in good yields.



Scheme 1. Synthesis of compounds **9a–e**. *Reagents and conditions*: a) HBr (1.2 equiv), toluene, reflux, 72 h; b) 3,4-2*H*-dihydropyran (1.2 equiv), PPTs (0.1 equiv), CH_2Cl_2, RT, 4 h; c) 1. PPh_3 (1 equiv), neat, 120 °C, 1 h, 2. LiHMDS, RCHO, 0 °C–RT, THF; d) H_2, Pd/C, MeOH, RT, 2 h; e) *p*-TsOH (1 equiv), MeOH, RT, 1 h; f) PCC (1 equiv), SiO_2 (1 weight equiv), CH_2Cl_2, RT, 3 h.

Final analogues **12a–e** were synthesized from demethylvancomycin (**2**) with the methods reported previously for the synthesis of derivatives **12f–v** (Scheme 2).[14] First, the N-terminal free amino group of **2** was protected with a 9-fluorenylmethoxycarbonyl (Fmoc) group in dioxane/H_2O (1:1) to afford **10** in 85% yield.[17] Then, compound **10** was reacted with aldehydes **9a–e** by reductive amination, using sodium cyanoborohydride as the reducing agent,



Scheme 2. Synthesis of compounds **12a–v**. *Reagents and conditions*: a) DIEA (2 equiv), Fmoc-Cl (1.1 equiv) in dioxane/H_2O (1:1 *v/v*), RT, 2.5 h; b) 1. aldehyde (5 equiv), DIEA (2 equiv)/DMF, RT, 1 h, 2. $NaCNBH_3$ (3 equiv), TFA (3 equiv) in MeOH, RT, 8–48 h; c) piperidine (15 equiv) in DMF, RT, 15 min.

Table 1. In vitro antibacterial activity of compounds **12a–v** against methicillin-resistant *Staphylococcus aureus* (MRSA) and gentamicin-resistant *Enterococcus* (GRE) strains.

	MIC [μg mL^{-1}][a]									
Compd	11001[b]	11002[b]	11004[b]	11005[b]	11006[b]	11031[c]	11032[c]	11033[c]	11034[c]	11035[c]
1	3.13	1.56	3.13	3.13	1.56	>50	>50	>50	3.13	3.13
2	0.78	1.56	3.13	3.13	1.56	>50	>50	>50	3.13	1.56
12a	1.56	0.78	0.78	0.78	0.78	12.5	12.5	12.5	0.78	0.78
12b	1.56	3.13	1.56	1.56	1.56	50	50	>50	1.56	1.56
12c	0.78	0.78	1.56	1.56	0.78	25	25	50	1.56	1.56
12d	1.56	1.56	1.56	1.56	0.78	50	50	50	3.13	0.78
12e	1.56	3.13	3.13	3.13	1.56	25	25	50	0.78	0.78
12f	3.13	3.13	3.13	3.13	3.13	>50	>50	>50	3.13	3.13
12g	3.13	3.13	3.13	3.13	3.13	>50	>50	>50	3.13	3.13
12h	1.56	3.13	1.56	1.56	1.56	>50	>50	>50	1.56	1.56
12i	3.13	6.25	6.25	3.13	3.13	>50	>50	>50	3.13	6.25
12j	6.25	6.25	12.5	12.5	6.25	>50	>50	>50	6.25	6.25
12k	3.13	3.13	3.13	6.25	3.13	>50	>50	>50	3.13	3.13
12l	3.13	3.13	3.13	6.25	3.13	>50	>50	>50	3.13	3.13
12m	3.13	6.25	6.25	6.25	3.13	50	50	50	3.13	1.56
12n	1.56	3.13	1.56	3.13	0.78	>50	50	>50	3.13	3.13
12o	6.25	6.25	6.25	25	6.25	50	>50	>50	6.25	6.25
12p	1.56	0.78	3.13	1.56	1.56	12.5	12.5	12.5	0.78	0.78
12q	3.13	3.13	3.13	3.13	3.13	25	25	25	3.13	3.13
12r	1.56	1.56	6.25	6.25	1.56	50	50	50	3.13	3.13
12s	3.13	3.13	3.13	3.13	3.13	>50	>50	>50	3.13	3.13
12t	3.13	3.13	6.25	6.25	3.13	>50	>50	>50	6.25	3.13
12u	3.13	3.13	3.13	3.13	3.13	>50	>50	>50	6.25	3.13
12v	12.5	12.5	12.5	25	12.5	>50	>50	>50	12.5	12.5

[a] Minimum inhibitory concentration; data are the mean of results from at least three independent experiments. [b] MRSA strain. [c] GRE strain.

to afford the corresponding N-alkylated Fmoc-demethylvancomycin derivatives **11a–e** in 80–90% yield.[18] Finally, after removing the Fmoc group on **11a–e** with piperidine in DMF at room temperature, compounds **12a–e** were obtained in good yields after purification by preparative high pressure liquid chromatography (HPLC). Their chemical structures were unambiguously confirmed by spectroscopic analyses, including ^{1}H NMR and MS (ESI-MS and HRESI-MS).

In vitro antibacterial activity

Along with vancomycin (**1**) and demethylvancomycin (**2**), new analogues **12a–e** and those we reported previously (i.e., **12f–v**) were evaluated in the current study for in vitro antibacterial activity against Gram-positive bacteria, including MRSA, GRE, MRS, and VRE. Minimum inhibitory concentration (MIC) values were determined using the agar twofold dilution method according to CLSI (Tables 1 and 2).

The assay results showed that **12a–e** exhibited very good antibacterial activities against both MRSA and GRE (Table 1). In fact, they were found to be much more potent than all other analogues tested in the current study except **12p**, suggesting that a substituted or unsubstituted phenyl group at the end of a seven-carbon linker is able to confer a stronger antibacterial effect than N-arylmethylene and N-aliphatic substitutions. This same activity profile was also observed when the analogues were tested against both MRS and VRE (Table 2), indicating that the linker length of a N-substituent is a pivotal determinant for antibacterial potency and is also consistent with our design rationale based on molecular docking (vide supra). It should also be noted that, among all the analogues tested, compound **12a** exhibited the most promising results in terms of antibacterial activity against all seventeen Gram-positive bacterial strains, and was two- to fourfold more potent than **1** and **2**. Interestingly, for analogues **12b–e**, the antibacterial activity decreased with aryl group substitutions. Thus, **12a** was chosen for further in vivo evaluation.

In vivo antibacterial activity

Compound **12a** was evaluated for in vivo antibacterial efficacy in a scalded rat model by smearing the burn wound once a day (Table 3). The results showed that **12a** significantly inhibited MRSA (26003) growth when compared with the scald MRSA control group at three different doses. Even at 20% of the dose, compound **12a** (80 μg mL^{-1}) still had a similar antibacterial efficacy to vancomycin (400 μg mL^{-1}).

The in vivo antibacterial activities of **12a** against MRSA (11001) and MRS (11061) in the mouse systemic infection model were shown in Table 4. Compound **12a** was found to be ~18-fold more efficacious than **1** (ED_{50}: 0.58 mg kg^{-1} versus 10.49 mg kg^{-1}) against MRSA; however, **12a** exhibited a similar efficacy (ED_{50}: 1.36 mg kg^{-1}) to **1** against MRS.

Pharmacokinetic profile of 12a

The highly potent compound **12a** was subjected to pharmacokinetic (PK) performance assessment in SD rats (Table 5). Com-

Table 2. In vitro antibacterial activity of compounds **12a**–**v** against methicillin-resistant *Streptococcus pneumoniae* (MRS) and vancomycin-resistant *Enterococcus faecalis* (VRE) strains.

Compd	MIC [μg mL^{-1}][a]						
	11061[b]	11062[b]	12031[c]	12032[c]	12033[c]	12034[c]	12035[c]
1	1.56	1.56	>50	>50	>50	>50	>50
2	1.56	1.56	50	50	>50	>50	50
12a	0.78	0.78	12.5	12.5	12.5	50	12.5
12b	1.56	0.78	50	>50	>50	50	50
12c	0.78	0.78	25	>50	50	25	25
12d	0.78	0.78	25	>50	>50	50	50
12e	0.195	0.78	25	50	50	50	50
12f	0.78	3.13	>50	>50	>50	>50	>50
12g	1.56	3.13	>50	>50	>50	>50	>50
12h	0.78	1.56	>50	>50	>50	>50	>50
12i	1.56	6.25	>50	>50	>50	>50	>50
12j	3.13	12.5	>50	>50	>50	>50	>50
12k	1.56	6.25	>50	>50	>50	>50	>50
12l	1.56	3.13	>50	>50	>50	>50	>50
12m	1.56	6.25	50	50	>50	>50	50
12n	0.78	3.13	>50	>50	>50	>50	>50
12o	6.25	12.5	50	>50	>50	>50	>50
12p	0.195	3.13	6.25	6.25	25	25	12.5
12q	3.13	3.13	25	25	>50	>50	25
12r	1.56	1.56	50	50	>50	>50	50
12s	3.13	6.25	50	50	>50	>50	>50
12t	1.56	6.25	>50	>50	>50	>50	>50
12u	6.25	6.25	>50	>50	>50	>50	>50
12v	6.25	25	>50	>50	>50	>50	>50

[a] Minimum inhibitory concentration; data are the mean of results from at least three independent experiments. [b] MRS strain. [c] VRE strain.

Table 3. *Staphylococcus aureus* counts under scab tissue.

Compd	Dose [μg mL^{-1}]	*n*	Log ± SD[a]			
			Day 3	Day 7	Day 10	Day 14
12a	80	12	5.31 ± 0.76	6.13 ± 0.71*	5.95 ± 0.46**	6.10 ± 0.97***
12a	40	12	5.291 ± 1.20	5.87 ± 0.38**	5.94 ± 0.96*	6.60 ± 0.50***
12a	20	12	6.05 ± 0.45*	6.77 ± 0.29*	7.63 ± 1.22	7.90 ± 0.39**
1	400	12	5.72 ± 0.46*	5.62 ± 0.96*	5.29 ± 2.11*	6.43 ± 0.91**
Scald group		12	4.19 ± 0.80	6.21 ± 0.10**	8.46 ± 1.74	8.66 ± 1.12
Scald MRSA group		12	4.87 ± 0.37	8.59 ± 0.95	9.53 ± 1.25	10.08 ± 0.61

[a] Significance compared with scald MRSA group: *$p<0.05$, **$p<0.01$, ***$p<0.001$ for n = number of animals in the group.

Table 4. In vivo antibacterial activity of compound **12a** in systemic infection mouse models.

Compd	ED_{50} [mg kg^{-1}][a]	
	MRSA (11001)	MRS (11061)
12a	0.58	1.36
1	10.49	1.56

[a] Systemic infection with methicillin-resistant *S. aureus* (MRSA) strain 11001 and methicillin-resistant *S. pneumoniae* (MRS) strain 11061 (see Experimental Section for details).

pound **12a** exhibited a favorable PK profile with a half-life of 5.11 ± 0.52 h, longer than that of vancomycin (4.3 ± 1.9 h).[19] The $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of **12a** were 971.24 ± 41.63 mg h L^{-1} and 1211.46 ± 27.16 mg h L^{-1}, respectively. The clearance was 0.07 L h^{-1} kg^{-1}, and the V_z was 0.49 ± 0.05 L kg^{-1}, which is also indicative of good PK performance.

Acute toxicity studies

The acute toxicity investigation of **12a** showed a regular dose-dependent increase in mortality in both sexes of mice after i.v. administration. The LD_{50} value for this compound was calculated to be 43.3 mg kg^{-1} (95% confidence limit: 41.139–45.859 mg kg^{-1}).

Pre-test study of long-term toxicity in rats

A two week preliminary toxicity study for **12a** (i.v. 10 mg kg^{-1}, once per day) showed no apparent toxicity due to drug-induced death. There were no abnormal changes in weight and food intake ($p>0.05$; Table 6). The percentage of neutrophils among leukocytes in the **12a**-treated male rats was decreased, and the percentage of monocytes and eosinophils was increased, especially the percentage of reticulocytes ($p<0.05$), whereas for the female rats, only reticulocytes were significantly increased ($p<0.01$; Table 7). There were no abnormal changes found in the liver, kidney function indicators, or urine.

Conclusions

In summary, a structure-based approach was taken in the current study to design five new N-substituted demethylvancomycin derivatives (**12a**–**e**) with an aromatic ring appended to a seven-carbon linker on the nitrogen of the amino sugar moiety. When evaluated for in vitro antibacterial activities against MRSA, GRE, MRS, and VRE, compound **12a** was found to show the most potent antibacterial activity among all compounds tested. When evaluated for in vivo antibacterial activity, compound **12a** was found to be ~18-fold more efficacious than vancomycin (**1**) against MRSA; however, the two compounds were found to exhibit similar efficacy against MRS.

Table 5. Pharmacokinetic parameters of compound **12a** determined in SD rats following i.v. compound administration.[a]

	$AUC_{(0-t)}$ [mg L^{-1} h^{-1}]	$AUC_{(0-\infty)}$ [mg L^{-1} h^{-1}]	$MRT_{(0-\infty)}$ [h]	$t_{1/2}$ [h]	t_{max} [h]	CL [L h^{-1} kg^{-1}]	V_z [L kg^{-1}]	C_{max} [mg L^{-1}]
Rat 1	923.96	1190.86	6.42	5.39	0.01	0.07	0.52	1283.68
Rat 2	1002.42	1201.28	5.05	4.51	0.01	0.07	0.43	1462.38
Rat 3	987.33	1242.23	5.96	5.43	0.01	0.06	0.50	1029.15
Mean	971.24	1211.46	5.81	5.11	0.01	0.07	0.49	1258.40
SD	41.63	27.16	0.70	0.52	0.00	0.00	0.05	217.72

[a] AUC = area under concentration–time curve; MRT = mean residence time.

Table 6. Changes in body weight of rats in a pre-test study of long-term toxicity.

Compd	Sex	*n*	Weight [g][a]					
			Day 0	Day 4	Day 8	Day 10	Day 12	Day 14
12a	F	10	163.0 ± 4.9	179.1 ± 4.4	190.1 ± 10.3	195.7 ± 12.3	204.3 ± 10.6	209.0 ± 13.7
	M	10	165.0 ± 4.4	191.6 ± 5.3	214.7 ± 5.5	234.6 ± 7.6	249.1 ± 8.9	247.1 ± 8.5
Control	F	10	164.5 ± 4.9	180.1 ± 4.7	193.3 ± 7.8	200.7 ± 9.1	209.1 ± 12.2	212.0 ± 12.7
	M	10	166.5 ± 4.0	194.4 ± 6.2	221.8 ± 11.2	238.8 ± 14.3	253.4 ± 14.1	261.7 ± 16.5

[a] Data represent the mean ± SD; *n* = number of animals per group.

Table 7. Blood test data for rats in a pre-test study of long-term toxicity.

	Sex	*n*	Cell type [%][a]					
			Reticulocyte	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophils
12a	F	10	20.7 ± 6.2**	19.67 ± 4.12	74.39 ± 4.46	4.99 ± 0.92	0.93 ± 0.39	0.06 ± 0.06
	M	10	23.8 ± 6.7*	14.56 ± 2.73**	78.72 ± 2.27	6.01 ± 1.40**	0.64 ± 0.30**	0.03 ± 0.03
Control	F	10	9.0 ± 3.7	22.79 ± 5.61	71.80 ± 4.90	4.58 ± 1.12	0.78 ± 0.36	0.06 ± 0.06
	M	10	17.3 ± 6.8	18.61 ± 2.86	77.28 ± 2.76	3.71 ± 0.86	0.36 ± 0.19	0.03 ± 0.03

[a] Data represent the mean ± SD; *n* = number of animals per group; significance compared with control group: *$p < 0.05$, **$p < 0.01$.

Compound **12a** also exhibited a favorable pharmacokinetic profile with a half-life of 5.11 ± 0.52 h, longer than that of vancomycin (4.3 ± 1.9 h). These findings strongly suggest that **12a** would be a promising antibacterial drug candidate for further preclinical evaluation.

Experimental Section

Chemistry

General: Reagents were purchased from Sigma–Aldrich and TCI Chemical companies. All solvents were purified and dried in accordance with standard procedures, unless otherwise indicated. Oxygen- and water-free operations were carried out under argon atmosphere in dried glassware unless otherwise noted. Reactions were monitored by TLC using Yantai (China) GF_{254} silica gel plates (5 × 10 cm). Silica gel column chromatography was performed on silica gel (300–400 mesh) from Yantai (China). ^{1}H NMR spectra were recorded on Bruker DRX-400 (400 MHz) and Bruker DRX-600 (600 MHz), and chemical shifts (δ) were recorded in ppm with coupling constants (*J*) in hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). ^{13}C NMR spectra data were recorded on Bruker DRX-400 (100 MHz) and Bruker DRX-600 (150 MHz) spectrometer at room temperature. A Shimadzu LCMS-2010EV was used for low-resolution mass spectra (ESI) and an IonSpec 4.7 Tesla FTMS (MALDI) or Bruker Daltonics APEXIII7.0 TESLA FMS (ESI) for high-resolution mass spectra.

6-Bromohexanol (4): 1,6-Hexanediol (**3**, 12.00 g, 66.27 mmol) was dissolved in toluene (100 mL), and the solution was heated at 120 °C before the dropwise addition of 48% hydrobromide (15 mL). After the addition was complete, the reaction mixture was stirred under reflux for 72 h. After removing the solvent under reduced pressure, the residue was purified by silica gel column chromatography (EtOAc/petroleum ether (PE), 1:4) to afford **4** as a light-yellow oil (11.76 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.64$ (t, $J = 6.9$ Hz, 2H), 3.41 (t, $J = 6.9$ Hz, 2H), 1.87 (m, 2H), 1.58 (m, 2H), 1.48 (m, 2H), 1.38 ppm (m, 2H); MS-ESI: *m/z* 181 $[M+H]^+$.

2-(6-Bromohexyloxy)tetrahydropyran (5): Compound **4** (13.5 g, 74.60 mmol), pyridinium *para*-toluenesulfonate (PPTs, 1.87 g, 7.46 mmol), and 3,4-dihydro-2*H*-pyran (10 mL, 111.84 mmol) were dissolved in CH_2Cl_2 (100 mL). After the reaction mixture was stirred at room temperature for 4 h, it was concentrated in vacuo. The obtained residue was purified by silica gel column chromatography (EtOAc/PE, 1:100) to give **5** as a light-yellow oil (18.36 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.56$ (d, $J = 4.1$ Hz, 1H), 3.85 (t, $J = 9.5$ Hz, 1H), 3.72 (dd, $J = 9.5$, 6.8 Hz, 1H), 3.49 (m, 1H), 3.38 (m, 3H), 1.84 (m, 3H), 1.70 (t, $J = 10.6$ Hz, 1H), 1.57 (m, 4H), 1.42 ppm (m, 4H); MS-ESI: *m/z* 265 $[M+H]^+$.

General procedure for the preparation of 9a–e: Compound **5** (1.0 g, 4.0 mmol) and triphenylphosphine (1.0 g, 4.0 mmol) were added to a dry three-necked flask (50 mL). After the mixture was melted at 120 °C and stirred for 1 h at this temperature, it was washed with hot toluene twice and dried to give a pale yellow phosphonium salt, to which was then added anhydrous THF (10 mL). The resulting cloudy solution was then stirred at 0 °C, then a solution of LiHMDS in THF (4.81 mL, 1 M) was added dropwise. After the addition was complete, the reaction mixture was further stirred at room temperature for 1 h, at which time the reaction mixture became clear. At this point, the corresponding aldehyde (2.67 mmol) was added at 0 °C, and after the addition was complete, the reaction mixture was allowed to warm to room temperature and was stirred for 1 h before quenching with H_2O. The reaction mixture was extracted with EtOAc twice, and the combined

organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The desired product (i.e., **6a–e**) was isolated from the resulting residue using silica gel column chromatography (CH_2Cl_2/PE, 1:5 to 1:4) to give colorless oil in 20–30% yields.

A solution of **6a–e** in MeOH (10 mL) and 10% Pd/C (100 mg) were added to a dried round-bottomed flask (25 mL). The reaction mixture was stirred for 2 h at room temperature under hydrogen. After filtering with a small amount of silica gel and washing with CH_2Cl_2, the solvent in the filtrate was removed in vacuo, and **7a–e** were obtained as colorless oils in 80–98% yields.

Compounds **7a–e** were dissolved in 10 mL MeOH (10 mL), and *p*-TsOH·H_2O (1 equiv) was added. After the reaction mixture was stirred at room temperature for 1 h, the solvent was removed in vacuo. Compounds **8a–e** were isolated from the resulting residues using silica gel column chromatography (CH_2Cl_2/MeOH, 100: 1) as colorless oils in 85–98% yields.

Pyridinium chlorochromate (1 equiv) and silica gel were added to a dry two-necked flask (25 mL). After the addition of a solution of **8a–e** in CH_2Cl_2 (2 mL), the reaction mixture was stirred at room temperature for 4 h before it was filtered through a small amount of silica gel and washed with CH_2Cl_2. The filtrate was evaporated in vacuo to give **9a–e** as colorless liquids (yields 85–98%), which were used directly in the next reaction.

7-Phenylheptanal (9a): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.68 (t, J = 2.2 Hz, 1H), 7.14 (m, 5H), 2.52 (t, J = 7.9 Hz, 2H), 2.33 (dt, J = 8.0, 2.2 Hz, 2H), 1.54 (m, 4H), 1.28 ppm (m, 4H); MS-ESI: *m/z* 191 $[M+H]^+$.

7-(*p*-Methoxyphenyl)heptanal (9b): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.67 (t, J = 2.2 Hz, 1H), 7.08 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 3.78 (s, 3H), 2.54 (m, 2H), 2.32 (m, 2H), 1.54 (m, 4H), 1.26 ppm (m, 4H); MS-ESI: *m/z* 221 $[M+H]^+$.

7-(*p*-Methylphenyl)heptanal (9c): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.67 (t, J = 2.2 Hz, 1H), 7.08 (d, J = 6.6 Hz, 2H), 6.64 (d, J = 6.6 Hz, 2H), 2.57 (m, 2H), 2.32 (m, 5H), 1.52 (m, 4H), 1.28 ppm (m, 4H); MS-ESI: *m/z* 205 $[M+H]^+$.

7-(*p*-Fluorophenyl)heptanal (9d): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.67 (t, J = 2.2 Hz, 1H), 7.11 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 2.52 (t, J = 6.6 Hz, 2H), 2.33 (m, 2H), 1.49 (m, 4H), 1.28 ppm (m, 4H); MS-ESI: *m/z* 209 $[M+H]^+$.

7-(2-Naphthyl)heptanal (9e): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.67 (t, J = 2.2 Hz, 1H), 7.78 (m, 3H), 7.61 (s, 1H), 7.43 (m, 2H), 7.33 (m, 1H), 2.78 (m, 2H), 2.33 (m, 2H), 1.71 (m, 2H), 1.57 (m, 2H), 1.38 ppm (m, 4H); MS-ESI: *m/z* 241 $[M+H]^+$.

General procedure for the preparation of 12a–e: Compound **2** (2.0 g, 1.35 mmol) and 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl; 384 mg, 1.49 mmol) were dissolved in dioxane/H_2O (1:1, 20 mL), then DIEA (0.45 mL, 2.70 mmol) was added. The resulting mixture was stirred at room temperature for 2 h before the addition of EtOAc. The resulting precipitate was filtered, washed with EtOAc, and dried in vacuo to give compound **10** in 85% yield. After dissolving compound **10** (99 mg, 0.06 mmol) in DMF (2 mL), DIEA (0.019 mL, 0.114 mmol) and aldehyde **9a–e** (4 equiv) were added. The mixture was stirred at room temperature for 1 h before the addition of $NaBH_3CN$ (10 mg, 0.17 mmol) and TFA (0.012 mL, 0.17 mmol). The reaction mixture was further stirred for 4 days before the addition of anhydrous ether (5 mL). The resulting precipitate was filtered, washed with EtOAc, and dried in vacuo to give compounds **11a–e** in 80–90% yields. Compound **11a–e** (0.05 mmol) was dissolved in DMF (2 mL), and piperidine (0.079 mL, 0.81 mmol) was added. After the reaction mixture was stirred for 15 min at room temperature, anhydrous ether (5 mL) was added. The resulting precipitate was filtered, washed with EtOAc, and dried in vacuo to give crude product. Further HPLC purification (gradient eluent: CH_3CN/H_2O, 5–70% in 0.1% TFA) provided the desired fractions. The combined fractions were concentrated to a volume of 20 mL, neutralized with saturated sodium bicarbonate, and extracted with *n*BuOH (3×20 mL). The organic layers were separated, washed with H_2O, and evaporated in vacuo to dryness to give pure compounds **12a–e** as off white solids.

***N*-(6-Phenylheptyl)demethylvancomycin (12a):** 23.6%; ^{1}H NMR (600 MHz, [D_6]DMSO): δ = 7.82 (s, 1H), 7.56 (m, 1H), 7.49 (s, 1H), 7.42 (m, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.12 (s, 1H), 6.74 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 6.37 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 2.2 Hz, 1H), 5.73 (s, 1H), 5.63 (s, 1H), 5.32 (d, J = 7.8 Hz, 1H), 5.27 (m, 1H), 5.11 (s, 1H), 5.08 (s, 2H), 4.79 (m, 1H), 4.57 (d, J = 6.4 Hz, 1H), 4.41 (m, 2H), 4.15 (s, 1H), 4.09 (m, 1H), 4.02 (m, 1H), 3.65 (d, J = 9.8 Hz, 1H), 3.55 (d, J = 9.8 Hz, 1H), 3.00–3.50 (5H), 2.65 (m, 2H), 2.58 (m, 1H), 2.51 (m, 1H), 1.80 (d, J = 13.0 Hz, 1H), 1.66 (d, J = 13.0, 1H), 1.58 (m, 1H), 1.53 (m, 1H), 1.45 (m, 1H), 1.34 (m, 4H), 1.29 (m, 3H), 1.06 (d, J = 6.4 Hz, 3H), 0.87 (m, 6H), 0.80 ppm (m, 6H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 172.9, 170.7, 169.5, 168.2, 158.7, 158.5, 157.6, 156.9, 155.4, 152.9, 151.6, 150.3, 148.7, 142.9, 142.6, 136.5, 136.1, 135.4, 132.2, 129.0, 128.7, 128.6, 127.9, 127.5, 126.9, 126.6, 126.1, 125.9, 124.7, 123.8, 121.9, 118.5, 116.6, 108.2, 106.1, 105.2, 102.8, 101.3, 96.9, 78.3, 77.4, 77.3, 72.1, 71.9, 70.6, 69.0, 63.6, 62.3, 61.6, 59.4, 59.1, 57.1, 55.5, 54.1, 51.8, 51.2, 49.0, 39.0, 35.5, 33.1, 31.2, 28.8, 28.8, 26.5, 23.9, 23.2, 22.3, 19.4, 17.3 ppm; MS-ESI: *m/z* 804.8 $[M+2H]^{2+}$; HRMS-ESI: *m/z* $[M+2H]^{2+}$ calcd for $C_{78}H_{91}Cl_2N_9O_{24}$: 804.7850, found: 804.7862.

***N*-(6-(*p*-Methoxyphenyl)heptyl)demethylvancomycin (12b):** 19.3%; ^{1}H NMR (600 MHz, [D_6]DMSO): δ = 7.85 (s, 1H), 7.61 (m, 1H), 7.49 (m, 1H), 7.45 (m, 1H), 7.31 (m, 1H), 7.20 (m, 1H), 7.16 (m, 1H), 7.08 (d, J = 8.6 Hz, 2H), 6.83 (m, 2H), 6.77 (m, 1H), 6.71 (m, 1H), 6.40 (d, J = 2.1 Hz, 1H), 6.26 (d, J = 2.1 Hz, 1H), 5.76 (m, 1H), 5.64 (m, 1H), 5.34 (s, 1H), 5.28 (m, 1H), 5.15 (m, 1H), 5.11 (m, 2H), 4.80 (m, 1H), 4.61 (m, 1H), 4.45 (m, 1H), 4.42 (m, 1H), 4.18 (m, 1H), 4.10 (m, 2H), 3.71 (s, 3H), 3.68 (m, 1H), 3.55 (m, 1H), 3.51 (m, 1H), 3.45 (m, 1H), 3.00–3.50 (4H), 2.77 (m, 1H), 2.68 (m, 1H), 2.40–2.60 (2H), 1.98 (m, 1H), 1.80 (m, 1H), 1.70 (m, 1H), 1.62 (m, 1H), 1.52 (m, 4H), 1.35 (s, 3H), 1.27 (m, 3H), 1.25 (m, 3H), 1.08 (d, J = 6.3 Hz, 3H), 0.91 ppm (m, 6H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 172.9, 170.7, 169.5, 168.2, 158.7, 158.5, 157.6, 156.9, 155.4, 152.9, 151.6, 150.3, 148.7, 142.9, 136.5, 136.1, 135.4, 134.5, 132.2, 129.6, 129.0, 127.9, 127.5, 126.9, 126.6, 125.9, 124.7, 123.8, 121.9, 118.5, 116.6, 114.1, 108.2, 106.1, 105.2, 102.8, 101.3, 96.9, 78.3, 77.4, 77.3, 72.1, 71.9, 70.6, 69.0, 63.6, 62.3, 61.6, 59.4, 59.1, 57.1, 55.5, 54.1, 51.8, 51.2, 49.0, 39.0, 35.5, 33.1, 31.2, 28.8, 28.8, 26.5, 23.9, 23.2, 22.3, 19.4, 17.3 ppm; MS-ESI: *m/z* 819.8 $[M+2H]^{2+}$; HRMS-ESI: *m/z* $[M+2H]^{2+}$ calcd for $C_{79}H_{93}Cl_2N_9O_{25}$: 819.7903, found: 819.7866.

***N*-(6-(*p*-Methylphenyl)heptyl)demethylvancomycin (12c):** 22.3%; ^{1}H NMR (600 MHz, [D_6]DMSO): δ = 7.85 (s, 1H), 7.60 (m, 1H), 7.51 (s, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.16 (s, 1H), 7.06 (q, J = 8.2 Hz, 3H), 6.77 (m, 1H), 6.71 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.5 Hz, 1H), 6.40 (d, J = 1.9 Hz, 1H), 6.26 (d, J = 1.9 Hz, 1H), 5.77 (m, 1H), 5.66 (s, 1H), 5.32 (m, 1H), 5.29 (s, 1H), 5.15 (s, 1H), 5.11 (s, 2H), 4.82 (m, 1H), 4.62 (d, J = 6.5 Hz, 1H), 4.46 (m, 1H), 4.43 (m, 1H), 4.19 (m, 1H), 4.13 (m, 1H), 4.06 (s, 1H), 3.69 (d, J = 10.2 Hz, 1H), 3.00–3.50 (6H), 2.76 (s, 1H), 2.69 (m, 1H), 2.40–2.60 (2H), 2.25 (s, 3H), 2.13 (m, 1H), 1.98 (m, 1H), 1.79 (d, J = 12.8 Hz, 1H), 1.69 (d, J = 12.8 Hz, 1H), 1.62 (m, 1H), 1.52 (m,

4H), 1.35 (s, 3H), 1.25 (m, 6H), 1.09 (m, 3H), 0.90 ppm (m, 6H); ^{13}C NMR (150 MHz, [D$_6$]DMSO) δ = 173.8, 171.5, 170.4, 169.1, 158.4, 157.8, 156.3, 153.7, 152.4, 151.0, 149.5, 143.8, 140.3, 137.3, 136.1, 135.7, 133.0, 130.0, 129.4, 128.6, 128.4, 127.7, 127.4, 126.7, 125.5, 124.6, 122.8, 119.3, 117.4, 109.0, 106.9, 106.0, 102.1, 97.7, 79.2, 78.3, 78.1, 72.8, 71.4, 69.9, 64.4, 63.1, 62.4, 60.3, 59.9, 57.9, 56.1, 54.9, 52.6, 52.1, 39.0, 35.9, 32.1, 29.6, 27.3, 24.7, 24.1, 23.1, 21.9, 20.3, 18.1 ppm; MS-ESI: *m/z* 811.8 $[M+2H]^{2+}$; HRMS-ESI: *m/z* $[M+2H]^{2+}$ calcd for $C_{79}H_{93}Cl_2N_9O_{24}$: 811.7928, found: 811.7905.

***N*-(6-(*p*-Fluorophenyl)heptyl)demethylvancomycin (12d):** 45%; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 7.85 (s, 1H), 7.59 (s, 1H), 7.51 (m, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.20 (m, 2H), 7.16 (m, 1H), 7.09 (m, 2H), 6.77 (d, *J* = 10.1 Hz, 1H), 6.70 (d, *J* = 10.1 Hz, 2H), 6.40 (d, *J* = 2.1 Hz, 1H), 6.26 (d, *J* = 2.1 Hz, 1H), 5.76 (m, 1H), 5.65 (s, 1H), 5.34 (m, 2H), 5.29 (s, 1H), 5.12 (m, 3H), 4.82 (m, 1H), 4.62 (d, *J* = 6.4 Hz, 1H), 4.44 (m, 2H), 4.19 (m, 1H), 4.01 (m, 1H), 3.68 (s, 1H), 3.54 (m, 2H), 3.46 (m, 1H), 3.00–3.40 (6H), 2.76 (s, 1H), 2.69 (s, 1H), 2.40–2.60 (2H), 1.98 (d, *J* = 13.0 Hz, 1H), 1.79 (d, *J* = 13.0 Hz, 1H), 1.70 (m, 1H), 1.62 (m, 1H), 1.55 (m, 4H), 1.35 (s, 3H), 1.26 (m, 6H), 1.08 (m, 3H), 0.89 ppm (m, 9H); ^{13}C NMR (150 MHz, [D$_6$]DMSO) δ = 173.0, 170.7, 169.6, 168.3, 161.7, 158.4, 158.2, 157.6, 157.0, 155.5, 152.9, 151.6, 150.2, 148.7, 143.0, 138.8, 136.5, 136.1, 132.2, 130.4, 130.3, 129.0, 127.8, 127.6, 126.9, 126.6, 125.9, 124.7, 123.8, 122.0, 118.5, 116.6, 115.4, 115.2, 108.2, 106.1, 105.1, 102.8, 101.3, 96.9, 78.4, 77.5, 77.3, 72.0, 70.6, 69.1, 63.6, 62.3, 61.6, 59.5, 59.1, 57.1, 55.3, 54.1, 51.7, 51.4, 46.1, 39.0, 34.6, 33.2, 31.3, 28.8, 28.8, 26.5, 23.9, 23.3, 22.3, 19.4, 17.3 ppm; MS-ESI: *m/z* 813.8 $[M+2H]^{2+}$; HRMS-ESI: *m/z* $[M+2H]^{2+}$ calcd for $C_{78}H_{90}Cl_2FN_9O_{24}$: 813.7814, found: 813.7781.

***N*-(6-(2-Naphthyl)heptyl)demethylvancomycin (12e):** 20.4%; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 7.85 (m, 4H), 7.67 (s, 1H), 7.63 (m, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.46 (m, 2H), 7.38 (d, *J* = 8.4, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.16 (s, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.40 (d, *J* = 2.0 Hz, 1H), 6.26 (d, *J* = 2.0 Hz, 1H), 5.77 (m, 1H), 5.65 (m, 1H), 5.33 (m, 2H), 5.29 (s, 1H), 5.12 (m, 3H), 4.82 (m, 1H), 4.61 (d, *J* = 6.6 Hz, 1H), 4.44 (m, 2H), 4.19 (m, 1H), 4.11 (m, 1H), 4.04 (m, 1H), 3.69 (d, *J* = 10.4 Hz, 1H), 3.56 (d, *J* = 10.4 Hz, 1H), 3.52 (m, 1H), 3.46 (m, 1H), 3.00–3.30 (5H), 2.75 (m, 1H), 2.70 (s, 1H), 2.40–2.60 (2H), 1.98 (d, *J* = 12.6 Hz, 1H), 1.80 (d, *J* = 12.6 Hz, 1H), 1.70 (m, 1H), 1.62 (m, 1H), 1.55 (m, 4H), 1.35 (s, 3H), 1.26 (m, 6H), 1.08 (m, 3H), 0.89 ppm (m, 9H); ^{13}C NMR (150 MHz, [D$_6$]DMSO) δ = 173.0, 170.7, 169.6, 168.2, 158.3, 158.1, 157.6, 157.0, 155.5, 152.9, 151.6, 150.2, 148.7, 142.9, 140.3, 136.5, 136.1, 133.6, 132.2, 132.0, 128.9, 128.1, 127.9, 127.8, 127.8, 127.6, 127.5, 126.9, 126.5, 126.4, 126.4, 125.9, 125.6, 124.7, 123.8, 122.0, 118.5, 116.6, 108.1, 106.1, 105.1, 102.8, 101.2, 96.9, 78.3, 77.5, 77.3, 72.0, 70.6, 69.0, 63.6, 62.2, 61.6, 59.5, 59.1, 57.1, 55.3, 54.1, 51.7, 51.3, 46.0, 39.0, 35.7, 31.1, 28.9, 28.9, 26.5, 23.9, 23.2, 22.4, 19.4, 17.4 ppm; MS-ESI: *m/z* 829.8 $[M+2H]^{2+}$; HRMS-ESI: *m/z* $[M+2H]^{2+}$ calcd for $C_{82}H_{93}Cl_2N_9O_{24}$: 829.7928, found: 829.7923.

Molecular docking and modeling

Sketching tools in the Discovery Studio 3.0 package were used to build 3D structures of the demethylvancomycin derivatives using the crystal structure of (**1**) as a template. Energy minimizations of demethylvancomycin derivatives were performed using the Powell method with Tripos force field and MMFF94 charge in SYBYL 6.9 for 1000 step iterations. The 3D structures of demethylvancomycin derivatives were characterized by an L-shape (Figure 2). Starting with a 1.8 Å resolution X-ray co-crystal structure of **1** and the peptidoglycan from VRSA, the 3D structure of peptidoglycan was constructed and optimized using the AMBER force field. Next, the automated molecular docking program AutoDock 4.2 (Morris et al., 1998) was used to dock **12a** into the peptidoglycan. By integrating all available experimental data, the docking pose of the peptidoglycan–**12a** complex was obtained, in which the appended phenyl group could extend to the surface of the peptidoglycan and form a favorable NH–π interaction with a Gly residue. This binding mode was consistent with the previous binding model. A series of the docking parameters were set: the number of generation, energy evaluation, and docking runs were set to 370000, 1500000, and 10, respectively. The atom types, generations, and the number of runs for the LGA algorithm were edited and assigned according to the requirement of the AMBER force field. Finally, the docked **12a**–peptidoglycan complex was selected by considering the geometrical complementarity and the lower binding energy. Specifically, the docked candidate complexes with the five lowest binding energies were firstly selected, then the conformations with rational geometrical matchings were chosen as the final complex. These geometrical matchings were consistent with those in the conserved interaction mode between vancomycin derivatives and peptidoglycan. These complexes were used as the initial conformation for further geometric optimization.

Biological evaluation

Minimum inhibitory concentration (MIC) measurement: The minimum inhibitory concentration (MIC) values were measured to determine the antibacterial activities of the test compounds against MRSA 11001–11006, GRE 11031–11035, MRS11061–11062, and VRE12031–12035. Vancomycin and demethylvancomycin were used as positive controls. MIC values were determined using an agar dilution method according to the methods of CLSI. Compound stock solutions (320 μg mL^{-1}) were prepared in DMSO/H$_2$O (50%). Serial twofold dilutions prepared from the stock solutions with sterile H$_2$O were further diluted tenfold with Mueller–Hinton (MH) agar medium to obtain a concentration range of 0.024–50 μg mL^{-1}. The test organisms were grown in MH broth medium at 35 °C for 8 h and were adjusted to a turbidity of 0.5 using the McFarland standard. The bacterial suspensions were inoculated onto the drug-supplemented MH agar plates with a multipoint inoculator and incubated at 35 °C for 16 h.

MRSA infection following scalding model: Sprague–Dawley (SD) rats whose back hairs were removed using a shaver and 10% sodium sulfide were housed in single cages before the experiment. Using a YLS-5Q super temperature control scalding device, the #3 vertebral bit hot head was applied to the skin (100 °C, 15 s, 500 g contact pressure) to cause a deep (II) to superficial (III) scald with an area of 4 cm^2. After 20 min, the scald was scribbled with 0.2 mL of an inoculum containing 10^9 CFU mL^{-1} of MRSA (26003). Four hours after the infection, SD rats were scribbled with test compound **12a** at doses of 20, 40, or 80 μg mL^{-1}; and vancomycin was used as a positive control. The scalding group and the scalding MRSA group were subsequently scribbled with saline (0.1 mL per day). After 3, 7, 10, or 14 days, three SD rats in each group were sacrificed, and the scald scab tissues were removed to prepare HE-stained specimens (0.3×0.2 cm), fixed with 10% neutral buffered formalin. The specimens were then homogenized with saline (100 mg mL^{-1}) and diluted tenfold with the same saline. A portion (0.5 mL) of the diluted liquid was dropped into MH medium and cultured for 20 h in a 37 °C incubator.

MRSA and MRS infection model: Mice (Kunming) weighing between 18 and 20 g, were used in the experiment, with 20 mice in each

group. A lethal systemic infection was given to the mice as 0.5 mL of an inoculum of MRSA (11001) or MRS (11061) (10^7–10^8 CFU mL^{-1}) via intraperitoneal injection. Compound **12a** and vancomycin were administered i.v. 1 h after infection. The ED_{50} values were calculated 7 days after treatment by the Bliss method.

Pharmacokinetic studies: Compound **12a** was examined for its PK profile in SD rats weighing 200–250 g. The compound was administered i.v. at a dose of 40 mg kg^{-1}. Serial specimens (0.3 mL) were collected via the retrobulbar vein, and quantification was performed by LC–MS. PK parameters were calculated from the mean plasma concentration by noncompartmental analysis.

Acute toxicity study in mice: Healthy Kunming mice of both sexes, weighing 18–20 g, were divided into 7 groups of 10 animals each, matched for weight and size. Compound **12a** was administered i.v. at doses of 200 mg kg^{-1}, 100 mg kg^{-1}, 50 mg kg^{-1}, 42.5 mg kg^{-1}, 36.1 mg kg^{-1}, 30.7 mg kg^{-1}, or 26.1 mg kg^{-1}. Behavioral and weight changes were monitored, and the death was recorded within 14 days. LD_{50} values were calculated using the Bliss method.

Pre-test study of long-term toxicity in rats: Forty SD rats of both sexes, weighing 160–180 g, were divided into two groups. Compound **12a** was administered i.v. at a dose of 10 mg kg^{-1} once per day for two weeks. Behavioral and weight changes in the rats, as well as changes in blood, urine, and biochemical indicators were recorded.

All animal experiments were previously approved by the Ethical Committee for Animal Experiments at the College of Pharmacy, Fudan University (No.: 2012010012).

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[1] R. F. Service, *Science* **1995**, *270*, 724 – 727.
[2] C. Walsh, *Nature* **2000**, *406*, 775 – 781.
[3] R. G. Finch, G. M. Eliopoulos, *J. Antimicrob. Chemother.* **2005**, *55 (Suppl. 2)*, 5 – 13.
[4] H. A. Kirst, D. G. Thompson, T. I. Nicas, *Antimicrob. Agents Chemother.* **1998**, *42*, 1303 – 1304.
[5] Y. Nitanai, T. Kikuchi, K. Kakoi, S. Hanamaki, I. Fujisawa, K. Aoki, *J. Mol. Biol.* **2009**, *385*, 1422 – 1432.
[6] S. S. Leung, J. Tirado-Rives, W. L. Jorgensen, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1236 – 1239.
[7] R. Nagarajan, A. A. Schabel, F. T. Occolowitz, F. T. Counter, J. L. Ott, A. M. Felty-Duckworth, *J. Antibiot.* **1989**, *42*, 63 – 72.
[8] A. Y. Pavlov, E. I. Lazhko, M. N. Preobrazhenskaya, *J. Antibiot.* **1997**, *50*, 509 – 513.
[9] D. A. Beauregard, D. H. Williams, M. N. Gwynn, D. J. Knowles, *Antimicrob. Agents Chemother.* **1995**, *39*, 781 – 785.
[10] G. J. Sharman, A. C. Try, R. J. Dancer, T. R. Cho, T. Staroske, B. Bardsley, A. J. Maguire, M. A. Cooper, D. P. O'Brien, D. H. William, *J. Am. Chem. Soc.* **1997**, *119*, 12041 – 12047.
[11] N. E. Allen, D. L. LeTourneau, J. N. Hobbs, Jr., R. C. Thompson, *Antimicrob. Agents Chemother.* **2002**, *46*, 2344 – 2348.
[12] L. Cegelski, D. Steuber, A. K. Mehta, D. W. Kulp, P. H. Axelsen, J. Scheafer, *J. Mol. Biol.* **2006**, *357*, 1253 – 1262.
[13] S. J. Kim, L. Cegelski, D. Steuber, M. Singh, E. Dietrich, K. S. E. Tanaka, T. R. Parr, Jr., A. R. Far, J. Schaefer, *J. Mol. Biol.* **2008**, *377*, 281 – 293.
[14] S.-J. Zhang, Q. Yang, L. Xu, J. Chang, X. Sun, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4942 – 4945.
[15] M. Schäfer, T. R. Schneider, G. M. Sheldrick, *Structure* **1996**, *4*, 1509 – 1515.
[16] G. Tóth, C. R. Watts, R. F. Murphy, S. Lovas, *Proteins Struct. Funct. Genet.* **2001**, *43*, 373 – 381.
[17] A. Y. Pavlov, O. V. Miroshnikova, S. S. Printsevskaya, E. N. Olsufyeva, M. N. Preobrazhenskaya, R. C. Goldman, A. A. Branstrom, E. R. Baizman, C. B. Longley, *J. Antibiot.* **2001**, *54*, 455 – 459.
[18] N. E. Allen, D. L. LeTourneau, J. N. Hobbs, Jr., *J. Antibiot.* **1997**, *50*, 677 – 684.
[19] N. Shibata, M. Ishida, Y. V. R. Prasad, W. Gao, Y. Yoshikawa, K. Takada, *J. Chromatogr. B* **2003**, *789*, 211 – 218.

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