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ARTICLE

N-Terminal guanidine derivatives of teicoplanin antibiotics strongly active against glycopeptide resistant *Enterococcus faecium*

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Abstract

Antibiotic resistance is one of the major challenges in healthcare of our time. To meet this challenge, we designed and prepared guanidine and lipophilic guanidine derivatives of the glycopeptide antibiotic teicoplanin to armed them with activity against the most threatening nosocomial bacteria, multiresistant enterococci. From teicoplanin and its pseudoaglycone, a series of *N*-terminal guanidine derivatives have been prepared with free and amide *C*-terminal parts. Six aliphatic and aromatic lipophilic carbodiimides were prepared and used for the synthesis of lipophilic guanidine teicoplanin conjugates. All new *N*-terminal guanidine antibiotics showed high activity against a standard panel of Gram-positive bacteria. Four selected derivatives displayed excellent antibacterial activity against a series of nosocomial VanA *Enterococcus faecium* strains.

Introduction

Annually 25,000 people are killed in Europe by drug-resistant bacteria [1]. Vancomycin-resistant enterococci (VRE) are the leading cause of nosocomial infections [2]. The hospital acquired infections are caused mostly by *Enterococcus faecalis* and only 20% of them by *Enterococcus faecium*. Nevertheless, 80% of *E. faecium* isolates were vancomycin-resistant in US hospitals [2–4]. According to a longitudinal analysis rates of *E. faecium* in Europe increased in the time interval of 2001–2014 from 1.4% (in 2001) to 4.3 % (in 2014), while VRE rates among the same strain changed from 4.7 to 20.3% [5]. VanA and VanB are the most frequent phenotypes of *E. faecium*, VanA types being resistant to vancomycin and teicoplanin [2]. Against this threat oritavancin, a semisynthetic glycopeptide antibiotic seems to give some safety, showing low MIC values against VanA *E. faecium* [5, 6].

In the framework of a systematic synthetic study on lipophilic modifications of teicoplanin and its (pseudo)aglycone (s) [7–9], we recently reported on a series of derivatives with simple lipophilic *N*-terminal substituents that possessed comparable in vitro activity with that of oritavancin against clinical isolates of VanA *E. faecium* [10, 11]. As a continuation of this study in the present paper, we describe the synthesis of some *N*-terminal guanidine derivatives of teicoplanin and its pseudoaglycone.

Guanidines are superbases, i.e. stronger bases than other nitrogen compounds [12]. Therefore, their enhanced Coulomb interactions as well as hydrogen bonds with proteins can be expected. Several guanidine derivatives possess antibacterial activity [13–16]. The mechanism of action of glycopeptide antibiotics is based on their strong interaction with bacterial peptidoglycan [17]. We hypothesized that

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introduction of a guanidino substituent into the teicoplanin molecule or into its derivatives will enhance their interactions with the bacterial target D-alanine containing peptides and as a consequence will improve the antibacterial activity. Since lipophilic substituents of teicoplanin derivatives also positively influence their bioactivity [7–11], various lipophilic guanidine moieties were also introduced into teicoplanin or its pseudoaglycone.

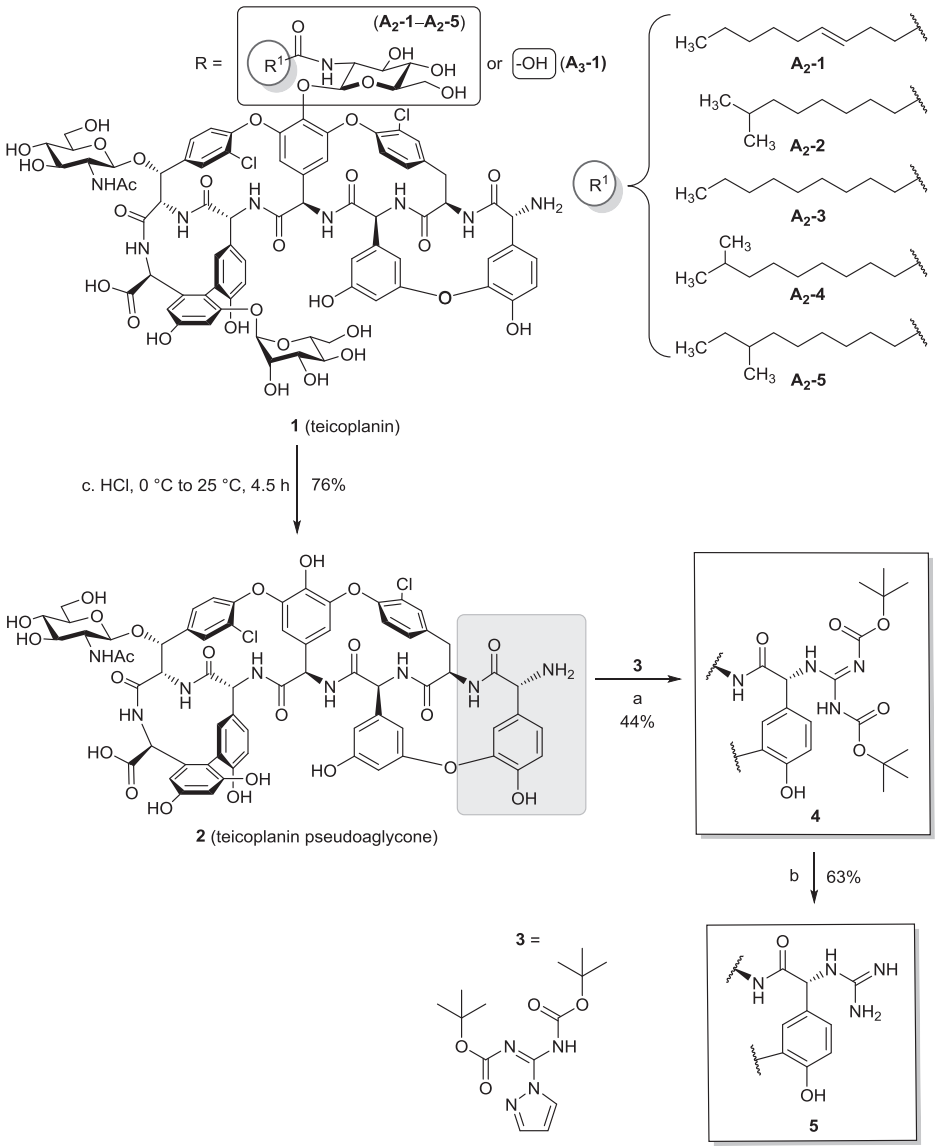
Results and discussion

Chemistry

For the introduction of guanidine moiety, the *N*-terminal primary amino group of teicoplanin (**1**) or its pseudoaglycone **2** (T-ΨAgl, also known as teicoplanin A_3-2) was used. To obtain **2** we elaborated a novel, simple deglycosidation method that involves treatment of **1** with concentrated hydrochloric acid. This new method is much cheaper, easier to carry out and considerably safer than the hydrogen-fluoride mediated hydrolytic process reported by the Boger group [18], which we have used in the past [7–10], while the yield of teicoplanin pseudoaglycone **2** is similarly excellent.

At first, a derivative of **2** with unsubstituted guanidino group was prepared using the readily available pyrazolecarboxamidine reagent **3**. The intermediate dicarbamate derivative **4** was deprotected to give **5** (Scheme 1).

One of the mildest ways of preparation of substituted guanidine derivatives is the addition of primary amines onto carbodiimides [19–21]. The preparation of derivatives carrying two lipophilic groups on the guanidine moiety required the synthesis of *N,N′*-disubstituted carbodiimides.

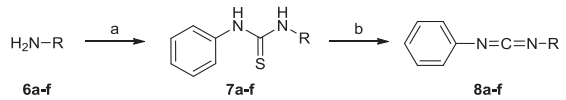


Scheme 1 Structure of starting teicoplanin **1**, teicoplanin pseudoaglycone **2** (T-ΨAgl), and transformation of pseudoaglycone **2** into guanidine derivatives **4** and **5**. Reagents and conditions: **a** dimethylformamide (DMF), *N,N′*-di-Boc-1*H*-pyrazole-1-carboxamidine, Et_3N, 40 °C, overnight; **b** trifluoroacetic acid (TFA), anisole, 25 °C, 1 h

Carbodiimides with two electron-donating alkyl groups display low reactivity to primary amines, but an aromatic substituent can enhance their reactivity. Therefore, we chose the phenyl group as one of the substituents of our carbodiimides. Six aliphatic and aromatic lipophilic amines **6a**–**f** were reacted with phenyl isocyanate producing thioureas **7a**–**f** in good yields. Subsequent treatment of the latter compounds with methanesulfonyl chloride and trimethylamine [22] resulted in carbodiimides **8a**–**f** (Scheme 2).

The synthetic steps of conjugation procedure of teicoplanin complex **1** and its pseudoaglycone **2** are summarized in Scheme 3 and the structure of products are shown in Table 1. The carboxylic group of **2** was protected in the form of diphenylmethyl ester, yielding **9**, which in the reactions with carbodiimides **8a**–**d** gave guanidines **13a**–**d**. The latter were deprotected by trifluoroacetic acid to provide final products **14a**–**d** (Scheme 3). From teicoplanin (**1**), which is a mixture of six different compounds ($\mathbf{A_2}$**-1**–$\mathbf{A_2}$**-5** and $\mathbf{A_3}$**-1**, see Scheme 1), a six-component dimethylaminopropyl amide mixture of similar composition (**11**) was obtained using PyBOP reagent, similarly to the dalbavancin molecule [23]. From **11** guanidino derivatives **16b**, **16e**, and **16f** were prepared, respectively, each in the form of inseparable mixture of six compounds. A diethylaminopropyl amide **10** of pseudoaglycone **2** was also obtained from **1** by amidation and subsequent hydrolytic removal of *N*-acylglucosamines and D-mannose. Reactions of **10** with carbodiimides gave guanidine amides **15a**–**d** (Scheme 3 and Table 1).

The structure of the new derivatives (see Table 1) was proved by 2D NMR techniques (^{1}H–^{1}H COSY, ^{1}H–^{1}H ROESY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC). The detailed NMR assignation of the new derivatives as well as the composition of **16b**, **16e**, and **16f** can be found in the supporting information.

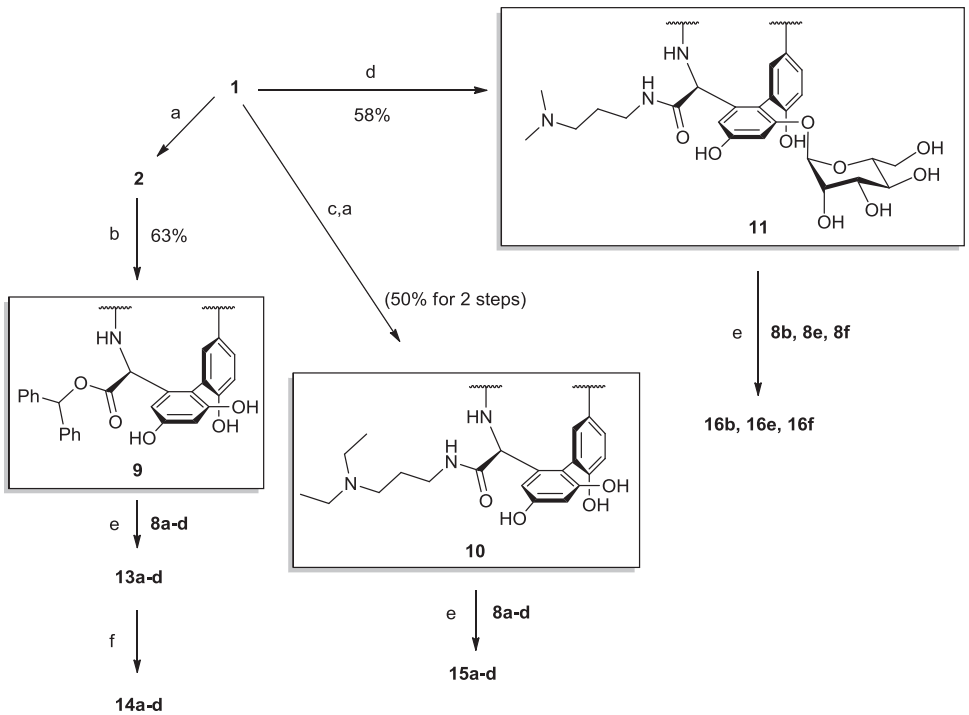


Starting amine		Thiourea (Yield)		Carbodiimide (Yield)	
6a	R = *n*-hexyl	**7a**	(65%)	**8a**	(86%)
6b	R = *n*-octyl	**7b**	(85%)	**8b**	(78%)
6c	R = *n*-decyl	**7c**	(90%)	**8c**	(40%)
6d	R = *n*-dodecyl	**7d**	(84%)	**8d**	(50%)
6e	R = 4-phenylbenzyl	**7e**	(92%)	**8e**	(45%)
6f	R = *N*-benzyl-piperidyl	**7f**	(97%)	**8f**	(80%)

Scheme 2 Synthesis of lipophilic carbodiimide reagents **8a**–**f**. Reagents and conditions: **a** dichloromethane (DCM), phenyl isothiocyanate, 4-dimethylaminopyridine (DMAP), 25 °C, 1 h; **b** DCM, methanesulfonyl chloride, Et_3N, 25 °C, 30 min

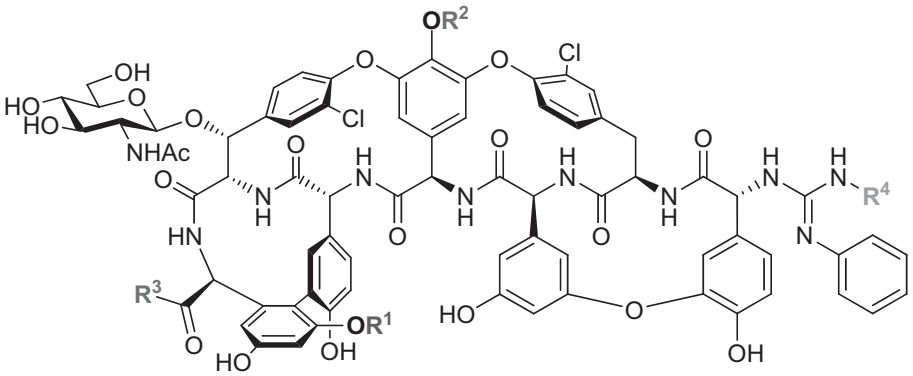
Antibacterial evaluation

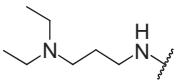
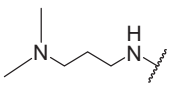
The antibacterial activity of the new teicoplanin derivatives was evaluated on a standard panel of eight Gram-positive bacterial strains for preliminary results (Table 2). The pseudoaglycone derivative **4** bearing a Boc protected



Scheme 3 Synthesis routes to lipophilic guanidine derivatives of teicoplanin pseudoaglycone (**13**–**15**) and teicoplanin (**16**). Ester (**9**) and amide (**10**, **11**) structures formed on the *C*-terminus are highlighted in boxes. The structures of lipophilic guanidine derivatives **13**–**16** are shown in Table 1. Reagents and conditions: **a** c. HCl, 0 °C to 25 °C, 4.5 h; **b** DMF, diphenyldiazomethane, *p*-toluenesulfonic acid, 25 °C, 16 h; **c** DMF, $Et_2N(CH_2)_3NH_2$, PyBOP, 25 °C, 5 h; **d** DMF, $Me_2N(CH_2)_3NH_2$, PyBOP, 25 °C, 3 h; **e** DMF, carbodiimides, 25 °C, 3–4 h; **f** TFA, 25 °C, 1 h

Table 1 Structure of lipophilic guanidine derivatives of teicoplanin pseudoaglycon (**13–15**) and teicoplanin (**16**)



Compound#		R^1	R^2	R^3	R^4
T-ΨAgl diphenylmethyl esters	**13a**	H	H	$OCHPh_2$	*n*-hexyl
	13b				*n*-octyl
	13c				*n*-decyl
	13d				*n*-dodecyl
T-ΨAgl free *C*-terminus	**14a**	H	H	H	*n*-hexyl
	14b				*n*-octyl
	14c				*n*-decyl
	14d				*n*-dodecyl
T-ΨAgl amides	**15a**	H	H		*n*-hexyl
	15b				*n*-octyl
	15c				*n*-decyl
	15d				*n*-dodecyl
Teicoplanin amides	**16b**	α-D-mannose	*N*-acyl-β-D-glucosamine		*n*-octyl
	16e				4-phenylbenzyl
	16f				*N*-benzyl-piperidyl

guanidine did not show any relevant activity, while its deprotected analogue **5** was highly active against every tested strain. This might very well be due to the capability of the guanidine moiety to gain a positive charge, as well as the ability to form extra hydrogen bonds as H-bond donor. The former is considered to be significant in the early stages of binding the D-Ala-D-Ala termini of cell wall precursors, while the latter might help to counteract the loss of the hydrogen bond due to the change of the terminal D-Ala to D-Lac in the peptidoglycan precursors of glycopeptide resistant enterococci.

Among the diphenylmethyl esters only the *n*-hexyl analogue **13a** seemed to have fairly good activity, all other analogues (**13b–d**) were essentially inactive, indicating that the structure and lipophilicity of substituents connecting to the *N*-terminus—in this case the guanidino group—have a strong influence on antibacterial activity. The latter is in accordance with previous findings of many different research groups [24–29], including our former results [7–10, 30] in this field. Analogues with the free carboxyl group (**14a–d**) gained significant activity against all strains, including the VRE strains. Two of them, **14a** and **14b**

Table 2 In vitro antibacterial activity (MIC) of guanidine derivatives of teicoplanins

		B. subtilis ATCC 6633	*S. aureus* MSSA ATCC 29213	*S. aureus* MRSA ATCC 33591	*S. epidermidis* ATCC 35984	*S. epidermidis* mecA	*E. faecalis* ATCC 29212	*E. faecalis* ATCC 51299 VanB	*E. faecalis* 15376 VanA
Teicoplanin		0.5	0.5	0.5	4.0	16	1.0	0.5	256
Vancomycin		0.5	0.5	0.5	2.0	4.0	1.0	128	256
4		32	16	16	16	16	32	32	64
5		0.8	0.4	0.4	0.4	0.4	0.8	0.4	0.8
13a		4.0	1.0	2.0	2.0	0.8	2.0	4.0	2.0
13b	T-ΨAgl diphenylmethyl esters	128	64	32	4.0	4.0	16	16	16
13c		128	256	128	64	32	64	128	64
13d		128	128	128	32	16	128	256	64
14a		6.4	0.8	0.4	0.2	0.2	0.2	0.5	0.4
14b	T-ΨAgl free *C*-terminus	1.6	0.2	0.4	0.05	0.05	0.2	0.2	0.8
14c		4.0	2.0	4.0	2.0	8.0	4.0	1.0	4.0
14d		4.0	8.0	4.0	0.8	0.8	2.0	32	8.0
15a		2.0	4.0	1.0	1.0	0.5	32	64	16
15b	T-ΨAgl amides	4.0	4.0	2.0	1.0	2.0	64	32	32
15c		1.0	2.0	2.0	4.0	0.5	2.0	64	32
15d		8.0	8.0	8.0	2.0	1.0	32	32	32
16b		0.8	0.8	0.8	0.4	0.4	0.8	1.6	0.8
16e	Teicoplanin amides	1.0	2.0	2.0	1.0	2.0	16	32	16
16f		0.5	0.5	1.0	0.5	0.5	32	32	16

MIC minimum inhibitory concentration (μg ml^{-1}), *ATCC* American Type Culture Collection, *MSSA* methicillin-sensitive *Staphylococcus aureus*, *MRSA* methicillin-resistant *Staphylococcus aureus*, *VSE* vancomycin-sensitive enterococcus, *mecA* mecA gene expression in *Staphylococcus*, *vanA*+ vanA gene positive, *vanB*+ vanB gene positive

bearing *n*-hexyl and *n*-octyl substituents on the guanidine moiety were highly active even against VRE. The pseudoaglycone amides **15a–d** although were as effective as their carboxyl analogues against staphylococci, a significant loss of activity was observed against enterococci. The antibacterial properties of the three amide derivatives obtained from the teicoplanin mixture (**16b**, **16e**, and **16f**) were about the same as the pseudoaglycone amides, except for the *n*-octyl analogue **16b**, which was highly active against every strain in the standard panel.

Four of the new derivatives **5**, **14a**, **14b**, and **16b** were selected for in vitro evaluation against a collection of VanA type enterococci isolated from patients at the Department of Medical Microbiology, University of Debrecen. The *n*-hexyl **14a** and *n*-octyl **14b** pseudoaglycone derivatives with a free *C*-terminus displayed about the same activity (Table 3), the MIC was below 0.5 μg ml^{-1} against most strains, outperforming oritavancin in multiple cases. The two isolates that seemed to be the most resistant to our derivatives were *E. faecium* 11408 and *E. faecium* 17980, which were still susceptible to oritavancin. The pseudoaglycone derivative **5** with the unsubstituted guanidine group had similar activity against VRE strains as **14a** and **14b**, with the exception of the *E. faecium* 38415 strain, which seemed to be resistant to this compound. The *n*-octyl analogue prepared from teicoplanin mixture displayed the same activity as **14a**, the only resistant strain was *E. faecium* 11408.

Conclusion

In summary, we have demonstrated a straightforward way to transform the *N*-terminal amino group of teicoplanin or its pseudoaglycone into unsubstituted or substituted guanidine groups. This modification led to derivatives with enhanced in vitro antibacterial activity against glycopeptide resistant Gram-positive bacteria including VanA type enterococci. Four of the new compounds has shown equal or higher activity than oritavancin against the 22 tested nosocomial VRE strains in vitro (typically 0.2–0.4 μg ml^{-1}). Not considering the *N*-terminal guanidine group, these three types of compounds have markedly different structures, leading to completely distinct ionization behavior and lipophilicity, hence water solubility. The fact that these similar but yet very different antibiotics have practically equally high activity, leaves us a wide range of opportunities for structural adjustments to the existing derivatives with the aim of tuning their in vivo behavior.

Experimental

General information

3-(Dimethylamino)-1-propylamine, 3-(diethylamino)-1-propylamine, pyrazolecarboxamidine (**3**) and *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine were purchased from Tokyo

Table 3 In vitro antibacterial activity (MIC) of selected guanidine derivatives against VanA enterococci

#	Strain	Source	TEI	VAN	ORI	14a	14b	16b	5
1	*E. faecium* 8663	Bronchus	256	256	2	0.1	0.1	0.1	0.2
2	*E. faecium* 22285	Urine	256	256	2	0.2	0.2	0.2	0.4
3	*E. faecium* 656	Wound	256	256	2	0.2	0.2	0.2	0.2
4	*E. faecium* 3452	Drain	256	256	1	0.4	0.8	0.8	0.4
5	*E. faecium* 4753	Decubitus	256	256	1	0.2	0.2	0.2	0.2
6	*E. faecium* 11408	Drain	256	256	<0.25	1.6	3.18	6.25	12.5
7	*E. faecalis* 17980	Urine	256	256	2	6.25	3.18	0.8	6.25
8	*E. faecium* 24581	Wound	256	256	0.5	0.2	0.4	0.2	0.2
9	*E. faecium* 25192	Haemoculture	256	256	0.5	0.4	0.4	0.4	0.2
10	*E. faecium* 29007	Urine	256	256	0.25	0.2	0.2	0.2	0.2
11	*E. faecium* 29810	Wound	256	256	0.5	0.4	0.4	0.2	0.4
12	*E. faecium* 29838	Urine	256	256	0.5	0.2	0.2	0.2	0.2
13	*E. faecium* 30458	Cannula	256	256	0.25	0.2	0.4	0.2	0.4
14	*E. faecium* 31482	Urine	256	256	0.25	0.2	0.2	0.2	0.2
15	*E. faecium* 32445	Cannula	256	256	0.5	0.2	0.2	0.2	0.2
16	*E. faecium* 35936	Urine	256	256	0.25	0.1	0.1	0.1	0.1
17	*E. faecium* 38276	Urine	256	256	0.25	0.2	0.2	0.2	0.2
18	*E. faecium* 38415	Wound	256	256	2	1.6	0.4	0.4	6.25
19	*E. faecium* 38522	Decubitus	256	256	1	0.4	0.2	0.8	1.6
20	*E. faecium* 39063	Wound	256	256	0.5	0.2	0.2	0.2	0.2
21	*E. faecium* 39759	Drain	256	256	0.25	0.2	0.2	0.2	0.8
22	*E. faecium* 42491	Urine	256	256	0.25	0.2	0.2	0.2	0.2
No. of MIC values above the breakpoint for teicoplanin (2 μg ml^{-1})						1	2	1	3
No. of MIC values above the breakpoint for vancomycin (4 μg ml^{-1})						1	0	1	3

MIC minimum inhibitory concentration (μg ml^{-1}), *TEI* teicoplanin, *VAN* vancomycin, *ORI* oritavancin

Chemical Industry Co., Ltd. Oritavancin was purchased from Xi'an Kerui Biotechnology Co., Ltd. (Xian, Shaanxi, China) and checked by MALDI-TOF MS, 1D and 2D NMR experiments. The vancomycin hydrochloride standard used for the antibacterial evaluations was a gift from TEVA Pharmaceutical Industries Ltd. (Debrecen, Hungary) and teicoplanin was purchased from Shaanxi Sciphar Biotechnology Co., Ltd (Xi'an, Shaanxi, China). Teicoplanin for synthetic purposes was purchased from Xi'an Sgonek Biological Technology Co., Ltd. (Weiyang Qu, Xian Shi, Shaanxi Sheng, China).

The antibacterial evaluations were carried out as it was described in our previous publication [8]. TLC was performed on Kieselgel 60 F_{254} (Merck) with detection either by immersing into ammonium molybdate-sulfuric acid solution followed by heating or by using Pauly's reagent for detection. Flash column chromatography was performed using Silica gel 60 (Merck 0.040–0.063 mm). The ^{1}H NMR (500 and 400 MHz), ^{13}C NMR (125 and 100 MHz) and 2D NMR spectra were recorded with a Bruker DRX-400 or a Bruker Avance II 500 spectrometer at 298 K or 310 K. Chemical shifts are referenced to Me_4Si (0.00 ppm for ^{1}H) and to the solvent residual signals. The spectra were evaluated using MestReNova and TopSpin softwares. ESI-HRMS spectra were recorded by a microTOF-Q type QqTOFMS mass spectrometer (Bruker) and a maXis II UHR ESI-QTOF MS instrument (Bruker) in positive ion mode using MeOH as solvent. In the case of maXis II UHR ESI-QTOF MS the following parameters were applied for the electrospray ion source: capillary voltage: 3.5 kV; end-plate offset: 500 V; nebulizer pressure: 0.8 bar; dry gas temperature: 200 °C and dry gas flow rate: 4.5 l min^{-1}. Constant background correction was applied for each spectrum, the background was recorded before each sample by injecting the blank sample matrix (solvent). Na-formate or tuning mix ESI-TOF (Agilent) calibrant was injected after each sample, which enabled internal calibration during data evaluation. Mass spectra were recorded by otofControl version 4.1 (build: 3.5, Bruker) and processed by Compass DataAnalysis version 4.4 (build: 200.55.2969). For analytical RP-HPLC a Waters 2695 Separations Module (Waters Corp., Milford, USA) was used. The separations were carried out on a VDSpher PUR 100 C18-M-SE, 5 μm, 150 × 4.6 mm column (Batch# VD173001) at an injection volume of 10 μl, using a flow rate of 1.0 ml min^{-1} with a Waters 2996 DADset at 254 nm and a Bruker MicroTOF-Q type Qq-TOF MS instrument (Bruker Daltonik, Bremen, Germany) as detectors. The following system was used for

the elutions: Solvent A: Water:MeCN 9:1 + 0.0025%v/v TFA, Solvent B: MeCN. Gradient: 20% B from 0 to 20 min, from 20% B to 80% B from 20 to 40 min, 80% B from 40 to 50 min, from 80% B to 20% B from 50 to 51 min. Solvent A: Water:MeCN 9:1 + 0.0025%v/v TFA, Solvent B: MeCN. The MicroTOF-Q mass spectrometer was equipped with an electrospray ion source. The mass spectrometer was operated in positive ion mode with a capillary voltage of 3.5 kV, an endplate offset of −500 V, nebulizer pressure of 1.8 bar, and N_2 as drying gas with a flow rate of 9.0 l min^{-1} at 200 °C. The mass spectra were recorded by means of a digitizer at a sampling rate of 2 GHz. The mass spectra were calibrated externally using the exact masses of clusters $[(NaTFA)_n + TFA]^-$ from the solution of sodium trifluoroacetate (NaTFA). The spectra were evaluated with the DataAnalysis 3.4 software from Bruker.

Synthesis

General procedure A for the synthesis of carbodiimides (Scheme 2)

Step a: To the corresponding amine dissolved in dry DCM, 1.0 equiv. of phenyl isothiocyanate was added, then the reaction mixture was stirred for 2 h at room temperature. The mixture was evaporated and the crude product was purified by flash column chromatography using *i*-hexanes:acetone mixtures as eluent. Step b: The obtained thiocarbamide intermediate was dissolved in dry DCM, then a catalytic amount of DMAP, 3.0 equiv. of TEA and 2.0 equiv. of MsCl were added while cooling in an ice bath. The mixture was allowed to warm to room temperature. After 30 min silica gel was added and the mixture was evaporated, then the crude product was purified by flash column chromatography using *i*-hexanes: ethyl acetate mixtures as eluent.

General procedure B for the deprotection of teicoplanin pseudoaglycone DPM esters (step f in Scheme 3)

The corresponding teicoplanin pseudoaglycone DPM ester was dissolved in dry TFA (1–2 ml) at room temperature and stirred for 1 h, then the mixture was co-evaporated with toluene (15 ml) two times. The crude product was dissolved in a MeCN:H_2O 1:1 mixture, silica gel was added, the mixture was evaporated and the product was purified by flash column chromatography.

Compound 2

After the treatment of **1** with concentrated hydrochloric acid ($c_{teicoplanin} = 100$ mg ml^{-1}) for about 4.5 h at room temperature, the pH of the reaction mixture was set to pH 5.5 by adding 2 N NaOH solution. The obtained white precipitate was filtered off and washed with water (pH 5.5). After normal phase column chromatography, using MeCN: H_2O gradient elution, **2** was obtained in 76% yield. The characterization data of **2** were identical to those previously reported in the literature [7].

Compound 4

Teicoplanin A_3-2 pseudoaglycone **2** (200 mg, 0.14 mmol) was dissolved in dry DMF (3 ml) and triethylamine (78 µl, 0.56 mmol, 4.0 equiv.) was added, followed by *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine (86.9 mg, 0.28 mmol, 2.0 equiv.). The reaction mixture was stirred at 40 °C for 24 h. After cooling down to room temperature, diethyl ether was added and the resulting white precipitate was filtered off and washed with additional diethyl ether. The crude product was purified by flash column chromatography eluting with MeCN:H_2O 97:3 → 95:5. Yield: 103 mg (44%) off-white powder. ESI-HRMS: *m/z* 1687.3951 $[M–H + 2Na]^+$; calcd: 1687.3976 ($C_{77}H_{75}Cl_2N_{10}O_{27}Na_2^+$).

Compound 5

Compound **4** (80 mg, 49 µmol) and anisole (10.6 µl, 2.0 equiv.) was dissolved in TFA (1 ml) and the mixture was stirred for 1 h at room temperature. The crude product was precipitated by adding diethyl ether. The precipitate was filtered off, washed, and dried. The crude product was purified by flash column chromatography eluting with MeCN:H_2O = 9:1. Yield: 44 mg (63%) off-white powder. ESI-HRMS: *m/z* 1465.3094 $[M + Na]^+$; calcd: 1465.3102 ($C_{67}H_{60}Cl_2N_{10}O_{23}Na^+$).

Synthesis of carbodiimides (Scheme 1)

N-Hexyl-*N*′-phenylcarbodiimide (8a)

See General procedure A. Reactants for Step a: *n*-hexylamine (2.64 ml, 20 mmol), phenyl isothiocyanate (2.7 g, 2.4 ml). Eluent: *i*-hexanes:acetone = 8:2. Yield of thiocarbamide intermediate **7a**: 3.08 g (65%). Step b: thiocarbamide (1010 mg, 5 mmol), DMAP (25 mg), trimethylamine (2.1 ml), methanesulfonyl chloride (774 µl). Eluent: *i*-hexanes → *i*-hexanes:EtOAc 98:2 → 97:3 → 96:4. Yield: 870 mg (56%, for two steps) yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.12–7.05 (m, 3H), 3.40 (t, $J = 6.8$ Hz, 2H), 1.67 (tt, $J = 7.0$ Hz, 2H), 1.46–1.37 (m, 2H), 1.31 (m, 4H), 0.89 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.90 (N = *C* = N), 129.44 (2C, 2 × *C*H_{Ar}), 124.64, 123.58 (2C, 2 × *C*H_{Ar}), 46.98 (N-*C*H_2), 31.47, 31.42, 26.59, 22.66, 14.11 (*C*H_3). ESI-HRMS: *m/z* 235.1809 $[M + CH_3OH + H]^+$; calcd: 235.1805 ($C_{13}H_{18}N_2 + CH_3OH + H^+$).

N-Octyl-*N*′-phenylcarbodiimide (8b)

See General procedure A. Reactants for Step a: *n*-octylamine (1.29 g, 1.65 ml, 10 mmol), phenyl isothiocyanate (1.35 g, 1.2 ml). Eluent: *i*-hexanes:acetone = 8:2. Yield of intermediate **7b**: 2.24 g (85%). Step b: thiocarbamide (1.32 g, 5 mmol), DMAP (25 mg), trimethylamine (2.09 ml), methanesulfonyl chloride (774 µl). Eluent: *i*-hexanes → *i*-hexanes:EtOAc 98:2 → 97:3 → 96:4. Yield: 900 mg (66%, for two steps) yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.32–7.23 (m, 2H), 7.14–7.03 (m, 3H), 3.40 (t, $J = 6.8$ Hz, 2H), 1.67 (tt, $J = 6.8$ Hz, 2H), 1.41 (tt, $J = 15.0$, 6.6 Hz, 2H), 1.35–1.20 (m, 8H), 0.87 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 140.91 (N=*C*=N), 136.14 (Ar-C_q), 129.43, 124.63, 123.58 (5C, 5 × Ar*C*H), 46.97 (N-CH_2), 31.88, 31.51, 29.29, 26.91, 22.76 (8C, 8 × CH_2), 14.20 (CH_3). ESI-HRMS: *m/z* 263.2118 $[M + CH_3OH + H]^+$; calcd: 263.2118 ($C_{15}H_{22}N_2 + CH_3OH + H^+$).

N-Decyl-*N*′-phenylcarbodiimide (8c)

See General procedure A. Reactants for Step a: *n*-decylamine (1573 µl, 8 mmol), phenyl isothiocyanate (955 µl). Eluent: *i*-hexanes:acetone = 8:2. Yield of thiocarbamide intermediate **7c**: 2.1 g (90%). Step b: thiocarbamide (1.00 g, 3.42 mmol), DMAP (20 mg), trimethylamine (1.43 ml), mesyl chloride (530 µl). Eluent: *i*-hexanes → *i*-hexanes:EtOAc 98:2 → 97:3 → 96:4. Yield: 350 mg (36%, for two steps) yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.32–7.23 (m, 2H), 7.14–7.03 (m, 3H), 3.40 (t, $J = 6.8$ Hz, 2H), 1.67 (tt, $J = 6.9$ Hz, 2H), 1.41 (tt, $J = 6.9$ Hz, 2H), 1.27 (d, $J = 9.4$ Hz, 12H), 0.88 (t, $J = 6.7$ Hz, 3v). ^{13}C NMR (91 MHz, $CDCl_3$) δ 140.92 (N=*C*=N), 136.13 (Ar-C_q), 129.43, 124.63, 123.58 (5C, 5 × Ar*C*H), 46.98 (N-CH_2), 32.01, 31.52, 29.43, 29.24, 26.92, 22.81 (8C, 8 × CH_2), 14.24 (CH_3). ESI-HRMS: *m/z* 291.2437 $[M + CH_3OH + H]^+$; calcd: 291.2431 ($C_{17}H_{26}N_2 + CH_3OH + H^+$).

N-Dodecyl-*N*′-phenylcarbodiimide (8d)

See General procedure A. Reactants for Step a: *n*-dodecylamine (1112 mg, 1380 µl, 6 mmol), phenyl isothiocyanate (811 mg, 718 µl). Eluent: *i*-hexanes:acetone = 8:2. Yield of intermediate **7d**: 1.33 g (69%). Step b: thiocarbamide (1.33 g, 4.15 mmol), DMAP (20 mg), trimethylamine (1.74 ml), methanesulfonyl chloride (642 µl). Eluent: *i*-hexanes → *i*-hexanes:EtOAc 98:2 → 97:3 → 96:4. Yield: 690 mg (40%, for two steps) yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.33–7.23 (m, 2H), 7.13–7.05 (m, 3H), 3.40 (t, $J = 6.8$ Hz, 2H), 1.67 (tt, $J = 6.9$ Hz, 2H), 1.46–1.35 (m, 2H), 1.27 (d, $J = 10.0$ Hz, 17H), 0.88 (t, $J = 6.7$ Hz, 3H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 140.92 (N=*C*=N), 136.14 (Ar-C_q), 129.44, 124.64, 123.59 (5C, 5 × Ar*C*H), 46.98 (N-CH_2), 32.06, 31.52, 29.77, 29.69, 29.65, 29.49, 29.25, 26.93, 22.83 (10C, 10 × CH_2), 14.25 (CH_3). ESI-HRMS: *m/z* 319.2753 $[M + CH_3OH + H]^+$; calcd: 319.2744 ($C_{19}H_{30}N_2 + CH_3OH + H^+$).

N-Phenyl-*N*′-4-phenylbenzyl carbodiimide (8e)

See General procedure A. Reactants for Step a: 4-phenylbenzylamine (275 mg, 1.5 mmol), phenyl isothiocyanate (180 µl). Eluent: *i*-hexanes:acetone = 8:2 → 7:3. Yield of intermediate **7f**: 440 mg (92%). Step b: thiocarbamide (420 mg, 1.32 mmol), DMAP (3 mg), trimethylamine (552 µl), mesyl chloride (204 µl). Eluent: *i*-hexanes → *i*-hexanes:EtOAc 98:2 → 97:3 → 96:4. Yield: 170 mg (42%, for two steps) yellowish solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.63–7.52 (m, 4H), 7.48–7.38 (m, 4H), 7.37–7.30 (m, 1H), 7.28–7.20 (m, 2 H), 7.12–7.05 (m, 1H), 7.04–6.97 (m, 2H), 4.59 (s, 2H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 140.88, 140.74, 140.07, 137.41, 136.98 (5C, 5 × C_q), 129.45, 128.91, 127.95, 127.63, 127.52, 127.20, 125.01, 123.80 (14C, 14 × Ar-*C*H), 50.34 (N-CH_2). ESI-HRMS: *m/z* 317.1662 $[M + CH_3OH + H]^+$; calcd: 317.1648 ($C_{20}H_{16}N_2 + CH_3OH + H^+$).

N-(1-Benzyl-4-piperidyl)-*N*′-phenylcarbodiimide (8f)

See General procedure A. Reactants for Step a: 4-amino-1-benzylpiperidine (611 µl, 3 mmol), phenyl isothiocyanate (358 µl). Eluent: *i*-hexanes:acetone = 7:3 → 6:4 + 0,1%V/V Et_3N. Yield of intermediate **7g**: 950 mg (97%). Step b: thiocarbamide (650 mg, 2 mmol), DMAP (10 mg), trimethylamine (836 µl), methanesulfonyl chloride (309 µl). Eluent: *i*-hexanes → *i*-hexanes:EtOAc 9:1 → 8:2 + 0,1%V/V Et_3N. Yield: 470 mg (78%, for two steps) pale yellow solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.45–7.34 (m, 2H), 7.32–7.22 (m, 6H), 7.20–7.14 (m, 2H), 4.39–4.25 (m, 1H), 3.46 (s, 2H), 2.74 (d, $J = 12.1$ Hz, 2H), 2.19–2.08 (m, 2H), 2.08–1.99 (m, 2H), 1.41 (dq, $J = 11.1$, 3.9 Hz, 2H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 138.31, 136.18 (2C, 2 × Ar-C_q), 130.22 (2C, 2 × Ar-*C*H), 129.10 (2C, 2 × Ar-*C*H), 128.24 (2C, 2 × Ar-*C*H), 127.19, 127.07 (2C, 2 × Ar-*C*H), 125.06 (2C, 2 × Ar-*C*H), 63.01 (C_q-CH_2), 52.09 (N-*C*H), 52.05 (2C, 2 × N-CH_2), 31.77 (2C, 2 × N-CH-CH_2).

Esters (Scheme 3)

Teicoplanin pseudoaglycone diphenylmethyl ester (9)

Teicoplanin A_3-2 (1.3 g, 0.93 mmol) was dissolved in DMF (8.0 ml) followed by the addition of *p*-toluenesulfonic acid (176 mg, 1.02 mmol, 1.1 equiv.) and freshly prepared diphenyldiazomethane (198 mg, 1.02 mmol, 1.1 equiv.).

After stirring at room temperature overnight, ether was added, the precipitated solid was filtered off and washed with additional ether, then redissolved in MeCN:H_2O 1:1 mixture, silica gel was added, the mixture was evaporated and the product was purified by flash column chromatography. Eluent: MeCN:H_2O = 95:5 → 90:10 → 85:15. Yield: 920 mg (63%) white powder. The product was used in the following steps without NMR characterization. ESI-HRMS: *m/z* 1589.3664 $[M + Na^+]$; calcd: 1589.3672 ($C_{79}H_{68}Cl_2N_8O_{23}Na^+$).

Compound 13a

Diphenylmethyl ester **9** (100 mg, 63.8 µmol) was dissolved in DMF (1.0 ml) and **8a** (16 mg, 1.2 equiv.) in DMF (1.0 ml) was added dropwise slowly. After 1 h of stirring at room temperature ether (75 ml) was added, and the precipitated solid was filtered off and washed with additional ether. The crude product was dissolved in methanol, silica gel was added, the mixture was evaporated and the product was purified by flash column chromatography. Eluent: MeCN:H_2O = 95:5 → 93:7. Yield: 47 mg (42%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1769.5303 $[M + H]^+$; calcd: 1769.5317 ($C_{92}H_{87}Cl_2N_{10}O_{23}^+$).

Compound 13b

The reaction of teicoplanin A_3-2 diphenylmethyl ester **9** (195 mg, 0.125 mmol) in DMF (1.5 ml) and **8b** (35 mg) was carried out as described for **13a**. Purification of **13b**: DCM: MeOH = 9:1 → 8:2. Yield: 99 mg (44%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1819.5469 $[M + Na]^+$; calcd: 1819.5450 ($C_{94}H_{90}Cl_2N_{10}O_{23}Na^+$).

Compound 13c

The reaction of teicoplanin A_3-2 diphenylmethyl ester **9** (195 mg, 0.125 mmol) in DMF (1.5 ml) and **8c** (38 mg) was carried out as described for **13a**. Purification of **13c**: DCM:MeOH = 90:10 → 85:15 → 80:20. Yield: 135 mg (59%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1825.5965 $[M + H]^+$; calcd: 1825.5943 ($C_{96}H_{95}Cl_2N_{10}O_{23}^+$).

Compound 13d

The reaction of teicoplanin A_3-2 diphenylmethyl ester **9** (157 mg, 0.1 mmol) in DMF (1.0 ml) and **8d** (35 mg) was carried out as described for **13a**. Purification of **13d**: DCM: MeOH = 9:1 → 8:2. Yield: 72 mg (38%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1853.6269 $[M + H]^+$; calcd: 1853.6256 ($C_{98}H_{98}Cl_2N_{10}O_{23}^+$).

Compound 14a

13a (37 mg, 20.9 µmol) was deprotected according to General procedure B. Eluent: MeCN:H_2O = 95:5 → 90:10 → 85:15. Yield: 28 mg (84%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1625.4360 $[M + Na]^+$; calcd: 1625.4354 ($C_{79}H_{76}Cl_2N_{10}O_{23}Na^+$).

Compound 14b

13b (81 mg, 45 µmol) was deprotected according to General procedure B. Eluent: MeCN:H_2O = 95:5 → 90:10 → 85:15. Yield: 33 mg (45%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1631.4857 $[M + H]^+$; calcd: 1631.4848 ($C_{81}H_{81}Cl_2N_{10}O_{23}^+$).

Compound 14c

13c (85 mg, 46.5 µmol) was deprotected according to General procedure B. Eluent: MeCN:H_2O = 95:5 → 90:10 → 85:15. Yield: 38 mg (49%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1659.5162 $[M + H]^+$; calcd: 1659.5161 ($C_{83}H_{85}Cl_2N_{10}O_{23}^+$).

Compound 14d

13d (62 mg, 33.4 µmol) was deprotected according to General procedure B. Eluent: Tol:MeOH = 1:1. Yield: 36 mg (63%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1709.5296 $[M + Na]^+$; calcd: 1709.5293 ($C_{85}H_{88}Cl_2N_{10}O_{23}Na^+$).

AMIDES (Scheme 3)

Compound 15a

Synthesis of diethylaminopropyl amide of teicoplanin A_3-2 pseudoaglycone (**10**) [31]: teicoplanin (1.2 g, ~0.64 mmol) was suspended in DMF (8.0 ml) followed by the addition of *N*,*N*-diethyl-1,3-propanediamine (252 µl, 1.6 mmol, 2.5 equiv.). After 10 min of stirring at room temperature, PyBOP® (333 mg, 1.0 equiv.) was added, then every half hour additional amine (50 µl, 0.32 mmol, 0.5 equiv.) was added for four times. By this time the mixture became homogenous. After 2 h an additional portion of PyBOP®

(166 mg, 0.32 mmol, 0.5 equiv.) was added, followed by another 3 h of stirring, after which diethyl ether (200 ml) was added to the mixture. The precipitated solid was filtered off and washed with ether, then dried in vacuo. The crude product was dissolved in c. HCl (5.0 ml) while cooling in an ice bath, then stirred for 4.5 h. The pH of the reaction mixture was set between 8 and 9 by adding 2 M NaOH while cooling in an ice bath, and stirring vigorously. The precipitated solid was filtered off and washed with a small amount of dilute NaOH solution pH = 8–9. The crude product was dissolved in a MeCN:H_2O 1:1 mixture, silica gel was added, the mixture was evaporated and the product was purified by flash column chromatography. Eluent: MeCN:H_2O = 85:15 → 80:20 → 75:25 → 70:30 → 65:35 (+0.2% V/V AcOH). Yield: 464 mg (50% for two steps) white powder. Synthesis of **15a**: teicoplanin A_3-2 diethylaminopropyl amide (92 mg, 60.8 µmol) was dissolved in DMF (1.0 ml) and Et_3N (17 µl, 121.5 µmol, 2.0 equiv.) was added. Then, *N*-phenyl-*N*′-hexylcarbodiimide (15 mg, 73 µmol, 1.2 equiv.) in DMF (1.0 ml) was added dropwise in 8–10 aliquots in the course of about 2 h. After another 2 h of stirring at room temperature diethyl ether (75 ml) was added, and the precipitated solid was filtered off and washed with additional diethyl ether. The crude product was dissolved in a MeCN:H_2O 1:1 mixture, silica gel was added, the mixture was evaporated and the product was purified by flash column chromatography. Eluent: MeCN:H_2O = 90:10 → 85:15 → 80:20 → 75:25 (+0.2%V/V AcOH). Yield: 30 mg (29%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1715.5894 $[M + H]^+$; calcd: 1715.5899 ($C_{86}H_{93}Cl_2N_{12}O_{22}^+$).

Compound 15b

Teicoplanin A_3-2 diethylaminopropyl amide (92 mg, 60.8 µmol) was dissolved in DMF (1.0 ml). The reaction and isolation was carried out as described for **15a**. Reactants: Et_3N (17 µl, 121.5 µmol), *N*-phenyl-*N*′-octylcarbodiimide (17 mg,73 µmol). Eluent: MeCN:H_2O = 90:10 → 85:15 → 80:20 → 75:25 (+0.2%V/V AcOH). Yield: 48 mg (46%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1743.6226 $[M + H]^+$; calcd: 1743.6212 ($C_{88}H_{97}Cl_2N_{12}O_{22}^+$).

Compound 15c

Teicoplanin A_3-2 pseudoaglycone diethylaminopropyl amide (95 mg, 62.7 µmol) was dissolved in DMF (1.5 ml). The reaction and isolation was carried out as described for **15a**. Reactants: Et_3N (17.4 µl, 125 µmol), *N*-phenyl-*N*′-decylcarbodiimide (20 mg, 75.4 µmol). Eluent: MeCN:H_2O = 90:10 → 85:15 → 80:20 → 75:25 (+0.2%V/V AcOH). Yield: 79 mg (71%). NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1771.6527 $[M + H]^+$; calcd: 1771.6525 ($C_{90}H_{101}Cl_2N_{12}O_{22}^+$).

Compound 15d

Teicoplanin A_3-2 pseudoaglycone diethylaminopropyl amide (140 mg, 92.5 µmol) was dissolved in DMF (1.5 ml). The reaction and isolation was carried out as described for **15a**. Reactants: Et_3N (26 µl), *N*-phenyl-*N*′-dodecylcarbodiimide (32 mg,0.11 mmol). Eluent: MeCN:H_2O = 90:10 → 85:15 → 80:20 → 75:25 (+0.1%V/V AcOH). Yield: 78 mg (47%) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1799.6839 $[M + H]^+$; calcd: 1799.6838 ($C_{92}H_{105}Cl_2N_{12}O_{22}^+$).

Compound 16b

Synthesis of dimethylaminopropyl amide of teicoplanin (**11**) [31]: teicoplanin (600 mg, ~0.32 mmol) was suspended in DMF (5.0 ml) followed by the addition of *N*,*N*-dimethyl-1,3-propanediamine (100 µl, 0.8 mmol, 2.5 equiv.). After 10 min of stirring at room temperature PyBOP® (166 mg, 1.0 equiv.) was added, then every half hour additional amine (20 µl) was added for four times. After the reaction mixture became homogenous, another portion of PyBOP® (83 mg, 0.16 mmol, 0.5 equiv.) was added. After 1 h of stirring ether:ethyl acetate = 1:1 mixture (100 ml) was added to precipitate the product, which was filtered off and washed further with ether. The crude product was dissolved in a MeCN:H_2O 1:1 mixture, silica gel was added, the mixture was evaporated and the product was purified by flash column chromatography. Eluent: MeCN:H_2O = 80:20 → 75:25 → 70:30 (+0.1%V/V AcOH). Yield: 362 mg (58%). Synthesis and isolation of the guanidine derivative: see **15a**. Reactants: 115 mg (58 µmol) teicoplanin dimethylaminopropyl amide, Et_3N (2.0 equiv., 116 µmol, 16 µl), *N*-phenyl-*N*′-octylcarbodiimide (1.2 equiv., 70 µmol, 16 mg). Eluent: MeCN:H_2O = 80:20 → 75:25 (+0.1%V/V AcOH). Yield: 55 mg (43 %) white powder. NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 2194.8557 $[M + H]^+$; calcd base peak: 2194.8495 (components A_2-2, 3) ($C_{108}H_{132}Cl_2N_{13}O_{32}^+$).

Compound 16e

See the synthesis and isolation of **16b** above. Reactants: teicoplanin dimethylaminopropyl amide (115 mg, 58 µmol), Et_3N (16 µl, 116 µmol, 2.0 equiv.), *N*-phenyl-*N*′-4-phenylbenzyl carbodiimide (20 mg, 70 µmol, 1.2 equiv.). Eluent: MeCN:H_2O = 80:20 → 75:25 (+0.1%V/V AcOH). Yield: 68 mg (52%) white powder. NMR data and spectra

can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1124.9060 $[M + 2H]^{2+}$; calcd base peak: 1124.9050 (components A_2-2,3) ($C_{113}H_{127}Cl_2N_{13}O_{32}^{2+}$).

Compound 16f

See the synthesis and isolation of **16b** above. Reactants: teicoplanin dimethylaminopropyl amide (115 mg, 58 µmol), Et_3N (16 µl, 116 µmol, 2.0 equiv.), *N*-phenyl-*N′*-(1-benzyl-4-piperidyl) carbodiimide (25 mg, 0.087 mmol, 1.5 equiv.). After 3 h sufficient conversion was achieved (checked by Cellulose F TLC—eluent: *n*-PrOH: c. NH_4OH = 7:3). ether: ethyl acetate = 1:1 mixture (100 ml) was added to precipitate the product, which was filtered off and washed with ether. The crude product was dissolved in MeCN:H_2O: acetone 1:1:1 mixture (1.0 ml), and applied to a column loaded with Sephadex® LH-20 gel in the same solvent mixture. The obtained product was purified further by flash column chromatography. Eluent: MeCN:H_2O = 80:20 → 75:25 → 70:30 (+0.1%V/V AcOH). Yield: 35 mg (27%). NMR data and spectra can be found in the supporting information (Table S1). ESI-HRMS: *m/z* 1128.4303 $[M + 2H]^{2+}$; calcd base peak: 1128.5078 (components A_2-2,3) ($C_{112}H_{132}Cl_2N_{14}O_{32}^{2+}$).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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