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ORIGINAL ARTICLE

Lipophilic teicoplanin pseudoaglycon derivatives are active against vancomycin- and teicoplanin-resistant enterococci

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A selection of nine derivatives of teicoplanin pseudoaglycon were tested *in vitro* against clinical vancomycin-resistant *Enterococcus* strains possessing *vanA*, *vanB* or both genes. The bacteria were characterized by PCR for the identification of their resistance genes. The tested compounds contain lipoic acid, different carbohydrates and aryl groups as lipophilic moieties. About one-third of the teicoplanin-resistant strains were shown to be susceptible to one or more of the glycopeptide derivatives.
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INTRODUCTION

In recent years, the emergence of widespread antibiotic resistance has been getting increasing publicity, while there is also a worldwide attempt to provide financial support for the research and development of new antibiotics.[1,2] Also, numerous events are organized to raise awareness to this global health-care crisis. Although the discovery of effective antibiotics against resistant Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, etc. are of main interest, many resistant Gram-positive bacteria (for example, *Clostridium difficile*, multidrug-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci) are also found on the list of the most dangerous organisms causing life-threatening infections,[3,4] therefore constant research in this area should not be neglected.

An enormous collection of derivatives of glycopeptide antibiotics has been synthesized by numerous research groups throughout the years[5–12] to yield compounds that are active against resistant Gram-positive bacteria, unlike the original molecules. In several cases, the chemical modifications did not only improve the antibacterial spectrum or activity but also many of them came out to be superior to the parent compounds. The successful development and approval of telavancin,[13] dalbavancin[14] and oritavancin[15] proves that semisynthetic work, especially lipophilic derivatization, is undoubtedly useful for preparing compounds with favorable biological characteristics.

Besides others, we have been working on the chemical transformations of glycopeptide antibiotics for a long time. Our synthetic work has essentially been focused on derivatives of the acid hydrolysis products of glycopeptides; that is, aglycons or teicoplanin pseudoaglycon. In order to obtain new derivatives with improved antibacterial activity, in the synthetic work we have carried out in this field, we introduced a large series of more or less lipophilic groups at the *N*-terminal position of aglycons or pseudoaglycons prepared from ristocetin, teicoplanin or dechloroteicoplanin.[16–19] Compounds obtained in this way form nanosized aggregates in water.[20,21] This phenomenon; that is, formation of multivalent clusters, can be one of the explanations of their improved antibacterial activity. In the sense of antibacterial activity, our best results were mostly achieved by starting from the *N*-acetylglucosaminyl teicoplanin aglycon. The antibacterial activity of these compounds has been evaluated on a standard panel of Gram-positive bacteria, including one vancomycin and one teicoplanin-resistant *Enterococcus* strain possessing resistance genes *vanB* or *vanA* accordingly, but none of the compounds have ever been tested against multiple clinical strains of vancomycin-resistant *Enterococcus* (VRE).

Although, as mentioned, several large systematic series of teicoplanin derivatives are found in the literature, the panel of bacteria for the measurement of bioactivity rarely includes more than a few glycopeptides-resistant *Enterococcus* strains and only a limited number of derivatives (mainly candidates for further development) are tested against larger collections of VRE. Here we wish to report the antibacterial activity of nine of our most active teicoplanin pseudoaglycon derivatives from recent years' work against a collection of clinical isolates of VRE.

MATERIALS AND METHODS

General information

Optical rotations were measured at room temperature with a Perkin-Elmer 241 automatic polarimeter (Perkin-Elmer, Waltham, MA, USA). TLC was performed on Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) with detection by immersing into 5% ethanolic sulfuric acid/ammonium molybdate solution followed by heating, or in the case of the teicoplanin derivatives, Pauly's reagent was used for detection. Flash column chromatography was performed on Silica

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gel 60 (Merck 0.040–0.063 mm). Organic solutions were dried over Na_2SO_4 and concentrated in vacuum. The ^1H NMR (360, 400 and 500 MHz) and ^{13}C NMR (91, 101 and 125.76 MHz) spectra were recorded with Bruker DRX-360, DRX-400 and Bruker Avance II 500 spectrometers at 25 °C. Chemical shifts are referenced to Me_4Si (0.00 p.p.m. for ^1H) and to the solvent signals (dimethylsulfoxide (DMSO)-d_6: 2.50 p.p.m. for ^1H and $CDCl_3$: 77.16 p.p.m., DMSO-d_6: 39.52 p.p.m. for ^{13}C). Matrix assisted laser desorption/ionization–time of flight MS (MALDI-TOF-MS) analyses of the compounds were carried out in the positive reflectron mode using a BIFLEX III mass spectrometer (Bruker, Bremen, Germany) equipped with delayed-ion extraction. 2,5-Dihydroxybenzoic acid was used as matrix and CF_3COONa as cationizing agent in dimethylformamide (DMF). ESI-TOF MS spectra were recorded by a microTOF-Q type QqTOFMS mass spectrometer (Bruker) in the positive ion mode using MeOH as the solvent. Elemental analysis was performed on an Elementar Vario MicroCube instrument. The log*P* values were calculated using the log*P* calculation plugin of Marvin Sketch (version 16.5.2) from ChemAxon (Budapest, Hungary) using the Consensus Method with electrolyte concentrations of 0.1 M.

Bacterial strains

The Department of Medical Microbiology, Semmelweis University, Budapest, Hungary cultured and characterized 44 VRE strains. All the strains were isolated from routine laboratory samples of patients suffering from wound, urinary tract or blood stream infections. Clinical samples were cultured on COS agar (Columbia agar+5% sheep blood, Biomérieux, Budapest, Hungary), and vancomycin-resistant strains were isolated on VRE chromogenic medium (Biomérieux). Species of strains were identified by MALDI-TOF-MS. Minimum inhibitory concentrations (MICs; $mg\,l^{-1}$) were determined for vancomycin and teicoplanin on Mueller Hinton E agar (Biomérieux using MIC strip tests (Liofilchem, Roseto degli Abruzzi, Italy), according to the manufacturer's instructions, and by using a direct colony suspension equivalent to a McFarland standard of 0.5. Testing conditions also included incubation at 35.5 °C for 24 h. All results were interpreted by using breakpoints for susceptibility and resistance according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[22] Concerning vancomycin/teicoplanin MIC breakpoints, strains with an MIC > 4 or 2 $mg\,l^{-1}$ were considered to be vancomycin- or teicoplanin-resistant, respectively.

MIC of lipophilic teicoplanin pseudoaglycon derivatives

The efficacy of the prepared compounds was determined with the broth microdilution method according to the EUCAST guideline. Bacterial strains were grown on COS agar at 35.5 °C overnight. Appropriate numbers of colonies were suspended in physiological saline in order to reach the density of 0.5 McFarland for inoculation.

Stock solutions containing the substances were prepared in distilled water and DMSO (1:1). These were two-fold serially diluted from 20 to 0.044 $mg\,l^{-1}$ in cation-adjusted Mueller–Hinton broth (Biolab, Budapest, Hungary) and 100 µl of each dilution was transferred into microplate holes. Inoculation was carried out with 10 µl of each bacterial suspension. Incubation was performed at 35 °C for 24 h and determination of MIC was made with the naked eyes on a black background.

Determination of *vanA* and *vanB* genes

Enterococcus strains were retrieved from storage at − 80 °C on Cryobank breads (Mast Diagnostica, Reinfeld, Germany), cultured on COS agar and incubated overnight at 35.5 °C. A turbid suspension of the subculture was made in PCR water and DNA was isolated with the GeneAll Ribospin Extraction Kit (Geneall Biotechnology, Seoul, Korea).

The presence of glycopeptides-resistance genes was investigated by PCR using primers for the identification of *vanA* or *vanB* sequence described by Dutka-Malen *et al.*[23] The full reaction mixture (25 µl) included 12.5 µl master mix (ImmoMix Red, BIOLINE, London, UK), 0.5 µl forward primer, 0.5 µl reverse primer, 6.5 µl PCR water and 5 µl DNA template. PCR thermal profile was 94 °C 2 min, (92 °C 1 min, 54 °C 1 min, 72 °C 1 min) 30 cycles, 72 °C 10 min. PCR products were detected by Gelgreen nucleic acid gel stain (BIOLINE) in 2% agarose under UV light.

Compound 1

Teicoplanin pseudoaglycon **10** (200 mg, 0.14 mmol) was dissolved in dry DMF (3 ml) and Et_3N (20 µl) and compound **11**[24] (57.6 mg, 0.19 mmol) was added. After 3 h, TLC showed complete disappearance of starting material **10**. The reaction mixture was concentrated and the residue was purified by flash column chromatography (toluene:MeOH 4:6+1% acetic acid) to give **1** (153 mg, 68%) as an off-white powder. MALDI-TOF-MS *m/z* 1611.35 $(M+Na)^+$; calcd. 1611.32 for $C_{74}H_{70}Cl_2N_8NaO_{24}S_2$.

Compound 2

Teicoplanin pseudoaglycon **10** (200 mg, 0.14 mmol) was dissolved in dry DMF (3 ml) and Et_3N (20 µl, 0.14 mmol) and compound **12**[24] (77.2 mg, 0.19 mmol) was added. After 3 h, TLC showed complete disappearance of starting material **10**. The reaction mixture was concentrated and the residue was purified by flash column chromatography (toluene:MeOH 1:1+1% acetic acid) to give **2** (166 mg, 68%) as an off-white powder. MALDI-TOF-MS *m/z* 1724.45 $(M+Na)^+$; calcd. 1724.41 for $C_{80}H_{81}Cl_2N_9NaO_{25}S_2$.

Methyl 2,3-di-*O*-*n*-butyl-4,6-*O*-*p*-methoxybenzylidene-α-D-glucopyranoside (14)

To the mixture of NaH (50% in mineral oil, 922 mg, 19.2 mmol) washed with hexane and dry DMF (30 ml), methyl 4,6-*O*-*p*-methoxybenzylidene-α-D-glucopyranoside **13**[25] (1.5 g, 4.8 mmol) was added, and the suspension was stirred for 45 min under argon. Then *n*-butyl bromide (1.55 ml, 14.4 mmol) was added in four portions in an hour. After two days, MeOH (2 ml) and distilled water (50 ml) was added carefully, and the mixture was stirred for half an hour. The precipitated solids were filtered off and purified by flash chromatography (*n*-hexane:acetone 9:1) to give **14** (1.30 g, 64%) as a white powder. $[\alpha]_D^{25}$ +46.7 (c 0.08, $CHCl_3$) MALDI-TOF-MS *m/z* 447.36 $(M+Na)^+$; calcd. 447.24 for $C_{23}H_{36}NaO_7$. ^1H NMR (400 MHz, $CDCl_3$) δ (p.p.m.): 7.43 (d, $J=8.7$ Hz, 2H, aromatic), 6.90 (d, 2H, $J=8.7$ Hz aromatic), 5.51 (s, 1H, H acetalic), 4.81 (d, $J=3.6$ Hz, 1H, H-1), 4.27 (dd, $J=9.8$, 4.5 Hz, 1H, CH_2), 3.85–3.60 (m, 10H), 3.50 (t, $J=9.3$ Hz, 1H), 3.45 (s, 3H, OMe), 3.37 (dd, $J=9.3$, 3.7 Hz, 1H, H-2), 1.63–1.52 (m, 4H, butyl CH_2), 1.46–1.33 (m, 4H, butyl CH_2), 0.94 (t, $J=7.4$Hz, 3H, butyl CH_3), 0.89 (t, $J=7.4$Hz, 3H, butyl CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (p.p.m.): 160.3, 130.0, 127.3, 113.5 (6C, aromatic), 101.2 (1C, acetalic), 99.1 (1C, C-1), 82.0, 80.4, 78.3 (3C, skeleton), 73.4, 72.0 (2C, butyl CH_2), 69.0 (1C, C-6), 62.4 (1C, C-5), 55.2 (2C, 2 OMe), 32.3, 32.1, 19.2, 19.1 (4C, butyl CH_2), 13.8 (2C, butyl CH_3).

Methyl 2,3-di-*O*-*n*-butyl-4-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (15)

p-Methoxybenzylidene derivative **14** (1.20 g, 2.24 mmol) was dissolved in a mixture of dry CH_2Cl_2 (20 ml) and dry Et_2O (7 ml) under an argon atmosphere. The stirred mixture was cooled to 0 °C, and $LiAlH_4$ (382 mg, 10.1 mmol) was added in four portions. Dry Et_2O (7 ml) was cooled to 0 °C by the use of drying tube filled with $CaCl_2$, and $AlCl_3$ (447 mg, 3.4 mmol) was added. The solution of $AlCl_3$ was stirred for 5 min, then it was added to the previously prepared mixture containing the *p*-methoxybenzylidene derivative and it was stirred at 0 °C under an argon atmosphere. After 1 h, ethyl acetate (45 ml) was added to the reaction mixture and it was stirred for 10 min, then water (10 ml) was added, and the mixture was stirred for further 10 min. The mixture was filtered through a Celite pad (Sigma Aldrich, Budapest, Hungary), and the solid part was washed with ethyl acetate. The filtrate was diluted with ethyl acetate (100 ml) and washed twice with water (2 × 40 ml). The organic solution was dried and concentrated. The residue was purified by flash column chromatography (*n*-hexane:acetone 8:2) to yield **15** (902 mg, 75%) as a colorless syrup. $[\alpha]_D^{25}$ +78.43 (c 0.07, $CHCl_3$); MALDI-TOF-MS *m/z* 449.36 $(M+Na)^+$; calcd. 449.25 for $C_{23}H_{38}NaO_7$. ^1H NMR (400 MHz, $CDCl_3$) δ (p.p.m.): 7.29 (d, $J=8.8$ Hz, 2H, aromatic), 6.90 (d, $J=8.6$ Hz, 2H, aromatic), 4.85 (d, $J=10.7$ Hz, 1H, benzyl CH_2), 4.78 (d, $J=3.5$ Hz, 1H, H-1), 4.60 (d, $J=10.7$ Hz, 1H, benzyl CH_2), 3.94–3.86 (m, 1H); 3.82 (s, 3H, OMe), 3.81–3.57 (m, 4H), 3.46–3.44 (m, 1H), 3.40 (s, 3H, OMe), 3.30 (dd, $J=9.6$, 3.6 Hz, 1H, H-2), 1.67–1.57 (m, 4H, butyl CH_2), 1.48–1.36 (m, 4H, butyl CH_2), 0.95 (t, 6H, $J=7.4$ Hz, butyl CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (p.p.m.): 159.3, 130.4, 129.8, 113.9 (6C, aromatic), 98.0 (1C, C-1), 81.6, 80.9, 77.3 (3C, skeleton), 74.6 (1C, benzyl CH_2), 73.4, 71.4

(2C, butyl *C*H$_2$), 70.6 (1C, C-5), 62.0 (1C, C-6), 55.2, 55.1 (2C, OMe), 32.7, 32.1, 19.4, 19.1 (4C, butyl *C*H$_2$), 14.1, 14.0 (2C, butyl *C*H$_3$).

Methyl 2,3-di-*O*-*n*-butyl-4-*O*-*p*-methoxybenzyl-6-*O*-(2,5,8,11, 14-pentaoxaheptadec-16-ynyl)-α-D-glucopyranoside (16)

To the solution of sugar derivative **15** (500 mg, 1.17 mmol) in dry DMF (20 ml), NaH was added (50% in mineral oil, 112 mg, 2.34 mmol), and the mixture was stirred for 20 min under an argon atmosphere. The solution of triethylene glycol 2-bromoethyl propargyl ether[26] (414 mg, 1.40 mmol) in dry DMF (2 ml) was added to the mixture and it was stirred overnight. Additional NaH (50%, 50 mg) and triethylene glycol 2-bromoethyl propargyl ether (50 mg) were added to the reaction mixture, and it was stirred at 60 °C for 2 days. The reaction was quenched by the addition of MeOH (2 ml) and water (2 ml) and the mixture was stirred for 10 min. After removing the solvents under vacuum, the residue was purified by flash chromatography (*n*-hexane: acetone 95:5) to yield **16** (360 mg, 48%) as a colorless syrup. $[\alpha]_D^{25}$ +63.40 (c 1.4, $CHCl_3$) ESI-TOF-MS *m/z* 663.3728 $(M+Na)^+$; calcd. 663.3715 for $C_{34}H_{56}NaO_{11}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ (p.p.m.) 7.31–7.23 (m, 2H, aromatic); 6.90–6.84 (m, 2H, aromatic); 4.83-4.75 (m, 2H, benzyl C*H*$_2$ and H-1); 4.57 (d, $J=10.5$ Hz, 1H, benzyl C*H*$_2$); 4.19 (d, $J=2.3$ Hz, 2H, propargyl C*H*$_2$); 3.89-3.81 (m, 1H); 3.80 (s, 3H, OMe); 3.76-3.53 (m, 23H); 3.49 (t, $J=9.2$ Hz, 1H); 3.38 (s, 3H, OMe); 3.30 (dd, $J=9.7$, 3.5 Hz, 1H); 2.43 (t, $J=2.3$ Hz, 1H, propargyl C*H*); 1.67-1.52 (m, 4H, butyl C*H*$_2$); 1.46-1.32 (m, 4H, butyl C*H*$_2$); 0.92-0.83 (m, 6H, butyl C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ (p.p.m.) 159.2, 130.7, 129.5, 113.7 (6C, aromatic), 98.0 (1C, C-1), 81.6, 80.7, 77.3, 70.0 (4C, skeleton), 74.6, 74.4, 73.3, 71.2, 70.8, 70.7, 70.6, 70.5, 70.4, 70.0, 69.8 (11C, O*C*H$_2$ tetraethyleneglycol, O*C*H$_2$ butyl, O*C*H$_2$ PMB (*p*-methoxybenzyl)), 58.3 (1C, C-6), 55.2, 55.0 (2C, OMe), 32.63, 32.02, 19.33, 19.05, (4C, *C*H$_2$ butyl), 14.0, 13.8 (2C, *C*H$_3$ butyl).

Methyl 2,3-di-*O*-*n*-butyl-6-*O*-(2,5,8,11,14-pentaoxaheptadec-16-ynyl)-α-D-glucopyranoside (17)

Compound **16** (225 mg, 0.35 mmol) and DDQ (2,3-dichloro-5,6-dicyano-1, 4-benzoquinone; 120 mg, 0.53 mmol) were dissolved in CH_2Cl_2:water 9:1 (25 ml), and the mixture was stirred vigorously. After 1 h, the reaction mixture was diluted with CH_2Cl_2 (300 ml), washed with saturated aq $NaHCO_3$ (2×50 ml) and with water (50 ml). The organic phase was dried and concentrated. The residue was purified by flash column chromatography to yield **17** (153 mg, 84%) as a colorless syrup. $[\alpha]_D^{25}$ +48.26 (c 0.09, $CHCl_3$); MALDI-TOF-MS *m/z* 655.45 $(M+Na)^+$; calcd. 655.44 for $C_{34}H_{64}NaO_{10}$. ^{1}H NMR (360 MHz, $CDCl_3$) δ (p.p.m.): 4.79 (d, $J=3.5$ Hz, 1H, H-1), 4.21 (d, $J=2.4$ Hz, 2H, propargyl CH_2), 3.91-3.82 (m, 1H), 3.80-3.48 (m, 25H); 3.41 (s, 3H, OMe), 3.29 (dd, $J=9.1$, 3.5 Hz, 1H, H-2), 2.45 (t, $J=2.4$ Hz, 1H, propargyl C*H*), 1.62-1.51 (m, 4H, butyl C*H*$_2$), 1.43-1.32 (m, 8H, butyl C*H*$_2$), 0.91 (t, $J=7.4$ Hz, 6H, C*H*$_3$). ^{13}C NMR (91 MHz, $CDCl_3$) δ (p.p.m.): 98.0 (1C, C-1), 80.9, 80.3, 70.0 (3C, skeleton) 74.4, 73.1, 70.9, 70.7, 70.3, 70.0 (O*C*H$_2$), 68.9 (1C, C-6), 58.2, 55.0 (1C, OMe), 32.3, 31.9, 19.1, 19.0 (4C, *C*H$_2$ butyl), 13.8, 13.7 (2C, *C*H$_3$ butyl).

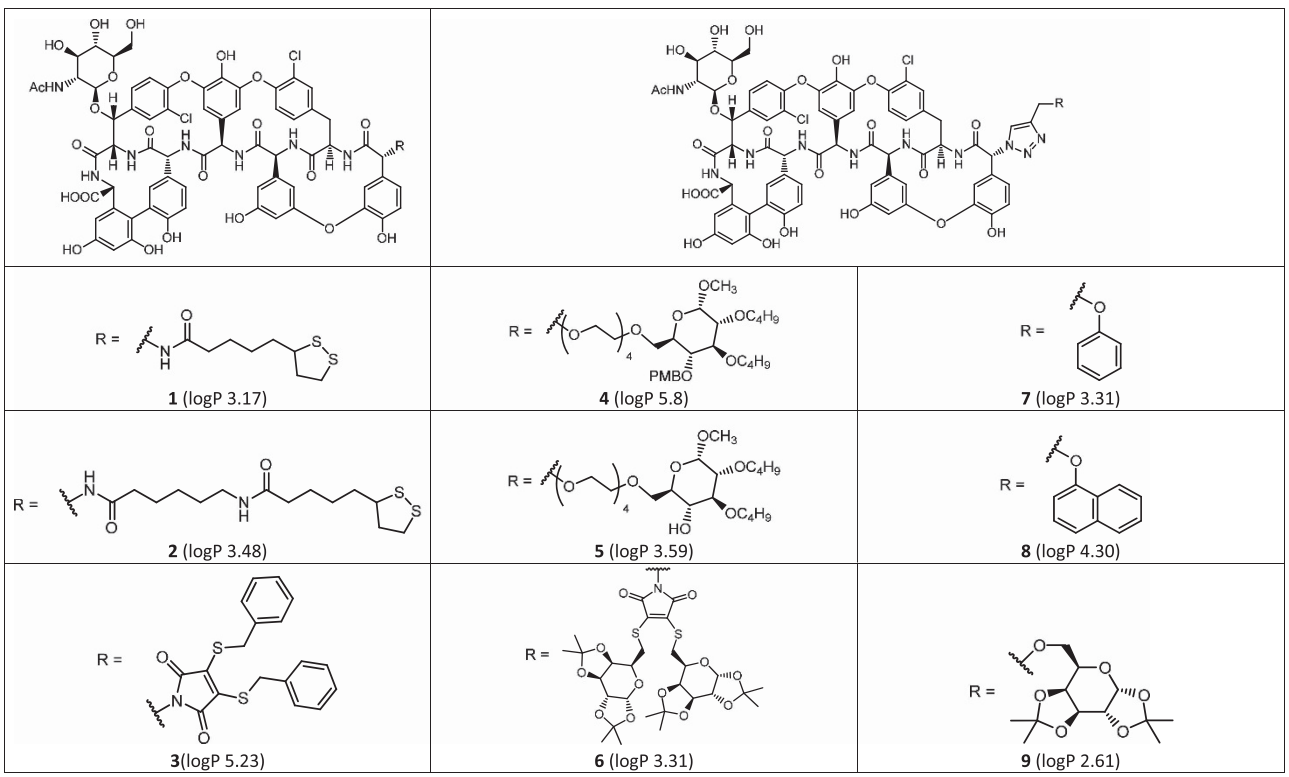
Compound 4

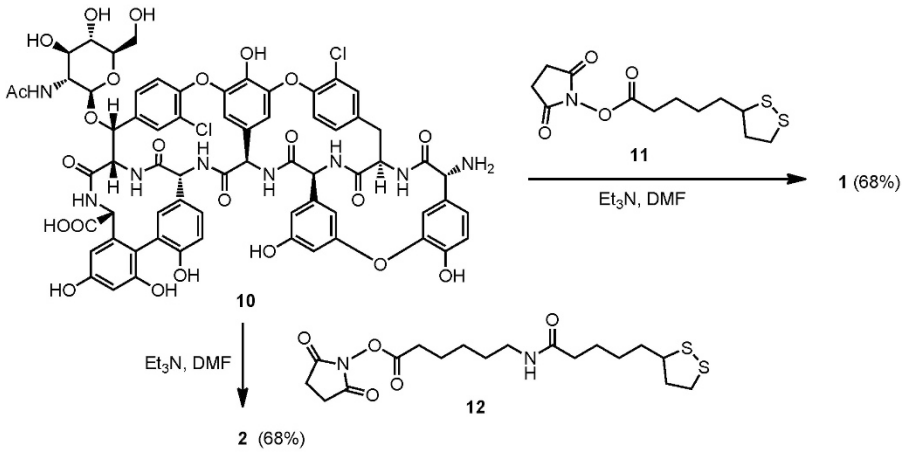
To the solution of azido teicoplanin pseudoaglycon **18**[20] (150 mg, 0.11 mmol) and the propargyl derivative **16** (81 mg, 0.126 mmol) in dry DMF (5 ml), CuI (2 mg, 0.011 mmol) and Et_3N (0.11 mmol, 15 µl) were added under an argon atmosphere and the mixture was stirred for 20 h. The mixture was concentrated and the residue was purified by flash column chromatography (toluene:MeOH 6:4+0.1% acetic acid) to yield **4** (84 mg, 39%) as a white powder. MALDI-TOF-MS *m/z* 2089.66 $(M+Na)^+$; calcd. 2089.66 for $C_{100}H_{112}Cl_2N_{10}NaO_{34}$.

Compound 5

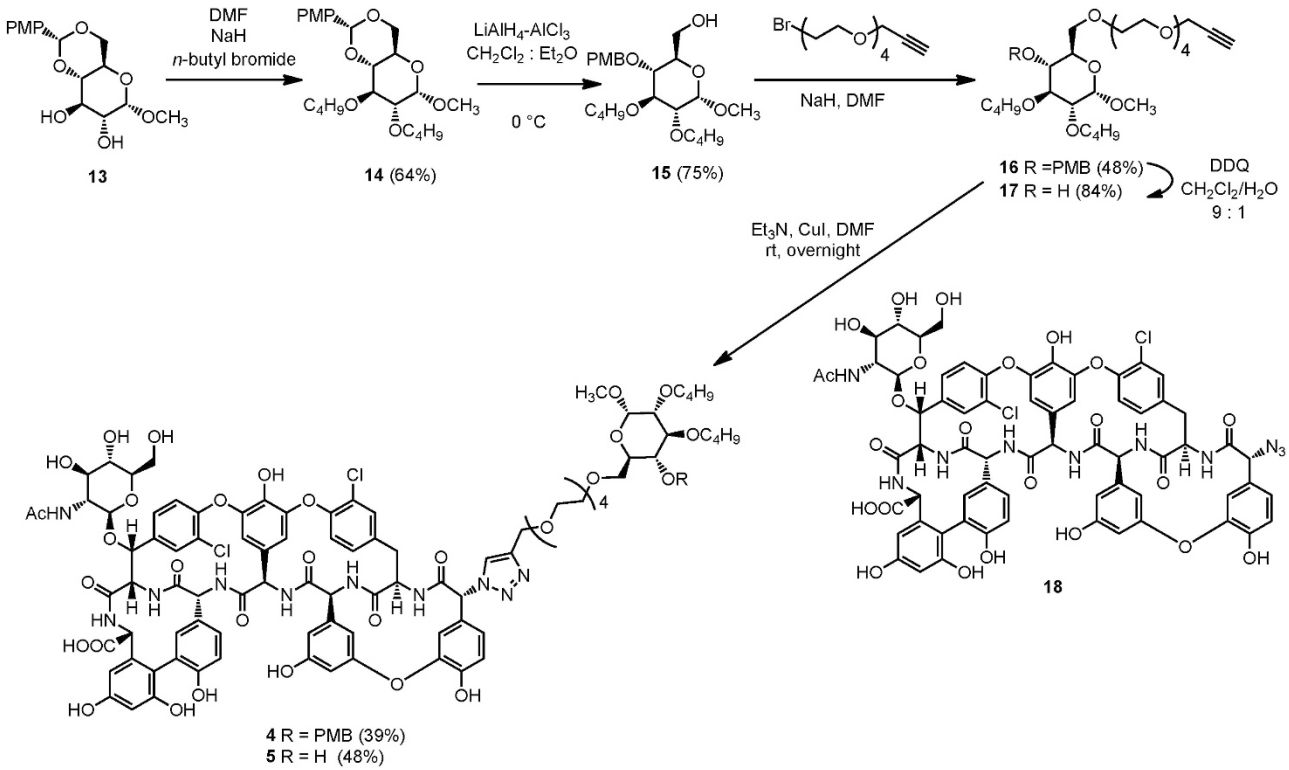
To the solution of azido teicoplanin pseudoaglycon **18** (150 mg, 0.11 mmol) and the propargyl derivative **17** (66 mg, 0.126 mmol) in dry DMF (5 ml), CuI (2 mg, 0.01 mmol) and Et_3N (0.11 mmol, 15 µl) were added under an argon atmosphere and the mixture was stirred for 2 days and at 60 °C for a day. The mixture was concentrated and the residue was purified by flash chromatography (toluene:MeOH 6:4+0.1% acetic acid) to yield **5** (98 mg, 48%) as a white powder. MALDI-TOF-MS *m/z* 1969.60 $(M+Na)^+$; calcd. 1969.60 for $C_{92}H_{104}Cl_2N_{10}NaO_{33}$.

Table 1 Structure and lipophilicity of teicoplanin pseudoaglycon derivatives 1–9

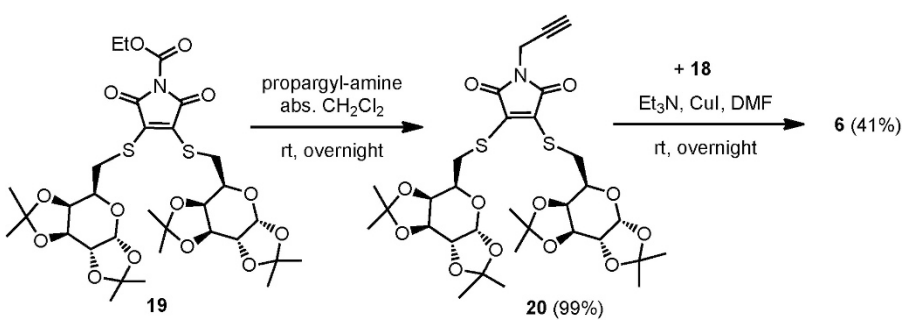




Scheme 1 Conjugation of lipoic acid derivatives **11** and **12** to the *N*-terminal part of teicoplanin pseudoaglycon.



Scheme 2 Derivatization of azido teicoplanin pseudoaglycon with sugar-based lipophilic moieties by CuAAC.



Scheme 3 Coupling of bis-(diisopropylidene-α-D-Galp-6-thio)-maleimide **20**–**18** by CuAAC.

Table 2 MIC of compounds 1–9 against VanB enterococci in μg ml^{-1}

Number	*Strain*	*PCR*	*Vancomycin*	*Teicoplanin*	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***
1	*E. faecium* 217/2014	vanB	48	1.5	1.25	5	1.25	1.25	5	5	1.25	1.25	5
2	*E. faecalis* 312/2014	vanB	48	1.5	0.625	10	0.625	2.5	5	1.25	2.5	1.25	20
3	*E. faecium* 821/2014	vanB	48	1.5	0.625	5	2.5	1.25	10	2.5	1.25	0.625	10
4	*E. faecium* 1256/2014	vanB	48	1	1.25	5	1.25	1.25	2.5	5	1.25	0.3125	10
5	*E. faecium* 3025/2014	vanB	12	2	1.25	5	1.25	1.25	5	1.25	0.625	0.625	5
6	*E. faecalis* 4035/2014	vanB	48	2	2.5	20	0.625	2.5	10	5	1.25	1.25	10
7	*E. faecium* 6644/2014	vanB	48	2	1.25	2.5	2.5	2.5	10	10	0.3125	0.3125	10
8	*E. faecium* 8976/2014	vanB	48	2	1.25	5	2.5	2.5	5	5	1.25	0.625	5

Abbreviation: MIC, minimum inhibitory concentration.

Table 3 MIC of compounds 1–9 against VanA enterococci in μg ml^{-1}

Number	*Strain*	*PCR*	*Vancomycin*	*Teicoplanin*	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***
9	*E. faecium* 276/2014	vanA	>96	96	>20	>20	>20	>20	>20	>20	>20	>20	>20
10	*E. faecium* 298/2014	vanA	>96	12	2.5	20	5	5	20	20	5	1.25	20
11	*E. faecalis* 675/2014	vanA	>96	>96	10	>20	2.5	20	>20	5	2.5	0.3125	>20
12	*E. faecalis* 863/2014	vanA	>96	32	>20	>20	>20	>20	>20	>20	>20	>20	>20
13	*E. faecalis* 1022/2014	vanA	>96	>96	>20	>20	10	10	>20	5	10	10	>20
14	*E. faecium* 1057/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
15	*E. faecium* 1788/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
16	*E. faecium* 1876/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
17	*E. faecium* 1997/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
18	*E. faecium* 2034/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
19	*E. faecium* 2076/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
20	*E. faecium* 3016/2014	vanA	>96	96	>20	>20	>20	>20	>20	>20	>20	>20	>20
21	*E. faecium* 4067/2014	vanA	>96	64	>20	>20	>20	>20	>20	>20	>20	>20	>20
22	*E. faecium* 4167/2014	vanA	>96	64	>20	>20	>20	>20	>20	>20	>20	>20	>20
23	*E. faecium* 4235/2014	vanA	>96	24	>20	5	10	5	>20	>20	2.5	0.3125	>20
24	*E. faecium* 5321/2014	vanA	>96	24	>20	>20	>20	>20	>20	>20	>20	>20	>20
25	*E. faecium* 5674/2014	vanA	>96	24	>20	>20	>20	>20	>20	>20	>20	>20	>20
26	*E. faecium* 6100/2014	vanA	>96	24	>20	>20	>20	>20	10	5	2.5	0.625	>20
27	*E. faecium* 6421/2014	vanA	>96	96	>20	>20	>20	>20	>20	>20	>20	>20	>20
28	*E. faecium* 6823/2014	vanA	>96	16	>20	>20	>20	>20	>20	>20	>20	>20	>20
29	*E. faecium* 7009/2014	vanA	>96	64	>20	>20	>20	>20	>20	>20	>20	>20	>20
30	*E. faecium* 8012/2014	vanA	>96	96	>20	>20	>20	>20	>20	>20	>20	>20	>20
31	*E. faecalis* 8046/2014	vanA	>96	64	20	10	5	10	5	2.5	2.5	0.3125	20
32	*E. faecium* 8122/2014	vanA	>96	64	>20	>20	>20	>20	>20	>20	>20	>20	>20
33	*E. faecium* 8341/2014	vanA	>96	64	>20	>20	>20	>20	>20	>20	>20	>20	>20
34	*E. faecium* 8598/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
35	*E. faecalis* 8673/2014	vanA	>96	>96	>20	10	20	5	10	>20	2.5	0.625	>20
36	*E. faecium* 9065/2014	vanA	>96	>96	20	20	10	5	5	20	5	1.25	>20
37	*E. faecium* 9181/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
38	*E. faecium* 9543/2014	vanA	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20
39	*E. faecalis* 9667/2014	vanA	>96	>96	0.3125	1.25	2.5	10	1.25	>20	0.3125	0.625	>20
40	*E. faecium* 9889/2014	vanA	>96	>96	2.5	5	2.5	5	5	20	1.25	0.625	10
41	*E. faecium* 10008/2014	vanA	>96	>96	5	20	2.5	5	20	20	2.5	1.25	20

Abbreviation: MIC, minimum inhibitory concentration.

Compound 20

To a stirred solution of compound **19**[27] (107 mg, 0.15 mmol) in CH_2Cl_2 (30 ml), propargyl amine (12 μl, 0.19 mmol) and Et_3N (26 μl, 0.19 mmol) were added under an argon atmosphere and stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 ml), washed with cc. aq NH_4Cl (20 ml) and water (2×20 ml), dried and concentrated. The crude product was purified by flash column chromatography (*n*-hexane:acetone 8:2) to give **20** (102 mg, 99%) as a yellow syrup. $[\alpha]_D^{25} = -87.5$ (c 0.11, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ (p.p.m.): 5.52 (d, J=5.0 Hz, 2H, 2xH-1), 4.70–4.52 (m, 2H), 4.44–4.13 (m, 4H), 4.06–3.85 (m, 2H), 3.62-3.56 (m, 2H), 3.46–3.30 (m, 2H), 2.17 (m, 1H), 1.63–1.51 (m, 2H), 1.46 (s, 6H), 1.44 (s, 6H), 1.33 (s, 6H), 1.32 (s, 6H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 165.3 (2×C=O), 127.6 (C=C), 113.8 (4 C, $C_{acetalic}$), 96.6 (2 C-1), 71.6, 71.0, 70.6, 68.0 (2×C-2,3,4,5), 31.7 (2×C-6), 26.0, 26.0, 24.9, 24.5 (8 C, $CH_{3,ip}$).

Compound 6

To the solution of azido teicoplanin pseudoaglycon **18** (105 mg, 0.075 mmol) and the propargyl derivative **20** (68 mg, 0.09 mmol) in dry DMF (5 ml), CuI (1.4 mg, 0.008 mmol) and Et_3N (10 μl, 0.075 mmol) were added under an argon atmosphere and the mixture was stirred overnight. The reaction mixture

Table 4 MIC of compounds 1–9 against VanA and VanB enterococci in μg ml^{-1}

Number	*Strain*	*PCR*	*Vancomycin*	*Teicoplanin*	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***
42	*E. faecium* 1333/2014	vanA, vanB	>96	12	>20	>20	>20	10	>20	>20	>20	1.25	>20
43	*E. faecium* 6713/2014	vanA, vanB	>96	32	>20	>20	>20	5	>20	>20	>20	5	>20
44	*E. faecium* 9698/2014	vanA, vanB	>96	>96	>20	>20	>20	>20	>20	>20	>20	>20	>20

Abbreviation: MIC, minimum inhibitory concentration.

Table 5 Number of vancomycin- and teicoplanin-resistant *Enterococcus* strains susceptible to teicoplanin pseudoaglycon derivatives 1–9

Compound	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***
Number of susceptible *Enterococcus* strains	5/36	5/35	9/36	11/36	6/36	4/36	11/36	13/36	2/36

was concentrated, and the crude product was purified by flash chromatography (toluene:MeOH 1:1+1% acetic acid) and gel filtration by Sephadex-LH 20 gel in methanol to give **6** (61 mg, 41%) as a yellow powder. MALDI-TOF-MS *m/z* 2132.62 $(M+Na)^+$; calcd. 2132.49 for $C_{97}H_{97}Cl_2N_{11}NaO_{35}S_2$.

RESULTS AND DISCUSSION

On the basis of the routine antibacterial screening (Supplementary information, Supplementary Table S1), teicoplanin pseudoaglycon derivatives **1–9** (Table 1) have been selected to the present antibacterial study. Synthesis of **3**[27] and **7–9** has been published recently.[28]

Lipoic acid derivatives **1** and **2** were prepared from *N*-acetylglucosaminyl teicoplanin aglycon[29] **10** by simple acylation reactions with hydroxysuccinimide active esters **11** and **12**[24] (Scheme 1).

In the framework of a systematic structure–anti-influenza virus activity study,[30] compounds **4** and **5** have been prepared by derivatization of azido teicoplanin pseudoaglycon. The synthesis of the substituents **16** and **17** started from methyl 4,6-*O*-*p*-methoxybenzylidene-α-D-glucopyranoside **13**.[25] Di-O-*n*-butylation of **13** followed by reductive opening of the 4,6-acetal ring[31,32] of the obtained **14** gave **15**. Subsequent alkylation of the liberated 6-hydroxyl group with a propargyl tetraethyleneglycol derivative produced **16**, conjugation of which to azido teicoplanin pseudoaglycon **18**[20] by the copper catalyzed azide–alkyne dipolar cycloaddition click reaction[33,34] furnished compound **4**. DDQ-mediated oxidative PMB deprotection of **16** resulted in **17**. Then compound **5** was obtained using click reaction of **17** and **18** (Scheme 2).

In a search for antibacterial and antiviral teicoplanin pseudoaglycon derivatives, we have synthesized compound **6** by the copper catalyzed azide–alkyne click reaction of azide **18** with alkyne **20** obtained from *N*-ethoxycarbonyl maleimide derivative **19**[27] (Scheme 3).

Our derivatives were characterized using calculated log*P* values (Table 1). According to these calculations, eight of the nine compounds displayed log*P* values >3 at their isoelectric point, showing their high lipophilicity.

Evaluation of antibacterial activity on a standard panel of Gram-positives showed that compounds **1–6** are very active against the two *Enterococcus* strains possessing resistance genes *vanA* or *vanB*. Compounds **7–9** that we have published earlier also displayed high activity against resistant enterococci (Supplementary Table S1).

In order to obtain structure–activity relationship information in this collection of teicoplanin pseudoaglycon derivatives, we evaluated the antibacterial activity of compounds **1–9** against a collection of 44 vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from routine laboratory samples of patients suffering from wound, urinary tract or blood stream infections. All the strains were characterized using PCR using primers for the identification of *vanA*- or *vanB*-resistance gene sequences. Eight of the strains proved to have *vanB*, 32 strains had *vanA* and interestingly 3 of them possessed both genes.[35] The prevalence of VREs carrying the *vanA* gene is higher owing to the enhanced mobility of the mentioned gene cluster.[36]

Against the first group of enterococci (the VanB group, Table 2, lines 1–8), all of our nine compounds exhibited low MIC values, mostly lower than that of teicoplanin. In the evaluation of antibacterial activities against the *vanA*-positive group of *E. faecalis* and *E. faecium* (Table 3, lines 9–41) and for the group of three strains possessing both resistance genes (Table 4, lines 42–44), the same compounds displayed diverse results. Four of them (**3**, **4**, **7** and **8**) proved to be active against about one-third of the *Enterococcus* strains; the other compounds had moderate or low activity. Analyzing the data further in Tables 3 and 4, it can be noticed that some of the strains are completely resistant to all of our teicoplanin pseudoaglycon derivatives, and some of them are more or less sensitive (see Table 5 for details). This phenomenon might be related to the different degrees of expression of the resistance genes in the different strains.[37]

The log*P* calculations for the whole collection of compounds revealed that all of them are lipophilic compounds with log*P* values close to or >3. Based on the results presented here and in our previous articles,[16–21,27,28] it seems that there is no linear correlation between the lipophilicity of glycopeptide antibiotics and their antibacterial activity against VRE strains, but the presence of a hydrophobic moiety on the antibiotic is critical in most cases.

It has been shown that other attributes than lipophilicity can also substantially influence the activity of glycopeptide derivatives; for example, the overall charge[38] and the capability of the compounds to dimerize[39] in the presence of their target, D-Ala-D-Ala, and obviously the structure of the sidechain itself. As all molecules presented here are of acidic character, they are uniformly negatively charged at the pH of the antibacterial tests (close to neutral pH). As the differences in activity owing to ionization characteristics can be ruled out and the dimerization of teicoplanin, its aglycon or pseudoaglycons was also shown to be insignificant,[40] we can conclude that among these derivatives the structure of the substituents is the most important factor in their activity against VRE. Considering these and some of our other results (Szucs Z, unpublished data) relatively simple aromatic substituents like phenyl, biphenyl, naphthyl, etc. seem to be very beneficial for high *in vitro* antibacterial activity.

In summary, evaluation of antibacterial activities of nine teicoplanin pseudoaglycon derivatives against a series of resistant enterococci demonstrated that introduction of hydrophobic side chains into the antibiotic molecule, in spite of the possible side effects of this structure

manipulation, is still a good strategy in fighting against bacterial resistance. Compounds **4**, **7** and **8** could be good lead derivatives for obtaining new highly active antibiotics against multiresistant enterococci.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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