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ORIGINAL ARTICLE

Synthesis and antibacterial evaluation of some teicoplanin pseudoaglycon derivatives containing alkyl- and arylthiosubstituted maleimides

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Bis-alkylthio maleimido derivatives have been prepared from teicoplanin pseudoaglycon by reaction of its primary amino group with *N*-ethoxycarbonyl bis-alkylthiomaleimides. Some of the new derivatives displayed excellent antibacterial activity against resistant bacteria.
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INTRODUCTION

Glycopeptide antibiotics exert their antibacterial activity by inhibiting two sequential enzymatic reactions—transglycosylation and transpeptidation—in the bacterial cell-wall biosynthesis. The antibiotics recognize and tightly bind to the L-Lys-D-Ala-D-Ala termini of peptidoglycan precursors at the external side of the developing bacterial membrane. In this way transglycosylation and transpeptidation are physically prevented, arresting cell-wall elongation and cross-linking and leading to cell lysis.[1] Due to the lack of cross-resistance to other antibacterial drugs, the glycopeptide antibiotics have become first-line drugs for the treatment of life-threatening multi-drug resistant infections by Gram-positive bacteria.[2]

The emergence and spread of glycopeptide-resistant enterococci and glycopeptide intermediate-resistant *Staphylococcus aureus*, as well as teicoplanin-resistant *Staphylococcus haemolyticus*[3] present a serious global challenge and have led to renewed interest in the development of novel, effective and safe antibacterials including new derivatives of glycopeptide antibiotics.[4–6]

Inspired by the high activity of the semisynthetic lipoglycopeptide antibiotics telavancin,[7] dalbavancin[8] and oritavancin[9] against vancomycin-resistant bacteria, we have started a program to produce new antibiotics by introducing lipophilic subtituents to the primary amino function of ristocetin aglycon and of teicoplanin pseudoaglycon. Applying various approaches including squaric acid conjugation method, azide-alkyne cycloaddition reaction or three-component isoindole formation, we have prepared a large set of new derivatives exhibiting high antibacterial[10–13] and, in some cases, robust anti-influenza virus activity.[14–17]

Recently, Caddick, Baker and coworkers[18–21] reported on applications of 3,4-dibromomaleimides for site-specific protein modification and bioconjugation. The method is based on addition–elimination reaction of thiols to the bromomaleimides leading to regeneration of the double bond resulting in thiomaleimide products (Scheme 1). Last year the group of Caddick and Baker published a simple method for the synthesis of *N*-functionalised bromo- and thiomaleimides through the corresponding *N*-ethoxycarbonyl maleimide derivatives.[22] Applying these recent results of maleimide chemistry we describe here derivatisation of teicoplanin pseudoaglycon with thiomaleimide substituents carrying two lipophilic alkyl or aryl sulfide side chains.

RESULTS AND DISCUSSION

Dibromomaleimide (**1**) that can be obtained by simple bromination of maleimide[23] has been allowed to react with a range of thiols including the 6-thio-D-galactose derivative **2a**, thiophenol **2b**, phenylmethanethiol **2c**, dodecanethiol **2d**, octanethiol **2e**, propanethiol **2f** and *t*-butyl mercaptane **2h**, representing a series of substituents of different lipophilicity.

The obtained sulfides **3a–g** have been then ethoxycarbonylated with ethyl chloroformate in the presence of potassium carbonate to provide **5a–g**, ready for a reaction with a primary amino group (Scheme 2). Direct methoxycarbonylation[22] of dibromomaleimide offers an alternative route for the synthesis of the targeted *N*-functionalized dithiomaleimide as it is illustrated by the synthesis of **6g**. We tested this route with several thiols such as **2d–2g**, however, the sulfide formation showed low efficacy in all cases.

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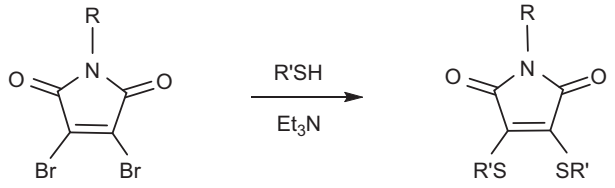
Next, teicoplanin pseudoaglycon **7**[16] has been reacted with *N*-ethoxycarbonyl maleimides **5a–g** and **6g** in the presence of triethylamine (Table 1). In these reactions bis-alkyl- or arylthiomaleimide **8a–f** were formed in moderate yields, together with the *N*-alkoxycarbonyl derivatives of the teicoplanin pseudoaglycon (**9** and **10**). The formation of **9** and **10** can be explained by the steric hindrance of the amino function of **7**. In the case of **5g** and **6g**, the undesired carbamate derivatives **9** and **10** were dominantly formed, probably due to the presence of bulky *t*-butyl substituents of the reagents.

Antibacterial activity of maleimido-teicoplanin-pseudoaglycons was evaluated on a panel of Gram-positive bacteria (Table 2). The D-galactose-containing **8a**, the bis-phenylthio derivative **8b** and the bis-benzylthio derivative **8c** displayed similar activities than teicoplanin pseudoaglycon **7** with one exception: the maleimido compounds **8a–c** were active against *Enterococcus faecalis* 15 376 having vanA resistance gene while teicoplanin and **7** were completely inactive against this bacterium strain.

The detected antibacterial activities of **8d**, **8e** and **8f** were related to the length of the alkyl chain substituents of their maleimide residues. The bis-dodecyl derivative **8d** was inactive, the bis-octyl derivative **8e** was a weak antibacterial and the bis-propylthio compound **8f** displayed very high activity. It can be supposed that a correlation exists between lipophilicity of the maleimide substituents and antibacterial activity, and the high lipophilicity erodes the activity. To test this hypothesis, logP (logarithm of partition coefficient between *n*-octanol and water) values were calculated for *N*-methyl maleimide derivatives **11a–f** and the calculated logP values corroborate our postulation (Table 3).

In conclusion we have utilized, for the first time, bis-sulfide derivatives of *N*-alkoxycarbonyl maleimide for versatile derivatisation of teicoplanin pseudoaglycon. It turned out that lipophilicity of substituents of the maleimide ring has strong influence on the antibacterial activity of these derivatives. Further synthetic tuning of these chemical structures hopefully will result in even more effective antibacterials.

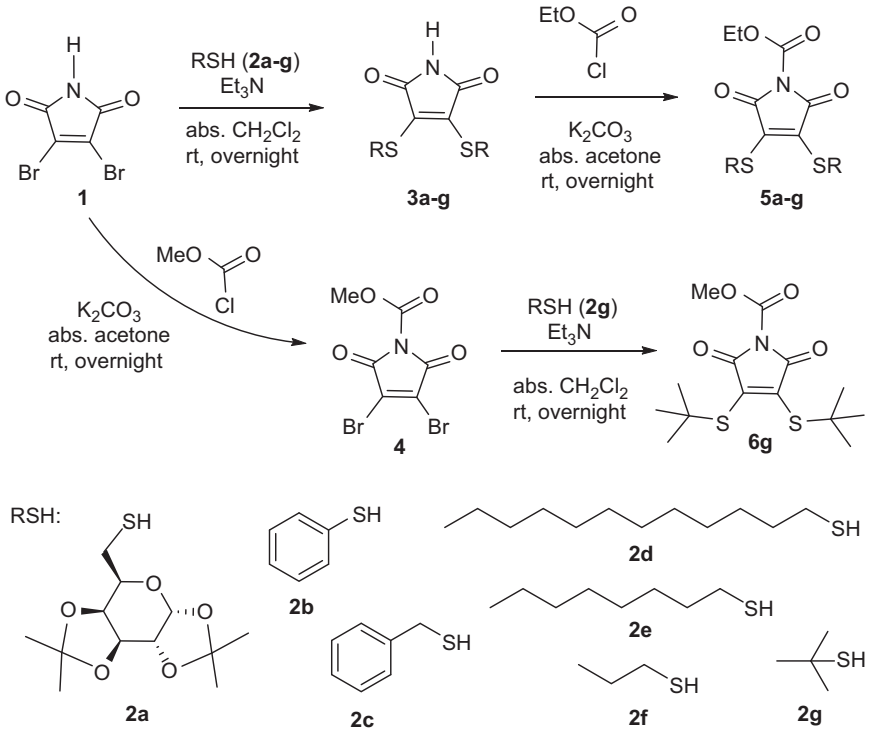


Scheme 1 Reaction of thiols with 3,4-dibromomaleimide.

EXPERIMENTAL PROCEDURE

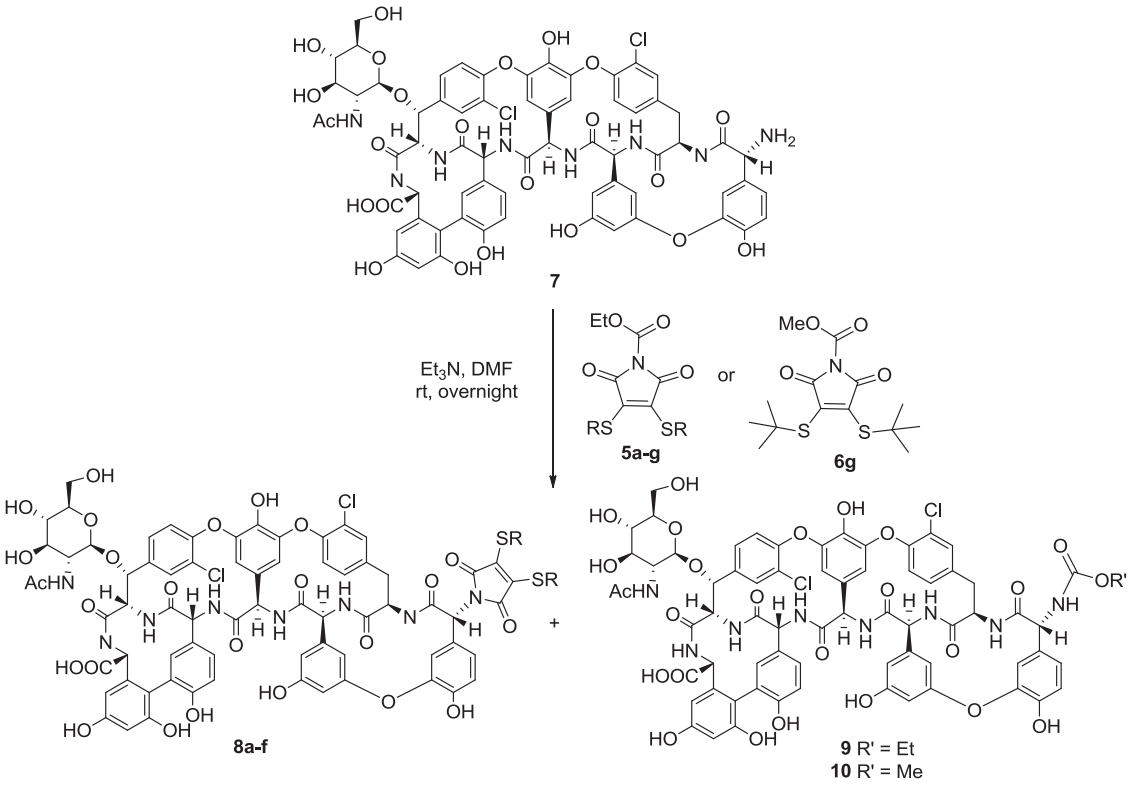
General information

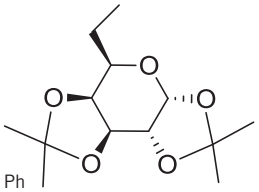
Maleimide and thiols **2b–2g** were purchased from Sigma-Aldrich Chemical (St Louis, MO, USA). 2,3-Dibromomaleimide **1**, 1,2:3,4-di-*O*-isopropylidene-6-deoxy-6-thio-α-D-galactopyranose **2a** and teicoplanin pseudoaglycon **7** were prepared according to literature procedures. TLC analysis was performed on Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) silica gel plates with visualization by immersing in ammonium-molibdate solution followed by heating or Pauly-reagent in the case of teicoplanin derivatives. Column chromatography was performed on silica gel 60 (Merck 0.063–0.200 mm), flash column chromatography was performed on silica gel 60 (Merck 0.040–0 0.063 mm). Organic solutions were dried over $MgSO_4$ and concentrated under vacuum. The ^1H (400 and 500 MHz) and ^{13}C NMR (100.28, 125.76 MHz) spectra were recorded with Bruker DRX-400 and Bruker Avance II 500 spectrometers. Chemical shifts are referenced to Me_4Si or DSS (2,2-Dimethyl-2-silapentane-5-sulfonate sodium salt) (0.00 p.p.m. for ^1H) and to solvent signals ($CDCl_3$:



Scheme 2 Synthesis of *N*-alkoxycarbonylated di-alkyl/arylthio-maleimide derivatives.

Table 1 Synthesis and structure of teicoplanin pseudoaglycon-maleimide conjugates



Reagent	*R*	*Products (yield %)*	
5a		**8a** (15)	**9**[a]
5b	Ph	**8b** (21)	**9** (41)
5c	Bn	**8c** (16)	**9** (44)
5d	*n*-dodecyl	**8d** (44)	**9**[a]
5e	*n*-octyl	**8e** (22)	**9**[a]
5f	*n*-propyl	**8f** (66)	**9**[a]
5g	*t*-butyl	**8g**[b]	**9** (59)
6g	*t*-butyl	**8g**[b]	**10** (48)

[a]Formation was observed (based on TLC), but it was not isolated.
[b]Identified by MS method but it could not be isolated in pure form.

77.00 p.p.m., DMSO-d_6: 39.51 p.p.m. for ^{13}C). MALDI-TOF MS analyses for the compounds **8b**, **8c**, **8e**, **9** and **10** were carried out in positive reflectron mode using a BIFLEX III mass spectrometer (Bruker, Bremen, Germany) equipped with delayed-ion extraction. In the case of **8a**, **8d** and **8f**, Matrix-Assisted Laser Desorption/Ionization Time-of-flight (MALDI-TOF) MS spectra were recorded by a Voyager-DE STR MALDI-TOF Biospectrometry Workstation (Applied Biosystems, Budapest, Hungary). 2,5-Dihydroxybenzoic acid was used as matrix and CF_3COONa as cationising agent in DMF. Elemental analysis (C, H, S) was performed on an Elementar Vario MicroCube instrument. The antibacterial activity of **8a–f**, **9** and **10** was tested against a panel of Gram-positive bacteria using broth microdilution method as described earlier.[24]

General method A for preparation maleimide bis-sulfides (3a–3g)

To a stirred solution of 2,3-dibromomaleimide[23] (1.0 mmol) in CH_2Cl_2 (20 ml) Et_3N (2.0 mmol) and thiol (2.1 mmol) were added under argon atmosphere and stirred for 3 h at room temperature. The reaction mixture was evaporated, and the crude product was purified by flash chromatography to give the desired compound.

General method B for preparation *N*-ethoxycarbonyl maleimide bis-sulfides (5a–5g)

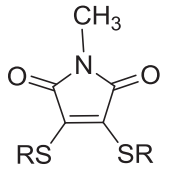
To a stirred solution of maleimide bis-sulfide (1.0 mmol) in dry acetone (20 ml) K_2CO_3 (1.2 mmol) and ethyl chloroformate (1.2 mmol) were added

Table 2 Antibacterial activity of compounds 7–10

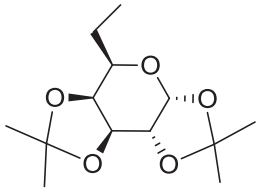
	Teicoplanin	*7*	*8a*	*8b*	*8c*	*8d*	*8e*	*8f*	*9*	*10*
Bacillus subtilis ATCC 6633	0.5/16	2/16	4/256	4/32	4/32	128/256	32/256	1/256	64/256	8/64
Staphylococcus aureus MSSA ATCC 29213	0.5/2	2/32	4/256	2/16	4./32	64/256	8/64	1/256	16/128	8/64
Staphylococcus aureus MRSA ATCC 33591	0.5/2	1/16	4/256	2/16	4/32	64/256	2/16	1/256	4/64	8/64
Staphylococcus epidermidis biofilm ATCC 35984	2/32	2/32	1/256	1/8	0.5/2	8/256	1/8	0.5/256	4/32	4/64
Enterococcus faecalis ATCC 29212	2/64	4/32	4/256	1/64	0.5/64	8/256	8/256	1/256	8/256	8/256
Staphylococcus epidermidis mecA	16/32	1/32	1/256	2/16	0.5/4	8/256	2/16	0.5/256	4/32	8/64
Enterococcus faecalis 15376 vanA	256/256	256/256	4/256	1/256	0.5/32	128/256	32/256	1/256	16/256	8/256
Enterococcus faecalis ATCC 51299 vanB	4/256	2/32	2/256	2/64	0.5/64	64/256	8/128	1/256	8/128	8/128

Abbreviations: ATCC, American type culture collection; mecA, *mecA* gene expression in *Staphylococcus*; MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*; vanA +, *vanA* gene positive; vanB +, *vanB* gene positive.

Table 3 Calculated logP for *N*-methyl maleimide derivatives 11a–f



11a-f

Compound	*R*	*LogP*
11a		0.54
11b	Ph	2.65
11c	Bn	2.78
11d	*n*-dodecyl	8.48
11e	*n*-octyl	5.14
11f	*n*-propyl	0.97

under argon atmosphere and stirred for 3 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, filtered through a pad of Celite and evaporated. The crude product was used for further step without purification.

General method C for the synthesis of teicoplanin pseudoaglycon derivatives (8a–8f)

To a stirred solution of teicoplanin pseudoaglycon[16] (0.1 mmol) in dry DMF (5 ml) *N*-ethoxycarbonyl maleimide bis-sulfides (0.14 mmol) and Et_3N (0.1 mmol) were added under argon atmosphere and stirred for overnight at room temperature. The reaction mixture was evaporated, and the crude product was purified by flash chromatography to give the desired compound.

Compound ***3a***. 2,3-Dibromomaleimide (255 mg, 1.0 mmol) was reacted with thiol **2a**[25] (580.4 mg, 2.1 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane:acetone = 8:2, to give **3a** (550 mg, 85%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (1H, s, N*H*), 5.51 (2H, d, $J_{1,2}$ = 0.3 Hz, 2 × H-1), 4.62 (2H, d, $J_{2,3}$ = 8.0 Hz, 2 × H-2), 4.32–4.30 (4H, m, 2 × H-3, 2 × H-4), 3.98–3.95 (2H, m, 2 × H-5), 3.57–3.36 (4H, m, 2 × H-6a,b), 1.48, 1.44, 1.33, 1.32 (24H, 4 × s, 8 × C*H*$_3$-ip); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8 (2C, 2 × C = O), 137.2, 136.9 (2C, C = C), 109.5, 108.7 (4C, 4 × C_q-ip), 96.5 (2C, 2 × C-1), 71.5, 70.9, 70.4, 67.9 (8C, skeleton carbons), 31.6 (2C, 2 × C-6), 25.9, 24.9, 24.4 (8C, 8 × *C*H$_3$); analysis calculated for $C_{28}H_{39}NO_{12}S_2$ C 52.08, H 6.09, N 2.17, O 29.73, S 9.93. Found: C 51.99, H 6.08, S 9.90.

Compound ***3b***. 2,3-Dibromomaleimide (255 mg, 1.0 mmol) was reacted with thiophenol **2b** (215 µl, 2.1 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane: acetone = 8:2, to give **3b** (310 mg, 98%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (1H, s, N*H*), 7.29–7.17 (10H, m, arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5 (2C, 2 × C = O), 136.8 (2C, C = C), 131.9, 129.1, 128.6 (10C, arom), 128.9 (2C, C_q arom); analysis calculated for $C_{16}H_{11}NO_2S_2$ C 61.32, H 3.54, N 4.47, O 10.21, S 20.46. Found: C 61.15, H 3.53, S 20.39.

Compound ***3c***. 2,3-Dibromomaleimide (510 mg, 2.0 mmol) was reacted with benzyl mercaptan **2c** (490 µl, 4.2 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane:acetone = 8:2, to give **3c** (460 mg, 67%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (1H, s, N*H*), 7.29–7.26 (10H, m, arom), 4.42 (4H, s, 2 × SC*H*$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 166.3 (2C, 2 × C = O), 136.5 (2C, C = C), 128.9, 128.8, 128.7, 127.7 (10C, arom), 36.2 (2C, 2 × SC*H*$_2$); analysis calculated for $C_{18}H_{15}NO_2S_2$ C 63.32, H 4.43, N 4.10, O 9.37, S 18.78. Found: C 63.19, H 4.45, S 18.69.

Compound ***3d***. 2,3-Dibromomaleimide (510 mg, 2.0 mmol) was reacted with dodecyl mercaptan **2d** (950 µl, 4.2 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane:ethyl acetate = 9:1, to give **3d** (670 mg, 67%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (1H, s, N*H*), 3.29–3.25 (4H, m, 2 × SC*H*$_2$), 1.64–1.25 (40H, m, 20 × C*H*$_2$), 0.89–0.86 (6H, m, 2 × C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8 (2C, 2 × C = O), 136.4 (2C, C = C), 31.5, 31.4, 30.2, 29.3, 29.1, 28.9, 28.7, 28.1 (20C, 20 × *C*H2), 22.3 (2C, 2 × SCH$_2$), 13.7 (2C, 2 × *C*H$_3$). Analysis calculated for $C_{28}H_{51}NO_2S_2$ C 67.55, H 10.33, N 2.81, O 6.43, S 12.88. Found: C 66.59, H 10.23, S 12.03.

Compound ***3e***. 2,3-Dibromomaleimide (255 mg, 1.0 mmol) was reacted with octyl mercaptan **2e** (364 µl, 2.1 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane:acetone = 8:2, to give **3e** (317 mg, 82%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (1H, s, N*H*), 3.28 (4H, t, *J* = 7.5 Hz, 2 × SC*H*$_2$), 1.69–1.60 (8H, m, 4 × C*H*$_2$), 1.43–1.27 (20H, m, 10 × C*H*$_2$), 0.88 (6H, t, *J* = 6.8 Hz, 2 × C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (2C, 2 × C = O), 136.7 (2C, C = C), 31.8, 30.5, 29.0, 28.5 (12C, 12 × *C*H$_2$), 22.6 (2C, 2 × SCH$_2$), 14.0 (2C, 2 × *C*H$_3$); analysis calculated for $C_{20}H_{35}NO_2S_2$ C 62.29, H 9.15, N 3.63, O 8.30, S 16.63. Found: C 61.03, H 9.08, S 16.08.

Compound ***3f***. 2,3-Dibromomaleimide (510 mg, 2.0 mmol) was reacted with propyl mercaptane **2f** (380 µl, 4.2 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane:ethyl acetate = 9:1, to give **3f** (430 mg, 87%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (1H, s, N*H*), 3.28–3.25 (4H, m, 2 × SCH_2), 1.73–1.66 (4H, m, 2 × CH_2), 1.06–1.02 (6H, m, 2 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (2C, 2 × C = O), 137.2 (2C, C = C), 33.6 (2C, 2 × CH_2), 23.8 (2C, 2 × SCH_2), 13.1 (2C, 2 × CH_3); analysis calculated for $C_{10}H_{15}NO_2S_2$ C 48.95, H 6.16, N 5.71, O 13.04, S 26.14. Found: C 48.18, H 5.70, S 26.01.

Compound ***3g***. 2,3-Dibromomaleimide (510 mg, 2.0 mmol) was reacted with *t*-butyl mercaptane **2g** (473 µl, 4.2 mmol) according to general method A. The crude product was purified by silica gel chromatography in *n*-hexane:ethyl acetate = 9:1, to give **3g** (432 mg, 80%) as a yellow sirup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (1H, s, N*H*), 1.54 (18H, s, 6 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (2C, 2 × C = O), 145.3 (2C, C = C), 51.9 (2C, 2 × SC_q), 32.2 (6C, 6 × CH_3); analysis calculated for $C_{12}H_{19}NO_2S_2$ C 52.71, H 7.00, N 5.12, O 11.70, S 23.46. Found: C 51.66, H 6.93, S 22.89.

Compound ***6g***. To a stirred solution of 2,3-dibromomaleimide (0.255 g, 1.0 mmol) in tetrahydrofuran (4 ml) *N*-methylmorpholine (76 µl, 1.1 mmol) and methyl chloroformate (85 µl, 1.1 mmol) were added at 0 °C. When TLC (*n*-hexane:acetone = 8:2) showed complete conversion of the starting material (3 h), the reaction mixture was diluted with CH_2Cl_2, filtered through a pad of Celite and evaporated. The obtained crude **4** (0.308 g) was reacted, without purification, with *t*-butyl mercaptan **2g** (237 µl, 2.1 mmol) according to general method A to give compound **6g** (0.150 g). The crude product was used for further step without purification.

Compound ***8a***. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) was reacted with compound **5a** (100 mg, 0.14 mmol) according to general method C. The crude product was purified by silica gel chromatography in toluene:methanol = 8:2, to give **8a** (30 mg, 15%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 2051.39$ *m/z*. Calcd for $C_{94}H_{94}Cl_2N_8O_{35}S_2Na$ 2051.45 *m/z*.

Compound ***8b***. Teicoplanin pseudoaglycon (140 mg 0.1 mmol) was reacted with compound **5b** (58 mg, 0.14 mmol) according to general method C. The crude product was purified by silica gel chromatography in toluene:methanol = 8:2, to give **8a** (35 mg, 21%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 1719.41$ *m/z*. Calcd for $C_{82}H_{66}Cl_2N_8O_{25}S_2Na$ 1719.29 *m/z*.

Compound ***8c***. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) was reacted with compound **5c** (41 mg, 0.14 mmol) according to general method C. The crude product was purified by silica gel chromatography in toluene:methanol = 7:3, to give **8c** (27 mg, 16%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 1747.47$ *m/z*. Calcd for $C_{84}H_{70}Cl_2N_8O_{25}S_2Na$ 1747.32 *m/z*.

Compound ***8d***. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) was reacted with compound **5d** (74 mg, 0.14 mmol) according to general method C. The crude product was purified by silica gel chromatography in toluene:methanol = 9:1, to give **8d** (85 mg, 44%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 1903.66$ *m/z*. Calcd for $C_{94}H_{106}Cl_2N_8O_{25}S_2Na$ 1903.60 *m/z*.

Compound ***8e***. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) was reacted with compound **5e** (69 mg, 0.14 mmol) according to general method C. The crude product was purified by silica gel chromatography in toluene:methanol = 8:2, to give **8d** (38 mg, 22%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 1791.64$ *m/z*. Calcd for $C_{86}H_{90}Cl_2N_8O_{25}S_2Na$ 1791.47 *m/z*.

Compound ***8f***. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) was reacted with compound **5f** (40 mg, 0.14 mmol) according to general method C. The crude product was purified by silica gel chromatography in toluene:methanol = 9:1, to give **8d** (110 mg, 66%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 1651.02$ *m/z*. Calcd for $C_{76}H_{70}Cl_2N_8O_{25}S_2Na$ 1651.32 *m/z*.

Compound ***9***. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) was reacted with compound **5g** (49 mg, 0.14 mmol) according to general method C. The

Table 4 ^{1}H and ^{13}C NMR data for compounds 8a, 8b, 8c and 8d (chemical shifts in ppm)

Assignment	*8a ^{13}C*	*8a ^{1}H*	*8b ^{13}C*	*8b ^{1}H*	*8c ^{13}C*	*8c ^{1}H*	*8d ^{13}C*	*8d ^{1}H*
x1	64.8	7.05	64.8	7.07	64.9	7.06	64.6	7.05
x2	55.6	4.98	55.9	4.98	55.9	4.99	55.5	4.98
x3	59.2	5.32	59.1	5.29	59.2	5.33	59.1	5.36
x4	54.8	5.59	54.8	5.58	54.9	5.59	54.8	5.64
z6	76.8	5.40	76.2	5.45	76.7	5.42	76.3	5.42
2f	131.5	7.68	131.6	7.67	131.5	7.65	131.8	7.69
3b	109.7	6.32	109.7	6.28	110.2	6.32	110.0	6.39
4b	107.9	5.57	108.2	5.53	108.3	5.55	108.2	5.55
4f	104.6	5.07	104.8	5.06	104.9	5.07	104.9	5.06
5b	136.3	7.09	136.6	7.09	136.6	7.09	136.5	7.11
GlcNAc 1	98.4	4.40	98.8	4.39	99.0	4.36	99.0	4.39
Maleimide 2	165.3		165.4		165.4		165.5	
Maleimide 3	135.3		135.3		136.8		134.2	
Maleimide 4	135.3		135.3		136.8		134.2	
Maleimide 5	165.3		165.4		165.4		165.5	
SCH_2					35.5	4.42–4.37	31.5	3.33–3.21
α-Galp 1	95.7	5.41						
α-Galp 2	69.7	4.30						
α-Galp 3	70.2	4.60						
α-Galp 4	70.9	4.22						
α-Galp 5	67.2	3.82						
α-Galp 6	31.2	3.37–3.27						
*i*P-C_q	108.8; 108.6							
*i*P-CH_3	31.5–24.2	1.40–1.23						
Ph			131.0–128.18	7.29–7.15				

Table 5 ^{1}H and ^{13}C NMR data for compounds 8e, 8f, 9 and 10 (chemical shifts in p.p.m.)

Assignment	*8e* ^{13}C	*8e* ^{1}H	*8f* ^{13}C	*8f* ^{1}H	*9* ^{13}C	*9* ^{1}H	*10* ^{13}C	*10* ^{1}H
x1	64.8	7.05	64.8	7.05	64.8	7.05	64.8	7.06
x2	56.0	4.99	55.7	4.99	55.7	4.98	56.0	4.98
x3	59.4	5.42	59.2	5.42	58.9	5.34	59.3	5.42
x4	54.9	5.57	54.8	5.58	54.8	5.62	54.8	5.59
z6	76.0	5.42	76.2	5.42	76.8	5.42	76.3	5.42
2f	131.3	7.64	131.0	7.64	131.8	7.63	131.5	7.66
3b	110.0	6.32	110.0	6.32	110.0	6.32	109.9	6.33
4b	108.3	5.57	108.1	5.54	107.6	5.53	108.0	5.51
4f	104.8	5.08	104.7	5.08	104.8	5.09	104.8	5.08
5b	136.6	7.09	136.3	7.09	136.2	7.2	136.1	7.09
GlcNAc 1	99.3	4.38	99.4	4.38	99.8	4.36	98.6	4.38
Maleimide 2	165.5		165.4					
Maleimide 3	138.5		135.4					
Maleimide 4	138.5		135.4					
Maleimide 5	165.5		165.4					
SCH_2	31.0	3.25–3.17	32.9	3.28–3.15				
CH_2	29.9–21.9	1.56–1.14	23.4	1.60–1.55				
CH_3	13.8	0.86–0.82	12.8	0.95–0.93				
NH 1						7.96		
CO 2					169.9		169.5	7.86
OCH_3							51.5	3.56
OCH_2					60.1	4.05–4.01		
CH_3					14.8	1.18–1.15		

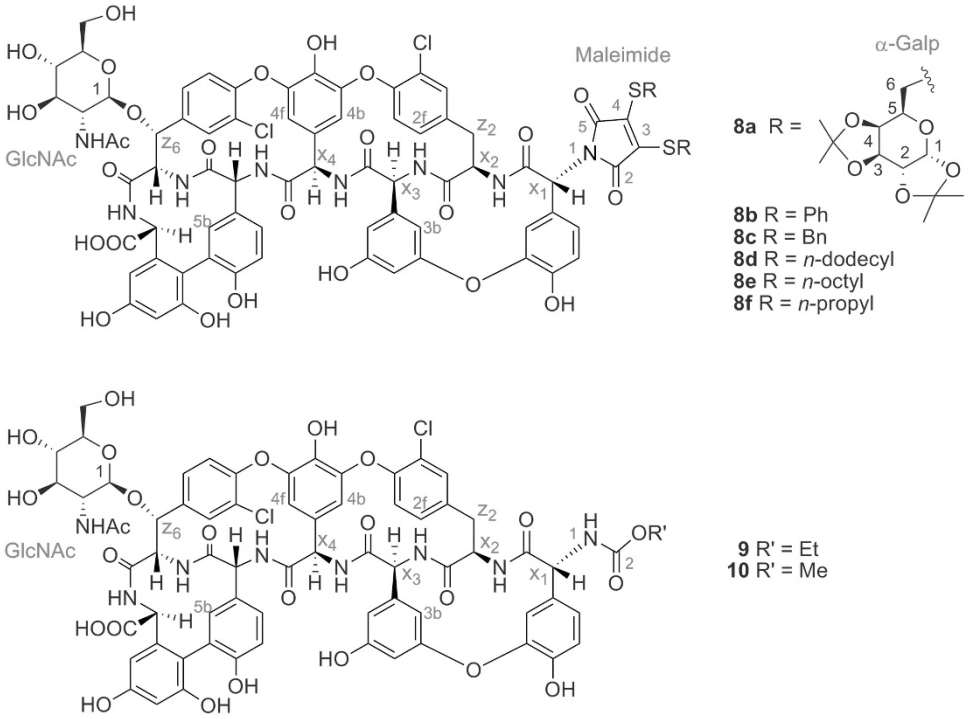


Figure 1 Structure and numbering for compounds **8a–f**, **9** and **10**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

crude product was purified by silica gel chromatography in toluene:methanol = 9:1, to give **9** (87 mg, 59%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+$ = 1495.34 *m/z*. Calcd for $C_{69}H_{62}Cl_2N_8O_{25}Na$ 1495.31 *m/z*.

Compound ***10***. Teicoplanin pseudoaglycon (210 mg, 0.15 mmol) was reacted with compound **6g** (70 mg, 0.21 mmol) according to general method C. The crude product was purified by silica gel chromatography

in toluene:methanol = 8:2, to give **10** (120 mg, 48%) as a yellow powder. MALDI-TOF MS: $[M+Na]^+ = 1481.51$ *m/z*. Calcd for $C_{68}H_{60}Cl_2N_8O_{25}Na$ 1481.29 *m/z*.

NMR analysis

The ^1H and ^{13}C NMR data of the teicoplanin derivatives **8a–f**, **9** and **10** are collected in Tables 4 and 5. The spectra were recorded at 500.13/125.76 MHz frequencies, respectively, at 300 K, using DMSO-d_6, as solvent. Numbering atoms in teicoplanin derivatives are given in Figure 1. Signal assignments were aided by 2D HSQC, TOCSY (15 and 60 ms mixing times) and HMBC (60 ms mixing time) experiments.

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