The Journal of Antibiotics (2017) 70, 152–157

www.nature.com/ja

ORIGINAL ARTICLE

Synthesis and biological evaluation of lipophilic teicoplanin pseudoaglycon derivatives containing a substituted triazole function

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A series of lipophilic teicoplanin pseudoaglycon derivatives, including alkyl-, aryl-, calixarene- and protected sugar-containing conjugates, were prepared using azide–alkyne click chemistry. Out of the conditions applied, the $CuSO_4$–ascorbate reagent system proved to be more efficient than the Cu(I)I–Et_3N-mediated reaction. Some of the new compounds have high *in vitro* activity against glycopeptide-resistant Gram-positive bacteria, including vanA-positive *Enterococcus faecalis*. A few of them also display promising *in vitro* anti-influenza activity.
The Journal of Antibiotics (2017) **70,** 152–157; doi:10.1038/ja.2016.80; published online 29 June 2016

INTRODUCTION

Glycopeptides are the drugs of first choice in cases of severe Gram-positive infections, which are mostly caused by pathogens, such as methicillin-resistant *Staphylococcus aureus*, coagulase-negative staphylococci and enterococci.[1] The site of action of these agents is the bacterial peptidoglycan. They bind to the L-Lys-D-Ala-D-Ala termini of peptidoglycan precursors by five hydrogen bonds. As a result, the transglycosylation and transpeptidation steps in cell wall synthesis are inhibited. It is well known that this lack of structural integrity of the cell wall ultimately leads to osmotic lysis of bacterial cells.[2]

Resistance against glycopeptide antibiotics has been emerging for a few decades now, probably as a result of their extensive use in both clinical and agricultural settings.[3,4] For many years, vancomycin was the only glycopeptide used clinically. In the 1980s, vancomycin resistance started to develop. A new glycopeptide, teicoplanin, was approved in the late 1980s in Europe (and later in Japan) and became an alternative treatment option. Later, teicoplanin resistance among vancomycin-resistant enterococci (VRE) and coagulase-negative staphylococci became more and more frequent.[5,6] The newest semisynthetic glycopeptides—dalbavancin, telavancin and oritavancin—are several times more efficient than their predecessors, with oritavancin being the most potent and active even against VRE.[7–9]

The strong activity of these new antibiotics proves that carefully selected lipophilic substituents can enhance the activity of the parent compounds by different ways thereby overcoming the problem of glycopeptide resistance. Our group has already prepared a variety of such semisynthetic glycopeptides from vancomycin aglycon, ristocetin aglycon and teicoplanin pseudoaglycon, by using different chemical approaches. These include the conjugation with a squaric acid moiety, isoindole formation and the incorporation of variously substituted maleimides.[10–12] Many of these compounds showed good *in vitro* activity against various bacteria, including VRE, and interestingly, some of them proved to be efficient against different strains of influenza virus.

Two teicoplanin pseudoaglycon derivatives that were prepared earlier by click chemistry showed outstanding antibacterial activity.[13] One of them had a 4-(decyloxymethyl)-1,2,3-triazole side chain derivatized from the terminal amino group of the molecule by a diazotransfer and a subsequent azide–alkyne click reaction. The other derivative had a biphenyl group instead of the *n*-decyl group.

In the present work, our aim was to obtain structure–activity relationships in this group of derivatives by synthesizing compounds with a larger variety of side chains, as it is known from the literature that an optimal length of substituents is required for maximum activity.[14] The preparation of new derivatives was accomplished by using the aforementioned CuAAC approach with varying alkyl and aryl side chain lengths and also with substituents that have more diverse chemical structures. Besides, we were interested in the potential anti-influenza activity of the new compounds.

RESULTS AND DISCUSSION

To form the various side chains on the teicoplanin pseudoaglycon, we used different alkynyl ethers. These included a series of propargyl ethers such as *n*-hexadecyl (**3a**),[15] *n*-dodecyl (**3b**),[16] *n*-decyl (**3c**),[13] *n*-octyl (**3d**),[16] *n*-hexyl (**3e**), phenyl (**3f**) and α-naphthyl propargyl

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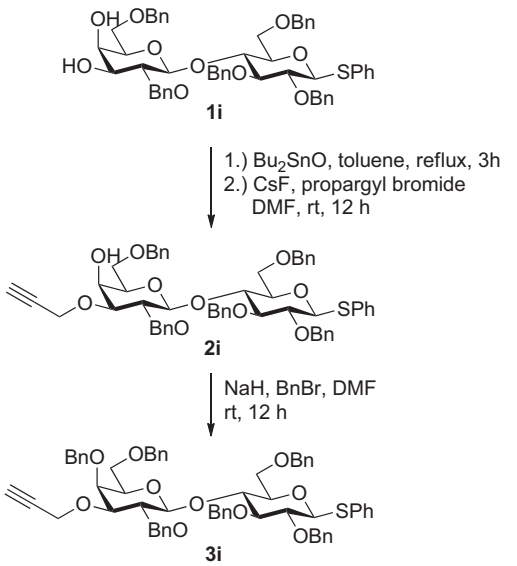
Received 4 April 2016; revised 24 May 2016; accepted 5 June 2016; published online 29 June 2016

ether (**3g**).[17] Compound **3c** was prepared to resynthesize the corresponding teicoplanin pseudoaglycon *n*-decyl derivative **5c**[13] for the current biological evaluations. Two protected carbohydrate derivatives, 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2-propargyl)-α-D-galactopyranose (**3h**)[18] and a highly bulky 3′-*O*-propargyl lactose derivative (**3i**) were also utilized as lipophilic moieties. Compound **3i** was prepared by stannylene acetal-mediated regioselective propargylation of the 3′-hydroxyl group of diol **1i**[19] using dibutyltin oxide and propargyl bromide followed by benzylation of the remaining free hydroxyl group of the resulting **2i** (Scheme 1). The calix[4]arene derivative (**3j**)[20] bearing a *n*-butynyl moiety was also incorporated within our studies.

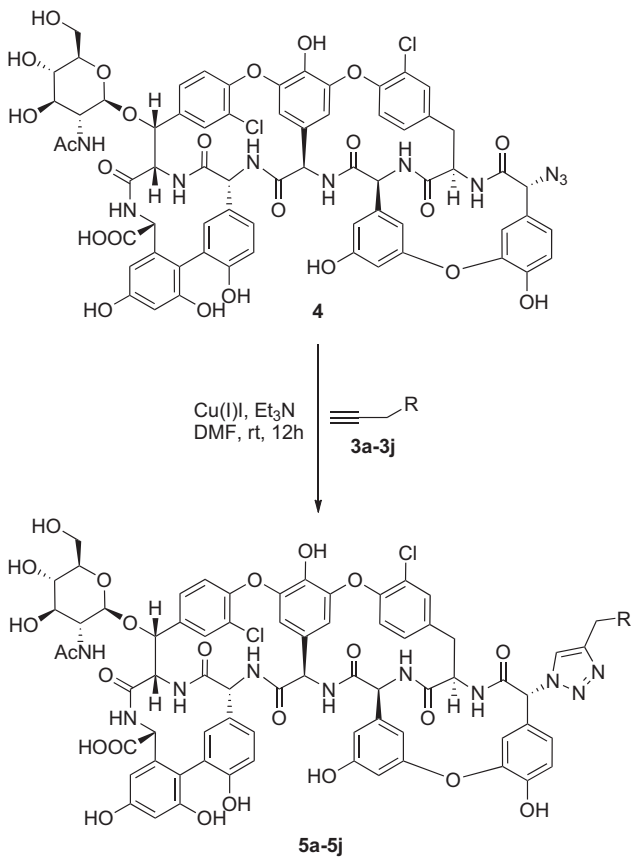
Teicoplanin pseudoaglycon azide (**4**)[13] was reacted with alkynyl ethers (**3a–3j**) in a Cu(I)-catalyzed azide–alkyne click reaction[21] to afford final products **5a–5j** in low-to-moderate yields (Scheme 2). Upon CuI-TEA catalysis in dimethylformamide (DMF), insufficient conversion of the reactants was observed and the yield of the corresponding triazole derivative after purification did not exceed 35%. We could achieve better conversions and significantly higher yields using the $CuSO_4$-L-ascorbate-mediated reaction in *t*BuOH—water, 1:1 (v/v). (see Table 1). However, there is a limiting factor when using the latter method, which is the solubility of the chosen reactants in the *t*BuOH-water mixture. All of our teicoplanin pseudoaglycon derivatives have lipophilic side chains, therefore calculated log*P* values for the alkynyl ethers **3a–j** can be informative for structure–biological activity studies (Table 1).

The antibacterial activity of the new derivatives was evaluated by the broth microdilution method[10,22] on a panel of Gram-positive bacteria (Table 2). Derivative **5a** containing the *n*-hexadecyl group turned out to be multiple times less active than teicoplanin or showed no activity at all. Compound **5b,** with the *n*-dodecyl side chain, was less active against both methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* than teicoplanin, but it had good MIC/MBC values in the case of both *S. epidermidis* strains. It showed moderate activity against the enterococci. Compound **5c** bearing the *n*-decyl side chain was more active against enterococci than the *n*-dodecyl derivative **5b**. Compound **5d** with the *n*-octyl side chain was highly active against all bacteria tested, showing excellent bactericidal effect. Although **5e** had similar or better bacteriostatic activity, it was not exceptionally bactericidal against enterococci. **5f** and **5g** with the aromatic substituents were even more active against resistant enterococci. Both of them displayed not only marked bacteriostatic but also noticeable bactericidal effect against the three *Enterococcus faecalis* strains (vancomycin sensitive/resistant/teicoplanin resistant). Derivative **5h** showed excellent activity against both *S. epidermidis* strains. Unfortunately, it was not particularly active against *E. faecalis*. **5i** was totally inactive against the tested bacteria, possibly owing to a shielding effect of the bulky benzyl groups, which prevents the peptide core from binding to the molecular target. The same phenomenon was proposed in the case of compound **5j**, which was not active against any of the bacteria tested.

As shown in Table 2, our recent measurement of MIC/MBC ratios are considerably different among derivatives with strikingly analogous structures. This fact may indicate the different activity of a compound against different bacterial strains or the different mode of action among derivatives against the same examined bacterial strain. The first case may result in the detectable different MIC/MBC ratios of one antibiotic examined in different strains from the same species.[23] The practice of clinical microbiology laboratories to support the selection of effective therapy is the determination of MIC for different antibiotics of the pathogen. The determination of MBC, postantibiotic effect or postantibiotic sub-MIC effect is not usual routine diagnostics; however, these characteristics are different against the antibiotics and strongly influence the process of treatment. Odenholt *et al.*[24] demonstrated high teicoplanin concentration-dependent killing of

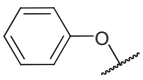
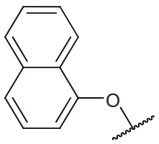
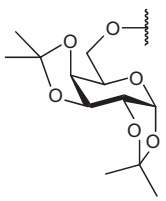
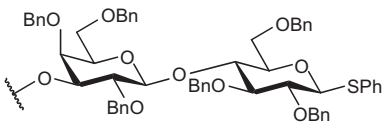
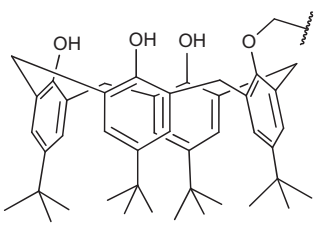


Scheme 1 Preparation of the lipophilic disaccharide **3i**.



Scheme 2 Lipophilic derivatization of teicoplanin pseudoaglycon by azide–alkyne click reaction.

Table 1 Structures of 3a–j and 5a–j, calculated log*P* values of the lipophilic moieties of 3a–j and yields for teicoplanin pseudoaglycon derivatives 5a–j

Compound	R	*log*P[a] *for 3a–j*	*Yields of final products 5a–j (%)*
3a **5a**	$OC_{16}H_{33}$ (*O-n*-hexadecyl)	5.85	33
3b **5b**	$OC_{12}H_{25}$ (*O-n*-dodecyl)	4.66	35
3c **5c**	$OC_{10}H_{21}$ (*O-n*-decyl)	3.87	61[b]
3d **5d**	OC_8H_{17} (*O-n*-octyl)	3.08	31
3e **5e**	OC_6H_{13} (*O-n*-hexyl)	2.82	28
3f **5f**		2.07	32
3g **5g**		3.07	48[b]
3h **5h**		1.55	50[b]
3i **5i**		12.57	28
3j **5j**		14.25	29

[a]Calculated using the log*P* calculation plugin of ChemAxon's Marvin Sketch.
[b]Reaction conditions: $CuSO_4$/L-ascorbate/tBuOH-H_2O, 1:1(v/v), room temperature, 12 h.

S. epidermidis and slight concentration-dependent killing of *S. aureus*, but for *Enterococcus faecium* only a bacteriostatic effect was noted. In that study, no or very short postantibiotic effects were noted for the investigated strains, but a lot of differences in regrowth were detected in the postantibiotic phase by sub-MICs against the different investigated species.[24] The different MIC/MBC ratios of our study may be caused by similar specificity of the investigated derivatives.

The results indicate that modification of the teicoplanin pseudoaglycon with highly lipophilic (**5a**) and/or very bulky (**5i**, **5j**) substituents eliminates the antibacterial effect. This clearly demonstrates that both the size/bulkiness and lipophilicity of the side chain have a strong influence on antibacterial activity, and the *n*-octyl, phenyl and α-naphthyl groups seem to be optimal. It is not clear whether aromaticity of the latter substituents has an impact on the activity, but the lipophilicity of the substituents in itself is clearly not the sole reason for biological properties of these compounds.

Oritavancin and other structurally related lipoglycopeptide derivatives are known to directly inhibit the transglycosylation step of peptidoglycan synthesis.[25] Also, oritavancin bearing a chlorophenylbenzyl group was shown to cause disruption of the bacterial membrane.[26] Moreover, semisynthetic lipoglycopeptides can form dimers more efficiently than vancomycin, and their enhanced antibacterial activity was attributed to their capability to dimerize, rather than their binding affinity to the cell-wall precursors.

Table 2 ***In vitro*** **antibacterial activity of new teicoplanin pseudoaglycon derivatives (MIC/MBC values in μg ml^{-1})**

	Teicoplanin	***5a***	***5b***	***5c***	***5d***	***5e***	***5f***	***5g***	***5h***	***5i***	***5j***
Bacillus subtilis ATCC 6633	0.5/16	16/128	2/8	2/16	0.5/4	0.5/16	0.5/4	1/8	2/16	128/256	128/256
Staphylococcus aureus MSSA ATCC 29213	0.5/2	4/32	4/32	2/8	0.5/4	0.16/4	0.5/4	0.5/2	0.5/2	32/128	32/256
Staphylococcus aureus MRSA ATCC 33591	0.5/2	4/32	2/32	2/8	0.5/4	0.16/4	0.5/4	0.5/2	0.5/1.25	32/256	16/128
Staphylococcus epidermidis biofilm forming ATCC 35984	2/32	8/32	0.5/8	2/16	0.5/8	0.16/2	0.5/4	0.5/1.25	0.16/0.63	32/256	32/256
Staphylococcus epidermidis mecA	16/32	4/32	0.5/2	4/16	0.5/4	0.16/2	0.5/4	0.5/1.25	0.32/0.63	32/256	16/64
Enterococcus faecalis ATCC 29212 (VSE)	2/64	64/256	2/32	4/16	1/8	1/64	0.5/4	1/8	4/32	128/256	32/256
Enterococcus faecalis ATCC 51299 vanB	4/256	64/256	4/16	2/8	2/8	2/64	1/8	1/4	4/32	128/256	64/256
Enterococcus faecalis 15376[a] vanA	256/256	256/256	4/32	4/16	1/8	1/128	1/8	2/4	4/32	256/256	256/256

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; vanB, vanB gene; VSE, vancomycin-sensitive Enterococcus.
[a]Clinical isolate.

Table 3 ***In vitro*** **anti-influenza activity of new teicoplanin pseudoaglycon derivatives**

	Cytotoxicity		*Antiviral EC_{50} (μM)*							
			Influenza A/H1N1 (A/PR/8)		*Influenza A/H1N1 (A/Virginia/ATCC3/2009)*		*Influenza A/H3N2 (A/HK/7/87)*		*Influenza B (B/HK/5/72)*	
Compound	*CC_{50} (μM)*	*MCC (μM)*	*Visual CPE*	*MTS*	*Visual CPE*	*MTS*	*Visual CPE*	*MTS*	*Visual CPE*	*MTS*
Teicoplanin	>100	>100	>100	>100	>100	23	>100	>100	>100	>100
5a	7.6	⩾4.0	1.6	1.8	1.8	1.8	⩽8.9	⩽1.6	1.8	1.3
5b	2.3	9.3	>100	>100	>100	>100	>100	>100	>100	>100
5c	3.7	4	>100	>100	>100	>100	>100	>100	>100	>100
5d	13	⩾4.0	>100	2.2	1.8	1.9	2.1	1.5	1.8	1.9
5e	53	⩾20	>100	>100	>100	>100	15	13	17	11
5f	41	⩾20	>100	>100	>100	>100	11	11	>100	>100
5g	47	20	11	11	11	4.4	15	8	15	6.6
5h	>100	⩾100	52	47	45	43	39	24	39	29
5i	>100	⩾20	>100	>100	8.9	7.3	8.9	20	6.6	4.0
5j	⩾58	⩾20	>100	43	8.9	6.7	>100	5.2	>100	5.7
Ribavirin	>100	⩾20	8.9	11	8.9	8.8	12	5.6	8.9	6.3
Zanamivir	>100	>100	2.3	1.5	19	6.4	2.3	9.9	0.019	0.018
Nucleozin	12	20	0.00075	0.00065	>20	>20	0.072	0.053	>20	>20

Abbreviations: CC_{50}, 50% cytotoxic concentration as determined by measuring the cell viability with the colorimetric formazan-based MTS assay; CPE, cytopathic effect; EC_{50}, 50% effective concentration or concentration producing 50% inhibition of virus-induced CPE as determined by visual scoring of the CPE or by measuring the cell viability with the colorimetric formazan-based MTS assay; MCC, minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

The membrane anchoring ability of such derivatives—which is due to their hydrophobic side chain—is also considered to be a relevant factor in the inhibition of VRE.[27] As the mode of action of semisynthetic glycopeptide antibiotics seems rather complex, it is difficult to hypothesize which factors are responsible for the efficacy of the compounds presented in this paper, as no extensive mechanistic studies have been carried out thus far.

In addition, we evaluated the anti-influenza virus activity of the new compounds against different strains of influenza virus, using an established assay in Madin Darby canine kidney cell cultures[28] (Table 3). Compound **5a** (with *n*-hexadecyl group) had strong activity against all influenza strains tested, albeit at antiviral EC_{50} values that were only approximately threefold lower than the concentrations giving cytotoxicity. When comparing compounds **5a**, **5b**, **5c, 5d** and **5e**, it was intriguing that the analogs having an *n*-hexadecyl (**5a**) or *n*-octyl side chain (**5d**) displayed robust and comparable antiviral activity, whereas the analogs having an *n*-dodecyl (**5b**) or *n*-decyl side chain (**5c**) were inactive. This is even more remarkable in light of the finding that these four analogs had very similar cytotoxic activity. Compound **5e** having an *n*-hexyl substituent displayed diminished activity against two of the four strains, while also being less cytotoxic than the other alkyl analogs, possibly owing to reduced lipophilicity of the substituent. This implies that the biochemical basis for the antiviral activity is critically dependent on the length of the aliphatic side chain and, second, that this antiviral activity is not strictly correlated to cytotoxicity. Although compound **5f** (having a phenyl group) was only slightly active against one of the four strains, **5g** (having a α-naphthyl group) exhibited more pronounced activity. These two compounds also had lower cytotoxicity than the alkyl substituted derivatives. On the other hand, the analogs **5i** and **5j** displayed not only reduced cytotoxicity but also lower antiviral activity that was less consistent for the four virus strains tested. Finally, among this compound series, **5h** had the lowest antiviral activity and cytotoxicity.

In summary, we have prepared a small series of semisynthetic teicoplanin pseudoaglycon derivatives having different lipophilic moieties on the *N*-terminal by click chemistry. The new compounds

possess significant *in vitro* biological activity. It is evident that the size and lipophilicity of the newly incorporated substituent are major determinants for activity against glycopeptide-resistant enterococci. In particular, the phenyl and α-naphthyl moieties were recognized as optimal substituents in this triazole functionalized group of derivatives. In the future, one or more of these candidates may be tested against a larger collection of VRE and in *ex vivo* or *in vivo* infection models.

Some of the new compounds show robust anti-influenza activity; however, they are also cytotoxic. Interestingly, while high hydrophobicity and bulkiness of side chains were detrimental for the antibacterial activity, these properties were favored to obtain anti-influenza effect. This anti-influenza activity critically depended on the length of the aliphatic side chain as *n*-octyl and *n*-hexadecyl provided equal activity but the analogs bearing *n*-dodecyl and *n*-decyl were inactive. We are currently investigating additional modifications with the hope to obtain compounds with improved antiviral activity and minimal cytotoxicity.

EXPERIMENTAL PROCEDURE

General information

Reagents were purchased from Sigma Aldrich Chemical Co., Budapest, Hungary, alkyne compounds **3a**,[15] **3b**,[16] **3c**,[13] **3d**,[16] **3e**, **3g**,[17] **3h**,[18] **3j**[20] and teicoplanin pseudoaglycon azide (**4**)[13] were prepared as described in other papers. Phenyl propargyl ether (**3f**) (technical, ⩾90%) was purchased from Sigma Aldrich Chemical Co. TLC analysis was performed on Kieselgel 60 F_{254} (Merck, Budapest, Hungary) silica gel plates with visualization by immersing in ammonium molybdate 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). Organic solutions were dried over $MgSO_4$ and concentrated under vacuum. The log*P* values of the alkynyl ethers **3a–j** were calculated using the log*P* calculation plugin of ChemAxon's Marvin Sketch (VG method, 0.1 M electrolyte concentration). The ^{1}H (360, 400 and 500 MHz) and ^{13}C NMR (90.54, 100.28, 125.76 MHz) spectra were recorded with Bruker DRX-360, Bruker DRX-400 and Bruker Avance II 500 spectrometers. Chemical shifts are referenced to Me_4Si or dextran sodium sulfate (0.00 p.p.m. for ^{1}H) and to solvent signals ($CDCl_3$: 77.00 p.p.m., CD_3OD: 49.15 p.p.m., DMSO-d_6: 39.51 p.p.m. for ^{13}C). MS (matrix assisted laser desorption/ionization time-of-flight MS (MALDI-TOF)) analysis was carried out in positive reflectron mode on a BIFLEX III mass spectrometer (Bruker, Bremen, Germany) with delayed-ion extraction. The matrix solution was a saturated solution of 2,4,6-trihydroxy-acetophenone in MeCN. 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 (C, H, N, S) was performed on an Elementar Vario Micro Cube (Elementar, Hanau, Germany) instrument. The antibacterial activity of **5a–5j** was tested against a panel of Gram-positive bacteria using broth microdilution method as described earlier.[22]

General method A for azide–alkyne click reaction

To a stirred solution of teicoplanin pseudoaglycon azide (**4**) in *N,N*-dimethylformamide, an alkyne compound (1.25 equiv.), Et_3N (1.0 equiv.) and Cu(I)I (0.5 equiv.) were added under argon atmosphere and stirred for overnight at room temperature. The solvents were evaporated and the crude product was purified by flash chromatography in toluene:methanol 8:2 → 1:1. The product obtained was passed through a column containing Sephadex LH-20 gel in methanol.

General method B for azide–alkyne click reaction

To a stirred solution of teicoplanin pseudoaglycon azide (**4**) in *t*-butanol: water = 1:1 (1 ml), an alkyne compound (1.25 equiv.), L-ascorbic acid (1.0 equiv.) and $CuSO_4 \times 5$ H_2O (0.1 equiv.) were added and stirred for overnight at room temperature. The solvents were evaporated and the crude product was purified by flash chromatography in toluene:methanol 8:2 → 1:1. The product obtained was passed through a column containing Sephadex LH-20 gel in methanol.

Compound 2i. Compound **1i** (1.148 g, 1.29 mmol) was dissolved in toluene (50 ml) and dibutyltin oxide (421 mg, 1.3 equiv.) was added. The reaction mixture was stirred for 3 h under reflux using a Dean–Stark apparatus. After evaporation, it was dissolved in DMF (20 ml), and then cesium fluoride (396 mg, 2 equiv.) and propargyl bromide (296 μl, 2 equiv.) were added. The reaction mixture was stirred overnight at room temperature. After evaporating the solvent, the residue was dissolved in CH_2Cl_2 (50 ml) and washed with distilled water three times (3 × 15 ml). The organic phase was dried, filtered and the solvents were evaporated. Then the crude product was purified by silica gel column chromatography in *n*-hexane–ethyl acetate, 1:1 (v/v) to give **2i** (830 mg, 69%), $[\alpha]_D$ −8.84 (*c* 0.18, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 7.65 (2H, m), 7.60–7.46 (5H, m), 7.45–7.34 (17H, m), 7.33–7.28 (6H, m), 5.14 (1H, d, *J* = 10.5 Hz), 4.91–4.80 (6H, m), 4.74 (1H, d, *J* = 10 Hz), 4.65 (1H, d, *J* = 12 Hz), 4.55 (3H, m), 4.50 (1H, d, *J* = 4.5 Hz), 4.47 (1H, s), 4.42 (2H, m), 4.18 (1H, s), 4.10 (1H, t, *J* = 9.5 Hz), 3.94–3.90 (1H, m), 3.84 (1H, d, *J* = 10.5 Hz), 3.76–3.69 (2H, m), 3.66 (1H, t, *J* = 8.5 Hz), 3.60–3.52 (3H, m), 3.48 (2H, t, *J* = 6 Hz), (2.47 1H, s). ^{13}C NMR (500 MHz, $CDCl_3$) *δ*: 138.9, 138.5, 138.4, 138.2, 138.1, 133.7, 132.0,128.8, 128.3, 128.2, 128.2, 128.1, 128.1, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.3, 102.5 (C-1′), 87.4 (C-1), 84.9, 80.9, 80.2, 79.9 ($C_q$propargyl), 79.3 (2C), 76.3, 75.5, 75.4, 75.2, 74.7, 73.4, 73.1, 72.8, 68.3, 68.4, 66.7, 57.8. Anal. calcd. for $C_{56}H_{58}O_{10}S$ C 72.86, H 6.33, S 3.47. Found: C 72.82, H 6.40, S 3.40.

Compound 3i. Sodium hydride (54 mg, 1.5 equiv.) was suspended in DMF (10 ml) in a flask, and the solution of **2i** (830 mg, 0.94 mmol) in DMF (20 ml) was added dropwise at 0 °C temperature. The mixture was stirred for 30 min, then benzyl bromide (145 μl, 1.3 equiv.) was added and the reaction mixture was stirred overnight at room temperature. Then a small amount of methanol was added and the solvents were evaporated. The residue was dissolved in CH_2Cl_2 (50 ml), washed with distilled water three times (3 × 15 ml). The organic phase was dried and filtered, the solvents were evaporated and the crude product was purified by silica gel column chromatography in *n*-hexane: ethyl acetate = 1:1 to give **3i** (500 mg, 58%), $[\alpha]_D$ −18.91 (*c* 0.17, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 7.58–7.53 (2H, m, arom.), 7.42–7.09 (33 H, m, arom.), 5.08 (1H, d, *J* = 11 Hz), 4.96 (1H, d, *J* = 11.5 Hz), 4.81–4.62 (6H, m), 4.54 (2H, t, *J* = 12 Hz), 4.45–4.38 (2H, m), 4.35–4.20 (6H, m), 3.98–3.90 (2H, m), 3.83–3.66 (4H, m), 3.60 (1H, t, *J* = 8,5 Hz), 3.52–3.32 (3H, m), 2.43 (1H, s, C*H*propargyl).^{13}C NMR (500 MHz, $CDCl_3$) *δ*: 139.0, 138.9, 138.7, 138.4, 138.3, 138.1, 133.7, 132.0, 129.7, 128.8, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.4, 127.3, 127.3, 127.1, 102.7 (C-1′), 87.4 (C-1), 84.9, 82.0, 80.3, 80.1, 80.0, 79.8, 79.3, 76.4, 75.5, 75.4, 75.2, 74.7, 74.3, 74.2, 73.3, 68.3, 68.1, 58.4. Anal. calcd. for $C_{63}H_{64}O_{10}S$ C 74.68, H 6.37, S 3.16. Found: C 74.75, H 6.40, S 3.14.

Compound 5a. Teicoplanin pseudoaglycon azide (**4**) (100 mg, 0.07 mmol) and compound **3a** (30 mg) were reacted according to general method A, yielding compound **5a** (40 mg, 33%) as a pale yellow powder. NMR data can be found in Supplementary Tables S1 and S2 (Supplementary Information). MS (MALDI-TOF) *m/z* 1729.56 ($M+Na^+$) (1729.57 calcd. for $C_{85}H_{92}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]). Anal. calcd. for $C_{85}H_{92}Cl_2N_{10}O_{24}$ C 59.75, H 5.43, N 8.20. Found: C 59.72, H 5.40, N 8.14.

Compound 5b. Compound **4** (100 mg, 0.07 mmol) and compound **3b** (24 mg) were reacted according to general method A, resulting in compound **5b** (40 mg, 35%) as a white powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z* 1673.48 ($M+Na^+$) (1673.49 calcd. for $C_{81}H_{84}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]). Anal. calcd. for $C_{81}H_{84}Cl_2N_{10}O_{24}$ C 58.87, H 5.12, N 8.48. Found: C 58.83, H 5.15, N 8.46.

Compound 5c. Compound **4** (100 mg, 0.07 mmol) and compound **3c** (17 mg) were reacted according to general method B. The reaction resulted in compound **5c** (70 mg, 61%) as a white powder. NMR, MS and elemental analysis properties corresponded to the data reported for the compound previously.[13]

Compound 5d. Compound **4** (100 mg, 0.07 mmol) was reacted with compound **3d** (14 mg) according to general method A, resulting in compound **5d** (20 mg, 18%) as a white powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z* 1617.43 ($M+Na^+$) (1617.43 calcd. for $C_{77}H_{76}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]). Anal. calcd. for $C_{77}H_{76}Cl_2N_{10}O_{24}$ C 57.93, H 4.80, N 8.77 Found: C 57.79, H 4.69, N 8.67.

Compound 5e. Compound **4** (100 mg, 0.07 mmol) was reacted with compound **3e** (12 mg) according to general method A, resulting in compound **5e** (31 mg, 28%) as a white powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z*1589.5 ($M+Na^+$) (1589.41 calcd. for $C_{75}H_{72}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]) Anal. calcd. for $C_{75}H_{72}Cl_2N_{10}O_{24}$ C 57.44, H 4.63, N 8.93 Found: C 57.40, H 4.68, N 8.87.

Compound 5f. Compound **4** (100 mg, 0.07 mmol) and compound **3f** (12.5 μl) were reacted according to general method A, yielding compound **5f** (35 mg, 32%) as a white powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z* 1581.34 ($M+Na^+$) (1581.34 calcd. for $C_{75}H_{64}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]). Anal. calcd. for $C_{75}H_{64}Cl_2N_{10}O_{24}$ C 57.73, H 4.13, N 8.98. Found: C 57.69, H 4.16, N 8.95.

Compound 5g. Azide–alkyne click reaction performed according to general method B using compound **4** (100 mg, 0.07 mmol) and compound **3g** (19 mg) resulted in compound **5g** (54 mg, 48%) in the form of a white powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z* 1631.35 ($M+Na^+$) (1631.35 calcd. for $C_{79}H_{66}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]). Anal. calcd. for $C_{79}H_{66}Cl_2N_{10}O_{24}$ C 58.92, H 4.13, N 8.70. Found: C 58.89, H 4.17, N 8.65.

Compound 5h. Compound **4** (100 mg, 0.07 mmol) and compound **3h** (32 mg) were reacted according to general method B, yielding compound **5h** (60 mg, 50%) as a white powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z* 1747.42 ($M+Na^+$) (1747.42 calcd. for $C_{81}H_{78}Cl_2N_{10}O_{24}Na$ [$M+Na^+$]). Anal. calcd. for $C_{81}H_{78}Cl_2N_{10}O_{24}$ C 56.35, H 4.55, N 8.11. Found: C 56.29, H 4.58, N 8.07.

Compound 5i. Compound **4** (100 mg, 0.07 mmol) and compound **3i** (80 mg) were reacted according to general method A, resulting in compound **5i** (49 mg, 28%) as a yellow powder. NMR data can be found in Supplementary Tables S1 and S2. MS (MALDI-TOF) *m/z* 2461.70 ($M+Na^+$) (2461.70 calcd. for $C_{129}H_{120}Cl_2N_{10}O_{33}SNa$ [$M+Na^+$]). Anal. calcd. for $C_{129}H_{120}Cl_2N_{10}O_{33}S$ C 63.46, H 4.95, N 5.74, S 1.31. Found: C 63.43, H 4.97, N 5.70, S 1.29.

Compound 5j. Compound **4** (107 mg, 0.075 mmol) and compound **3j** (65 mg) were reacted according to general method A, yielding compound **5j** (46 mg, 29%) as a yellow powder. NMR data can be found in Supplementary Tables S1 and S2. MS (ESI-TOF) *m/z* 2127.76 ($M+H^+$) (2127.75 calcd. for $C_{114}H_{117}Cl_2N_{10}O_{27}$ [$M+H^+$]).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the financial support from the Hungarian Research Fund (OTKA K 109208). This research was also supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.2B-15/1/KONV-2015-0001.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)