Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Medicinal Chemistry 157 (2018) 1017–1030

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Structure-activity relationship studies of lipophilic teicoplanin pseudoaglycon derivatives as new anti-influenza virus agents

Zsolt Szűcs [a], Viktor Kelemen [a], Son Le Thai [a], Magdolna Csávás [a], Erzsébet Rőth [a], Gyula Batta [b], Annelies Stevaert [c], Evelien Vanderlinden [c], Lieve Naesens [c, *], Pál Herczegh [a, **], Anikó Borbás [a, ***]

[a] Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary
[b] Department of Organic Chemistry, University of Debrecen, H-4032 Debrecen, Hungary
[c] Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 7 June 2018
Received in revised form
20 August 2018
Accepted 21 August 2018
Available online 22 August 2018

Keywords:
Teicoplanin
Lipoglycopeptide
Maleimide
Sulfonamide
Influenza virus inhibitor
Coronavirus

ABSTRACT

Six series of semisynthetic lipophilic glycopeptide antibiotic derivatives were evaluated for *in vitro* activity against influenza A and B viruses. The new teicoplanin pseudoaglycon-derived lipoglycopeptides were prepared by coupling one or two side chains to the *N*-terminus of the glycopeptide core, using various conjugation methods. Three series of derivatives bearing two lipophilic groups were synthesized by attaching bis-alkylthio maleimides directly or through linkers of different lengths to the glycopeptide. Access to the fourth and fifth series of compounds was achieved by click chemistry, introducing single alkyl/aryl chains directly or through a tetraethylene glycol linker to the same position. A sixth group of semisynthetic derivatives was obtained by sulfonylation of the *N*-terminus. Of the 42 lipophilic teicoplanin pseudoaglycon derivatives tested, about half showed broad activity against influenza A and B viruses, with some of them having reasonable or no cytotoxicity. Minor differences in the side chain length as well as lipophilicity appeared to have significant impact on antiviral activity and cytotoxicity. Several lipoglycopeptides were also found to be active against human coronavirus.

1. Introduction

Human influenza A and B viruses cause the annual influenza epidemics and sporadic pandemics associated with high fatality rate [1]. The viral envelope contains two glycoproteins with a crucial role in virus replication. The hemagglutinin (HA) is responsible for initial attachment of the virus to sialylated cell surface glycans, and fusion of the viral and endosomal membranes after endocytosis of the virus particle [2]. The influenza virus neuraminidase (NA) catalyzes release of newly formed virions at the end of the viral life cycle. Currently available influenza virus blockers are the NA inhibitors oseltamivir and zanamivir, and the M2 ion channel blockers amantadine and rimantadine [3]. The latter two are rarely used nowadays because of global viral resistance against them [4]. The increasing awareness of potential oseltamivir resistance [5,6] advocates the need for new anti-influenza medications. Clinical trials are ongoing for a few HA-targeting approaches, i.e. the fusion inhibitor arbidol and diverse broadly neutralizing anti-HA antibodies [3]. In addition, the possibility to target a host factor involved in HA functioning is tested with the HA maturation inhibitor nitazoxanide and a receptor-destroying sialidase enzyme, besides various concepts in the preclinical stage [7].

Antiviral drugs are also required for pandemic preparedness against zoonotic and highly virulent influenza viruses [8]. In this context, antiviral glycopeptide analogues seem particularly

Abbreviations: DCM, dichloromethane; DMF, dimethylformamide; CPE, cytopathic effect; Et_3N, triethylamine; Galp, galactopyranose; HA, hemagglutinin; HIV, human immunodeficiency virus; logP, logarithm of the partition coefficient; MCC, minimum cytotoxic concentration; MDCK, Madin–Darby Canine Kidney; M2, Matrix-2; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NA, neuraminidase; Ph, phenyl; PMB, *p*-methoxybenzyl; SARS-CoV, severe acute respiratory syndrome coronavirus; SI, selectivity index; SEM, standard error of the mean; TEG, tetraethylene glycol; TLC, thin layer chromatography; tosyl, *p*-toluenesulfonyl.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: lieve.naesens@kuleuven.be (L. Naesens), herczeghp@gmail.com (P. Herczegh), borbas.aniko@pharm.unideb.hu (A. Borbás).

https://doi.org/10.1016/j.ejmech.2018.08.058
0223-5234/

relevant since they often display broad activity against influenza plus some other emerging viruses. Derivatives of teicoplanin or related antibiotics were reported to inhibit, among others, influenza virus [9]; coronaviruses [10] including SARS-CoV (severe acute respiratory syndrome coronavirus) [11]; Ebola pseudovirus [12]; HIV [13]; or hepatitis C virus [14]. Our focus of the last years was to investigate the structure-activity relationship and mechanism of action for influenza virus. We reported lipophilic derivatives of ristocetin aglycon modified on the *N*-terminal part of the molecule, which proved to be strong inhibitors of influenza virus replication in cell culture [15]. Mechanistic studies with the lead compound demonstrated that it interferes with influenza virus endocytosis [16]. Its favorable selectivity index encouraged us to prepare a series of analogues to gain further insight into structure activity relationships [17,18]. The outstanding antiviral properties were lost in analogues containing teicoplanin aglycon or pseudoaglycon, meaning that minor structural differences between the aglycon of teicoplanin and that of ristocetin, have major impact on antiviral activity [19].

Next, we found that a special lipophilic modification of teicoplanin pseudoaglycon also resulted in derivatives with high anti-influenza virus activity [20]. These derivatives contain a sugar unit carrying two *n*-octyl chains, and this lipophilic auxiliary is attached to teicoplanin pseudoaglycon through a tetraethylene glycol chain and a triazole ring (**1a** and **1b**, Fig. 1). Although the antiviral mode of action of **1a** and **1b** remains to be elucidated, we observed that these dually octylated teicoplanin pseudoaglycon derivatives inhibit influenza virus-induced hemagglutination, suggesting that they interfere with the binding interaction between the viral HA and sialylated host cell receptors. We also found that the lipophilic side chains in these molecules are essential for anti-influenza virus activity, since changing these octyl chains to methyl groups (**1c**) completely abolished the antiviral effect [20]. Unfortunately, the strong activity of **1a** and **1b** was accompanied by high cytotoxicity, while the inactive and less amphiphilic **1c** was only moderately toxic.

Surprisingly, we recently found that teicoplanin pseudoaglycon achieves anti-influenza virus properties by a simple lipophilic modification based on the well-known azide-alkyne cycloaddition click reaction (**2a-j**) [21]. The activity oddly correlated to the structure, since it disappeared and then reappeared by increasing the length of the lipophilic alkyl chains. Changes in cytotoxicity, however, were more consistent and indicated that the addition of longer alkyl substituents resulted in higher cytotoxicity. Although compounds **2a** and **2d** showed excellent inhibitory activity against influenza virus, their selectivity indices were unfavorable [21]. Noteworthy, some of these derivatives with amphiphilic, bulky substituents (**2i**, **2j**) displayed anti-influenza virus activity without significant cytotoxicity, despite their high calculated logP values.

With the aim of achieving derivatives with more favorable biological properties, we decided to carry out a systematic structure-activity relationship analysis of teicoplanin pseudoaglycon derivatives resembling **1a**, **1b**, or e.g. **2d**. A simple conjugation method for protein and peptide modification was reported [22], involving rapid and clean reaction of 3,4-dibromomaleimides and thiols, and giving bis-alkylthio maleimides in high yields through an addition-elimination mechanism. We envisioned the application of maleimide chemistry in combination with azide-alkyne

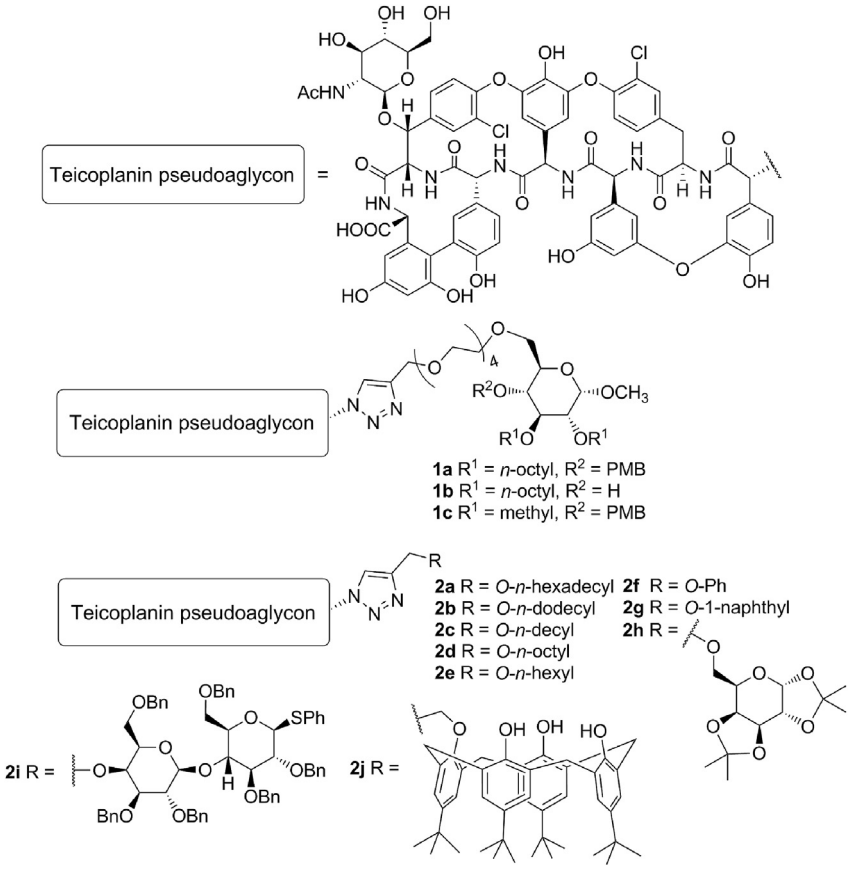


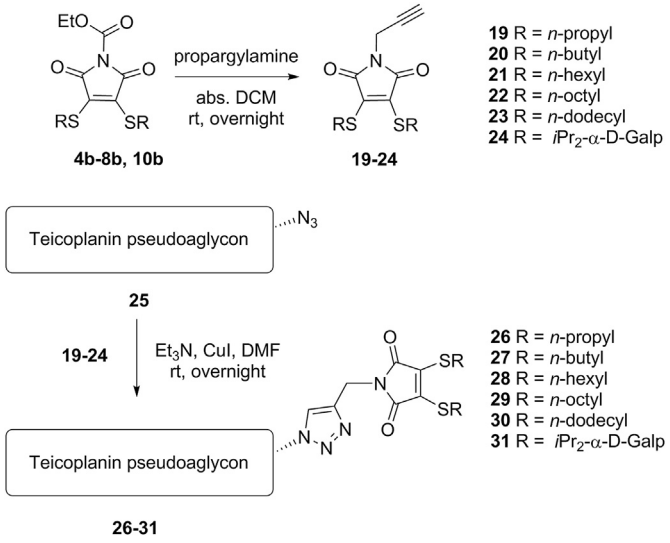
Fig. 1. Structure of previously synthesized teicoplanin derivatives. Compounds **1a**, **1b**, **2a** and **2d** showed promising anti-influenza virus activity; **1c**, **2b**, and **2c** were inactive; and **2e** showed modest activity [20,21]. (PMB = *p*-methoxybenzyl).

click chemistry, to produce three sets of teicoplanin derivatives equipped with two lipophilic substituents similarly as in **1a** and **1b**. (In the Discussion, these derivatives are denoted as Series 1–3). Compounds **2a-j** are referred to in the SAR analysis as Series 4. Two further sets of derivatives (Series 5 and 6) comparable to **2a-g**, which contain only one lipophilic alkyl chain, were prepared by azide-alkyne 1,3-dipolar cycloaddition reaction and by *N*-sulfonylation. Here we describe the synthesis and antiviral investigation of these compounds.

2. Results and discussion

Chemistry. The first group of derivatives (**12**–**18**) was prepared by synthesizing various bis-alkylthio maleimides **4a-10a** from 3,4-dibromomaleimide **3**, followed by subsequent ethoxycarbonylation of the maleimide NH group [23] making compounds **4b-10b** suitable for conjugation to the *N*-terminus of teicoplanin pseudoaglycon **11** (Scheme 1). Although we have described this path earlier [24] for the synthesis of compounds **4**, **7**, **8**, **10**, **12**, **15**, **16**, **18**, this time we also prepared the bis-*n*-butylthio and bis-*n*-hexylthio maleimide derivatives (**5** and **6**) for the sake of the systematic approach in this study. We also prepared the asymmetrically substituted maleimide variant **9** that has an *n*-propyl and *n*-dodecyl function.

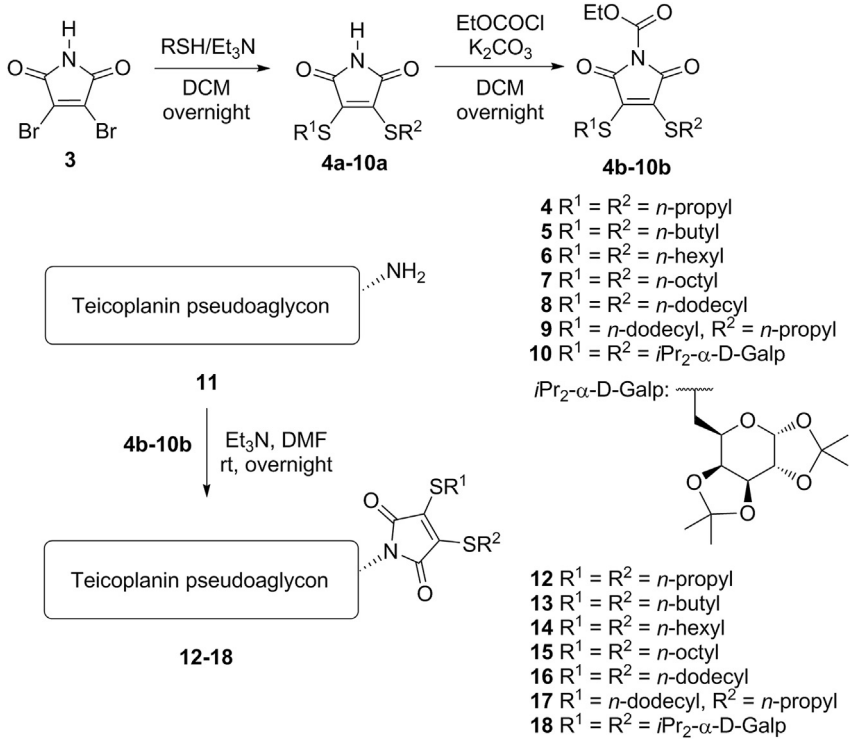
The second series of derivatives contains similar maleimide-derived side chains as the previous group, but these are attached through a triazole linker to the glycopeptide core, in order to increase the distance of the lipophilic chains from the pseudoaglycon. In this synthetic procedure we prepared *N*-propargyl maleimides **19**–**24** by reaction of the corresponding *N*-ethoxycarbonyl maleimides (**4b-8b**, **10b**) with propargylamine. Finally, an azide-alkyne click reaction was carried out between azido teicoplanin pseudoaglycon **25** [9] and maleimides **19**–**24**, yielding final products **26**–**31** (Scheme 2).



Scheme 2. Conjugation of the bis-alkylthio maleimide derivatives **19**–**24** to azido teicoplanin pseudoaglycon **25** through a triazole moiety (Series 2).

The third group of derivatives carrying maleimide substituents differs from the previous one in the structure of the linker, which in this case contains a tetraethylene glycol segment besides the triazole ring as in compounds **1a** and **1b**. By introduction of the tetraethylene glycol fragment, the overall lipophilicity of the compounds is reduced, while the crucial lipophilic substituents are still incorporated in the structure. This linker modification proved to have a substantial effect on the biological profile of the molecules (see below).

The compounds suitable for this type of modification (**33**–**39**) were prepared by the simple reaction of *N*-ethoxycarbonyl



Scheme 1. Synthesis of maleimide derivatives **12**–**18** (Series 1) with double lipophilic tails by direct coupling of **4**–**10** to teicoplanin pseudoaglycon **11**.

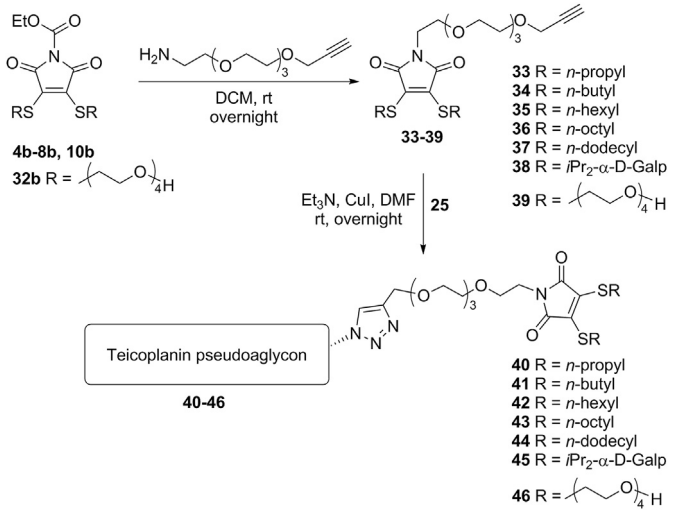
maleimides **4b-8b**, **10b**, **32b** and t**riethylene glycol 2-aminoethyl propargyl ether**. Subsequent click reaction with azido teicoplanin pseudoaglycon **25** gave glycopeptides **40**–**46** (Scheme 3).

The fourth type of modification we describe here was based on our previous results mentioned above. Although compounds **2a** and **2d** possessing single, relatively long alkyl chains were highly active against influenza virus, they also displayed considerable cytotoxicity [21]. (For the sake of clarity, these compounds are listed as a separate series, i.e. Series 4 in Table 1. The structures can be found in Fig. 1.)

We speculated that this high cytotoxicity might be related to a combination of two factors, the first being high lipophilicity of the side chains in contrast to the hydrophilic pseudoaglycon part, which may cause a membrane-disrupting effect. This might be enhanced by the simple structure of the alkyl chains, which could facilitate insertion of these molecules into biological membranes. In our previous work [21] we attached two very bulky substituents (a perbenzylated disaccharide and a calix[4]arene carrying *tert*-butyl-substituents) to the same pseudoaglycon (**2i** and **2j**). Calculated logP values for these compounds indicated higher lipophilicity than e.g. the *n*-hexadecyl or *n*-octyl derivatives **2a** and **2d**. These derivatives did not produce profound cytotoxic effect, supporting our hypothesis that cytotoxicity is determined by the combination of the lipophilicity and bulkiness of the substituents. It needs to be mentioned here, that in the A2 components of teicoplanin the ß-D-glucosamine on the D-ring is *N*-acylated by similarly simple acyl chains, yet these compounds seem to be harmless to mammalian cells. Therefore, the overall lipophilicity of the molecules may also influence cytotoxicity.

Since the introduction of alkyl chains proved to be promising in terms of antiviral activity, we decided to insert the tetraethylene glycol linker between the core and these substituents, in order to decrease lipophilicity as in the case of maleimide derivatives, which again caused a significant change in biological properties.

First, the mono-azido derivative of tetraethylene glycol **47** was utilized in a click reaction with alkyl propargyl ethers **48**–**51**, yielding triazole derivatives **52**–**55**. These were O-alkylated with propargyl bromide to provide compounds **56**–**59**, which were used in the last step in a CuAAC reaction with azido teicoplanin pseudoaglycon **25** to obtain derivatives **60**–**63** (Scheme 4).



Scheme 3. Conjugation of bis-alkylthio maleimide derivatives equipped with an *N*-TEG-propargyl moiety (**33**–**39**) to azido teicoplanin pseudoaglycon **25** gave Series 3 (**40**–**46**).

Finally, by the reaction of teicoplanin pseudoaglycon with different sulfonyl chlorides **64**–**71** we prepared sulfonamide derivatives (**72**–**79**) (Scheme 5). This type of derivatization was attractive mainly because of its simplicity and high stability of the sulfonamide bond. Despite this, to our knowledge no one has thus far reported this type of derivatives of teicoplanin or related glycopeptides.

Biological evaluation. The anti-influenza virus activity was determined in Madin-Darby canine kidney (MDCK) cells using a reported cytopathic effect (CPE) reduction method, in which CPE is assessed by microscopy plus formazan-based cell viability assay [25]. In parallel, cytotoxicity was determined in mock-infected cells. Table 1 summarizes the data for three human influenza A strains (including an A/H1N1 2009 pandemic strain) plus one influenza B strain.

Series 1, maleimide derivatives **12**–**18**, showed variable activity against the four influenza virus strains tested, but neither of them proved as potent as compound **1a**. Only compounds **17** and **18** displayed quite consistent activity against the four strains. Curiously enough, neither the double *n*-propyl nor the double *n*-dodecyl substituted maleimide moieties yielded active compounds, while the combination of *n*-propyl and *n*-dodecyl chains (compound **17**) led to notable activity. The fair activity of compound **18** carrying a double galactose substituted maleimide was accompanied by modest selectivity with an SI of 5 [selectivity index (SI): ratio of MCC to average EC_{50}]. This indicated the need for structural modification in order to reduce the cytotoxicity.

Series 2 (**26**–**31**) containing the triazole linker was, overall, more successful against influenza virus with somewhat lower cytotoxicity than the first series. Namely, antiviral activity was seen in the case of compounds **27**, **28** and **29** which contain double *n*-butyl, *n*-hexyl and *n*-octyl chains, respectively. The presence of the linker highly altered the behavior of these compounds, since the analogous derivatives in the previous series (i.e. no triazole linker) with the same alkyl chain lengths were inactive. **29** displayed robust antiviral activity and a favorable SI of 10, whereas **27** and in particular **28** still had poor selectivity. **30** with the double *n*-dodecyl moiety remained inactive, but became less cytotoxic, which was a general finding in Series 2. Sadly, the double galactose substituted derivative lost its activity on introduction of the triazole linker.

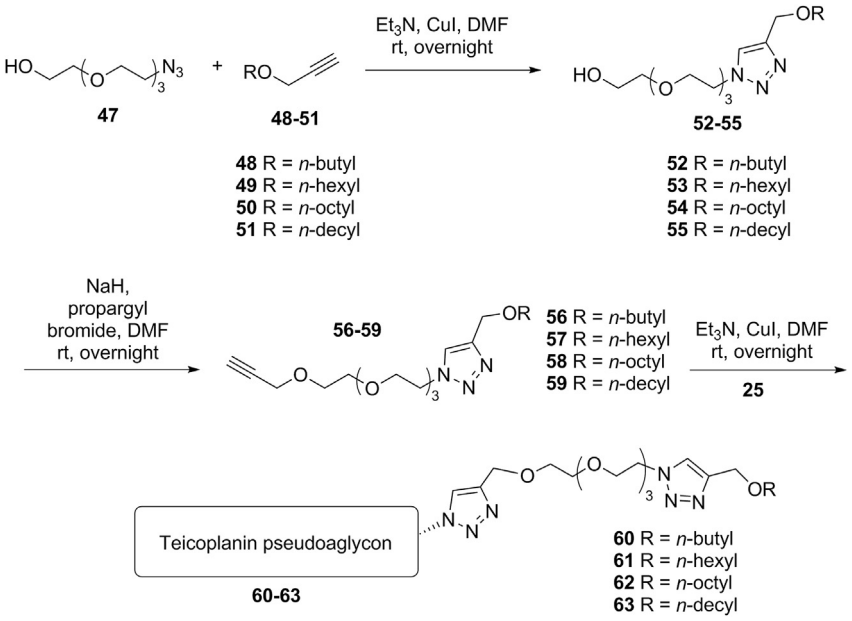
The biological results for Series 3 (**40**–**46**) demonstrated that by introducing the tetraethylene glycol linker, cytotoxicity was generally reduced, while the anti-influenza virus activity was retained in the *n*-butyl (**41**) and *n*-hexyl (**42**) derivatives, and even increased in the *n*-octyl (**43**) analogue. Since the tetraethylene glycol element seemed beneficial, we also prepared a derivative with double tetraethylene glycol side chains (**46**), but this modification did not yield an active compound, probably because it misses the lipophilic moiety that proved to be essential for anti-influenza virus activity (see conclusions). Surprisingly, despite the lack of lipophilic moieties, compound **46** proved to be highly cytotoxic. Ultimately, **41** stood out as the most promising compound in this group, with very low cytotoxicity and robust activity against all tested strains, yielding a nice SI of 16.

The activities of triazole derivatives (**2a-j**) in Series 4 have already been published [21] elsewhere. These data are shown again, considering the systematic approach in our present report and the structural analogy to some of the compounds in this work. Surprisingly, among the alkyl substituted compounds, only the *n*-hexadecyl (**2a**) and *n*-octyl (**2d**) derivatives showed high activity, while the attachment of *n*-dodecyl (**2b**) and *n*-decyl (**2c**) side chains led to inactive compounds. However, all four derivatives had very high cytotoxicity. **2e** with a *n*-hexyl side chain and compounds with aromatic substituents (**2f**, **2g**) were only mildly active with

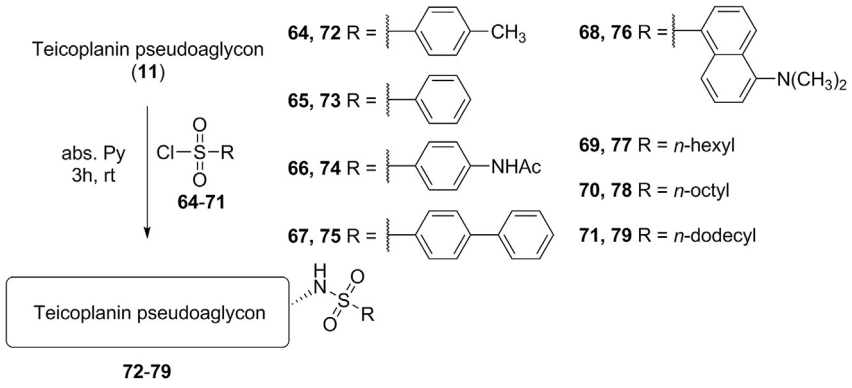
Table 1
Activity in influenza virus-infected MDCK[a] cells.

Compound*	Antiviral EC_{50}^{b}								Cytotoxicity[c]	
	A/H1N1		A/H1N1pdm		A/H3N2		Influenza B			
	CPE	MTS	CPE	MTS	CPE	MTS	CPE	MTS	CC_{50}	MCC
	(μM)									
1a	0.80	1.2	1.4	<0.80	1.8	1.2	>100#	>100#	11	20
Series 1: Maleimide derivatives										
12 (2x C_3)	>100	>100	1.8	2.0	>100	>100	>100	7.2	18	20
13 (2x C_4)	>100	>100	2.9	2.6	>100	>100	>100	>100	8.6	4
14 (2x C_6)	>100	>100	>100	>100	>100	>100	>100	>100	9.8	4
15 (2x C_8)	>100	>100	2.1	1.9	>100	>100	>100#	>100#	11	20
16 (2x C_{12})	>100	>100	>100	>100	>100	>100	>100	>100	≤1.1	≤4.0
17 (C_3, C_{12})	>100	10	5.1	8.2	7.3	6.8	8.9	7.3	29	≥20
18 (2 x Galp)	>100	9.1	2.1	2.2	2.3	1.7	>100	4.2	26	20
Series 2: Maleimide derivatives with a triazole linker										
26 (2x C_3)	>100	>100	>100	>100	>100	>100	>100#	>100#	22	20
27 (2x C_4)	2.1	3.2	9.8	7.4	>100	4.9	2.5	3.5	28	20
28 (2x C_6)	3.1	>100	4.0	2.9	1.9	1.9	2.3	2.7	12	≥4
29 (2x C_8)	9.2	12	4.4	3.4	8.9	6.3	6.7	7.2	49	73
30 (2x C_{12})	>100	>100	>100	>100	>100	>100	>100#	>100#	75	100
31 (2 x Galp)	>100	>100	>100	>100	>100	>100	>100#	>100#	18	20
Series 3: Maleimide derivatives with a triazolyl TEG linker										
40 (2x C_3)	>100	>100	>100	7.8	6.2	≤7.8	>100#	>100#	38	20
41 (2x C_4)	4.8	5.8	9.5	8.6	5.3	6.0	5.5	4.9	>100	100
42 (2x C_6)	2.0	3.5	2.7	6.0	2.0	2.9	1.8	3.6	65	20
43 (2x C_8)	1.3	2.0	≤1.4	≤0.80	0.92	≤0.80	>20#	>20#	19	≥4.0
44 (2x C_{12})	>100	13	>100	2.8	>100	2.4	>100	>100	79	20
45 (2x TEG)	>100	>100	>100	>100	>100	>100	>100	>100	3.0	4.0
46 (2 x Galp)	>100	>100	>100	>100	>100	23	>100	42	≥32	>100
Series 4: Triazole derivatives [21]										
2a (C_{16})	1.6	1.8	1.8	1.8	≤8.9	≤1.6	1.8#	1.3#	7.6	≥4.0
2b (C_{12})	>100	>100	>100	>100	>100	>100	>100#	>100#	2.3	9.3
2c (C_{10})	>100	>100	>100	>100	>100	>100	>100#	>100#	3.7	4
2d (C_8)	>100	2.2	1.8	1.9	2.1	1.5	1.8#	1.9#	13	≥4.0
2e (C_6)	>100	>100	>100	>100	15	13	17#	11#	53	≥20
2f (Ph)	>100	>100	>100	>100	11	11	>100#	>100#	41	≥20
2g (α-Np)	11	11	11	4.4	15	8	15#	6.6#	47	20
2h (Galp)	52	47	45	43	39	24	39#	29#	>100	≥100
2i (lactose)	>100	>100	8.9	7.3	8.9	20	6.6#	4.0#	>100	≥20
2j (calixarene)	>100	43	8.9	6.7	>100	5.2	>100#	5.7#	≥58	≥20
Series 5: Triazole derivatives with a TEG linker and single alkyl chains										
60 (C_4)	>100	51	>100	47	>100	31	>100	28	>100	100
61 (C_6)	>100	>100	>100	>100	>100	>100	>100	>100	51	100
62 (C_8)	>100	>100	>100	>100	>100	>100	>100	>100	19	20
63 (C_{10})	1.6	1.5	1.8	1.8	1.6	1.3	>100	<0.80	≥82	≥20
Series 6: Sulfonamide derivatives										
72 (toluyl)	9	11	30	32	41	19	11	12	>100	100
73 (Ph)	16	27	46	52	25	23	51	21	>100	100
74 (Ph*N*HAc)	>100	>100	>100	>100	>100	>100	>100	47	60	100
75 (biphenyl)	1.8	1.7	2.0	2.0	1.5	1.6	1.8	2.4	15	11
76 (dansyl)	4.9	7.0	6.3	5.9	8.3	2.3	6.7	5.3	52	20
77 (C_6)	8.6	9.6	8.9	9.1	8.3	8.9	8.9	10.3	54	100
78 (C_8)	1.9	2.5	>100	>100	2.0	2.4	4.0	>100	14	20
79 (C_{12})	0.4	0.8	>100	1.6	<0.8	<0.8	<0.8	<0.8	4.5	4.0
Zanamivir	0.041	0.19	1.9	30	20	3.2	0.0079	0.0062	>100	>100
Ribavirin	8.4	9.1	10	8.5	13	5.9	2.3	2.2	>100	>100
Amantadine	>500	>500	>500	>500	11	1.9	>500	>500	>500	>500
Rimantadine	>500	>500	>500	>500	0.20	0.17	>500	>500	>500	>500

*Lipophilic substituents are specified in brackets.
[a]MDCK, Madin-Darby canine kidney cells.
[b]Antiviral activity expressed as the EC_{50}, i.e. the compound concentration producing 50% inhibition of virus replication, as estimated by microscopic scoring of the cytopathic effect (CPE) or by measuring cell viability in the formazan-based MTS assay. Influenza strains: A/PR/8/34 (A/H1N1); A/Virginia/ATCC3/2009 (A/H1N1pdm); A/HK/7/87 (A/H3N2); B/Ned/537/05 or B/HK/5/72 (data marked with #).
[c]Cytotoxicity expressed as the minimum cytotoxic concentration (MCC; compound concentration producing minimal changes in cell morphology, as estimated by microscopy) or the 50% cytotoxic concentration (CC_{50}; estimated by the MTS cell viability assay).
Data represent the means of two to five independent tests.



Scheme 4. Another variant (Series 5) of teicoplanin pseudoaglycon modification (**60**–**63**).



Scheme 5. Synthesis of sulfonamide derivatives (Series 6) of teicoplanin pseudoaglycon (**72**–**79**).

moderate cytotoxicity. Interestingly, **2h** and **2i** (a perbenzylated lactose and a calix[4]arene derivative) displayed moderately high activity which was accompanied by only modest cytotoxicity, despite their very high calculated logP values indicating much higher lipophilicity when compared to **2a**. As stated above, the explanation could be that, although these molecules are very lipophilic, their bulky structures may lead to an inability to disrupt cellular membranes as is the case for alkyl derivatives.

To further investigate how the tetraethylene glycol linker impacts the cytotoxicity, we synthesized Series 5, derivatives **60**–**63**, which are analogous to the alkyl chain-containing triazole derivatives in Series 4. We speculated that introduction of the linker would boost the antiviral activity and reduce the cytotoxicity compared to the published compounds. Indeed, compound **63** (an analogue of **2c**) showed excellent antiviral activity and reasonable cytotoxicity compared to **2c** which is highly cytotoxic and devoid of anti-influenza virus activity. Interestingly, lowering the length of the alkyl chain (analogues **60**–**62**) did not yield effective compounds.

Also for the sulfonamide derivatives (Series 6), the size and type of lipophilic substituents seemed to play an important role. The less hydrophobic tosyl (**72**), benzenesulfonyl (**73**), and *p*-acetamidobenzenesulfonyl (**74**) derivatives had rather low or no anti-influenza virus activity and were also not cytotoxic. However, the more lipophilic aryl substituted compounds, such as the biphenylsulfonyl (**75**) and dansyl (**76**) derivatives, displayed good activity. Unfortunately, they were also cytotoxic in the MDCK cells. The relationship between alkyl chain length, cytotoxicity and antiviral activity was very clear in case of alkylsulfonates **77**–**79**. The hexanesulfonyl derivative (**77**) was the best compound showing moderate cytotoxicity and very consistent anti-influenza virus activity. With growing alkyl chain length (**78**, **79**), the activity rapidly increased, but so did the cytotoxicity.

Inhibition of influenza HA- and NA-bearing lentiviral pseudoparticles. We previously published the anti-influenza virus mechanism of action of a lipophilic derivative of aglycoristocetin denoted SA-19 [16]. This compound proved to have nice activity in an assay with green fluorescent protein (GFP)-expressing lentiviral

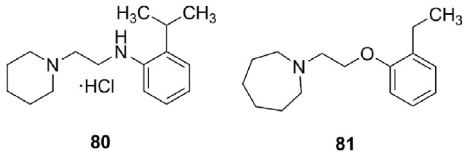


Fig. 2. Structure of aniline-based influenza virus fusion inhibitors **80** and **81**. (See ref. [26]. compounds **9d** and **14a**, respectively).

pseudoparticles bearing influenza virus HA and NA. This was consistent with other findings that SA-19 interferes with HA-mediated endocytosis. We now used the same procedure for two of the most potent teicoplanin pseudoaglycon derivatives (**1a**, **63**). Strong and dose-dependent inhibition was seen with all four control compounds (Fig. 3), i.e. SA-19; chloroquine (an inhibitor of endosomal acidification); and two aniline-based influenza fusion inhibitors (**80**, **81**, Fig. 2) which inhibit the conformational refolding of H1 HA at low endosomal pH [26]. With **63**, the inhibition of pseudoparticle entry, as deduced from the reduction in GFP signal, was 92% at 10 μM and 56% at 2 μM. Likewise, **1a** produced 54% reduction at 2 μM.

Activity against human coronavirus 229E. Given that teicoplanin and related glycopeptides were reported to have anti-coronavirus activity [10,11], we evaluated a subset of the newly synthesized compounds against human coronavirus 229E, using two complementary methods, i.e. CPE reduction in human embryonic lung (HEL) fibroblast cells and virus yield reduction in human lung carcinoma A549 cells. Among Series 1, 2 and 3, we tested the analogues carrying alkyl groups of intermediate length, i.e. *n*-butyl and *n*-hexyl; for Series 6, the entire series was tested. The antiviral activity values obtained (see Table S6 in Supporting Information) in HEL and A549 cells showed nice agreement. In A549 cells, several compounds produced 2-$\log_{10}$ (i.e. 100-fold) reduction in coronavirus yield at a concentration of ~10 μM, with no or minimal cytotoxicity at 50 μM. The alkanesulfonamide derivatives **77** and **79** displayed potent anti-coronavirus activity in terms of EC_{50} (~2 μM) and SI (ratio of MCC to EC_{50}: 11 for **77** and 13 for **79**). Within the same series, the *p*-toluenesulfonamide analogue **72** was also nicely active and selective (SI: 19).

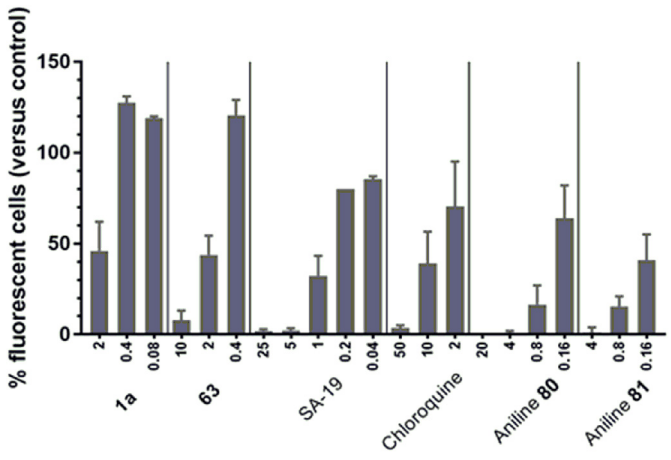


Fig. 3. Inhibitory effect on cell entry of influenza virus HA- and NA-bearing lentiviral pseudoparticles. The GFP-expressing particles carrying H1-HA and N1-NA (from A/PR/8/34) were transduced into MDCK cells in the presence of compounds (X-axis: concentrations in μM), and GFP expression was quantified after three days incubation. The two test compounds, **1a** and **63**, were tested in parallel with four control compounds, i.e. SA-19, a lipophilic derivative of aglycoristocetin [16]; chloroquine; and two aniline-based influenza fusion inhibitors, **80** and **81** [26]. Data are the mean ± SEM of four independent experiments.

3. Conclusion

In summary, we synthesized and evaluated several derivatives of teicoplanin pseudoaglycon in a systematic manner to obtain structure-activity relationships concerning the anti-influenza virus activity of the compounds. Many of the lipoglycopeptides exhibit remarkable activity against both influenza A and B viruses and showing, occasionally, favorable selectivity index.

Based on the biological data, it became evident that inhibition of influenza virus replication is not primarily dependent on the type or complexity of the chemical bond or functional group between the glycopeptide *N*-terminus and the newly introduced fragments. The structure of the side chains, but even more the overall lipophilicity of the compounds are the most influential on biological properties.

Hence, the presence of a lipophilic side chain is definitely a requirement for antiviral activity. The optimal length of alkyl substituents, which is somewhat dependent on the structure of the linker, is usually equivalent to 6–10 methylene groups. If the side chain carries two alkyl groups, reduced length might be enough, e.g. double butyl substitution. The incorporation of longer alkyl groups (>14 carbon atoms, Series 4) might also lead to active compounds, however most of these proved to be highly cytotoxic. The latter was even noted for some compounds with the optimum side chain lengths (e.g. 8–10). Reducing the cytotoxicity with preservation of antiviral activity was achieved by incorporation of the tetraethylene glycol linker. In some cases, this modification led to an improvement, while other derivatives became less active, probably due to a loss of required lipophilicity.

Analyzing the influence of the aryl substitution is more difficult, since this type of substituent is only present in two compound series (Series 4 and 6) in a smaller number. Nevertheless, these analogues seem to display a similar relationship between lipophilicity, anti-influenza virus activity and cytotoxicity, as the alkyl substituted compounds. Compounds containing one aromatic ring are usually not very active nor cytotoxic. Two aromatic rings seem to boost antiviral activity because of increased lipophilicity, but cytotoxicity also becomes prominent. Interestingly, the two compounds that carried numerous aromatic rings in the form of a highly bulky, lipo/hydrophilic side chain were effective against one or more influenza virus strains without causing serious cytotoxicity.

As mentioned earlier, it is likely that cytotoxicity of the lipoglycopeptides increases with the compounds' ability to solubilize cellular membranes and with net lipophilicity. With bulky and amphiphilic side chains, this effect might be weaker compared to more simple structures such as alkyl chains which could easily insert into lipid membranes. This could explain the apparent contradiction that some analogues displayed very high lipophilicity without severe cytotoxicity.

It is evident that the major challenge lies in designing the optimal lipoglycopeptide structures to provide sufficient lipophilicity for anti-influenza virus activity without causing serious cytotoxicity. As we have demonstrated, high activity goes hand in hand with high toxicity in many instances. Still, our study proved that once an active lead compound has been identified, it is possible to diminish its cytotoxicity by applying appropriate structural adjustments. Besides the modifications described here, there are unlimited variations that could lead to derivatives with excellent biological profiles. Moreover, as we have seen, the promising antiviral activity is not limited to influenza virus since some of our derivatives also displayed activity against human coronavirus. This makes this class of lipoglycopeptides a relevant class for further investigation [27].

4. Experimental section

4.1. General information

Propargylamine and alkyl thiols were purchased from Sigma Aldrich Chemical Co., dansyl chloride, benzenesulfonyl chloride, *p*-acetamidobenzenesulfonyl chloride, biphenylsulfonyl chloride were purchased from Tokyo Chemical Industry Co., Ltd., alkyl sulfonyl chlorides were prepared from the corresponding thiols using a literature method [28], **triethylene glycol 2-aminoethyl propargyl ether**, [29] **1-mercapto-11-hydroxy-3,6,9-trioxaundecane**, [30] **1-azido-11-hydroxy-3,6,9-trioxaundecane**, [31] 2,3-dibromomaleimide **3** [32], teicoplanin pseudoaglycon **11** [9] and azido teicoplanin pseudoaglycon **25** [9] were also prepared according to literature procedures. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) silica gel plates with visualization by immersing in ammonium-molibdate solution followed by heating or Pauly-reagent in the case of teicoplanin-derivatives. Flash column chromatography was performed on silica gel 60 (Merck 0.04–0.063 mm). Organic solutions were dried over Na_2SO_4 and concentrated under vacuum. The ^1H (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 DSS (0.00 ppm for ^1H) and to solvent signals ($CDCl_3$: 77.16 ppm, DMSO-d_6: 39.52 ppm for ^{13}C). MS (MALDI-TOF) analysis was carried out in positive reflectron mode on a BIFLEX III mass spectrometer (Bruker, Germany) with delayed-ion extraction. The matrix solution was a saturated solution of 2,4,6-trihydroxyacetophenone (2,4,6-THAP) in DMF. Elemental analysis (C, H, N, S) was performed on an Elementar Vario MicroCube instrument.

4.2. General method A for the preparation of N-ethoxycarbonyl bis-alkylthio maleimides (**5b**, **6b**, **9b**, **32b**)

To a stirred solution of bis-alkylthio maleimide (1.0 equiv.) in dry acetone (20 mL) K_2CO_3 (1.2 equiv.) and ethyl chloroformate (1.2 equiv.) were added under an 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 concentrated. The crude product was used for further steps without purification.

4.3. General method B for preparing N-propargyl-bis-alkylthio maleimides or N-TEG-propargyl-bis-alkylthio maleimides

To a stirred solution of *N*-ethoxycarbonyl bis-alkylthio maleimide [24] (1.0 equiv.) in CH_2Cl_2 (30 mL) propargylamine or triethylene glycol 2-aminoethyl propargyl ether (1.25 equiv.) and Et_3N (1.25 equiv.) were added under an argon atmosphere and stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with cc. NH_4Cl and water twice, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography to give the desired compound.

4.4. General method C for azide-alkyne click reaction

To a stirred solution of azide (1.0 equiv.) in dry DMF (5 mL) the alkyne compound (1.0–1.5 equiv.), Et_3N (1.0 equiv.) and Cu(I)I (20–30 mol%) were added under an argon atmosphere and stirred for overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by flash chromatography. In the case of teicoplanin derivatives toluene/methanol (+1.0 v/v% acetic acid) or acetonitrile/water mixtures were used as eluent.

4.5. General method D for alkylation

To a stirred suspension of NaH (2.0 equiv.) in dry DMF (5 mL) the alcohol (1 equiv.) was added dropwise at 0 °C. After 30 min alkylbromide (1.5 equiv.) was added dropwise and stirred for 3 h. The reaction mixture was quenched with methanol, concentrated, diluted with DCM, washed twice with water, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography to give the desired compound.

4.6. General method E for the preparation of sulfonamides

To a stirred solution of teicoplanin pseudoaglycon (1.0 equiv.) in dry pyridine (3–5 mL) the corresponding sulfonyl chloride (1.3–1.4 equiv.) was added at once and the reaction mixture was allowed to stir at room temperature for 4 h. Afterwards, methanol was added to quench the reaction, the solvent was evaporated, and the crude product was purified by flash chromatography using gradient elution starting from 100% acetonitrile to 90% acetonitrile 10% water.

Compounds 5a and 5b. To a stirred solution of 2,3-dibromomaleimide **3** [32] (255 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) Et_3N (279 μL, 2.0 mmol) and *n*-butyl-mercaptan (226 μl, 2.1 mmol) were added under an argon atmosphere and stirred for 3 h at room temperature. The reaction mixture was evaporated, and the crude product was purified by flash chromatography (hexanes:acetone = 9:1) to give the desired compound **5a** (243 mg, 89%) as a yellow powder. ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H, NH), 3.29 (t, J = 7.3 Hz, 4H, 2 x S-CH_2), 1.69–1.58 (m, 4H), 1.52–1.35 (m, 4H), 0.93 (t, J = 7.3 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.7 (2C, 2x *C*=O); 136.8 (*C*=*C*); 32.6, 31.6, 21.7 (6C, 6x CH_2); 13.7 (2C, 2x CH_3). MS (ESI): *m/z* calculated for $C_{12}H_{19}NO_2S_2$ + Na^+ [M + Na^+]: 296.075. Found: 296.073. Compound **5a** was converted into the *N*-ethoxycarbonyl compound according to general method **A**. The crude compound **5b** was used in further steps without purification.

Compounds 6a and 6b. To a stirred solution of 2,3-dibromomaleimide (510 mg, 2.0 mmol) in CH_2Cl_2 (20 mL) Et_3N (558 μL, 4.0 mmol) and *n*-hexyl-mercaptan (600 μl, 4.2 mmol) were added under an argon atmosphere and stirred for 3 h at room temperature. The reaction mixture was evaporated, and the crude product was purified by flash chromatography (hexanes:ethyl acetate = 9:1) to give compound **6a** (526 mg, 80%) as a yellow powder. ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H), 3.28 (t, J = 7.4 Hz, 4H, 2 x -SCH_2), 1.70–1.59 (m, 4H, CH_2), 1.47–1.37 (m, 4H), 1.35–1.24 (m, 8H), 0.89 (t, J = 6.7 Hz, 6H, 2x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (2C); 136.8 (*C*=*C*); 31.9, 31.4, 30.6, 28.3 (8C, 8x CH_2); 22.6 (2C, 2 x CH_2S); 14.1 (2C, 2x CH_3). MS (ESI): *m/z* calculated for $C_{16}H_{27}NO_2S_2$ + Na^+ [M + Na^+]: 352.138. Found: 352.201. Compound **6a** was converted into *N*-ethoxycarbonyl compound according to general method **A**. The crude compound **6b** was used in further steps without purification.

Compounds 9a and 9b. To a stirred solution of 2,3-dibromomaleimide (510 mg, 2.0 mmol) in CH_2Cl_2 (20 mL) Et_3N (277 μl, 2.0 mmol) was added, then cooled to 0 °C and *n*-propyl-mercaptan (186 μl, 2.0 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise under an argon atmosphere and stirred for 5 h. The reaction mixture was concentrated, and the crude product was purified by flash chromatography (hexanes:ethyl acetate = 9:1) to give the desired intermediate (261 mg, 52%) as a yellow powder. To a stirred solution of the intermediate (261 mg, 1.04 mmol) in CH_2Cl_2 (20 mL) Et_3N (160 μl, 1.1 mmol) and *n*-dodecyl-mercaptan (264 μl, 1.1 mmol) were added under an argon atmosphere and stirred for 30 min. The reaction mixture was concentrated, and the crude product was purified by flash chromatography (hexanes:ethyl

acetate = 95:5) to give compound **9a** (273 mg, 71%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (s, 1H, N*H*), 3.34–3.22 (m, 4H, 2x S-CH_2), 1.74–1.59 (m, 4H), 1.45–1.37 (m, 2H), 1.30–1.21 (m, 16H), 1.03 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.61, 166.40, (2C, 2 x C=O) 136.98, 136.71, (2C, 2 x C-S) 33.79, 32.04, 31.95, 30.59, 29.76, 29.69, 29.60, 29.48, 29.24, 28.62, 24.03, 22.82, (13C, 13 x CH_2) 14.26, 13.23. (2C, 2 x CH_3). MS (MALDI-TOF): *m/z* calculated for $C_{19}H_{33}NO_2S_2 + Na^+$ [M + Na^+]: 394.18. Found: 394.25. Compound **9a** was converted into *N*-ethoxycarbonyl compound according to general method **A**. The crude compound **9b** was used in further steps without purification.

Compound 13. To a stirred solution of teicoplanin pseudoaglycon [9] (224 mg, 0.16 mmol) in dry DMF (5 mL) *N*-ethoxycarbonyl bis-alkylthio maleimide **5b** (43 mg, 0.16 mmol) and Et_3N (22 µl, 0.16 mmol) were added under an argon atmosphere and stirred for overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by flash chromatography (toluene:methanol = 1:1) to give **13** (68 mg, 26%), as a yellow powder. NMR data can be found in Table S1(supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{78}H_{74}Cl_2N_8O_{25}S_2 + Na^+$ [M + Na^+]: 1679.35. Found: 1679.5.

Compound 14. Compound **6b** (53 mg, 0.16 mmol) was coupled to teicoplanin pseudoaglycon (224 mg, 0.16 mmol) according to the procedure described for compound **13**. The crude product was purified by flash chromatography (toluene:methanol = 1:1) to give **14** (61 mg, 22%), as a yellow powder. NMR data can be found in Table S1(supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{82}H_{82}Cl_2N_8O_{25}S_2 + Na^+$ [M + Na^+]: 1735.41. Found: 1734.99.

Compound 17. Compound **9b** (27 mg, 71 µmol) was coupled to teicoplanin pseudoaglycon (100 mg, 71 µmol) according to the procedure described for compound **13**. The crude product was purified by flash chromatography (toluene:methanol = 1:1) to give **17** (23 mg, 18%), as a yellow powder. NMR data can be found in Table S1 (supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{85}H_{88}Cl_2N_8O_{25}S_2 + Na^+$ [M + Na^+]: 1777.46. Found: 1777.47.

Compound 19. The title compound was prepared by the reaction of **4b** (63 mg, 0.2 mmol) and propargylamine according to general method **B**. The crude product was purified by flash chromatography in hexanes:acetone = 9:1, to give **19** (48 mg, 85%) as a yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 4.27 (d, J = 2.5 Hz, 2H, NCH_2), 3.38–3.16 (m, 4H, 2 x SCH_2), 2.23 (t, J = 2.5 Hz, CH_2CC*H*), 1.80–1.60 (m, 4H, 2 x CH_2), 1.03 (t, J = 7.3 Hz, 6H, 2 x CH_3); ^{13}C NMR (91 MHz, $CDCl_3$): δ 165.3 (2C, 2 x C=O), 136.1 (2C, 2 x C=C), 71.6 (1C, *C*H), 33.8 (2C, 2 x S*C*H_2) 27.6 (N*C*H_2), 24.0 (1C, *C*H_2), 13.2 (2C, 2x *C*H_3). Elemental analysis calculated (%) for $C_{13}H_{17}NO_2S_2$: C 55.10, H 6.25, N 4.94, S 22.62. Found: C 54.96, H 6.42, N 4.79, S 22.45.

Compound 20. Compound **20** was prepared by the reaction of **5b** (117 mg, 0.34 mmol) and propargylamine according to general method **B**. The crude product was purified by flash chromatography in hexanes:ethyl acetate = 9:1, to give the title compound (89 mg, 84%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27 (d, J = 3.3 Hz, 2H), 3.31 (t, J = 7.4 Hz, 4H), 2.23 (t, J = 2.5 Hz, 1H), 1.69–1.59 (m, 4H), 1.50–1.40 (m, 4H), 0.93 (t, J = 7.4 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.4 (2C, 2x *C*=O); 136.1 (*C*=*C*); 71.6 (1C, C≡*C*H) 32.6, 31.7 (4C, 4x *C*H_2); 27.6 (1C, N-*C*H_2); 21.8 (2C, 2 x *C*H_2S); 13.7 (2C, 2x *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{15}H_{21}NO_2S_2 + Na^+$ [M + Na^+]: 334.09. Found: 334.12.

Compound 21. Compound **6b** (230 mg, 0.57 mmol) and propargylamine were reacted according to general method **B**. The crude product was purified by flash chromatography in hexanes:ethylacetate = 9:1, to give **21** (155 mg, 74%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27 (d, J = 2.3 Hz, 2H), 3.30 (t, J = 7.4 Hz, 4H), 2.22 (t, J = 2.3 Hz, 1H), 1.65 (m, 4H), 1.42 (m, 4H), 1.35–1.24 (m, 8H), 0.89 (t, J = 6.8 Hz, 6H). ^{13}C (101 MHz, $CDCl_3$): δ 165.4 (2C 2x *C*=O); 136.1 (*C*=*C*); 71.6 (1C, C≡*C*H) 32.0, 31.4, 30.5, 28.3, (8C, 8x *C*H_2); 27.6 (1C, N-*C*H_2); 22.6 (2C, 2 x *C*H_2S); 14.1 (2C, 2x *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{19}H_{29}NO_2S_2 + Na^+$ [M + Na^+]: 390.15. Found: 390.3.

Compound 22. The desired compound was prepared by the reaction of **7b** (341 mg, 0.74 mmol) and propargylamine according to general method **B**. The crude product was purified by silica gel chromatography in *n*-hexanes:ethyl acetate = 9:1, to give **22** (172 mg, 55%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27 (4H, d, J = 2.4 Hz, 2 x C*H*$_2$), 3.34–3.26 (4H, m, 2 x SC*H*$_2$), 2.22 (1H, t, J = 2.5 Hz, C*H*), 1.69–1.60 (4H, m, 2 x C*H*$_2$), 1.45–1.36 (4H, m, 2 x C*H*$_2$), 1.27 (16H, br s, 8 x C*H*$_2$), 0.88 (6H, t, J = 6.8 Hz, 2 x C*H*$_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.4 (2 x C=O), 136.1 (C=C), 71.6 (*C*H), 32.0 (N-*C*H_2), 31.9, 30.6, 29.2, 29.2, 28.6, 27.5 (*C*H_2), 22.7 (S-*C*H_2), 14.2 (*C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{23}H_{37}NO_2S_2 + Na^+$ [M + Na^+]: 446.22. Found: 446.19.

Compound 23. The reaction between compound **8b** (114 mg, 0.2 mmol) and propargylamine was carried out according to general method **B**, followed by flash chromatography (hexanes:acetone = 8:2), which gave compound **23** (83 mg, 78%) as a yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 4.27 (d, J = 2.5 Hz, 2H), 3.36–3.22 (m, 4H), 2.21 (t, J = 2.5 Hz, 1H), 1.64 (dq, J = 13.6, 6.5, 5.9 Hz, 4H), 1.48–1.36 (m, 4H), 1.26 (s, 32H), 0.88 (t, J = 6.7 Hz, 6H). Elemental analysis calculated (%) for $C_{31}H_{53}NO_2S_2$: C 69.48, H 9.97, N 2.61, S 11.97. Found: C 69.32, H 10.18, N 2.55, S 11.86.

Compound 24. Compound **10b** (107 mg, 0.15 mmol) was reacted with propargylamine and worked up according to general method **B**. The crude product was used in the next step without further purification (see compound **31** below).

Compound 26. Compound **19** (25 mg, 0.09 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) were reacted according to general method **C** to give **26** (54 mg, 42%) as a yellow powder. NMR data can be found in Table S2 (supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{79}H_{73}Cl_2N_{11}O_{25}S_2 + Na^+$ [M + Na^+]: 1732.35. Found: 1732.62.

Compound 27. The reaction of compound **20** (31 mg, 0.10 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) according to general method **C** gave compound **27** (54 mg, 41%) as a yellow powder after purification. NMR data can be found in Table S2 (supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{81}H_{77}Cl_2N_{11}O_{25}S_2 + Na^+$ [M + Na^+]: 1760.38. Found: 1760.60.

Compound 28. The glycopeptide **28** was prepared by the reaction of compound **21** (37 mg, 0.10 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) according to general method **C**. After purification the title compound was obtained as a yellow powder (56 mg, 42%). NMR data can be found in Table S2 (supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{85}H_{85}Cl_2N_{11}O_{25}S_2 + Na^+$ [M + Na^+]: 1816.44. Found: 1815.99.

Compound 29. Compound **22** (43 mg, 0.1 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) were reacted according to general method **C** to give **29** (57 mg, 41%) as a yellow powder. NMR data can be found in Table S2 (supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{89}H_{93}Cl_2N_{11}O_{25}S_2 + Na^+$ [M + Na^+]: 1872.51. Found: 1872.63.

Compound 30. The reaction of compound **23** (48 mg, 0.09 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) according to general method **C** gave **30** (58 mg, 39%) as a yellow powder. NMR data can be found in Table S2. MS (MALDI-TOF): *m/z* calculated for $C_{97}H_{109}Cl_2N_{11}O_{25}S_2 + Na^+$ [M + Na^+]: 1984.63. Found: 1984.93.

Compound 31. Compound **24** (70 mg, ~0.09 mmol) was reacted with azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) according to general method **C** to give **31** (61 mg, 39%) as a yellow powder. NMR data can be found in Table S2 (supporting information). MS (MALDI-TOF): *m/z* calculated for $C_{97}H_{97}Cl_2N_{11}O_{35}S_2 + Na^+$

[M + Na^+]: 2132.49. Found: 2132.62.

Compounds 32a and b. To a stirred solution of 2,3-dibromomaleimide (255 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) Et_3N (2.0 mmol, 278 μL) and 1-mercapto-11-hydroxy-3,6,9-trioxaundecane (402 μl, 2.1 mmol) were added under an argon atmosphere and stirred for 3 h at room temperature. The reaction mixture was evaporated, and the crude product was converted into *N*-ethoxycarbonyl compound and worked up according to general method **A** yielding compound **32b** which was used in further steps without purification.

Compound 33. The reaction of **4b** (150 mg, 0.47 mmol) and triethylene glycol 2-aminoethyl propargyl ether was carried out according to general method **B**. After flash chromatography in hexanes:acetone = 9:1 the desired compound **33** was obtained (186 mg, 86%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.26–4.14 (m, 2H, CH_2), 3.76–3.56 (m, 16H, 8 x CH_2), 3.26 (t, *J* = 7.3 Hz, 4H, 2 x SCH_2), 2.48–2.41 (m, 1H, C*H*), 1.75–1.61 (m, 4H, 2 x CH_2), 1.03 (t, *J* = 7.3 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (2C, 2 x *C*=O), 135.9 (2C, 2 x *C*=*C*), 74.6 (*C*H≡C), 70.7, 70.7, 70.5, 70.1, 69.2, 67.9, 58.5 (8C, 8 x OCH_2), 37.8 (NCH_2), 33.8 (2C, 2 x SCH_2), 24.0 (2C, 2 x *C*H_2), 13.2 (2C, 2 x *C*H_3). Elemental analysis calculated (%) for $C_{21}H_{33}NO_6S_2$: C 54.88, H 7.24, N 3.05, S 13.95. Found: C 54.80, H 7.35, N 2.99, S 13.78.

Compound 34. Compound **5b** (117 mg, 0.34 mmol) was reacted with triethylene glycol 2-aminoethyl propargyl ether according to general method **B**. The crude product was purified by flash chromatography in hexanes:ethyl acetate = 7:3, to give **34** (154 mg, 93%) as a yellow syrup. ^{1}H: (400 MHz, $CDCl_3$): δ 4.21 (d, *J* = 2.4 Hz, 2H), 3.73–3.58 (m, 16H), 3.28 (t, *J* = 7.4 Hz, 4H), 2.44 (t, *J* = 2.4 Hz, 1H), 1.69–1.58 (m, 4H), 1.51–1.39 (m, 4H), 0.93 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (2C, 2x *C*=O); 135.8 (2C, 2 x *C*=*C*); 74.6 (*C*H≡C), 70.7, 70.6, 70.5, 70.1, 69.2, 67.9, 58.5 (8C, 8 x OCH_2), 37.8 (NCH_2), 32.6, 31.6 (4C, 4x *C*H_2); 21.7 (2C, 2 x *C*H_2*S*); 13.7 (2C, 2x *C*H_3) MS (ESI): *m/z* calculated for $C_{23}H_{37}NO_6S_2$ + Na^+ [M + Na^+]: 510.20. Found: 510.21.

Compound 35. Compound **35** was prepared by the reaction of **6b** (230 mg, 0.57 mmol) and triethylene glycol 2-aminoethyl propargyl ether according to general method **B**. The crude product was purified by flash chromatography in hexanes:ethyl acetate = 85:15, yielding the title compound (236 mg, 76%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.20 (d, *J* = 2.4 Hz, 2H), 3.73–3.59 (m, 16H), 3.31–3.24 (m, 4H), 2.44 (t, *J* = 2.3 Hz, 1H), 1.64 (dt, *J* = 15.1, 7.4 Hz, 4H), 1.42 (dt, *J* = 14.3, 7.2 Hz, 4H), 1.36–1.23 (m, 8H), 0.89 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.6 (2C, *C*=O), 135.7 (2C, 2 x *C*=*C*), 74.6 (*C*H≡C), 70.6, 70.6, 70.4, 70.0, 69.1, 67.9, 58.4 (8C, 8 x OCH_2), 37.8 (NCH_2), 31.9, 31.3, 30.4, 28.2, 22.5 (10C, 10 x *C*H_2), 14.0 (2C, 2 x *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{27}H_{45}NO_6S_2$ + Na^+ [M + Na^+]: 566.26. Found: 566.25.

Compound 36. Compound **7b** (150 mg, 0.33 mmol) and triethylene glycol 2-aminoethyl propargyl ether were reacted according to general method **B**. The crude product was purified by flash chromatography in hexanes:acetone = 8:2, to give **36** (112 mg, 57%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21 (d, *J* = 2.4 Hz, 2H), 3.72–3.59 (m, 16H), 3.32–3.23 (m, 4H), 2.43 (t, *J* = 2.4 Hz, 1H), 1.64 (dt, *J* = 15.0, 7.4 Hz, 4H), 1.46–1.36 (m, 4H), 1.30 (dt, *J* = 8.7, 5.1 Hz, 16H), 0.88 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.7 (2C, 2 x *C*=O), 135.9 (2C, 2 x *C*=*C*), 74.6 (*C*H≡C), 70.8, 70.7, 70.5, 70.1, 69.3, 68.0, 58.5 (8C, 8 x OCH_2), 37.8 (*N*CH_2), 32.0, 31.9 (2C, 2 x SCH_2), 30.6, 29.3, 29.3, 28.7, 22.8 (10C, 10 x *C*H_2), 14.2 (2C, 2 x *C*H_3). Elemental analysis calculated (%) for $C_{31}H_{53}NO_6S_2$: C 62.07, H 8.91, N 2.33, S 10.69. Found: C 61.85, H 9.07, N 2.25, S 10.60.

Compound 37. Compound **8b** (150 mg, 0.26 mmol) and triethylene glycol 2-aminoethyl propargyl ether were reacted according to general method **B**. The crude product was purified by silica gel chromatography in hexanes:acetone = 8:2, to give **37** (135 mg, 72%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21 (d, *J* = 2.4 Hz, 2H), 3.73–3.57 (m, 16H), 3.27 (t, *J* = 7.4 Hz, 4H), 2.43 (t, *J* = 2.4 Hz, 1H), 1.69–1.59 (m, 4H), 1.40 (dd, *J* = 13.2, 7.5 Hz, 4H), 1.34–1.20 (m, 32H), 0.88 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.7 (2C, 2 x *C*=O), 135.8 (2C, 2 x *C*=*C*), 74.6 (*C*H≡C), 70.7, 70.7, 70.5, 70.1, 69.2, 67.9, 58.5 (8C, 8 x OCH_2), 37.6 (NCH_2), 32.0 (2C, 2 x SCH_2), 30.6, 29.8, 29.7, 29.6, 29.5, 29.2, 28.7, 22.8 (20C, 20 x *C*H_2), 14.2 (2C, 2 x *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{39}H_{69}NO_6S_2$ + Na^+ [M + Na^+]: 734.45. Found: 735.01.

Compound 38. The reaction of **10b** (381 mg, 0.53 mmol) and triethylene glycol 2-aminoethyl propargyl ether according to general method **B** gave a crude product that was purified by flash chromatography in hexanes:acetone = 7:3, yielding **38** (340 mg, 75%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.51 (d, *J* = 4.9 Hz, 2H, *H*-1), 4.62 (dd, *J* = 7.9, 2.1 Hz, 2H), 4.35–4.27 (m, 4H), 4.21 (d, *J* = 2.0 Hz, 2H), 3.98 (t, *J* = 6.8 Hz, 2H), 3.73–3.57 (m, 16H), 3.57–3.34 (m, 4H), 2.45 (t, *J* = 2.4 Hz, 1H, C*H*), 1.46 (d, *J* = 14.6 Hz, 12H), 1.32 (d, *J* = 3.9 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.3 (2C, 2 x *C*=O), 135.9 (2C, 2 x *C*=*C*), 109.5, 108.8 (4C, 4 x C_q), 96.6 (2C, 2 x *C*H), 74.5 (1C, *C*H), 71.6, 71.0 (4C, 4 x *C*H), 70.7, 70.6 (3C, 3 x *C*H_2) 70.5 (2C, 2 x *C*H), 70.5 (1C, *C*H_2), 70.1, 69.2 (2C, 2 x *C*H_2), 68.0 (2C, 2 x *C*H), 67.8 (1C, *C*H_2), 58.5 (1C, *C*H_2) 37.8 (1C, NCH_2), 31.8 (2C, 2 x *C*-6,6'), 26.0 (4C, 4 x *C*H_3), 25.0, 24.5 (4C, 4 x *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{39}H_{57}NO_{16}S_2$ + Na^+ [M + Na^+]: 882.30. Found: 882.30.

Compound 39. To a stirred solution of **32b** (62 mg, 0.11 mmol) triethylene glycol 2-aminoethyl propargyl ether was added and the reaction was carried out according to general method **B**. The reaction was worked up and used in its crude form for the synthesis of the final product **46**.

Compound 40 The reaction between compound **33** (46 mg, 0.1 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) was carried out according to general method **C** to give **40** (59 mg, 42%) as a yellow powder. NMR data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{87}H_{89}Cl_2N_{11}O_{29}S_2$ + Na^+ [M + Na^+]: 1908.45. Found: 1908.60.

Compound 41. The reaction between compound **34** (49 mg, 0.1 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) was carried out according to general method **C** to give **41** (34 mg, 24%) as a yellow powder. NMR data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{89}H_{93}Cl_2N_{11}O_{29}S_2$ + Na^+ [M + Na^+]: 1936.49. Found: 1936.70.

Compound 42. The reaction between compound **35** (54 mg, 0.1 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) was carried out according to general method **C** to give **42** (27 mg, 18%) as a yellow powder. NMR data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{93}H_{101}Cl_2N_{11}O_{29}S_2$ + Na^+ [M + Na^+]: 1992.55. Found: 1991.9.

Compound 43. Glycopeptide derivative **43** was obtained by carrying out the reaction between compound **36** (60 mg, 0.1 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) according to general method **C**, giving **43** (65 mg, 43%) as a yellow powder. NMR data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{97}H_{109}Cl_2N_{11}O_{29}S_2$ + Na^+ [M + Na^+]: 2048.61. Found: 2048.68.

Compound 44. Compound **37** (71 mg, 0.1 mmol) and azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol) were reacted according to general method **C** to give **44** (44 mg, 27%) as a yellow powder. NMR data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{105}H_{125}Cl_2N_{11}O_{29}S_2$ + Na^+ [M + Na^+]: 2160.74. Found: 2160.89.

Compound 45. To a stirred solution of azido teicoplanin pseudoaglycon **25** (107 mg, 0.075 mmol)in DMF, compound **38** (86 mg, 0.1 mmol) was added and the reaction was carried out according to general method **C** to give **45** (57 mg, 33%) as a yellow powder. NMR

data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{105}H_{113}Cl_2N_{11}O_{39}S_2 + Na^+$ [M + Na^+]: 2308.59. Found: 2308.62.

Compound 46. Compound **39** (42 mg, 57 μmol) and azido teicoplanin pseudoaglycon **25** (82 mg, 57 μmol) were reacted according to general method **C** to give **46** (27 mg, 22%) as a yellow powder. NMR data can be found in Table S3. MS (MALDI-TOF): *m/z* calculated for $C_{97}H_{109}Cl_2N_{11}O_{37}S_2 + Na^+$ [M + Na^+]: 2176.57. Found: 2176.86.

Compound 52. 1-Azido-11-hydroxy-3,6,9-trioxaundecane **47** (413 mg, 1.89 mmol) was coupled with propargyl ether **48** (265 mg, 2.36 mmol) according to general method **C**. The crude product was purified by flash chromatography (hexanes:acetone = 6:4) resulting in **52** (405 mg, 52%) as a yellowish syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.78 (s, 1H, C=C*H*), 4.62 (s, 2H, OCH_2C), 4.58–4.51 (m, 2H), 3.88 (t, *J* = 5.1 Hz, 2H), 3.76–3.70 (m, 2H) 3.67 (dd, *J* = 6.0, 2.7 Hz, 2H), 3.61 (q, *J* = 4.5 Hz, 8H), 3.53 (t, *J* = 6.6 Hz, 2H), 2.94 (s, 1H, O*H*), 1.58 (dt, *J* = 14.5, 6.7 Hz, 2H), 1.37 (dq, *J* = 14.5, 7.3 Hz, 2H), 0.91 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (91 MHz, $CDCl_3$): δ 145.3 (1C, *C*=CH), 123.7 (1C, C=*C*H), 72.6, 70.6, 70.5, 70.4, 70.3, 69.5, 64.3 (8C, 8 x OCH_2), 61.6 (1C, *C*H_2OH), 50.2 (1C, NCH_2), 31.7 (1C, *C*H_2), 19.3 (1C, *C*H_2), 13.9 (1C, *C*H_3). MS (ESI): *m/z* calculated for $C_{15}H_{29}N_3O_5 + Na^+$ [M + Na^+]: 354.200. Found: 354.201.

Compound 53. Compound **47** (444 mg, 2.03 mmol) and propargyl ether **49** (284 mg, 2.03 mmol) were reacted according to general method **C**. The crude product was purified by flash chromatography (hexanes:acetone = 6:4) resulting in **53** (542 mg, 74%) as a yellowish syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.79 (s, 1H, C=C*H*), 4.62 (s, 2H, OCH_2), 4.55 (t, *J* = 5.0 Hz, 2H), 3.92–3.84 (m, 2H, OCH_2), 3.76–3.69 (m, 2H), 3.69–3.57 (m, 10H), 3.52 (t, *J* = 6.7 Hz, 2H), 3.09 (s, 1H, O*H*), 1.66–1.53 (m, 2H), 1.41–1.24 (m, 6H) 0.88 (t, *J* = 6.6 Hz, 3H, *C*H_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.2 (1C, *C*=CH), 123.8 (1C, C=*C*H), 72.6, 70.8, 70.5, 70.4, 70.2, 69.3, 64.2 (8C, 8 x OCH_2), 61.5 (1C, *C*H_2OH), 50.2 (1C, NCH_2), 31.6, 29.6, 25.7, 22.6 (4C, 4 x *C*H_2), 14.0 (1C, *C*H_3). MS (ESI): *m/z* calculated for $C_{17}H_{33}N_3O_5 + Na^+$ [M + Na^+]: 382.231. Found: 382.226.

Compound 54. Compound **47** (400 mg, 1.83 mmol) and propargyl ether **50** (462 mg, 2.75 mmol) according to general method **C**. After purification by flash chromatography (hexanes:acetone = 7:3) **54** was obtained (480 mg, 68%) as a yellowish syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.77 (s, 1H, C=C*H*), 4.62 (s, 2H, OCH_2), 4.55 (t, *J* = 5.0 Hz, 2H), 3.95–3.82 (m, 2H, OCH_2), 3.75–3.70 (m, 2H), 3.69–3.56 (m, 10H), 3.51 (t, *J* = 6.7 Hz, 2H), 2.94 (s, 1H, O*H*), 1.66–1.51 (m, 2H), 1.39–1.18 (m, 10H), 0.87 (t, *J* = 6.7 Hz, 3H, *C*H_3). ^{13}C NMR (91 MHz, $CDCl_3$): δ 145.3 (1C, *C*=CH), 123.7 (1C, C=*C*H), 72.6, 70.8, 70.6, 70.5, 70.3, 69.6, 64.3 (8C, 8 x OCH_2), 61.7 (1C, *C*H_2OH), 50.2 (1C, NCH_2), 31.8, 29.7, 29.5, 29.3, 26.1, 22.7 (6C, 6 x *C*H_2), 14.1 (1C, *C*H_3). MS (ESI): *m/z* calculated for $C_{19}H_{37}N_3O_5 + Na^+$ [M + Na^+]: 410.263. Found: 410.258.

Compound 55. Compound **47** (260 mg, 1.19 mmol) was coupled with propargyl ether **51** (235 mg, 1.30 mmol) according to general method **C**. The crude product was purified by flash chromatography (hexanes:acetone = 7:3) resulting in **55** (463 mg, 86%) as a light yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.79 (s, 1H, C=C*H*), 4.62 (s, 2H, OCH_2), 4.55 (t, *J* = 5.0 Hz, 2H), 3.88 (t, *J* = 5.0 Hz, 2H), 3.75–3.70 (m, 2H, OCH_2), 3.68–3.64 (m, 2H), 3.65–3.57 (m, 8H), 3.52 (t, *J* = 6.7 Hz, 2H), 1.58 (dq, *J* = 10.1, 5.0, 3.4 Hz, 2H), 1.26 (br s, 14H, 7 x *C*H_2), 0.88 (t, *J* = 6.8 Hz, 3H, *C*H_3). ^{13}C NMR (91 MHz, $CDCl_3$): δ 72.6, 70.9, 70.5, 70.4, 70.3, 69.6, 64.3 (8C, 8 x OCH_2), 61.7 (1C, *C*H_2OH), 50.3 (1C, NCH_2), 32.0, 29.8, 29.7, 29.6, 29.4, 29.2, 26.2, 22.7 (8C, 8 x *C*H_2), 14.2 (1C, *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{21}H_{41}N_3O_5 + Na^+$ [M + Na^+]: 438.29. Found: 438.35.

Compound 56. Compound **52** (140 mg, 0.42 mmol) was *O*-propargylated according to general method **D**. The crude product was purified by flash chromatography (hexanes:acetone = 7:3) resulting in **56** (48 mg, 31%) as a light yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.73 (s, 1H, C*H*=C), 4.61 (s, 2H, OCH_2), 4.53 (t, *J* = 5.1 Hz, 2H), 4.18 (d, *J* = 2.3 Hz, 2H), 3.86 (t, *J* = 5.1 Hz, 2H), 3.71–3.57 (m, 12H, 6 x OCH_2), 3.51 (t, *J* = 6.7 Hz, 2H), 2.43 (t, *J* = 2.3 Hz, 1H), 1.57 (dt, *J* = 14.6, 6.7 Hz, 2H), 1.41–1.31 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (91 MHz, $CDCl_3$): δ 145.4 (1C, *C*=CH), 123.7 (1C, C=*C*H), 74.6 (1C, *C*H), 70.7, 70.6, 70.5, 69.6, 64.3 (9C, 9 x OCH_2), 58.5 (1C, OCH_2C≡CH), 50.3 (1C, NCH_2), 31.8, 19.4 (2C, 2x *C*H_2), 14.0 (1C, *C*H_3). MS (ESI): *m/z* calculated for $C_{18}H_{31}N_3O_5 + Na^+$ [M + Na^+]: 392.216. Found: 392.216.

Compound 57. Compound **53** (140 mg, 0.39 mmol) was *O*-propargylated according to general method **D**. The crude product was purified by flash chromatography (hexanes:acetone = 7:3) resulting in **57** (60 mg, 39%) as a yellowish syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.73 (s, 1H, C=C*H*), 4.61 (s, 2H, OCH_2), 4.54 (t, *J* = 5.1 Hz, 2H), 4.19 (d, *J* = 2.3 Hz, 2H), 3.88 (t, *J* = 5.1 Hz, 2H), 3.73–3.59 (m, 12H, 6 x OCH_2), 3.51 (t, *J* = 6.7 Hz, 2H), 2.44 (t, *J* = 2.4 Hz, 1H), 1.59 (p, *J* = 6.9 Hz, 2H), 1.40–1.21 (m, 6H, 3 x *C*H_2), 0.88 (t, *J* = 6.7 Hz, 3H, *C*H_3). ^{13}C NMR (91 MHz, $CDCl_3$): δ 145.3 (1C, *C*=CH), 123.5 (1C, C=*C*H), 74.6 (1C, *C*H), 70.8, 70.6, 70.5, 70.4, 69.5, 64.3 (9C, 9 x OCH_2), 58.4 (1C, OCH_2C≡CH), 50.3 (1C, NCH_2), 31.7, 29.7, 25.8, 22.6 (4C, 4 x *C*H_2), 14.1 (1C, *C*H_3). MS (ESI): *m/z* calculated for $C_{20}H_{35}N_3O_5 + Na^+$ [M + Na^+]: 420.247. Found: 420.249.

Compound 58. Compound **54** (150 mg, 0.39 mmol) was *O*-propargylated according to general method **D**. The crude product was purified by flash chromatography (hexanes:acetone = 75:25) resulting in **58** (60 mg, 36%) as a yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.74 (s, 1H, C=C*H*), 4.61 (s, 2H, OCH_2), 4.54 (t, *J* = 5.1 Hz, 2H), 4.19 (d, *J* = 2.4 Hz, 2H), 3.92–3.84 (m, 2H), 3.73–3.57 (m, 12H, 6 x OCH_2), 3.51 (t, *J* = 6.7 Hz, 2H), 2.44 (t, *J* = 2.3 Hz, 1H, C≡C*H*), 1.59 (dt, *J* = 14.4, 6.8 Hz, 2H), 1.38–1.21 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H, *C*H_3). ^{13}C NMR (91 MHz, $CDCl_3$): δ 123.6 (1C, C=CH), 74.6 (*C*H), 70.8, 70.6, 70.5, 70.4, 69.5, 69.2, 64.3 (9C, 9 x OCH_2), 58.4 (1C, OCH_2C≡CH), 50.3 (1C, NCH_2), 31.8, 29.7, 29.5, 29.3, 26.2, 22.7 (6C, 6 x *C*H_2), 14.1 (1C, *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{22}H_{39}N_3O_5 + Na^+$ [M + Na^+]: 448.28. Found: 448.30.

Compound 59. Compound **55** (300 mg, 0.72 mmol) was *O*-propargylated according to general method **D**. The crude product was purified by flash chromatography (hexanes:acetone = 7:3) resulting in **59** (284 mg, 87%) as a yellow syrup. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.73 (s, 1H, C=C*H*), 4.62 (s, 2H, OCH_2), 4.54 (t, *J* = 5.1 Hz, 2H), 4.20 (d, *J* = 2.4 Hz, 2H), 3.94–3.82 (m, 2H), 3.78–3.57 (m, 12H, 6 x OCH_2), 3.51 (t, *J* = 6.7 Hz, 2H), 2.44 (t, *J* = 2.4 Hz, 1H, C≡C*H*), 1.59 (p, *J* = 6.6 Hz, 2H), 1.26 (s, 14H), 0.88 (t, *J* = 6.7 Hz, 3H, *C*H_3). ^{13}C NMR (91 MHz, $CDCl_3$): δ 145.4 (1C, *C*=CH), 123.5 (1C, C=*C*H), 70.9, 70.6, 70.5, 69.6, 69.2, 64.4 (9C, 9 x OCH_2), 58.5 (1C, OCH_2C≡CH), 50.3 (1C, NCH_2), 32.0, 29.7, 29.4, 26.2, 22.8 (8C, 8 x *C*H_2), 14.2 (1C, *C*H_3). MS (MALDI-TOF): *m/z* calculated for $C_{24}H_{43}N_3O_5 + Na^+$ [M + Na^+]: 476.31. Found: 476.30.

Compound 60. Azido teicoplanin pseudoaglycon **25** (86 mg, 0.06 mmol) and propargyl ether **56** (37 mg, 0.10 mmol) were allowed to react according to general method **C**. The crude product was purified by flash chromatography (acetonitrile:water = 9:1) resulting in **60** (28 mg, 26%) as a white powder. NMR data can be found in Table S4. MS (MALDI-TOF): *m/z* calculated for $C_{84}H_{87}Cl_2N_{13}O_{28} + Na^+$ [M + Na^+]: 1818.51. Found: 1818.4.

Compound 61. Azido teicoplanin pseudoaglycon **25** (86 mg, 0.06 mmol) and propargyl ether **57** (40 mg, 0.10 mmol) were allowed to react according to general method **C**. The crude product was purified by flash chromatography (acetonitrile:water = 9:1) resulting in **61** (39 mg, 36%) as a white powder. NMR data can be found in Table S4. MS (MALDI-TOF): *m/z* calculated for $C_{86}H_{91}Cl_2N_{13}O_{28} + Na^+$ [M + Na^+]: 1846.54. Found: 1846.6.

Compound 62. Azido teicoplanin pseudoaglycon **25** (86 mg, 0.06 mmol) and propargyl ether **58** (43 mg, 0.1 mmol) were allowed to react according to general method **C**. The crude product

was purified by flash chromatography (acetonitrile:water = 9:1) resulting in **62** (22 mg, 20%) as a white powder. NMR data can be found in Table S4. MS (MALDI-TOF): *m/z* calculated for $C_{88}H_{95}Cl_2N_{13}O_{28}$ + Na^+ [M + Na^+]: 1874.57. Found: 1874.6.

Compound 63. Compound **25** (100 mg, 0.07 mmol) azide was coupled with propargyl ether **59** (41 mg, 0.09 mmol) according to general method **C**. The crude product was purified by flash chromatography (toluene:MeOH = 1:1 + 1%v/v AcOH) resulting in **63** (35 mg, 27%). NMR data can be found in Table S4. MS (MALDI-TOF): *m/z* calculated for $C_{90}H_{99}Cl_2N_{13}O_{28}$ + Na^+ [M + Na^+]: 1902.60. Found: 1902.60.

Compound 72. Teicoplanin pseudoaglycon **11** (100 mg, 0.071 mmol) and *p*-toluenesulfonyl chloride **64** (18 mg, 0.13 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **72** (59 mg, 54%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{73}H_{64}Cl_2N_8O_{25}S$ + Na^+ [M + Na^+]: 1577.30. Found: 1577.4.

Compound 73. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) and benzenesulfonyl chloride **65** (18 μL, 0.14 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **73** (82 mg, 53%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{72}H_{62}Cl_2N_8O_{25}S$ + Na^+ [M + Na^+]: 1563.28. Found: 1563.6.

Compound 74. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) and *p*-acetamidobenzenesulfonyl chloride **66** (33 mg, 0.14 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **74** (78 mg, 49%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{74}H_{65}Cl_2N_9O_{26}S$ + Na^+ [M + Na^+]: 1620.30. Found: 1620.7.

Compound 75. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) and biphenylsulfonyl chloride **67** (35 mg, 0.14 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **75** (73 mg, 46%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{78}H_{66}Cl_2N_8O_{25}S$ + Na^+ [M + Na^+]: 1639.31. Found: 1639.7.

Compound 76. Teicoplanin pseudoaglycon (140 mg, 0.1 mmol) and dansyl chloride **68** (38 mg, 0.14 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **76** (92 mg, 56%) as a yellow powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{78}H_{69}Cl_2N_9O_{25}S$ + Na^+ [M + Na^+]: 1656.34. Found: 1656.8.

Compound 77. Teicoplanin pseudoaglycon (100 mg, 0.071 mmol) and hexanesulfonyl chloride **69** (16 μL, 0.1 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **77** (28 mg, 25%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{72}H_{70}Cl_2N_8O_{25}S$ + Na^+ [M + Na^+]: 1571.34. Found: 1571.7.

Compound 78. Teicoplanin pseudoaglycon (100 mg, 0.071 mmol) and octanesulfonyl chloride **70** (20 μL, 0.1 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **78** (32 mg, 29%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{74}H_{74}Cl_2N_8O_{25}S$ + Na^+ [M + Na^+]: 1599.38. Found: 1599.4.

Compound 79. Teicoplanin pseudoaglycon (100 mg, 0.071 mmol) and dodecanesulfonyl chloride **71** (27 mg, 0.1 mmol) were allowed to react under the conditions described in general method **E**. The crude product was purified by flash chromatography to give **79** (36 mg, 31%) as a white powder. NMR data can be found in Table S5. MS (MALDI-TOF): *m/z* calculated for $C_{78}H_{82}Cl_2N_8O_{25}S$ + Na^+ [M + Na^+]: 1655.44. Found: 1655.9.

4.7. Influenza virus experiments

All details on the CPE reduction assay for influenza virus can be found in previous publications [16,25]. The virus strains were: A/PR/8/34 (A/H1N1); A/Virginia/ATCC3/2009 (A/H1N1pdm); A/HK/7/87 (A/H3N2); B/Ned/537/05; and B/HK/5/72. The infection medium consisted of Ultra-MDCK® medium (Lonza), supplemented with 0.0225% sodium bicarbonate, 2 mM L-glutamine and 2 μg/mL tosyl phenylalanyl chloromethyl ketone-treated trypsin. On day 0, semi-confluent cultures of Madin-Darby canine kidney (MDCK) cells in 96-well plates were infected with influenza virus at a multiplicity of infection (MOI) of 0.0004 plaque forming units (PFU) per cell. After three days incubation at 35 °C, virus-induced CPE and compound cytotoxicity were scored by microscopy, after which the data were confirmed by formazan-based MTS cell viability assay (CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay from Promega). The antiviral effect was expressed as the compound concentration producing 50% inhibition of the virus-induced CPE (EC_{50}). Compound cytotoxicity was expressed as the compound concentration causing minimal changes in cell morphology (MCC), and 50% cytotoxic concentration based on MTS (CC_{50}).

The plasmids for the lentiviral pseudoparticle assay [described in full detail elsewhere [16]] were: pCGGagPol (a gift from G. Maertens [33]); pQCXIP-AcGFP (kind gift from D. Daelemans); and two pCAGEN plasmids, i.e. pCAGEN backbone [kindly provided by C. Cepko (Boston, MA) via Addgene (plasmid No. 11160[34]] into which we cloned the A/PR/8/34 HA and NA cDNA sequences. The four plasmids were transfected into human HEK-293T cells to produce GFP-expressing lentiviral particles. Two days after transfection, the supernatant was collected and trypsin was added to activate the influenza HA0 protein [16] For lentiviral transduction, MDCK cells were seeded at 7500 cells per well in 96-well plates, and incubated one day later with lentiviral particles together with test compounds. After three days incubation, the cells were trypsinized, fixated with paraformaldehyde, and submitted to flow cytometry for GFP detection, using a BD FACSCanto II apparatus.

4.8. Coronavirus experiments

Human embryonic lung fibroblast (HEL) 299 cells; human lung carcinoma A549 cells; and human coronavirus (HCoV) 229E were obtained from ATCC. During virus infections, the medium contained 2% fetal calf serum. To conduct the CPE reduction assay, confluent HEL cell cultures in 96-well plates were exposed to HCoV-229E (MOI: 100 $CCID_{50}$ per well) together with the test compounds. After 5 days incubation at 35 °C, microscopy was performed to determine the EC_{50} and MCC values (same definition as above).

To perform the qRT-PCR-based coronavirus yield assay, the materials were: HCoV-229E nucleoprotein-derived forward primer (5′-TTAGAGAGCGTGTTGAAGGTG-3′); reverse primer (5′-GTTCTGAATTCTTGCGCCTAAC-3′); probe (5′-6-FAM-TCTGGGTTG/ZEN/CTGTTGATGGTGCTA-IBFQ-3′; and a standardization plasmid with a 294-bp sequence of the HCoV-229E N-gene. As reference compound, the HCoV RNA synthesis inhibitor K22[35] (from ChemDiv) was used.

On day 0, confluent A549 cells in 96-well plates were exposed to the compounds together with HCoV-229E. One hour after infection, the virus inoculum was removed and the cells were further incubated at 35 °C in the presence of compounds. At day 3 p.i., the supernatants were frozen at −80 °C. Before qRT-PCR, virions were lysed by treating 2 μl sample with 10 μl resuspension buffer and 1 μl

lysis enhancer (CellsDirect One-Step qRT-PCR kit; Invitrogen), and 10 min incubation at 75 °C. Next, quantification of CoV genome copies was performed by one-step qRT-PCR. The program was run on an Applied Biosystems 7500 Fast instrument and consisted of 15 min at 50 °C; 2 min at 95 °C; and 40 cycles of 15 s at 95 °C followed by 45 s at 60 °C. The EC_{99} and EC_{90} values were calculated by interpolation and defined as the compound concentration producing respectively a 2-$\log_{10}$ and 1-$\log_{10}$ reduction in viral RNA copy number, as compared to the virus control receiving no compound. To monitor compound cytotoxicity, microscopy was done on mock-infected cells incubated with the compounds for three days; the MCC value was defined as above.

Acknowledgments

The synthetic work was supported by National Research, Development and Innovation Office of Hungary (K119509, and TÉT_15_IN-1-2016-0071) and the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008. The project was further supported by the János Bolyai Fellowship of the Hungarian Academy of Sciences (M. Csávás) and the Gedeon Richter's Talentum Foundation (1103 Budapest, Gyömrői út 19–21) for financial support. The research was also supported by the EU and co-financed by the European Regional Development Fund and European Social Fund under the project „Debrecen Venture Catapult Program" EFOP-3.6.1-16-2016-00022. L.N. acknowledges excellent technical assistance from Talitha Boogaerts and Benjamin Van Loy. We thank S. Vazquez (University of Barcelona) for the kind gift of the influenza virus fusion inhibitors 80 and 81, and G. Maertens and D. Daelemans for providing plasmid materials for the pseudoparticle assay.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.08.058.

References

[1] B.L. Coleman, S.A. Fadel, T. Fitzpatrick, S.M. Thomas, Risk factors for serious outcomes associated with influenza illness in high- versus low- and middle-income countries: systematic literature review and meta-analysis, Influenza Other Respir. Viruses. 12 (2018) 22–29.
[2] E. Vanderlinden, L. Naesens, Emerging antiviral strategies to interfere with influenza virus entry, Med. Res. Rev. 34 (2014) 301–339.
[3] L. Naesens, A. Stevaert, E. Vanderlinden, Antiviral therapies on the horizon for influenza, Curr. Opin. Pharmacol. 30 (2016) 106–115.
[4] G. Dong, C. Peng, J. Luo, C. Wang, L. Han, B. Wu, G. Ji, H. He, Adamantane-resistant influenza A viruses in the world (1902-2013): frequency and distribution of M2 gene mutations, PLoS One 10 (2015), e0119115.
[5] M. Samson, A. Pizzorno, Y. Abed, G. Boivin, Influenza virus resistance to neuraminidase inhibitors, Antivir. Res. 98 (2013) 174–185.
[6] A. Moscona, Global transmission of oseltamivir-resistant influenza, N. Engl. J. Med. 360 (2009) 953–956.
[7] L.Y. Zeng, J. Yang, S. Liu, Investigational hemagglutinin-targeted influenza virus inhibitors, Expet Opin. Invest. Drugs 26 (2017) 63–73.
[8] F.P. Havers, A.P. Campbell, T.M. Uyeki, A.M. Fry, Commentary: a historical review of Centers for Disease Control and Prevention antiviral treatment and postexposure chemoprophylaxis guidance for human infections with novel influenza A viruses associated with severe human disease, J. Infect. Dis. 216 (2017) S575–S580.
[9] G. Pintér, G. Batta, S. Kéki, A. Mándi, I. Komáromi, K. Takács-Novák, F. Sztaricskai, E. Rőth, E. Ostorházi, F. Rozgonyi, L. Naesens, P. Herczegh, Diazo transfer–click reaction route to new, lipophilic teicoplanin and ristocetin aglycon derivatives with high antibacterial and anti-influenza virus activity: an aggregation and receptor binding study, J. Med. Chem. 52 (2009) 6053–6061.
[10] J. Balzarini, E. Keyaerts, L. Vijgen, H. Egberink, E. De Clercq, M. Van Ranst, S.S. Printsevskaya, E.N. Olsufyeva, S.E. Solovieva, M.N. Preobrazhenskaya, Inhibition of feline (FIPV) and human (SARS) coronavirus by semisynthetic derivatives of glycopeptide antibiotics, Antivir. Res. 72 (2006) 20–33.
[11] N. Zhou, T. Pan, J. Zhang, Q. Li, X. Zhang, C. Bai, F. Huang, T. Peng, J. Zhang, C. Liu, L. Tao, H. Zhang, Glycopeptide antibiotics potently inhibit cathepsin L in the late endosome/lysosome and block the entry of Ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV), J. Biol. Chem. 291 (2016) 9218–9232.
[12] Y. Wang, R. Cui, G. Li, Q. Gao, S. Yuan, R. Altmeyer, et al., Teicoplanin inhibits Ebola pseudovirus infection in cell culture, Antivir. Res. 125 (2016) 1–7.
[13] J. Balzarini, C. Pannecouque, E. De Clercq, A.Y. Pavlov, S.S. Printsevskaya, O.V. Miroshnikova, M.I. Reznikova, M.N. Preobrazhenskaya, Antiretroviral activity of semisynthetic derivatives of glycopeptide antibiotics, J. Med. Chem. 46 (2003) 2755–2764.
[14] S. Obeid, S.S. Printsevskaya, E.N. Olsufyeva, K. Dallmeier, D. Durantel, F. Zoulim, M.N. Preobrazhenskaya, J. Neyts, J. Paeshuyse, Inhibition of hepatitis C virus replication by semi-synthetic derivatives of glycopeptide antibiotics, J. Antimicrob. Chemother. 66 (2011) 1287–1294.
[15] L. Naesens, E. Vanderlinden, E. Rőth, J. Jekő, G. Andrei, R. Snoeck, C. Pannecouque, E. Illyés, G. Batta, P. Herczegh, F. Sztaricskai, Anti-influenza virus activity and structure–activity relationship of aglycoristocetin derivatives with cyclobutenedione carrying hydrophobic chains, Antivir. Res. 82 (2009) 89–94.
[16] E. Vanderlinden, E. Vanstreels, E. Boons, W. ter Veer, A. Huckriede, D. Daelemans, A. Van Lommel, E. Rőth, F. Sztaricskai, P. Herczegh, L. Naesens, Intracytoplasmic trapping of influenza virus by a lipophilic derivative of aglycoristocetin, J. Virol. 86 (2012) 9416–9431.
[17] A. Sipos, G. Máté, E. Rőth, A. Borbás, G. Batta, I. Bereczki, S. Kéki, I. Jóna, E. Ostorházi, F. Rozgonyi, E. Vanderlinden, L. Naesens, P. Herczegh, Synthesis of fluorescent ristocetin aglycon derivatives with remarkable antibacterial and antiviral activities, Eur. J. Med. Chem. 58 (2012) 361–367.
[18] A. Sipos, Z. Török, E. Rőth, A. Kiss-Szikszai, G. Batta, I. Bereczki, Z. Fejes, A. Borbás, E. Ostorházi, F. Rozgonyi, L. Naesens, P. Herczegh, Synthesis of isoindole and benzoisoindole derivatives of teicoplanin pseudoaglycon with remarkable antibacterial and antiviral activities, Bioorg. Med. Chem. Lett 22 (2012) 7092–7096.
[19] I. Bereczki, A. Mándi, E. Rőth, A. Borbás, Á. Fizil, I. Komáromi, A. Sipos, T. Kurtán, G. Batta, E. Ostorházi, F. Rozgonyi, E. Vanderlinden, L. Naesens, F. Sztaricskai, P. Herczegh, A few atoms make the difference: synthetic, CD, NMR and computational studies on antiviral and antibacterial activities of glycopeptide antibiotic aglycon derivatives, Eur. J. Med. Chem. 94 (2015) 73–86.
[20] I. Bereczki, M. Kicsák, L. Dobray, A. Borbás, G. Batta, S. Kéki, É. Nemes Nikodém, E. Ostorházi, F. Rozgonyi, E. Vanderlinden, L. Naesens, P. Herczegh, Semi-synthetic teicoplanin derivatives as new influenza virus binding inhibitors: synthesis and antiviral studies, Bioorg. Med. Chem. Lett 24 (2014) 3251–3254.
[21] Z. Szűcs, M. Csávás, E. Rőth, A. Borbás, G. Batta, F. Perret, E. Ostorházi, R. Szatmári, E. Vanderlinden, L. Naesens, P. Herczegh, Synthesis and biological evaluation of lipophilic teicoplanin pseudoaglycon derivatives containing a substituted triazole function, J. Antibiot. 70 (2017) 152–157.
[22] M.E.B. Smith, F.F. Schumacher, C.P. Ryan, L.M. Tedaldi, D. Papaioannou, G. Waksman, S. Caddick, J.R. Baker, Protein modification, bioconjugation, and disulfide bridging using bromomaleimides, J. Am. Chem. Soc. 132 (2010) 1960–1965.
[23] L. Castañeda, Z.V.F. Wright, C. Marculescu, T.M. Tran, V. Chudasama, A. Maruani, E.A. Hull, J.P.M. Nunes, R.J. Fitzmaurice, M.E.B. Smith, L.H. Jones, S. Caddick, J.R. Baker, A mild synthesis of N-functionalised bromomaleimides, thiomaleimides and bromopyridazinediones, Tetrahedron Lett. 54 (2013) 3493–3495.
[24] M. Csávás, A. Miskovics, Z. Szűcs, E. Rőth, Z.L. Nagy, I. Bereczki, M. Herczeg, G. Batta, É. Nemes-Nikodém, E. Ostorházi, F. Rozgonyi, A. Borbás, P. Herczegh, Synthesis and antibacterial evaluation of some teicoplanin pseudoaglycon derivatives containing alkyl- and arylthiosubstituted maleimides, J. Antibiot. 68 (2015) 579–585.
[25] E. Vanderlinden, F. Göktas, Z. Cesur, M. Froeyen, M.L. Reed, C.J. Russell, N. Cesur, L. Naesens, Novel inhibitors of influenza virus fusion: structure-activity relationship and interaction with the viral hemagglutinin, J. Virol. 84 (2010) 4277–4288.
[26] R. Leiva, M. Barniol-Xicota, S. Codony, T. Ginex, E. Vanderlinden, M. Montes, M. Caffrey, F.J. Luque, L. Naesens, S. Vázquez, Aniline-based inhibitors of influenza H1N1 virus acting on hemagglutinin-mediated fusion, J. Med. Chem. 61 (2018) 98–118.
[27] P. Colson, D. Raoult, Fighting viruses with antibiotics: an overlooked path, Int. J. Antimicrob. Agents 48 (2016) 349–352.
[28] K. Bahrami, M.M. Khodaei, M. Soheilizad, A novel, practical synthesis of sulfonyl chlorides from thiol and disulfide derivatives, Synlett 17 (2009) 2773–2776.
[29] F. Tran, A.V. Odell, G.E. Ward, N.J. Westwood, A modular approach to triazole-containing chemical inducers of dimerization for yeast three-hybrid screening, Molecules 18 (2013) 11639–11657.
[30] J.L. Lau, M.M. Baksh, J.D. Fiedler, S.D. Brown, A. Kussrow, D.J. Bornhop, P. Ordoukhanian, M.G. Finn, Evolution and protein packaging of small-molecule RNA aptamers, ACS Nano 5 (2011) 7722–7729.
[31] M. Bollini, K.M. Frey, J.A. Cisneros, K.A. Spasov, K. Das, J.D. Bauman, E. Arnold, K.S. Anderson, W.L. Jorgensen, Extension into the entrance channel of HIV-1 reverse transcriptase-Crystallography and enhanced solubility, Bioorg. Med. Chem. Lett 23 (2013) 5209–5212.
[32] C. Marminon, A. Pierré, B. Pfeiffer, V. Pérez, S. Léonce, P. Renard, M. Prudhomme, Syntheses and antiproliferative activities of rebeccamycin analogues bearing two 7-azaindole moieties, Bioorg. Med. Chem. 11 (2003)

679–687.
[33] J.W. Ulm, M. Perron, J. Sodroski, R.C. Mulligan, Complex determinants within the Moloney murine leukemia virus capsid modulate susceptibility of the virus to Fv1 and Ref1-mediated restriction, Virology 363 (2007) 245–255.
[34] T. Matsuda, C. Cepko, Electroporation and RNA interference in the rodent retina in vivo and in vitro, Proc. Natl. Acad. Sci. Unit. States Am. 101 (2004) 16–22.
[35] A. Lundin, R. Dijkman, T. Bergström, N. Kann, B. Adamiak, C. Hannoun, E. Kindler, H.R. Jónsdóttir, D. Muth, J. Kint, M. Forlenza, M.A. Müller, C. Drosten, V. Thiel, E. Trybala, Targeting membrane-bound viral RNA synthesis reveals potent inhibition of diverse coronaviruses including the middle East respiratory syndrome virus, PLoS Pathog. 10 (2014), e1004166.