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Semisynthetic teicoplanin derivatives as new influenza virus binding inhibitors: Synthesis and antiviral studies

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ABSTRACT

In order to obtain new, cluster-forming antibiotic compounds, teicoplanin pseudoaglycone derivatives containing two lipophilic *n*-octyl chains have been synthesized. The compounds proved to be poor antibacterials, but, surprisingly, they exhibited potent anti-influenza virus activity against influenza A strains. This antiviral action was related to inhibition of the binding interaction between the virus and the host cell. Related analogs bearing methyl substituents in lieu of the octyl chains, displayed no anti-influenza virus activity. Hence, an interaction between the active, dually *n*-octylated compounds and the lipid bilayer of the host cell can be postulated, to explain the observed inhibition of influenza virus attachment.

Human influenza A and B viruses cause the annual influenza epidemics and sporadic pandemics, giving a considerable number of fatalities.[1] The influenza virus has two important antigenic membrane glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Both are involved in virus replication. HA is responsible for initial attachment of the virus to sialic acid-containing receptors on the host cell, and membrane fusion after virus uptake by endocytosis. NA performs the release of newly formed virions at the end of the viral life cycle.

Vaccination is an important preventive strategy but, because of the antigenic drift of influenza viruses, vaccines must be reformulated and readministered every year.[2]

An alternative and potentially life-saving intervention is the use of anti-influenza virus drugs. The M2 proton channel inhibitors amantadine and rimantadine have become of less importance since the widespread appearance of resistant viral strains.[3] The neuraminidase inhibitors oseltamivir and zanamivir are the standard drugs for treatment and prevention of influenza, but increasing viral resistance (against oseltamivir in particular[4,5]) urges development of new anti-influenza medications.

Viral HA is one of the potential targets for drug development.[6,7] To obtain potent HA inhibitors, sialoclusters, multivalent analogs of HA ligands, have been synthesized by covalent attachment of sialic acid or its oligosaccharide derivatives to polyacrylamids,[8–10] liposomes,[11,12] chitosan[13] or polymersomes.[14] Another strategy is to prepare sialoconjugates capable of forming self-assembled multivalent ligands.[15,16] Recently, it has been discovered that some synthetic peptides can be used as influenza virus HA traps.[17–20]

In the course of a detailed study on chemical transformations of the aglycones of glycopeptide antibiotics, we have identified several lipophilic aglycoristocetin derivatives possessing high anti-influenza virus activity.[21–25] Examination of the antiviral mode of action of one of the most potent derivatives revealed that it traps the virus in a distinct vesicular compartment, thereby preventing entry of the virus into the nucleus of the host cell.

Parallel with the aglycoristocetin transformations we did similar reactions with teicoplanin pseudoaglycone,[23,26] but despite the close structural similarities between the two series of semisynthetic antibiotics, the teicoplanin derivatives did not exhibit anti-influenza virus activity. However, most of them proved to be strong antibacterials with outstanding activities against multidrug-resistant bacteria.[23,26]

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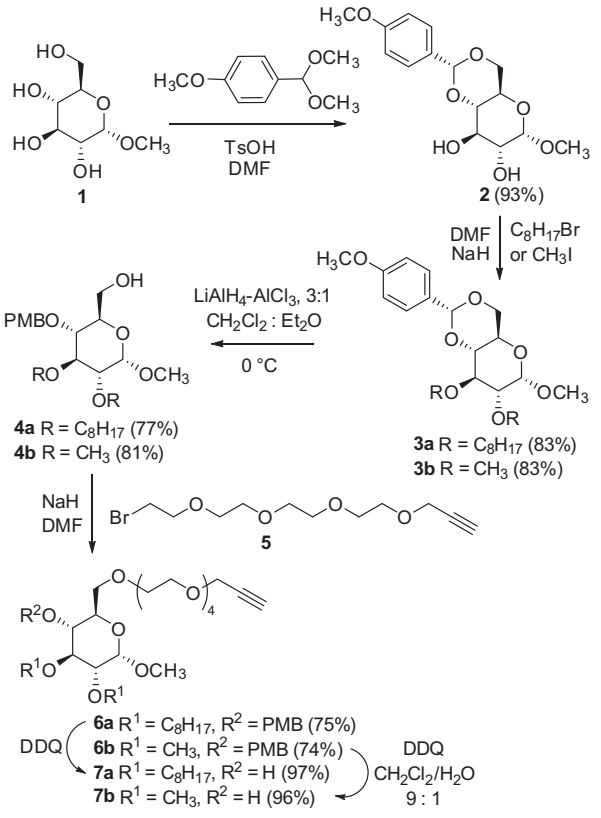
All of our lipophilic antibiotic derivatives have amphiphilic properties and, therefore, the tendency to form large, nano-sized clusters in water. We postulated that such aggregates can act as multivalent ligands of surface receptors of bacteria and viruses, explaining their enhanced biological activities.

In this work, we report on a novel, saccharide-based, versatile, lipophilic derivatisation of teicoplanin pseudo-aglycone with the aim of obtaining new antibacterial agents. The new compounds were found to display unexpected inhibitory activity towards influenza virus by interfering with virus binding to the host cell.

The synthesis of the carbohydrate auxiliary started from methyl α-D-glucopyranoside **1** (Scheme 1). *p*-Methoxybenzylidenation of **1** by transacetalisation afforded diol **2**,[27] the two hydroxyls of which were *n*-octylated to result in **3a**. Regioselective reductive cleavage of the 4,6-*O*-acetal ring using the $LiAlH_4$–$AlCl_3$ reagent combination in a 3:1 ratio (forming AlH_3)[28] resulted in exclusively the 4-*O*-*p*-metoxybenzyl (PMB) ether **4a**. The liberated 6-OH of **4a** was etherified with propargylated bromo-tetraethyleneglycol **5**[29] to give **6a** from which the *p*-methoxybenzyl group was removed by oxidative cleavage using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as the reagent[28a] to afford **7a**. Both latter compounds were coupled with an azido derivative of teicoplanin pseudoaglycone **8**[23] using a Cu(I) catalyzed 1,3-dipolar cycloaddition click reaction providing amphiphiles **9** and **10** (Scheme 2). To obtain reference compounds for the biological and aggregation studies, synthesis of teicoplanin derivatives **11** and **12** containing *O*-methyl substituents on the D-glucose unit was also carried out. We supposed that due to the lower lipophilicity of methyl groups than that of *n*-octyls the tendency to form clusters will be less pronounced for **11** and **12** than for **9** and **10**, and we also hypothesized that the lower cluster-forming ability will be accompanied by lower antibacterial activity. Hence, synthesis of **11** and **12** was performed starting from **2** in a similar reaction sequence which was applied for **9** and **10**. First, instead of octylation, methylation was carried out to furnish **3b**,[30] which was transformed to **4b** by reductive opening of the 4,6-*O*-acetal ring. Introduction of the propargyl-containing linker to the primary position afforded **6b**, which was transformed to **7b** by removal of the *p*-methoxybenzyl protecting group (Scheme 1). Finally, the glucose units **6b** and **7b** were conjugated to teicoplanin ψ-aglycone via azide–alkyne click reaction to provide **11** and **12**, respectively (Scheme 2).



Scheme 1. Functionalization of methyl α-D-glucopyranoside with lipophilic side chains and a propargyl-containing linker.

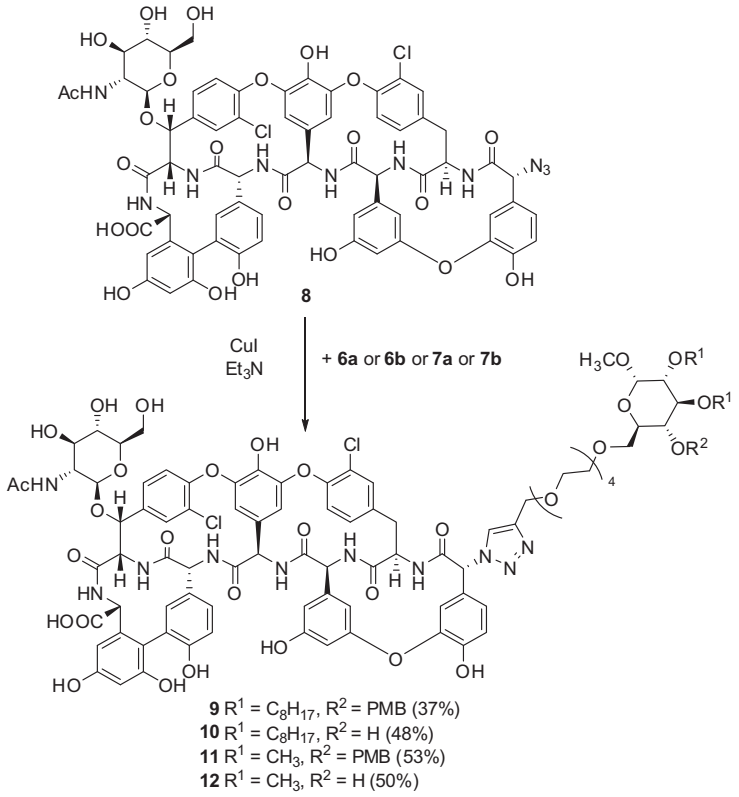
The aggregation of **9**–**12** in water was studied by dynamic light scattering. Formations of clusters with bimodal size-distribution were observed for **9** and **10**. Effective diameter of these clusters was 62 nm for **9** and 21 nm for **10**. Contrary to our expectations, **11** and **12** turned out to be even more prone to form clusters. Effective diameters of aggregates were 160 nm and 203 nm for **11** and **12**, respectively.

When evaluated for antibacterial activity against a panel of bacterial strains, compound **10** exhibited modest antibacterial activities, **9** was totally inactive, and **11** and **12** turned out to be very modest antibacterials (see Supporting information). These data were surprising when we consider the favorable antibacterial activity of **13** (Fig. 1), a structurally similar teicoplanin ψ-aglycone derivative bearing one lipophilic *n*-decyl side chain.[23]

Even more surprising, we observed that **9** exhibited strong antiviral activity against three strains of influenza A virus, including the 2009 pandemic virus A/H1N1 Virginia/ATCC3/2009. However, it was inactive against influenza B virus (data not shown). Compound **10** proved to be highly active against two out of the three investigated influenza A strains. In the course of our extensive studies on teicoplanin aglycone derivatives,[23,26] the herein reported **9** and **10** were the first compounds possessing anti-influenza virus activity. Their antiviral EC_{50} values were in the range of 1–2 μM (Table 1) and 4- to 20-fold lower than the compound concentrations causing cytotoxic effects, as estimated by microscopic examination or cell viability testing (see MCC and CC_{50} values, respectively, in Table 1). The methyl-functionalized derivatives **11** and **12** showed no inhibitory activity against any of the influenza strains used in this study (data not shown).

In the presence of **9** and **10**, influenza virus-induced hemagglutination was inhibited. The concentration to achieve this inhibition increased with the amount of virus (expressed in hemagglutinating units) (Table 2). This result suggests that compounds **9** and **10** interfere with virus binding to the host cell. In the absence of virus, **9** and **10** caused hemagglutination at 50 μM. Based on the results of the hemagglutination assay, we carried out a binding assay on MDCK cells.[22] As shown in Figure 2, a dose-dependent inhibition of influenza virus binding to the host cells was observed; the concentration giving 50% inhibition of virus binding was 4.7 μM for **9** and 7.0 μM for **10**.

In conclusion, two teicoplanin ψ-aglycone derivatives with excellent anti-influenza virus activity have been prepared. The antiviral mode of action appears to be based on inhibition of the binding interaction between the virus and the host cell. It is noteworthy that **9** and **10** as well their *O*-methyl analogs **11** and **12** can form nanoaggregates in water. However, the striking difference in anti-influenza virus activity of these pairs of compounds leads to the conclusion that the antiviral action of **9** and **10** cannot be explained by their self-assembled multivalency. Instead, the observed activity may be explained by the potential interaction of their double, long alkyl chains with the lipid bilayer of the host cell membrane. We hypothesize that this anchoring of **9** and **10** on the surface of the host cell creates a strong shielding effect and/or a multivalent interaction with the viral HA. As a consequence, virus attachment is prevented. The short methyl groups of **11** and **12** are not capable of interaction with the host cell membrane, explaining their lack of antiviral activity.



Scheme 2. Synthesis of teicoplanin ψ-aglycone derivatives **9**–**12** bearing variously substituted glucose unit at the N-terminal of the glycopeptide core.

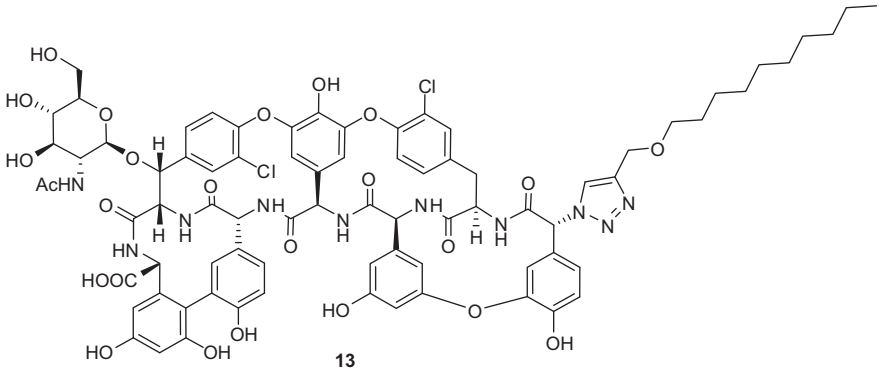


Figure 1. Previously prepared teicoplanin pseudoaglycone derivative with high antibacterial activity.[23]

Table 1
Anti-influenza virus activity of **9** and **10** in MDCK cell cultures

Compound	Cytotoxicity (μM)		Antiviral EC_{50} (μM)					
			Influenza A/H1N1 (A/PR/8/34)		Influenza A/H1N1 (A/Virginia/ATCC3/2009)		Influenza A/H3N2 (A/HK/7/87)	
	CC_{50}	MCC	CPE	MTS	CPE	MTS	CPE	MTS
9	11	20	0.80	1.2	1.4	<0.80	1.8	1.2
10	12	⩾4.0	>100	>100	1.8	1.3	2.3	2.2
Zanamivir	>100	>100	0.47	0.50	45	20	8.9	9.0
Ribavirin	>100	⩾20	6.8	21	12	11	6.8	7.3
Amantadine	>500	>500	212	111	>500	>500	0.45	0.50
Rimantadine	>500	⩾500	4.6	5.3	>500	>500	0.051	0.046

Abbreviations used: MDCK, Madin–Darby canine kidney; EC_{50}, 50% effective concentration, or compound concentration required to give 50% inhibition of virus replication, as estimated by microscopic scoring of the cytopathic effect (CPE) or the colorimetric TS cell viability assay; TS, tetrazolium salt; CC_{50}, 50% cytotoxic concentration, that is, the concentration that reduces cell viability by 50% according to the colorimetric MTS assay; MTS, 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)tetrazolium, inner salt; MCC, minimal cytotoxic concentration, that is, the compound concentration that causes minimal alterations in cell morphology, as assessed by microscopy.

Table 2
Inhibitory effect of **9** and **10** on influenza virus-induced hemagglutination

Compound	RBC incubated with virus at			RBC incubated without virus
	4 HA-U	2 HA-U	1 HA-U	
	Minimal concentration (μM),causing hemagglutination inhibition			Minimal concentration (μM), causing hemagglutination
9	12	6.3	0.78	50
10	3.1	0.78	0.39	50

Abbreviations used: RBC, red blood cells (from chicken); HA-U, hemagglutinating units.

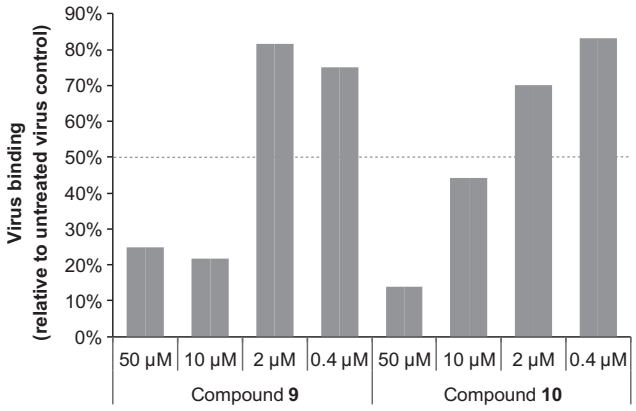


Figure 2. Inhibitory effect of **9** and **10** on influenza virus binding to MDCK cells.

Therefore, compounds **9** and **10** can be regarded as promising lead compounds to develop novel influenza virus inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.018.

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