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Click reaction synthesis of carbohydrate derivatives from ristocetin aglycon with antibacterial and antiviral activity

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ABSTRACT

New sugar derivatives of ristocetin were prepared by copper-catalyzed 1,3-dipolar cycloaddition reaction using azido-ristocetin aglycon and various propargyl glycosides. Some of them were found to be active against Gram-positive bacteria and showed favorable antiviral activity against the H1N1 subtype of influenza A virus.

Ristocetin A (**1**) is a glycopeptide-type antibiotic produced by *Nocardia lurida*.[1] The ristocetin molecule carries 6 sugar molecules (2 D-mannose, D-glucose, D-arabinose, L-rhamnose and L-ristosamine) linked to the aglycon. In spite of its good antibacterial activity against Gram-positive strains including MRSA,[2] this antibiotic has not been used in therapy due to its ability to cause aggregation of blood platelets.[3] Williams et al. reported[4] that cleavage of L-rhamnose from ristocetin A removes this aggregating property. In a systematic study with aglyco-ristocetin[5] (**2**) (Scheme 1) derivatives we found that deglycosylation of **1** retains the antibacterial activity, however **2** is not suitable for platelet aggregation tests due to its very low solubility in water. To improve solubility, we prepared derivatives of **2** containing β-D-glucopyranosyl and β-D-maltosyl substituents attached through an *N*-glycosylthiourea moiety.[6] These derivatives possessed good antibacterial activity and did not induce platelet aggregation. These promising results prompted us to perform a systematic study on the influence of various sugar substituents on the biological activity of derivatives of **2**. In this work we here report on the synthesis and biological activity of several new sugar derivatives of **2**. For conjugation of **1** we have chosen D-glucose, D-mannose and L-rhamnose, since the ristocetin molecule also contains these saccharides. Another glycopeptide antibiotic, teicoplanin has *N*-acetyl-D-glucosamine substituent, consequently this sugar was also chosen for the derivatisation of **1**. Following our diazo-transfer-click reaction sequence[7] **3** was chosen as the starting compound. Copper-catalyzed 1,3-dipolar cycloaddition reaction ('click reaction')[8] of the latter with per-O-acetylated propargyl glycosides[9] resulted in the carbohydrate-substituted aglycons **4a**–**8a**. Zemplén deacetylation[10] of the sugar acetate moieties led to the glycosyl–oxymethyl–triazolyl derivatives of the ristocetin aglycon carrying saccharides with α-D-glucopyranosyl (**4b**), α-D-mannopyranosyl (**5b**), α-D-maltopyranosyl (**6b**), α-D-*N*-acetylglucosaminyl (**7b**) and α-L-rhamnopyranosyl (**8b**) configuration (Scheme 2).

Evaluation of the biological properties of these derivatives (including the per-*O*-acetates) against Gram-positive bacteria demonstrated that introduction of the sugar moieties at the N-terminus into the ristocetin A aglycon molecule had a beneficial effect on the antibacterial activity. From an analysis of the data summarized in Table 1, it can be seen that the most of the new derivatives displayed higher activity than **1**. The sugar derivatives **4**–**7** had good to excellent bacteriostatic activity against the test organisms, including glycopeptide-resistant enterococci. Interestingly, in this small group of compounds, the configuration of the sugar part (D-sugars) exhibited only a slight influence on the MIC values.

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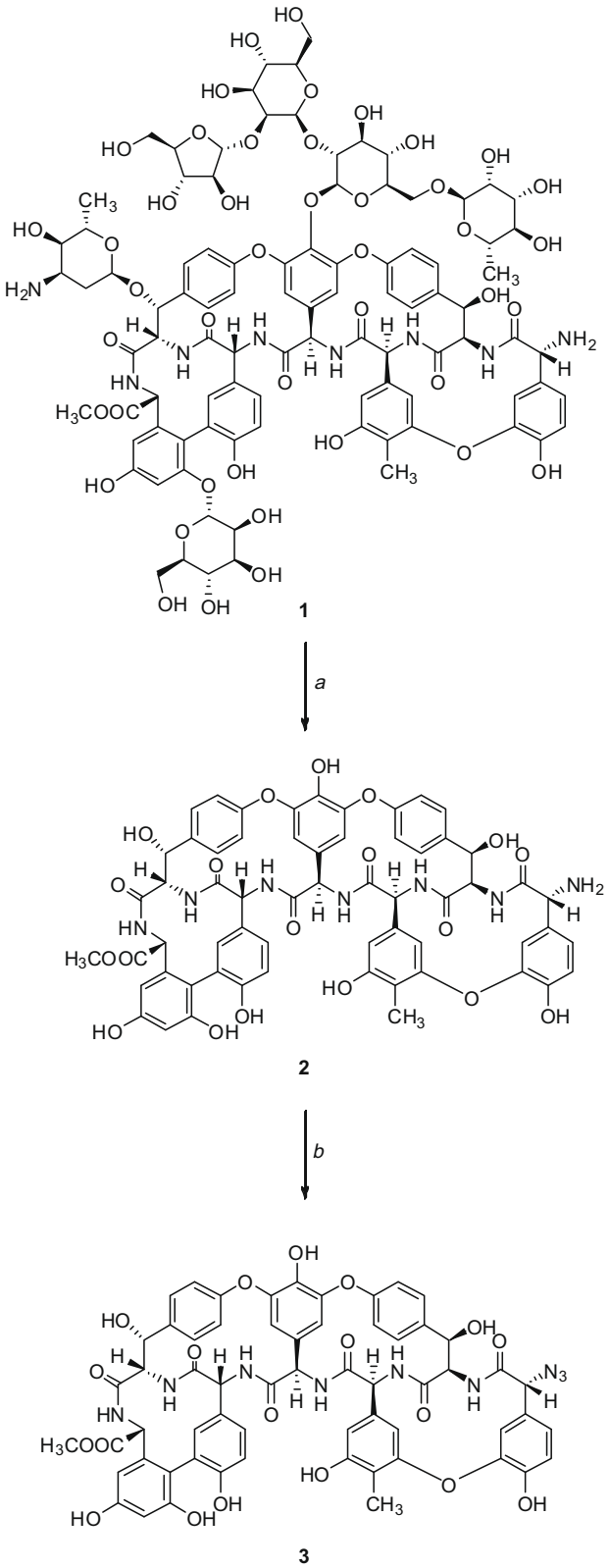
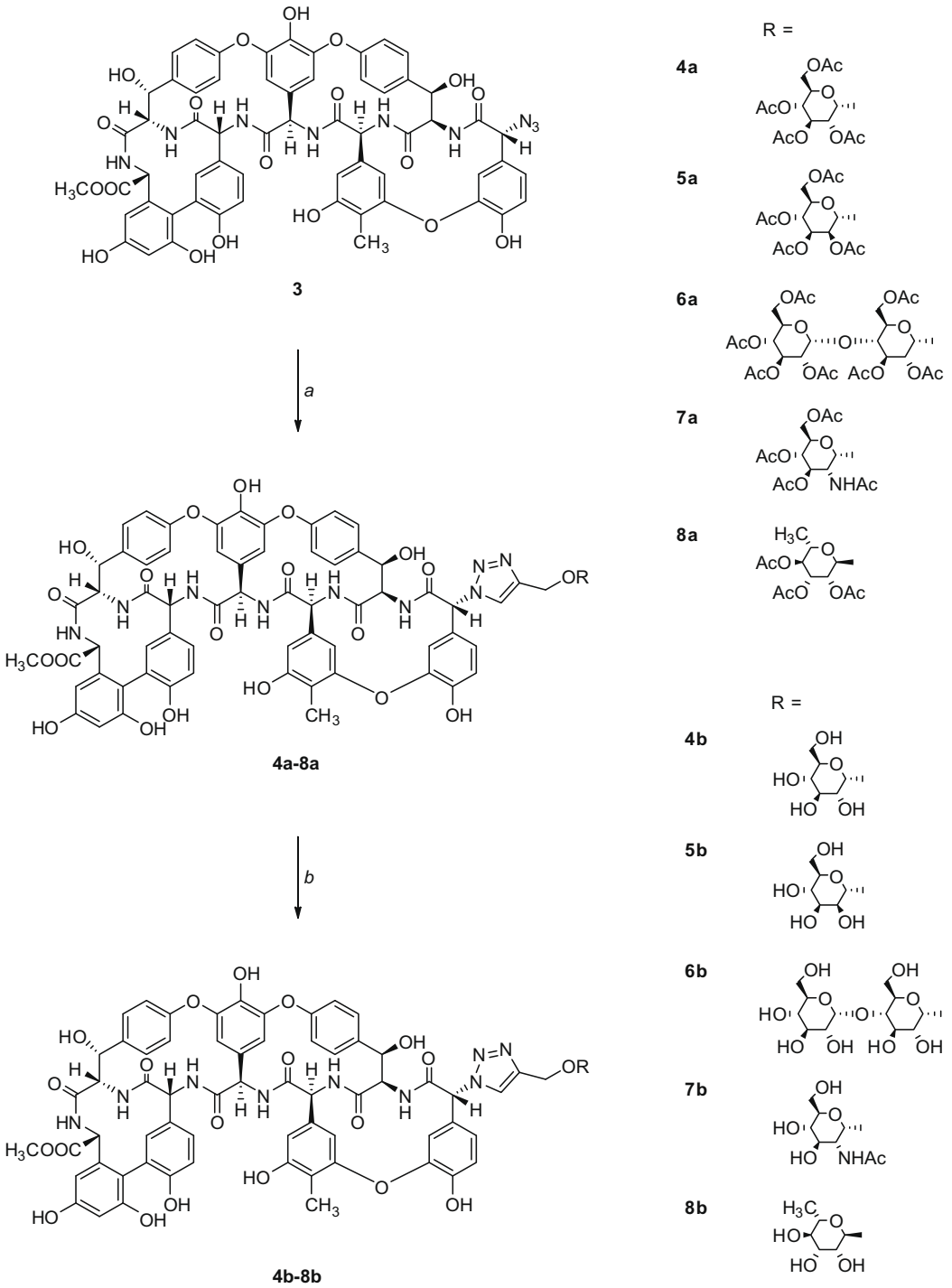


Scheme 1. Synthesis of the azido-ristocetin aglycon **3**. Reagents and conditions: (a) HF, anisole, −18 °C, 2 h, 61%; (b) TfN_3, pyridine, $CuSO_4$, Et_3N, room temperature, overnight, 58%.

Derivatives **8a** and **8b** containing rhamnosyl glycosides of the L-sugar series possessed much lower activity indicating the role of the stereochemistry in the antibacterial activity. It has to be noted that there is only a slight difference between the MIC values of compounds having acetylated (a series) or free sugars (b series), which can be explained by a possible deacetylation in the culture media by aspecific esterases. The anti-influenza virus activity of the prepared substances was also evaluated, since we



Scheme 2. Synthesis of the per-O-acetylated and free sugar derivatives of the ristocetin aglycon. Reagents and conditions: (a) per-O-acetylated propargyl glycoside, CuI, Et_3N, dry DMF, room temperature, overnight, 38–53%; (b) NaOMe, MeOH, room temperature, overnight, 87–96%.

recently discovered good to excellent activities of some derivatives of **1**.[11] The cell-culture based assays to determine the inhibitory effect on influenza virus replication against, have been published elsewhere.[11] Briefly, the antiviral activity was expressed as the 50% effective concentration (EC_{50}), or compound concentration producing 50% inhibition of influenza virus replication (as determined by microscopic or spectrophotometric assays). These experiments also allowed to estimate the cytotoxicity of the test compounds. As shown in Table 2, compounds **4a**,**b**–**7a**,**b** exhibited antiviral activity against influenza virus, with the highest activity being noted for the A/H1N1 subtype (antiviral EC_{50} values in the range of 6–16 μM). These four compounds produced minimal, if any, cytotoxicity at 70–100 μM. We have also studied the influence of the presence of sugar moieties on blood platelet aggregation properties. In about 0.1 mg/mL concentration the D-glucose and D-maltose analogs **4b** and **6b** inhibited the ristocetin induced platelet aggregation. The D-mannose and D-glucosamine derivatives **5b** and **7b** had no influence, but the L-rhamnose derivative **8b** increased the intensity of aggregation.

Table 1
Antibacterial activity of the new sugar derivatives of the ristocetin aglycon

Test microorganism	MIC/MBC (μg/mL)											
	Ristocetin	**3**	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**	**8a**	**8b**
ATCC 6633 *B. subtilis*	2/4	1/4	8/16	2/8	4/8	4/4	8/8	4/4	2/8	4/8	32/32	16/16
ATCC 33591 MSSA	16/64	1/2	4/8	2/128	2/4	4/16	1/4	2/256	1/64	4/4	16/32	8/16
ATCC 29213 MRSA	16/64	2/2	4/8	2/256	2/4	2/216	1/4	4/256	0.5/256	2/2	16/32	8/8
ATCC 35984 *S. epidermidis* Biofilm-positive	32/64	8/16	2/4	4/4	4/8	4/8	4/8	8/8	2/2	4/4	16/16	16/16
S. epidermidis ATCC 29212 *E. faecalis* Vanco: S Teico: S	4/8	16/16	2/4	4/16	4/4	4/8	4/4	4/8	1/2	2/4	16/32	16/16
	4/256	8/16	8/256	4/256	4/128	8/256	4/256	4/256	4/256	8/256	32/256	32/256
ATCC 51299 *E. faecalis* Vanco: R Teico: S VanB +	128/256	64/128	16/256	8/256	8/256	16/256	8/256	32/256	4/256	16/256	128/256	64/128
15376[a] *E. faecalis* Vanco: R Teico: R VanA +	128/256	>256	8/256	4/256	2/128	16/64	2/256	8/256	2/256	8/256	16/256	16/256

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, ATCC: American Typed Culture Collection, MSSA: Methicillin Sensitive *Staphylococcus aureus*, MRSA: Methicillin Resistant *Staphylococcus aureus*, Vanco/Teico R: Vancomycin/Teicoplanin Resistant, Vanco/Teico S: Vancomycin/Teicoplanin Sensitive, vanA +: vanA gene positive, vanB +: vanB gene positive.
[a] Ref. 12.

Table 2
Anti-influenza virus activity of the new sugar derivatives of the ristocetin aglycon

Compound	Concentration unit	Cytotoxicity		Antiviral EC_{50}[c]					
		CC_{50}[a]	Minimum cytotoxic concentration[b]	Influenza A/H1N1		Influenza A/H3N2		Influenza B	
				CPE	MTS	CPE	MTS	CPE	MTS
3	μM	⩾53	100	9.5	8.8	⩾20	⩾9	>100	>100
4a	μM	⩾87	⩾100	9.5	8.2	>100	>100	>100	>100
4b	μM	>100	⩾100	14	16	⩾45	⩾30	32	33
5a	μM	>100	20	4.0	5.0	>100	>100	>100	>100
5b	μM	>100	⩾100	10	22	⩾45	⩾46	⩾45	⩾50
6a	μM	60	20	>100	>100	>100	>100	>100	>100
6b	μM	>100	⩾100	16	20	>100	>100	52	61
7a	μM	⩾70	100	8.0	5.8	>100	>100	>100	>100
7b	μM	>100	⩾100	9.0	8.7	⩾45	⩾72	32	40
8a	μM	>100	>100	>100	>100	>100	>100	>100	>100
8b	μM	>100	>100	>100	>100	>100	>100	>100	>100
Oseltamivir carboxylate	μM	>100	>100	12	11	32	38	23	27
Ribavirin	μM	>100	>100	9.0	11	9.0	8.7	4.0	4.7
Amantadine	μM	>500	>500	20	19	7.0	9.2	>500	>500
Rimantadine	μM	>500	>500	224	283	4.0	5.6	>500	>500

CPE: cytopathic effect.
MTS: methyltetrazolium salt.
Data shown are the mean of two independent tests.
[a] 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c] 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

In summary, it is demonstrated that resubstitution of aglycoristocetin with saccharides by the 'click reaction' dramatically improves the antibacterial activity against glycopeptide-resistant enterococci and leads to anti-influenza virus derivatives. It turned out that the platelet aggregation activity can be effectively modified by introduction of various saccharides into the ristocetin aglycon molecule.

In order to improve and extend the antibacterial and antiviral activity, derivatisation of aglyco-ristocetin with further mono- and oligosaccharides with D and L configuration, as well as deoxy and deoxyamino sugars and other side chains with hydrophilic character is under way in our laboratory.

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