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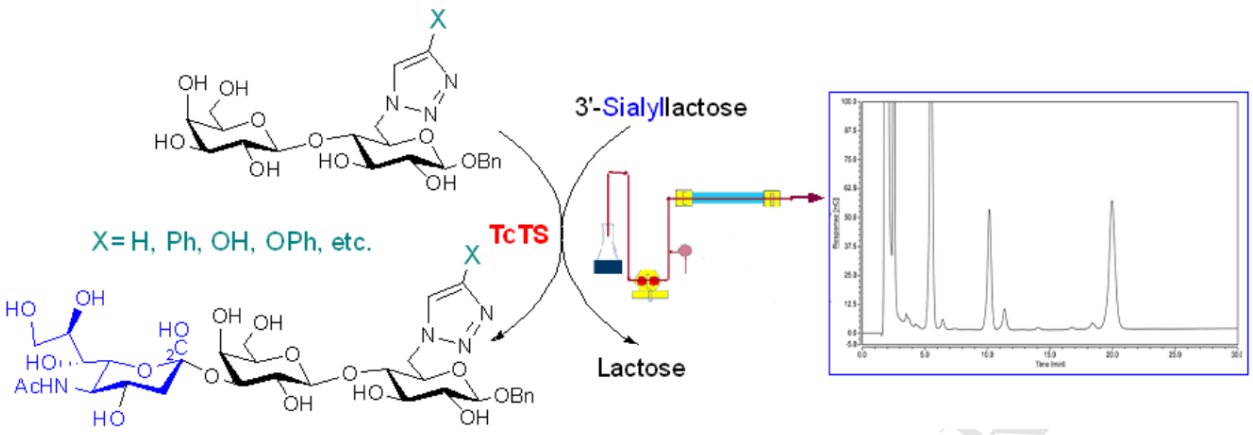

Trypanosoma cruzi trans-sialidase alternative substrates: Study of the effect of substitution in C-6 in benzyl β-lactoside.

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Abstract

Trypanosoma cruzi trans-sialidase (TcTS) is a cell surface protein that participates in the adhesion and invasion mechanisms of the parasite into the host cells, making it an attractive target for inhibitors design. In order to contribute to the knowledge of the interaction between TcTS and their acceptor substrates, we design and synthesized a library of 20 benzyl lactosides substituted in C-6 of the glucose residue with a series of 1,2,3-triazole derivatives containing different aromatic substituents in the C-4 position. The library was prepared by alkyne-azide cycloaddition reaction catalyzed by Cu(I) ("click chemistry") between a benzyl β-lactoside functionalized with an azide group in the C-6 position and a series of 2-propargyl phenyl ethers. Herein we analyzed the chromatographic behavior on high performance anion exchange chromatography (HPAEC) of the triazoyl-lactose derivatives and their activity as acceptors of TcTS and inhibitors of the sialylation of *N*-acetyllactosamine. The triazoyl derivatives were obtained with excellent yields and all of them behaved as moderate alternative substrates. The presence of bulky hydrophobic substituents dramatically increased the retention times in HPAEC but did not affect significantly their acceptor properties toward TcTS.

Keywords: *Trypanosoma cruzi* trans-sialidase, click chemistry, HPAEC, acceptor substrates, triazole.

1. Introduction

Chagas disease, or American trypanosomiasis, is one of the most prevalent neglected tropical diseases [1]. It is occasioned by the protozoan parasite *Trypanosoma cruzi* and disseminated by the triatomine insect vector. The parasite can also be transmitted by blood or organ donation; or from infected mothers onto their babies during pregnancy [2]. There are currently only two drugs available to treat this disease, nifurtimox and benznidazole, both displaying low efficacy in the chronic phase and several toxic side effects [3]. In order to address this issue, specific enzymes of the parasite biochemical pathways, such as cysteine proteases, trypanothione reductase, and trans-sialidase, are considered to be potential drug targets [4].

Trypanosoma cruzi trans-sialidase (TcTS) is a retaining glycosyltransferase anchored to the parasite membrane by glycosylphosphatidylinositol (GPI) [5,6], which primary function consists in the transfer of sialic acid residues from mammalian host glycoconjugates to terminal β-Gal*p* residues on the mucins that cover the parasite cell surface, generating (α2→3)-sialylated β-galactopyranose units [7–9]. By altering the membrane profile, TcTS provides direct protection from recognition by the host's immune system [10], resistance to the complement [11] and recognition of and attachment to host cells [12–15]. The enzyme follows a ping-pong bi-bi kinetic mechanism with formation of a sialyl-enzyme intermediate. The active site shows two distinct sites, one responsible for the interaction with the sialic acid residue (donor site) and the other with the β-galactose acceptor molecule (acceptor site). The donor site contains an arginine triad (Arg35, Arg245 and Arg314) that contributes to the

stabilization of the transition state, two acidic amino acids (Asp59 and Glu230) that function as acid-base catalysts and Tyr342 that binds the sialic acid to form the sialyl-enzyme intermediate. The acceptor site is comprised mainly by two aromatic amino acids (Tyr119 and Trp312) which forms an aromatic sandwich that binds the β-galactose-containing acceptor by π-CH and π-OH interactions [16,17]. Asp59 (common to both sites) and Glu362 cooperate in the catalysis of the reverse reaction in which the acceptor substrate attacks the sialyl-enzyme intermediate [18].

In order to exploit TcTS as a drug target for chemotherapy use, it is essential to understand the characteristics of the enzyme/substrates interaction. Numerous approaches had been taken to address this point, including spectroscopic techniques [19–22], X-ray crystallography [18,23,24], virtual screening [4,25–28], natural product library screening [29] and chemical synthesis of transition state or substrate analogues [7,30–38].

TcTS inhibitors are usually classified in two main groups according to their binding site target: sialic acid mimetics or acceptor substrate analogues. 2-Deoxy-2,3-didehydro-D-*N*-acetylneuraminic acid (DANA), a transition state analogue, is a potent inhibitor of the Influenza neuraminidase but a poor inhibitor of TcTS [39]. Regarding the inhibitors that target the acceptor binding site, lactitol and other lactose derivatives prevent sialylation of *N*-acetyllactosamine by behaving as a better acceptor substrate than the conventional acceptor [30,32,40,41]. Acceptor substrate studies performed on a series of systematically modified octyl galactosides and octyl *N*-acetyllactosamines determined that TcTS activity largely diminished if galactose is substituted at 2 and 4 positions but substitution at 6 position is well tolerated

[37]. Giorgi *et al* covalently bound oseltamivir, a well-known sialidase inhibitor, with lactose and lactobionolactone with the aim to obtain a bi-substrate potential inhibitor. The synthesized compounds were shown to be moderate acceptors and inhibitors of TcTS [31].

In the last years a growing interest to the synthesis of 1,2,3-triazole derivatives of carbohydrates has been developed due to the improved stability and physical properties of the triazole ring when compared to an amide, ester or ether linkage [42]. Besides, Cu(I)-assisted 1,3-dipolar azide–alkyne cycloaddition (CuAAC) is a fast, selective and easily executed reaction that allows the introduction of a variety of substituents from the proper azide and alkyne precursors. In this respect, Carvalho *et al* [36] synthesized a library of substrate analogues based on 1,4-disubstituted 1,2,3-triazole derivatives of galactose modified at either the C-1 or C-6 positions. They behaved as poor inhibitors of TcTS even though they were able to act as acceptor substrates and even had in vitro trypanocidal activity. More recently, mixed multivalent cyclic and linear triazole–linked oligomers were synthesized that showed to be acceptor substrates and blocked macrophage invasion by *T. cruzi*.[43]. Multivalent β-thio-galactopyranosides and β-thio-lactosides were used as acceptor substrates using 3'-sialyllactose as the sialic acid donor and allowing the chemoenzymatic synthesis of polysialoside glycoclusters [44,45].

With the aim to explore the influence of the presence of aromatic substituents in the acceptor substrates, taking into account the key function of the aromatic sandwich in the binding site of TcTS, herein we report the synthesis of a library of lactose analogues modified at C-6 of the glucose ring with 1,2,3-triazole derivatives substituted at C-4 with different aromatic groups.

The ability of the synthesized compounds to act as substrates of TcTS and inhibitors of the sialylation of *N*-acetyllactosamine, as well as their chromatographic behavior on high performance anion exchange chromatography was studied.

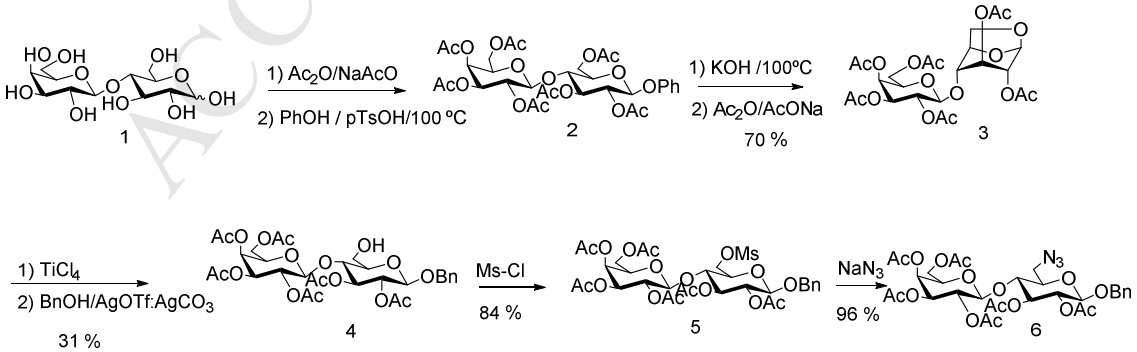
2. Results and discussion

For the synthesis of lactose analogues modified at C-6 with different substituents, it was important to develop a method to selectively modify the C-6 of the glucose residue with high yield. Two different pathways were taken for the synthesis of the key 6-azido lactose derivative: the first one involved the utilization of a lactosan intermediate while the other proceeds via the 4',6'-benzyliden lactose derivative.

2.1. Synthesis of benzyl peracetyl-6-azido-6-deoxy-β-lactoside

2.1.1 Lactosan pathway: per-acetyllactosan, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**3**) was synthesized from lactose (**1**) according to the procedure described by Tejima with minor modifications (Scheme 1) [46]. Lactose was peracetylated and glycosylated with phenol in the presence of *p*-TsOH/Ac_2O to obtain phenyl hepta-*O*-acetyl-lactoside (**2)** as pure β anomer according to its NMR spectra (δ 5.06, $J_{1,2}$ 7.4 Hz H-1; δ 4.51. $J_{1',2'}$ 7.8 Hz, H-1'; δ 98.7, C-1; δ 101.1, C-1'). For the synthesis of the hexa-*O*-acetyl-lactosan **3**, alkaline treatment was performed on the β-phenyl lactoside since the anhydrosugar formation occurred

exclusively on the β anomer. After acetylation, the attainment of **3** was confirmed by the observation of two broad singlets at 5.47 and 5.17 ppm in the ^{1}H-NMR spectrum, corresponding respectively to H-1 and H-2 of the anhydro residue. The opening of the anhydro bridge was achieved by treatment with $TiCl_4$. Further glycosylation with benzyl alcohol required catalytic amounts of silver triflate added to the silver carbonate used as promoter, affording **4** as a single anomer. Compound **4** was a convenient intermediate for the introduction of the azide group at the 6-position of the Glc residue. The NMR-^{1}H spectra of **4** showed two signals at 4.63 and 4.59 ppm corresponding to the anomeric protons of the galactose and glucose residues, respectively. Additionally, in the ^{13}C-NMR spectrum only two signals appeared in the anomeric region at 100.9 and 99.6 ppm for the C-1 of galactose and glucose, respectively. The azide substituent in the C-6 position was introduced converting the free hydroxyl into an appropriate leaving group by standard mesylation (**5**) followed by displacement of the mesylate with sodium azide. The ^{1}H-NMR spectrum of **6** showed the signals of H-6 at higher fields (3.4 and 3.5 ppm) in comparison with the analogous signals in the mesylate **5**. The same behavior was observed for C-6, which appeared upfield at 50.5 ppm.



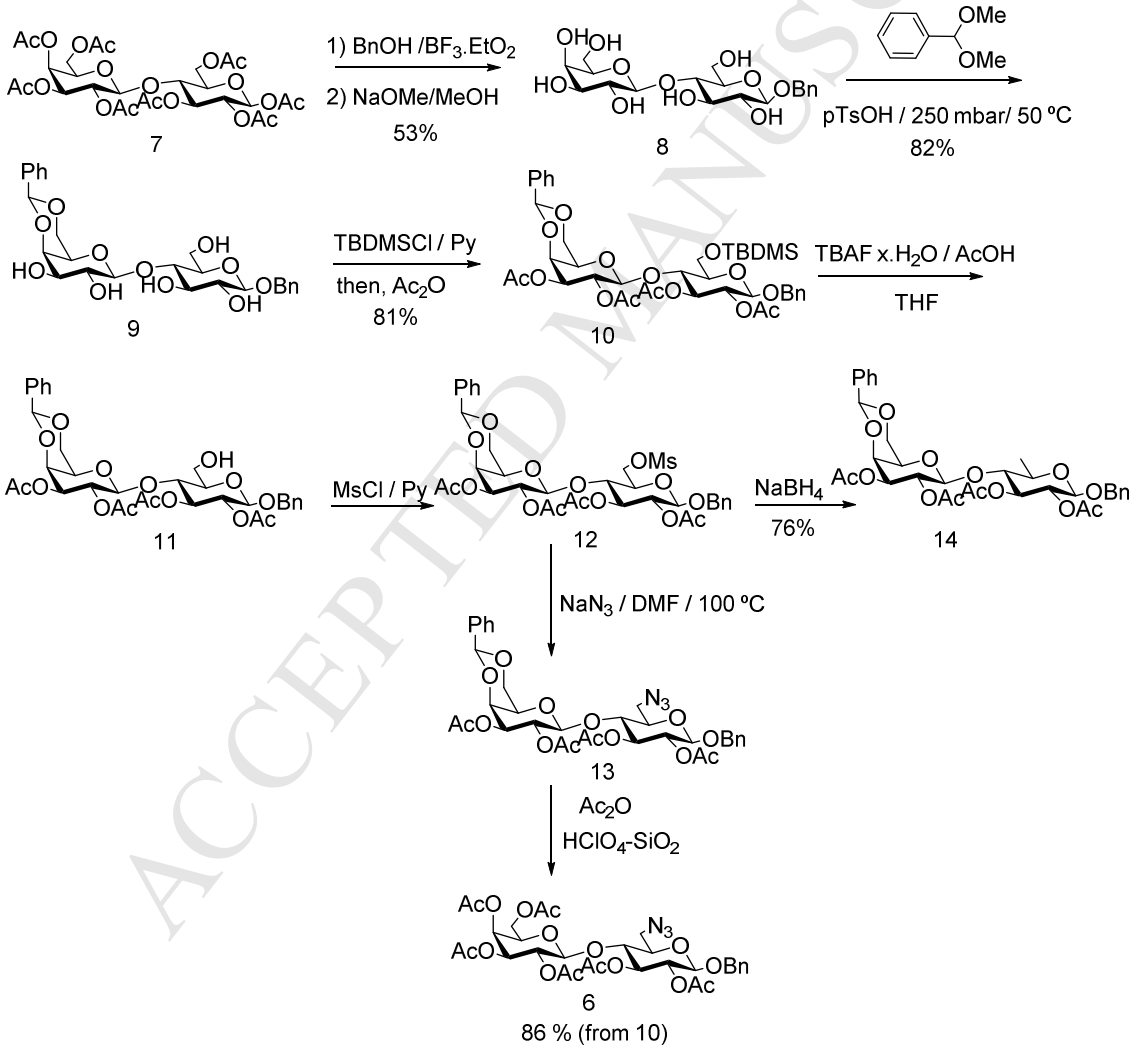
Scheme 1- Lactosan pathway for the synthesis of the azido lactoside **6**

2.1.2. Benzyliden pathway: The alternative pathway showed in Scheme 2 was explored in the search of a more readily synthetic procedure to obtain the key precursor **6**. In the first place, benzyl β-D-lactoside (**8**) was synthesized from octa-*O*-acetyl-lactose (**7**) by glycosylation with benzyl alcohol in the presence of $BF_3.Et_2O$ followed by deacetylation. The 4':6'-benzyliden acetal (**9**) was obtained in very good yield (81%) from **8** using dimethoxytoluene and *p*-TsOH as acid catalyst and allowing the reaction to proceed in a rotavap, following the procedure described for phenyl β-D-lactoside [47]. The *in situ* evaporation of the solvent led to the precipitation of the product and the concomitant displacement of the equilibrium reaction, accounting for the good yield obtained. As direct sulphonylation of the 6-OH was shown to give poor yields for the glucose configuration [48], **9** was regioselectively protected with a *tert*-butyldimethylsilyl group, and then acetylated in all free positions to obtain **10**. The silyl protecting group was then removed with tetrabutylammonium fluoride to give **11.** After mesylation and displacement with sodium azide, **13** was obtained. Benzylidene acetal cleavage and *in situ* acetylation of the diol was performed using perchloric acid immobilized on silica as catalyst [49]. The reaction progressed fast and with excellent yield and the solid catalyst could be removed by filtration, avoiding tedious work up, to give **6** in an overall yield from **10** of 86% without in between purifications. The spectroscopic data of **6** obtained through this sequence matched exactly to the observed for the same compound obtained by the lactosan pathway.

For the synthesis of the triazole derivatives library, we selected this last pathway since the total yield for the synthesis of the azide derivative **6** was 5-fold superior than the one summarize in Scheme 1. The lactosan pathway, on

the other hand, allowed us to obtain compounds **2** and **3** to be analyzed as substrates of TcTS after deacetylation.

Mesylated derivative **12** was also used for the synthesis of the 6-deoxy-β-lactoside derivative. Treatment of **12** with sodium borohydride in DMF afforded **14**, which NMR spectra showed the characteristic signals for the 6-deoxy sugar; a doublet at 1.37 ppm in the ^{1}H-NMR spectrum, and a peak at 17.3 ppm in the ^{13}C-NMR spectrum corresponding to the 6-CH_3. C-5 was also shifted 4 ppm upfield.



Scheme 2- Benzyliden pathway for the synthesis of the azido lactoside **6**

2.2. Synthesis of benzyl β-lactosides modified at C-6 with 1,4-substituted-1,2,3-triazoles

CuAAC reactions on the 6-azido modified benzyl lactoside **6** were carried out with a set of terminal alkynes (**a**-**r**, Figure S1), either commercially available or synthesized according to the procedure described by Wang *et al* [50]. Cycloadditions were performed under microwave irradiation and proceeded with excellent yields (85-96 %) and complete regioselectivity to obtained 1,4-disubstituted triazoles (**15a**-**15r**, Figure 1), as certified by NMR spectroscopy. Cycloaddition with propiolic acid (**a**) required 2 microwave cycles at 120 °C and afforded the monosubstituted triazole derivative **15a** with a moderate yield (51 %). To our knowledge, this is the first report of a successful alkyne-azide cycloaddition using propiolic acid in a carbohydrate derivative, followed by decarboxylation. Finally, compounds **15a**-**15r** were deacetylated with catalytic NaOMe in methanol, desalted with a cation exchange resin and the deprotected products recovered with high yields and characterized by NMR and HR-MS. Carboxymethyl ester-containing compounds **16q** and **16r** were further saponified with aqueous NaOH to afford the carboxylic acid derivatives **16s** and **16t**, respectively. Thus, a library of 20 triazole lactosides (**16a**-**16t**) was obtained, to be analyzed as potential TcTS substrates.

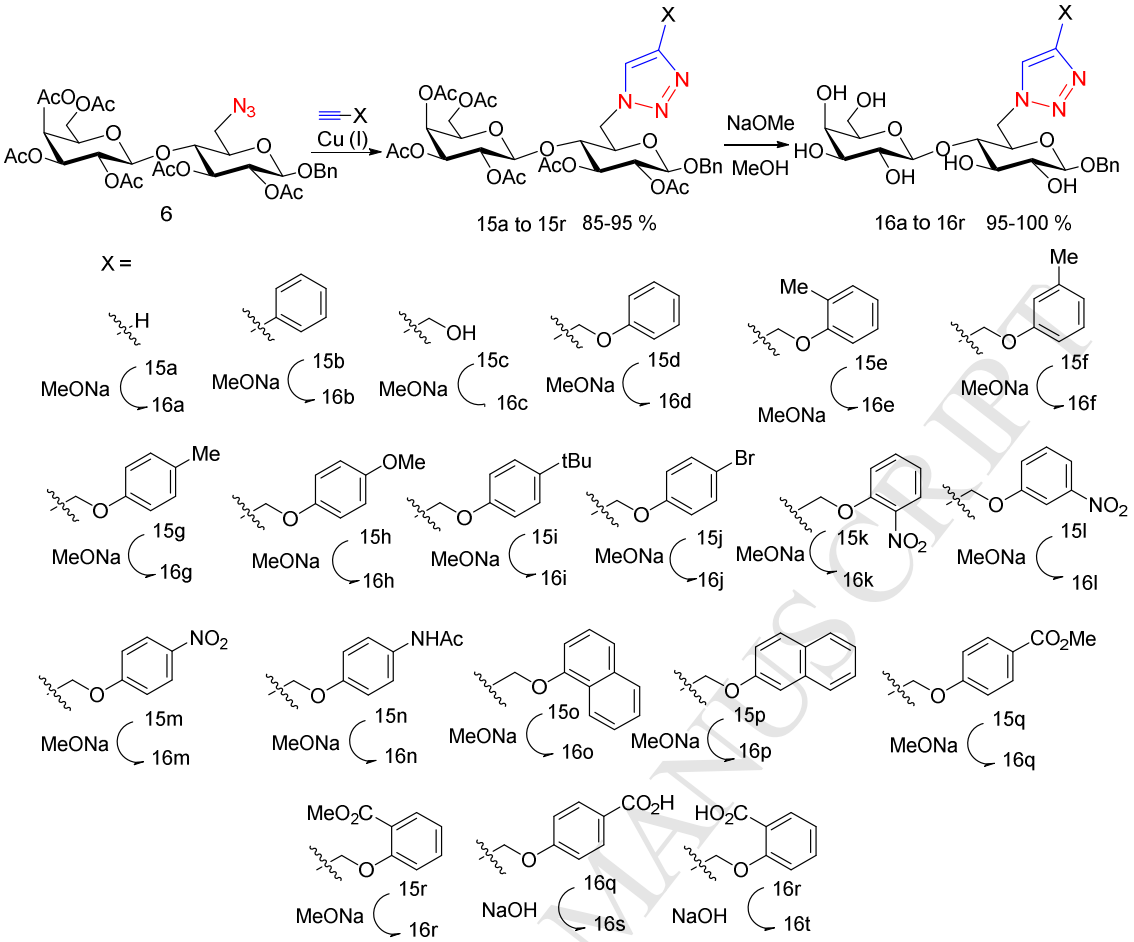


Figure 1- Synthesis of benzyl β-lactosides modified at C-6 with 1,4-substituted-1,2,3-triazoles

2.3. Analysis of lactose derivatives by HPAEC

Compounds **2,3,5,6** were deacetylated with catalytic sodium methoxide in methanol, and desalted with a cation exchange resin to afford compounds **17-20**, respectively (Figure 2). Compound **14** was deprotected by one-pot removal of the benzylidene acetal and the acetate groups yielding compound **21**. All products were recovered with high yields and characterized by NMR and HR-MS. These free compounds, together with the library of 20 triazole lactosides (compounds **16a-16t)** provided a suitable set of lactose-related products to be

studied by HPAEC, prior to the investigation of their activity towards the TcTS enzyme.

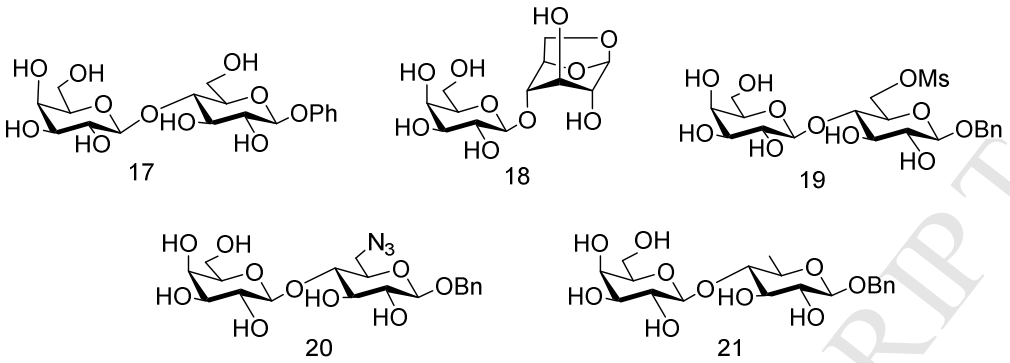
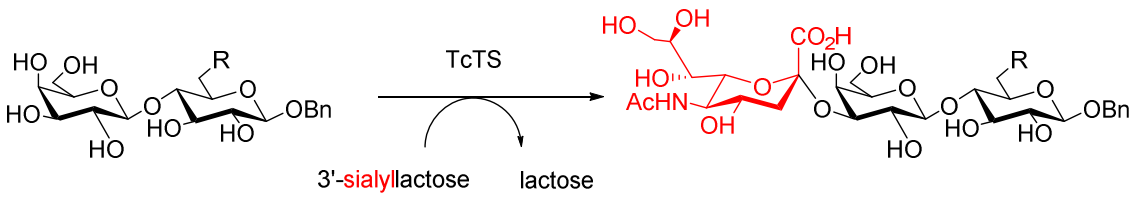


Figure 2- Lactose derivatives analyzed by HPAEC

The chromatographic resolution of lactose derivatives was studied using a Carbopac PA-10 analytical column under Condition A, as described in the Experimental Section. Retention times of the triazole derivatives highly increased with the incorporation of an aromatic ring (Table 1, see below). Moreover, compounds **16o** and **16p**, with naphtyl substituents were highly retained and eluted after 70 min under these conditions. Among the phenyl-substituted derivatives, compounds containing bulky hydrophobic substituents, such as *tert*-butyl and bromine (compounds **16i** and **16j**) were more retained in the chromatographic column than those with polar substituents -OMe, -NO_2, -NHAc (compounds **16h**, **16m**, **16n**). Benzoic acid derivatives, compounds **16s** and **16t**, were highly retained due to the presence of the acid and this effect was more pronounced for *para* isomer. The chromatographic behavior of their ester precursors, compounds **16q** and **16r**, could not be assessed since they hydrolyzed under the alkaline condition of the chromatography.

2.4. Lactose derivatives as acceptors of TcTS. Analysis by HPAEC

The 6-modified lactose derivatives **17-21** and the 6-triazolyl lactose derivatives **16a**-**16t** were analyzed as acceptor substrates of TcTS. The reaction is shown in Scheme 3. Incubations were performed as previously described [32] using 1 mM 3'-SL as donor, 1 mM of the potential substrate and recombinant TcTS. The incubation mixtures were analyzed by HPAEC (PA10, various conditions, Table 1). The CarboPac PA-10 column was chosen for this experiment since sialylated derivatives of the most hydrophobic compounds (**16o** and **16p**) were highly retained in a PA100 column. The percentage of sialic acid transference in each case was calculated from the amount of sialylated product with respect to the sum of all sialylated species present in the reaction mixture [51]. The results are included in Table 1 (fourth column). As an example, the chromatograms obtained for lactosan (**18**), and the click derivatives with propargylic alcohol (**16c**) and *p-tert*-butylphenyl propargyl ether (**16i**) are depicted in Figure 3. Control experiments without the addition of trans-sialidase were performed for all the samples to discard the occurrence of other peaks, besides the sialylated species. Moreover, the presence of the sialylated derivative of compound **21** was verified by MALDI-TOF mass spectrometry of the incubation mixture (Figure Sx). Peaks at m/z 752.326 and m/z 730.348 corresponding to the $[M-H+2Na]^+$ and $[M+Na]^+$ confirmed the presence of the sialylated product in the incubation mixture.



Scheme 3- Sialylation of lactose derivatives by *T. cruzi* trans-sialidase

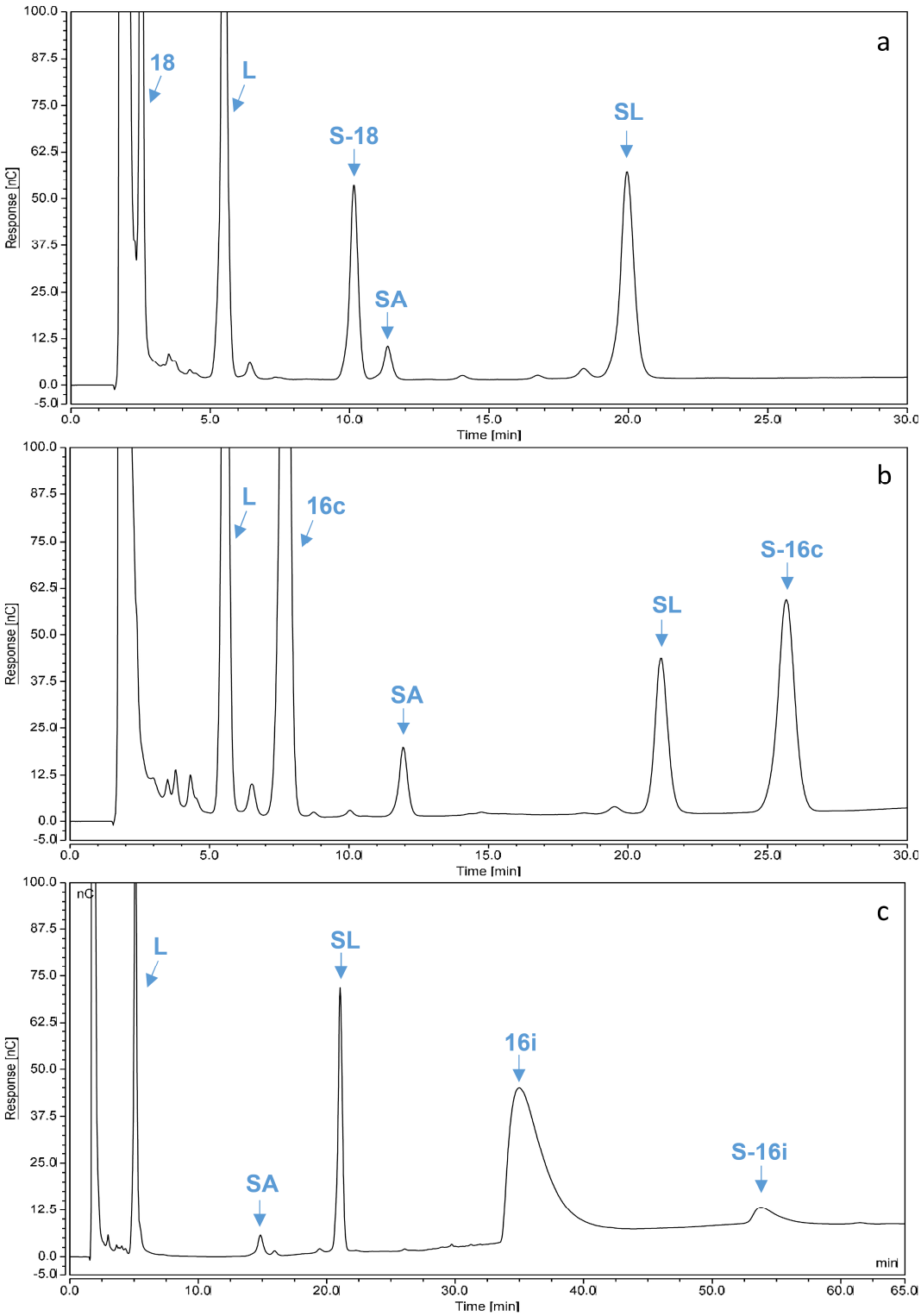


Figure 3- HPAEC of the incubations of compounds **18** (panel **a**), **16c** (panel **b**) and **16i** (panel **c**) with TcTS (300 ng) in 20 mM Tris–HCl, pH 7.6 buffer, 30 mM NaCl, containing 1 mM 3'-sialyllactose as a sialic acid donor. The mixtures were analyzed using a Carbopac PA-10 column under conditions C (panels **a** and **b**) and A (panel **c**). HPAEC conditions A-C are detailed in Experimental. L, lactose; SA, sialic acid; SL, 3'-sialyllactose; S-**18**, sialylated compound **18**; S-**16c**, sialylated compound **16c**; S-**16i**, sialylated compound **16i**.

All compounds were good acceptors of sialic acid showing that the presence of the triazoyl substituent did not impair their acceptor capacity, unless substituted with hydrophobic bulky groups such as *tert*-butylphenyl (**16i**) or β-naphtyl (**16p**). When comparing compounds with similar characteristics, more polar derivatives were better as sialic acid acceptors. Compound **16c**, containing only an $–CH_2OH$ substituent in the triazoyl moiety, was among the best substrates and inhibitors tested (see below). Additionally, the *p*-methylphenyl derivative **16g** is a worse acceptor than the unsubstituted phenyl (**16d**) but a better one than the *tert*-butyl derivative **16i**. The three positional isomers of the nitrophenyl derivatives behaved similarly being the *o*-nitrophenyl **16k** a better substrate than the *m*- and *p*-isomers **16l** and **16m**. Interestingly, even though the glucose residue in compound **18** is conformationally locked in a $^1C_4$ conformation, **18** still showed a moderate activity as acceptor of sialic acid. Percentages of transference were similar than those obtained for other lactose derivatives previously synthesized [31,32,52,53].

2.5. Lactose derivatives as inhibitors of sialylation of *N*-acetyllactosamine by TcTS.

All synthesized compounds were analyzed as inhibitors of sialylation of *N*-acetyllactosamine by the enzyme, a model reaction that can be considered as representative of the natural process that occurs during infection with the parasite. Incubations were performed as mentioned above with the addition of 1 mM *N*-acetyllactosamine. Inhibition percentages were calculated as the

decrease in the percentage of sialyl-*N*-acetyllactosamine in comparison with the value obtained in a control experiment performed in absence of the proposed inhibitor. Reaction mixtures were also analyzed by HPAEC and the results are shown in Table 1, fifth column.

The analysis of the values obtained in the inhibition experiments suggests that the presence of the triazole ring generally improves inhibition; taking into account the values obtained for the non-triazoyl containing derivatives (cf. **17**, **19**-**21** with the **16** series). On the other hand, interestingly, compound **21**, which lacks the oxygenated functionality at C-6, resulted as active as the benzyl glycoside **8**, which can be considered as reference compound to evaluate the activity behavior of our library.

Compound **16c**, containing only an $–CH_2OH$ substituent in the triazoyl moiety, was among the best substrates and inhibitors tested. On the other hand, **16d**, which poses a $–CH_2OPh$ in the same position, shows a similar activity to that of **16c**, a fact that suggests that steric effects are tolerable to a certain extent. On the other hand, the incorporation of a *o*-methyl group in the aromatic ring (compound **16e**), causes a substantial drop in the inhibition (cf. entries 10 and 11, Table 1), also observed for the *meta* and *para* isomers (compounds **16f** and **16g)**. This effect is more pronounced in the case of **16i**, having the bulky and hydrophobic *p-tert*-butylphenyl methyl ether, which rendered the worst acceptor and inhibition results.

In summary, all compounds resulted good to moderate inhibitors, with the exception of the *p-tert*-butylphenyl derivative **16i**, that showed only 4.9% of inhibition. It is noticeable that the β-naphtyl derivative **16p** was among the worst

substrates tested but showed the highest inhibitory value, suggesting that an alternative mode of action is taking place.

Table 1- Chromatographic behavior and activity towards TcTs of the 6-substituted lactose derivatives.

Entry	Compound	R_t (min) (condition A) PA-10	% T[a]	% I[b]	HPAEC Condition PA-10
1	**8**	5.5	46.0	43.2	C
2	**17**	5.7	48.7	32.9	C
3	**18**	2.2	33.5	42.5	C
4	**19**	6.3	38.0	34.5	C
5	**20**	7.2	51.0	39.8	C
6	**21**	2.9	48.4	44.8	C
7	**16a**	5.1	42.3	50.4	C
8	**16b**	25.7	42.0	45.3	A
9	**16c**	5.4	58.8	59.6	C
10	**16d**	22.1	53.3	60.2	B
11	**16e**	24.7	44.8	35.4	A
12	**16f**	25.5	45.5	53.6	A
13	**16g**	25.4	29.2	49.5	A
14	**16h**	21.6	48.5	56.5	B
15	**16i**	35.9	22.5	4.9	A
16	**16j**	37.2	39.3	55.2	A
17	**16k**	24.5	48.3	42.7	B
18	**16l**	25.7	39.0	60.2	A
19	**16m**	23.4	37.7	44.0	A
20	**16n**	20.6	32.3	35.2	B
21	**16o**	88.4	44.5	50.9	A
22	**16p**	74.1	30.1	68.7	A
23	**16q**	- (i)	35.8	40.7	A
24	**16r**	- (i)	30.7	42.5	A
25	**16s**	62.5	35.1	45.8	A
26	**16t**	41.2	33.2	32.8	A

(i) Not determined because the ester hydrolyzed under the alkaline chromatographic conditions. [a]%T, Percentage of transference: lactose derivatives were incubated with TcTS (300 ng) in 20 mM Tris–HCl, pH 7.6 buffer, 30 mM NaCl, containing 1 mM 3'-sialyllactose as a sialic acid donor and the mixture analyzed by HPAEC in the indicated conditions A-C. [b]%I, Percentage of inhibition: each compound (1 mM) was incubated with TcTS in 20 mM Tris–HCl, 30 mM NaCl, pH 7 buffer (20 µL), containing 1 mM 3'-SL as donor and 1 mM *N*-acetyllactosamine for 15 min at room temperature. % Transfer and inhibition were calculated by integration of the chromatographic peaks. HPAEC conditions A-C are detailed in Experimental.

3. Conclusions

A library of 20 benzyl lactosides substituted in C-6 of the glucose residue with a series of 1,2,3-triazole derivatives containing mostly aromatic substituents was synthesized and each component was analyzed as substrate

of TcTs and inhibitor of the sialylation of *N*-acetyllactosamine. A nine step synthesis starting from readily available benzyl β-lactoside (**8**) was carried out allowing the obtaining of highly pure lactose derivatives with yields ranging from 50 to 55 %. Additionally, the synthetic routes explored for the synthesis of **6** provided some interesting derivatives (compounds **17**-**21**) that were also included in this study.

Regarding the chromatographic conditions used for the analysis of the enzymatic determinations, the PA-100 column generally used for sialylated species was not appropriate for this set of compounds. As the hydrophobicity of the substituents increased, the retention times for the sialylated derivatives became too high to be analyzed. The Carbopac PA-10 column, with smaller particle size, proved to be more effective. Even so, four different chromatographic conditions had to be used in order to allow the resolution of the different sialylated species, required for the inhibition determinations.

In general, the 6-triazoyl lactose derivatives showed better inhibitory properties than the previously described 6-substituted β-galactosides, although a different inhibitory assay was employed in this case (based on MuNANA hydrolysis) [36]. The inhibition properties of compounds **16a**-**16t** were in the same range than the *S*- and *N*-lactosides previously studied by us, having a triazoyl ring in the anomeric substituent [44] These results provided additional information on the selectivity of the enzyme and the structural features that modulate the effective transfer of sialic acid residues. , the development of active small structures is the first necessary approach for the development of more complex conjugates with greater bioavailability or activity

These results open the possibility for the incorporation of a variety of other groups (carbohydrate or even peptide residues) through the position 6- of the glucose residue, towards the development of new acceptors of sialic acid. Moreover, by combination with the CuAAC click reaction, even multivalent species can be obtained, an approach that has proved to be effective in this respect [45].

4. **Experimental***General Information*

Reagents were purchased from commercial suppliers and were used without further purification. Solvents were treated according to standard procedures [54]. TLC was performed on Silica Gel 60 F254 plates (Merck) and visualization was accomplished using UV light or by charring at ~150°C with 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230-400 mesh). Optical rotations were measured at 25 °C in a 1 dm cell with a Perkin-Elmer 343 polarimeter. Microwave irradiation was carried out in a CEM Discover MW instrument with a System Internal IR probe type at 70 ºC (power max 300 W). High resolution mass spectra (HRMS) were obtained by Electrospray Ionization (ESI) and Q-TOF detection with a BRUKER microTOF-Q II ESI-Qq-TOF spectrometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 25 °C at 500 and 126 MHz, respectively, using a Bruker Avance II 500 spectrometer. For ^{1}H, ^{13}C nuclear magnetic resonance (NMR) spectra, chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CDCl_3$: ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.2 ppm, DMSO-d_6:

^{1}H: δ = 3.33 ppm, ^{13}C: δ = 39.5 ppm , D_2O: ^{1}H: δ = 4.79 ppm). Assignments of ^{1}H and ^{13}C were assisted by 2D ^{1}H–COSY and 2D ^{1}H–^{13}C experiments. For the sialylation experiments a recombinant TcTS expressed in *Escherichia coli* was kindly provided by the group of O. Campetella from Universidad Nacional General San Martin (Buenos Aires, Argentina).

Analysis by HPAEC was performed using a Dionex ICS 5000 HPLC system equipped with a pulse amperometric detector. A CarboPac PA-10 ion exchange analytical column (4 × 250 mm) equipped with a guard column PA-10 (4 × 50 mm) was used eluted with one of the following solvent systems. *Condition A*: 100 mM NaOH 20 mM AcONa for 10 minutes followed by a gradient system from 100 mM NaOH-20 mM AcONa to 100 mM NaOH-500 mM AcONa in 50 min at a flow rate of 0.9 ml/min at 25 ºC. *Condition B*: 50 mM NaOH 20 mM AcONa for 10 minutes followed by a gradient system from 50 mM NaOH-20 mM AcONa to 50 mM NaOH-500 mM AcONa in 50 min at a flow rate of 0.9 ml/min at 25 ºC. *Condition C*: gradient system from 150 mM NaOH to 150 mM NaOH-200 mM AcONa in 60 min at a flow rate of 0.9 ml/min at 25 ºC.

4.2. General procedure for the click reaction

Sugar derivative **6**, containing an azide group at C-6 (0.21 mmol) was dissolved in dioxane (2.6 mL) in a microwave flask equipped with a stirring bar. After dissolution, one of the acetylene derivatives **b**-**t** (0.23 mmol, Figure S1) was added. Then, sodium ascorbate (0.23 mmol), $CuSO_4$ (0.11 mmol) and water (0.48 mL) were added and the tube was sealed. The mixture was stirred at 85 °C (5 W) for 1 h in the microwave, unless stated otherwise. The reaction

was followed by TLC and after completion, the solvent was evaporated and the residue chromatographed in a silica gel column (toluene/ethyl acetate as mobile phase). Yields ranged between 85 and 96% (For a complete characterization of products **15b**-**15r** see Supporting information).

4.3. Click reaction with propiolic acid

The azido lactose derivative **6** (0.17 g, 0.24 mmol) was dissolved in anhydrous DMF (3 mL) in a microwave flask equipped with a stirring bar. After dissolution, the propiolic acid (**a**, 0.024 mL, 0.4 mmol) was added together with sodium ascorbate (0.1 mmol), CuI (0.03 g, 0.16 mmol) and DBU (0.02 mL, 0.13 mmol) and the tube was sealed. The mixture was stirred at 120 °C (7 W) for 1 h. Then, another addition of propiolic acid, sodium ascorbate, CuI and DBU in the same amounts as before was made and the reaction stirred at the microwave for another 2 h. After TLC showed no further advance in the reaction, the mixture was purified as described above affording 0.09 g of the monosubstituted triazole derivative **15a** (51 % yield, Supporting information).

4.4. General procedure for deacetylation

Deacetylation was performed with sodium methoxide, unless otherwise indicated. The acetylated compound was dissolved in CH_3OH (10 mL) and 0.1 M $NaOCH_3$-CH_3OH (1.5 mL) and stirred for 3 h. The mixture was desalted with an Amberlite IR-120 (H^+) column and concentrated.

*4.5. 1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazole (**16a**).*

From 71 mg of **15a**, 45 mg of **16a** were obtained (97 % yield); $[\alpha]_D^{25}$ -17.4 (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 7.85, 7.76 (2H, 2s, Trz-*H*), 7.31 (3H, m, aromatics), 7.14 (2H, m, aromatics), 4.93 (1H, m, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.5, H-6a), 4.59 (1H, dd, $J_{5,6b}$ 8.6 Hz, H-6b), 4.59, 4.48 (2H, 2d, *J* 11.7 Hz, CH_2Ph), 4.50 (1H, d, $J_{1',2'}$ 7.5 Hz, H-1'), 4.29 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 3.86 (1H, m, $J_{3',4'}$ 3.4 Hz, H-4'), 3.82 (1H, ddd, $J_{4,5}$ = $J_{5,6b}$ 9.0 Hz, H-5), 3.75-3.63 (3H, m, H-5', H-6'), 3.62 (1H, dd, $J_{2',3'}$ 9.6, H-2'), 3.57–3.48 (3H, m, H-3, H-4, H-3'), 33.26 (1H, dd, $J_{2,3}$ 8.6 Hz, H-2). ^{13}C NMR (D_2O, 126 MHz) δ 133.9, 126.8 (Trz *C*-H x 2), 136.1, 128.7 x 2, 128.7 x 2, 128.5 (aromatics), 103.0 (C-1'), 100.4 (C-1), 80.1 (C-4), 75.5 (C-5'), 74.2 (C-3), 72.8 (C-3'), 72.7 (C-2), 72.5 (C-5), 71.4 (C-2'), 70.9 (Ph*C*H_2), 68.5 (C-4'), 61.0 (C-6'), 50.4 (C-6). HRMS (ESI) m/z calcd. for $C_{21}H_{30}N_3O_{10}$ $[M + H]^+$: 484.1926; found: 484.1920.

4.6. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-benzene (**16b**).*

Starting from 98 mg of **15b**, 61 mg of **16b** were obtained (91 % yield); $[\alpha]_D^{25}$ -12.9 (*c* 1, DMSO); mp 239-240 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.56 (1H, s, Trz-*H*), 7.84 (2H, m, aromatics), 7.45 (2H, m, aromatics), 7.34 (1H, m, aromatic), 7.25 (3H, m, aromatics), 7.15 (2H, m, aromatics), 5.07 (1H, m, $J_{5,6a}$ 2.6 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.56 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.56, 4.39 (2H, 2d, *J* 12.3 Hz, CH_2Ph), 4.40 (1H, d, $J_{1',2'}$ 7.3 Hz, H-1'), 4.25 (1H, d, $J_{1,2}$

7.9 Hz, H-1), 3.77 (1H, ddd, $J_{4,5}$ = $J_{5,6b}$ 9.0 Hz, H-5), 3.66 (1H, m, $J_{3',4'}$ 3.7 Hz, H-4'), 3.59-3.47 (3H, m, H-5', H-6'), 3.45 (1H, dd, $J_{2',3'}$ 9.6, H-2'), 3.42–3.39 (2H, m, H-3, H-4), 3.37 (1H, dd, H-3'), 3.13 (1H, dd, $J_{2,3}$ 8.9 Hz, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 146.6 (Trz C-*C*-R), 137.8, 131.2, 129.4 x 2, 128.6 x 2, 128.3 x 2, 128.2 x 2, 128.0, 122.9 (aromatics), 125.5 (Trz C-*C*-H), 104.0 (C-1'), 101.6 (C-1), 81.7 (C-4), 76.1 (C-5'), 75.0 (C-3), 73.5 (C-3'), 73.3 (C-2), 72.8 (C-5), 70.7 (C-2'), 69.9 (Ph*C*H_2), 68.5 (C-4'), 60.9 (C-6'), 51.0 (C-6); HRMS (ESI) m/z calcd. for $C_{27}H_{34}N_3O_{10}$ $[M + H]^+$: 560.2239; found: 560.2244.

4.7. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol (**15c**).*

Starting from 100 mg of **15c**, 64 mg of **16c** were obtained (96 % yield); $[\alpha]_D^{25}$ -13.7 (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 7.83 (1H, s, Trz-*H*), 7.29 (3H, m, aromatics), 7.13 (2H, dd, J 4.8, 1.9 Hz, aromatics), 4.88 (1H, m, H-6a), 4.64 (2H, m, Trz-CH_2), 4.58, 4.48 (2H, 2d, *J* 12.0 Hz, CH_2Ph), 4.57 (1H, m, H-6b), 4.49 (1H, d, $J_{1',2'}$ 8.1 Hz, H-1'), 4.28 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 3.85 (1H, m, H-4'), 3.80 (1H, m, H-5), 3.68 (3H, m, H-5', H-6'), 3.61 (1H, dd, $J_{2',3'}$ 6.5 Hz, $J_{3',4'}$ 2.5 Hz, H-3'), 3.50 (2H, m, H-3, H-2'), 3.47 (1H, m, H-4), 3.25 (1H, dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2); ^{13}C NMR (D_2O, 126 MHz) δ 146.7 (Trz C-*C*-R), 136.1, 128.6 x 4, 128.5 (aromatics), 125.2 (Trz C-*C*-H), 103.0 (C-1'), 100.4 (C-1), 80.1 (C-4), 75.5 (C-5'), 74.1 (C-3), 72.7 (C-5), 72.6 (C-2), 72.5 (C-3'), 71.3 (Ph*C*H_2), 70.9 (C-2'), 68.5 (C-4'), 61.0 (C-6'), 54.5 (Trz-*C*H_2), 50.6 (C-6); HRMS (ESI) m/z calcd. for $C_{22}H_{32}N_3O_{11}$ $[M + H]^+$: 514.2031; found: 514.2029; calcd. for $C_{22}H_{31}N_3NaO_{11}$ $[M + Na]^+$: 536.1851; found: 536.1858.

4.8. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-phenyl ether (**16d**).*

Starting from 100 mg of **15d**, 62 mg of **16d** were obtained (89 % yield); $[\alpha]_D^{25}$ -13.2 (*c* 1, DMSO); mp 208-209 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz) δ 8.16 (1H, s, Trz-*H*), 7.29-7.23 (5H, m, aromatics), 7.20 (2H, m, aromatics), 6.98 (2H, m, aromatics), 6.91 (1H, m, aromatic), 5.13 (2H, m, Trz-C*H*$_2$OH), 5.03 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.53, 4.34 (2H, 2d, *J* 12.3 Hz, C*H*$_2$Ph), 4.51 (1H, dd, $J_{5,6b}$ 8.6 Hz, H-6b), 4.34 (1H, d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.22 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.71 (1H, ddd, $J_{4,5}$ 8.8 Hz, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.2 Hz, H-4'), 3.55-3.48 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.7 Hz, H-2'), 3, 38 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.5 Hz, H-3), 3.35 (1H, dd, H-3'), 3.33 (1H, t, H-4), 3.10 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz) δ 143.0 (Trz C-*C*-R), 158.5, 137.9, 130.0 x 2, 128.6 x 2, 128.2 x 2, 128.0, 121.3, 115.1 x 2 (aromatics), 126.0 (Trz C-*C*-H), 104.1 (C-1'), 101.7 (C-1), 81.8 (C-4), 76.1 (C-5'), 74.9 (C-3), 73.5 (C-3'), 73.3 (C-2), 72.8 (C-5), 70.7 (C-2'), 70.0 (Ph*C*H$_2$), 68.5 (C-4'), 61.4 (Trz-*C*H$_2$OH), 60.8 (C-6'), 50.8 (C-6); HRMS (ESI) m/z calcd. for $C_{28}H_{36}N_3O_{11}$ $[M + H]^+$: 590.2344; found: 590.2367; calcd. for $C_{28}H_{35}N_3NaO_{11}$ $[M + Na]^+$: 612.2164; found: 612.2194.

4.9. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(o-methylphenyl) ether (**16e**)*

Starting from 100 mg of **15e**, 68 mg of **15e** were obtained (96 % yield); $[\alpha]_D^{25}$ -12.8 (*c* 1, DMSO); mp 109-110 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.11 (1H, s, Trz-*H*), 7.30-7.20 (3H, m, aromatics), 7.15-7.02 (5H, m, aromatics), 6.80 (1H, m, aromatic), 5.12 (2H, m, Trz-CH_2OH), 5.00 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.51 (1H, dd, $J_{5,6b}$ 8.6 Hz, H-6b), 4.46, 4.29 (2H, 2d, *J* 12.0 Hz, CH_2Ph), 4.32 (1H, d, $J_{1',2'}$ 7.6 Hz, H-1'), 4.18 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.70 (1H, td, $J_{4,5}$ 9.2 Hz H-5), 3.63 (1H, d, $J_{3',4'}$ 3.5 Hz, H-4'), 3.55-3.46 (3H, m, H-5', H-6'), 3.39 (1H, dd, $J_{2',3'}$ 9.6 Hz, H-2'), 3.33 (1H, dd, $J_{2,3}$= $J_{3,4}$ 8.5 Hz, H-3), 3.34 (1H, dd, H-3'), 3.31 (1H, dd, H-4), 3.09 (1H, dd, H-2), 1.99 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 143.5 (Trz C-*C*-R), 156.5, 137.7, 131.1, 128.7 x 2, 128.4 x 2, 128.2, 127.5, 126.5, 121.2, 112.3 (aromatics), 126.5 (Trz C-*C*-H), 104.1 (C-1'), 101.6 (C-1), 81.8 (C-4), 76.1 (C-5'), 74.9 (C-3), 73.4 (C-3'), 73.2 (C-2), 72.8 (C-5), 70.8 (C-2'), 70.1 (Ph*C*H_2), 68.6 (C-4'), 61.7 (Trz-*C*H_2OH), 60.9 (C-6'), 50.9 (C-6), 16.4 (*C*H_3); HRMS (ESI) m/z calcd. for $C_{29}H_{38}N_3O_{11}$ $[M + H]^+$: 604.2501; found: 604.2509.

4.10. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(m-methylphenyl) ether (**16f**)*

Starting from 101 mg of **15f**, 67 mg of **16f** were obtained (96 % yield); $[\alpha]_D^{25}$ -13.1 (*c* 1, DMSO); mp 184-185 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.15 (1H, s, Trz-*H*), 7.32-7.24 (3H, m, aromatics), 7.23-7.17 (2H, m, aromatics), 7.21 (1H, m, aromatic), 6.79-6.69 (3H, m, aromatics), 5.10 (2H, m, Trz-CH_2OH), 5.03 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.53, 4.33 (2H, 2d, *J* 11.9 Hz, CH_2Ph), 4.51 (1H, dd, $J_{5,6b}$ 8.9 Hz, H-6b), 4.34 (1H, d, $J_{1',2'}$ 7.6 Hz, H-

1'), 4.22 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.71 (1H, td, $J_{4,5}$ 9.2 Hz H-5), 3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.54-3.46 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.6 Hz, H-2'), 3.38 (1H, dd, $J_{2,3}$= $J_{3,4}$ 8.8 Hz, H-3), 3.34 (1H, dd, H-3'), 3.33 (1H, dd, H-4), 3.10 (1H, dd, H-2), 2.21 (3H, s, C*H*$_3$); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 142.9 (Trz C-*C*-R), 158.2, 139.2, 137.6, 129.4, 128.4 x 2, 128.0 x 2, 127.7, 121.8, 115.5, 111.7 (aromatics), 125.7 (Trz C-*C*-H), 103.8 (C-1'), 101.4 (C-1), 81.5 (C-4), 75.8 (C-5'), 74.6 (C-3), 73.2 (C-3'), 73.0 (C-2), 72.5 (C-5), 70.5 (C-2'), 69.7 (Ph*C*H_2), 68.2 (C-4'), 61.1 (Trz-*C*H_2OH), 60.6 (C-6'), 50.6 (C-6), 21.3 (*C*H_3); HRMS (ESI) m/z calcd. for $C_{29}H_{38}N_3O_{11}$ $[M + H]^+$: 604.2501; found: 604.2494.

4.11. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-methylphenyl) ether (**16g**)*

Starting from 100 mg of **15g**, 62 mg of **16g** were obtained (88 % yield); $[\alpha]_D^{25}$ -8.4 (*c* 1, DMSO); mp 125 °C (dec., ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.16 (1H, s, Trz-*H*), 7.30-7.23 (3H, m, aromatics), 7.20 (2H, m, aromatics), 7.01 (2H, m, aromatics), 6.84 (2H, m, aromatic), 5.08 (2H, m, Trz-C*H*$_2$OH), 5.00 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.49, 4.31 (2H, 2d, *J* 12.3 Hz, C*H*$_2$Ph), 4.51 (1H, dd, $J_{5,6b}$ 8.6 Hz, H-6b), 4.33 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1'), 4.19 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.69 (1H, m, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.55-3.46 (3H, m, H-5', H-6'), 3.40 (1H, dd, $J_{2',3'}$ 9.2 Hz, H-2'), 3, 38-3.33 (2H, m, H-3, 3', 3.31 (1H, dd, $J_{3,4}$ 9.4 Hz, $J_{4,5}$ 8.7 Hz, H-4), 3.09 (1H, dd, $J_{2,3}$ 8.3 Hz, H-2), 2.16 (3H, s, C*H*$_3$); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 143.2 (Trz C-*C*-R), 158.2, 137.7, 130.2 x 2, 130.0, 128.6 x 2, 128.2 x 2, 128.0, 114.9 x 2 (aromatics), 125.9 (Trz C-*C*-H), 104.0 (C-1'), 101.5 (C-1), 81.7 (C-4), 76.0 (C-

5'), 74.8 (C-3), 73.3 (C-3'), 73.2 (C-2), 72.7 (C-5), 70.7 (C-2'), 70.0 (Ph*C*H_2), 68.5 (C-4'), 61.3 (Trz-*C*H_2OH), 60.8 (C-6'), 50.8 (C-6), 20.4 (*C*H_3); HRMS (ESI) m/z calcd. for $C_{29}H_{38}N_3O_{11}$ $[M + H]^+$: 604.2501; found: 604.2493; calcd. for $C_{29}H_{37}N_3NaO_{11}$ $[M + Na]^+$: 626.2320; found: 626.2316.

4.12. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-methoxyphenyl) ether (**16h**)*

Starting from 102 mg of **15h**, 67 mg of **16h** were obtained (93 % yield); $[\alpha]_D^{25}$ -8.0 (*c* 1, DMSO); mp 209-210 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.13 (1H, s, Trz-*H*), 7.31-7.25 (3H, m, aromatics), 7.23-7.16 (2H, m, aromatics), 6.91 (2H, d, *J* 9.1 Hz, aromatics), 6.80 (2H, d, *J* 9.1 Hz, aromatics), 5.07 (2H, m, Trz-C*H*$_2$OH), 5.02 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.53, 4.34 (2H, 2d, *J* 12.1 Hz, C*H*$_2$Ph), 4.51 (1H, dd, $J_{5,6b}$ 8.8 Hz, H-6b), 4.34 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1'), 4.22 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.71 (1H, m, H-5), 3.65 (3H, s, OC*H*$_3$),.3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.55-3.46 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.6 Hz, H-2'), 3.38 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.6 Hz, H-3), 3.34 (1H, dd, H-3'), 3.32 (1H, dd, $J_{4,5}$ 9.0 Hz, H-4), 3.10 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 142.9 (Trz C-*C*-R), 153.6, 152.2, 137.6, 128.3 x 2, 127.9 x 2, 127.7, 115.8 x 2, 114.7 x 2 (aromatics), 125.6 (Trz C-*C*-H), 103.8 (C-1'), 101.4 (C-1), 81.5 (C-4), 75.8 (C-5'), 74.6 (C-3), 73.2 (C-3'), 72.9 (C-2), 72.4 (C-5), 70.4 (C-2'), 69.7 (Ph*C*H_2), 68.2 (C-4'), 61.7 (Trz-*C*H_2OH), 60.5 (C-6'), 55.4 (O*C*H_3), 50.5 (C-6); HRMS (ESI) m/z calcd. for $C_{29}H_{38}N_3O_{12}$ $[M + H]^+$: 620.2450; found: 620.2473; calcd. for $C_{29}H_{37}N_3NaO_{12}$ $[M + Na]^+$: 642.2269; found: 642.2290.

4.13. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-terbutylphenyl) ether (**16i**)*

Starting from 99 mg of **15i**, 61 mg of **16i** were obtained (85 % yield); $[\alpha]_D^{25}$ -13.1 (*c* 1, DMSO); mp 220-221 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.15 (1H, s, Trz-*H*), 7.31-7.24 (3H, m, aromatics), 7.24 (2H, m, aromatics), 7.22-7.16 (2H, m, aromatics), 6.90 (2H, m, aromatic), 5.11 (2H, m, Trz-CH_2OH), 5.03 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.53, 4.33 (2H, 2d, *J* 12.3 Hz, CH_2Ph), 4.51 (1H, dd, $J_{5,6b}$ 8.1 Hz, H-6b), 4.34 (1H, d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.21 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.71 (1H, m, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.54-3.46 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.5 Hz, H-2'), 3.39 (1H, dd, $J_{2,3}$ 7.7 Hz, $J_{3,4}$ 8.8 Hz, H-3), 3.34 (1H, dd, H-3'), 3.33 (1H, dd, $J_{4,5}$ 8.7 Hz, H-4), 3.10 (1H, dd, H-2), 1.20 (9H, s, CH_3 x 3); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 143.2 (Trz C-*C*-R), 156.0, 143.0, 137.5, 128.3 x 2, 128.0 x 2, 127.7, 126.2 x 2, 114.3 x 2 (aromatics), 125.6 (Trz C-*C*-H), 103.8 (C-1'), 101.3 (C-1), 81.5 (C-4), 75.8 (C-5'), 74.6 (C-3), 73.2 (C-3'), 73.0 (C-2), 72.5 (C-5), 70.4 (C-2'), 69.7 (Ph*C*H_2), 68.2 (C-4'), 61.2 (Trz-*C*H_2OH), 60.6 (C-6'), 50.6 (C-6), 33.9 (*C*$(CH_3)_3$), 31.5 (*C*H_3 X 3); HRMS (ESI) m/z calcd. for $C_{32}H_{44}N_3O_{11}$ $[M + H]^+$: 646.2970; found: 646.2969; calcd. for $C_{32}H_{43}N_3NaO_{11}$ $[M + Na]^+$: 668.2790; found: 668.2783.

4.14. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-bromo-phenyl) ether (**16j**)*

Starting from 100 mg of **15j**, 69 mg of **16j** were obtained (95 % yield); $[\alpha]_D^{25}$ -6.4 (*c* 1, DMSO); mp 215-216 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.12 (1H, s, Trz-*H*), 7.36 (2H, d, *J* 9.0 Hz aromatics), 7.29-7.22 (3H, m, aromatics), 7.15 (2H, m, aromatics), 6.93 (2H, d, *J* 9.0 Hz aromatics), 5.12 (2H, m, Trz-CH_2OH), 4.99 (1H, dd, $J_{5,6a}$ 2.6 Hz, $J_{6a,6b}$ 14.4, H-6a), 4.48, 4.29 (2H, 2d, *J* 12.1 Hz, CH_2Ph), 4.50 (1H, dd, $J_{5,6b}$ 8.8 Hz, H-6b), 4.33 (1H, d, $J_{1',2'}$ 7.6 Hz, H-1'), 4.17 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.68 (1H, ddd, $J_{4,5}$ 8.8 Hz, H-5), 3.63 (1H, d, $J_{3',4'}$ 2.7 Hz, H-4'), 3.53-3.47 (3H, m, H-5', H-6'), 3.39 (1H, dd, $J_{2',3'}$ 9.6 Hz, H-2'), 3, 38 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.5 Hz, H-3), 3.36 (1H, dd, H-3'), 3.33 (1H, t, H-4), 3.09 (1H, dd, H-2); (DMSO-d_6/D_2O, 126 MHz): δ 142.8 (Trz C-*C*-R), 157.7, 137.7, 132.6 x 2, 128.7 x 2, 128.3 x 2, 128.1, 117.5 x 2, 112.8 (aromatics), 126.2 (Trz C-*C*-H), 104.0 (C-1'), 101.6 (C-1), 81.7 (C-4), 76.1 (C-5'), 74.9 (C-3), 73.4 (C-3'), 73.2 (C-2), 72.8 (C-5), 70.8 (C-2'), 70.1 (Ph*C*H_2), 68.6 (C-4'), 61.6 (Trz-*C*H_2OH), 60.9 (C-6'), 50.9 (C-6); HRMS (ESI) m/z calcd. for $C_{28}H_{35}BrN_3O_{11}$ $[M + H]^+$: 668.1445; found: 668.1474.

4.15. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(2-nitro-phenyl) ether (**16k**)*

Starting from 100 mg of **15k**, 65 mg of **16k** were obtained (91 % yield); $[\alpha]_D^{25}$ -14.4 (*c* 1, DMSO); mp 117 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.18 (1H, s, Trz-*H*), 7.80 (1H, dd, *J* 8.1, 1.6 Hz, aromatic), 7.63 (1H, ddd, *J* 8.8, 7.2, 1.7 Hz, aromatic), 7.58 (1H, dd, *J* 8.6, 1.2 Hz, aromatic), 7.25 (3H, m, aromatics), 7.19–7.13 (2H, m, aromatics), 7.13–7.06 (1H, m, aromatics), 5.36 (2H, m, Trz-CH_2OH), 5.04 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.4, H-

6a), 4.53 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.51, 4.31 (2H, 2d, *J* 12.2 Hz, C*H*$_2$Ph), 4.34 (1H, d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.21 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.73 (1H, ddd, $J_{4,5}$ 9.2 Hz, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.2 Hz, H-4'), 3.52-3.49 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.6 Hz, H-2'), 3.37 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.9 Hz, H-3), 3.34 (1H, dd, H-3'), 3.32 (1H, t, H-4), 3.09 (1H, dd, H-2); (DMSO-d_6/D_2O, 126 MHz): δ 141.8 (Trz C-*C*-R), 150.7, 139.9, 137.5, 134.5, 128.3 x 2, 127.9 x 2, 127.6, 125.1, 121.1, 115.7 (aromatics), 126.0 (Trz C-*C*-H), 103.8 (C-1'), 101.4 (C-1), 81.5 (C-4), 75.8 (C-5'), 74.6 (C-3), 73.2 (C-3'), 72.9 (C-2), 72.4 (C-5), 70.4 (C-2'), 69.7 (Ph*C*H_2), 68.2 (C-4'), 62.7 (Trz-*C*H_2OH), 60.5 (C-6'), 50.6 (C-6); HRMS (ESI) m/z calcd. for $C_{28}H_{35}N_4O_{13}$ $[M + H]^+$: 635.2195; found: 635.2226.

4.16. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(3-nitro-phenyl) ether (**16l**)*

Starting from 102 mg of **15l**, 64 mg of **16l** were obtained (88 % yield); $[\alpha]_D^{25}$ -4.5 (*c* 1, DMSO); mp 189-190 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.21 (1H, s, Trz-*H*), 7.81–7.73 (2H, m, aromatics), 7.53 (1H, t, *J* 8.2 Hz, aromatic), 7.43 (1H, ddd, *J* 8.3, 2.5, 0.9 Hz, aromatic), 7.30– 7.19 (3H, m, aromatics), 7.17–7.11 (2H, m, aromatics), 5.30 (2H, m, Trz-C*H*$_2$OH), 5.03 (1H, dd, $J_{5,6a}$ 2.6 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.52 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.48, 4.29 (2H, 2d, *J* 12.1 Hz, C*H*$_2$Ph), 4.34 (1H, d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.19 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.71 (1H, ddd, $J_{4,5}$ 9.1 Hz, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.59-3.45 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.7 Hz, H-2'), 3.37 (1H, t, $J_{2,3}$=$J_{3,4}$ 9.0 Hz, H-3), 3.34 (1H, dd, H-3'), 3.33 (1H, t, H-4), 3.09 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 142.1 (Trz C-*C*-R), 158.7, 148.9, 137.5, 130.9,

128.4 x 2, 128.0 x 2, 127.8, 122.2, 116.0, 109.4 (aromatics), 126.2 (Trz C-*C*-H), 103.8 (C-1'), 101.4 (C-1), 81.6 (C-4), 75.9 (C-5'), 74.7 (C-3), 73.2 (C-3'), 73.0 (C-2), 72.5 (C-5), 70.5 (C-2'), 69.7 (Ph*C*H_2), 68.3 (C-4'), 61.9 (Trz-*C*H_2OH), 60.6 (C-6'), 50.7 (C-6); HRMS (ESI) m/z calcd. for $C_{40}H_{47}N_4O_{19}$ $[M + H]^+$: 635.2195; found: 635.2187.

4.17. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-nitro-phenyl) ether (**16m**)*

Starting from 100 mg of **15m**, 65 mg of **16m** were obtained (90 % yield); $[\alpha]_D^{25}$ -17.0 (*c* 1, DMSO); mp 211-212 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.24 (1H, s, Trz-*H*), 8.14 (2H, d, *J* 9.3 Hz aromatics), 7.29-7.23 (3H, m, aromatics), 7.19 (2H, d, *J* 9.3 Hz aromatics), 7.18-7.12 (2H, m, aromatics), 5.33 (2H, m, Trz-C*H*$_2$OH), 5.04 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.52 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.48, 4.29 (2H, 2d, *J* 12.0 Hz, C*H*$_2$Ph), 4.34 (1H, d, $J_{1',2'}$ 7.8 Hz, H-1'), 4.21 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 3.72 (1H, ddd, $J_{4,5}$ 9.1 Hz, H-5), 3.63 (1H, d, $J_{3',4'}$ 2.7 Hz, H-4'), 3.54-3.44 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.6 Hz, H-2'), 3.38 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.7 Hz, H-3), 3.34 (1H, dd, H-3'), 3.34 (1H, t, H-4), 3.09 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 141.7 (Trz C-*C*-R), 163.4, 141.1, 137.4, 128.3 x 2, 127.9 x 2, 127.7, 126 x 2, 115.4 x 2 (aromatics), 126.2 (Trz C-*C*-H), 103.8 (C-1'), 101.3 (C-1), 81.5 (C-4), 75.8 (C-5'), 74.6 (C-3), 73.2 (C-3'), 72.9 (C-2), 72.4 (C-5), 70.4 (C-2'), 69.6 (Ph*C*H_2), 68.2 (C-4'), 62.0 (Trz-*C*H_2OH), 60.5 (C-6'), 50.6 (C-6); HRMS (ESI) m/z calcd. for $C_{28}H_{35}N_4O_{13}$ $[M + H]^+$: 635.2195; found: 635.2169.

4.18. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(p-acetamido-phenyl) ether (**16n**)*

Starting from 100 mg of **15n**, 65 mg of **16n** were obtained (91 % yield); $[\alpha]_D^{25}$ -8.4 (*c* 1, DMSO); mp 197-198 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.13 (1H, s, Trz-*H*), 7.43 (2H, d, *J* 9.0 Hz, aromatics), 7.30-7.23 (3H, m, aromatics), 7.18 (2H, m, aromatics), 6.90 (2H, d, *J* 9.0 Hz, aromatics), 5.08 (2H, m, Trz-CH_2OH), 5.01 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.3, H-6a), 4.52, 4.34 (2H, 2d, *J* 11.8 Hz, CH_2Ph), 4.51 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.34 (1H, d, $J_{1',2'}$ 7.6 Hz, H-1'), 4.21 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.70 (1H, ddd, $J_{4,5}$ 9.2 Hz, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.55-3.46 (3H, m, H-5', H-6'), 3.40 (1H, dd, $J_{2',3'}$ 9.4 Hz, H-2'), 3.37 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.9 Hz, H-3), 3.34 (1H, dd, H-3'), 3.32 (1H, t, H-4), 3.10 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 168.2 (NH*C*OCH_3), 154.1, 137.6, 132.8, 128.4 x 2, 128.0 x 2, 127.8, 120.8 x 2, 114.9 x 2 (aromatics), 142.9 (Trz C-*C*-R), 125.8 (Trz C-*C*-H), 103.9 (C-1'), 101.4 (C-1), 81.6 (C-4), 75.9 (C-5'), 74.7 (C-3), 73.2 (C-3'), 73.1 (C-2), 72.6 (C-5), 70.5 (C-2'), 69.8 (Ph*C*H_2), 68.3 (C-4'), 61.4 (Trz-*C*H_2OH), 60.7 (C-6'), 50.6 (C-6), 23.9 (NHCO*C*H_3); HRMS (ESI) m/z calcd. for $C_{30}H_{39}N_4O_{12}$ $[M + H]^+$: 647.2559; found: 647.2543.

4.19. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-α-naphtyl ether (**16o**)*

Starting from 100 mg of **15o**, 64 mg of **16o** were obtained (89 % yield); $[\alpha]_D^{25}$ -10.7 (*c* 1, DMSO); mp 130 °C (dec., ethanol); ^{1}H NMR (DMSO-d_6/D_2O,

500 MHz): δ 8.27 (1H, s, Trz-*H*), 8.02 (1H, d, *J* 8.4 Hz, aromatic), 7.82 (1H, d, *J* 8.2 Hz, aromatic), 7.45 (2H, d, *J* 7.8 Hz, aromatics), 7.39 (1H, t, *J* 7.9 Hz, aromatic), 7.30 (1H, t, *J* 7.7 Hz, aromatic), 7.22–7.14 (4H, m, aromatics), 7.11 (2H, d, *J* 7.5 Hz, aromatic), 5.33 (2H, m, Trz-CH_2OH), 5.05 (1H, dd, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 14.4, H-6a), 4.54 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.50, 4.32 (2H, 2d, *J* 12.0 Hz, CH_2Ph), 4.34 (1H, d, $J_{1',2'}$ 8.2 Hz, H-1'), 4.21 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.75 (1H, m, H-5), 3.65 (1H, d, $J_{3',4'}$ 3.2 Hz, H-4'), 3.55-3.47 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.5 Hz, H-2'), 3.39 (1H, dd, $J_{2,3}$ 7.8 Hz, $J_{3,4}$ 6.8 Hz, H-3), 3.36-3.32 (2H, m, H-3', H-4), 3.10 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 143.0 (Trz C-*C*-R), 153.8, 137.5, 134.3, 128.5 x 2, 128.2 x 2, 127.9, 127.8, 126.8, 126.5, 125.6, 125.1, 121.7, 120.7, 106.0 (aromatics), 125.9 (Trz C-*C*-H), 104.0 (C-1'), 101.4 (C-1), 81.7 (C-4), 76.0 (C-5'), 74.8 (C-3), 73.3 (C-3'), 73.1 (C-2), 72.6 (C-5), 70.6 (C-2'), 69.8 (Ph*C*H_2), 68.4 (C-4'), 61.9 (Trz-*C*H_2OH), 60.7 (C-6'), 50.8 (C-6); HRMS (ESI) m/z calcd. for $C_{32}H_{38}N_3O_{11}$ $[M + H]^+$: 640.2501; found: 640.2473; calcd. for $C_{32}H_{37}N_3NaO_{11}$ $[M + Na]^+$: 662.2320; found: 662.2293.

4.20. *[{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-β-naphtyl ether (**16p**)*

Starting from 105 mg of **15p**, 66 mg of **16p** were obtained (88 % yield); $[\alpha]_D^{25}$ -9.2 (*c* 1, DMSO); mp 234-235 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.21 (1H, s, Trz-*H*), 7.77 (3H, m, aromatics), 7.48 (1H, d, *J* 2.5 Hz, aromatic), 7.44 (1H, ddd, *J* 8.2, 6.9, 1.3 Hz, aromatic), 7.34 (1H, m, aromatic), 7.25 (3H, m, aromatics), 7.16 (2H, d, *J* 1.9 Hz, aromatic), 7.08 (1H, dd, *J* 8.9,

2.6 Hz, aromatic), 5.26 (2H, m, Trz-CH_2OH), 5.04 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.4, H-6a), 4.53 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.49, 4.31 (2H, 2d, J 11.9 Hz, CH_2Ph), 4.34 (1H, d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.20 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.71 (1H, m, H-5), 3.63 (1H, d, $J_{3',4'}$ 3.2 Hz, H-4'), 3.55-3.47 (3H, m, H-5', H-6'), 3.41 (1H, dd, $J_{2',3'}$ 9.7 Hz, H-2'), 3.38 (1H, dd, $J_{2,3}$=$J_{3,4}$ 8.7 Hz, H-3), 3.34 (1H, dd, H-3'), 3.34 (1H, dd, $J_{4,5}$ 8.7 Hz, H-4), 3.10 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 142.7 (Trz C-*C*-R), 156.2, 137.6, 134.4, 129.6, 128.8, 128.4 x 2, 128.0 x 2, 127.8 x 2, 127.0, 126.8, 124.1, 118.8, 107.4 (aromatics), 126.0 (Trz C-*C*-H), 103.9 (C-1'), 101.4 (C-1), 81.6 (C-4), 75.9 (C-5'), 74.7 (C-3), 73.3 (C-3'), 73.1 (C-2), 72.6 (C-5), 70.6 (C-2'), 69.8 (Ph*C*H_2), 68.3 (C-4'), 61.4 (Trz-*C*H_2OH), 60.7 (C-6'), 50.7 (C-6); HRMS (ESI) m/z calcd. for $C_{32}H_{38}N_3O_{11}$ [M + H]$^+$: 640.2501; found: 640.2494; calcd. for $C_{32}H_{37}N_3NaO_{11}$ [M + Na]$^+$: 662.2320; found: 662.2309.

4.21. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-carboxymethyl-phenyl) ether (**16q**)*

Starting from 101 mg of **15q**, 68 mg of **16q** were obtained (93 % yield); $[\alpha]_D^{25}$ -13.7 (*c* 1, DMSO); mp 210-211 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.18 (1H, s, Trz-*H*), 7.85 (2H, d, *J* 8.9 Hz, aromatics), 7.28-7.24 (3H, m, aromatics), 7.16-7.14 (2H, m, aromatics), 7.08 (2H, d, *J* 8.9 Hz, aromatics), 5.23 (2H, m, Trz-CH_2OH), 5.02 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.5, H-6a), 4.50 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.48, 4.28 (2H, 2d, *J* 12.1 Hz, CH_2Ph), 4.33 (1H, d, $J_{1',2'}$ 7.7 Hz, H-1'), 4.19 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.78 (3H, s, OCH_3), 3.70 (1H, ddd, $J_{4,5}$ 9.2 Hz, H-5), 3.54-3.47 (4H, m, H-4', H-5', H-6'), 3.40 (1H,

dd, $J_{2',3'}$ 9.5 Hz, H-2'), 3.34 (1H, t, $J_{3,4}$=$J_{4,5}$ 9.2, H-4), 3.34 (1H, dd, $J_{3',4'}$ 3.4 Hz H-3'), 3.33 (1H, t, $J_{2,3}$ 8.8 Hz, H-3), 3.09 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 166.3 (CO_2CH_3), 162.1, 137.6, 131.5 x 2, 128.5 x 2, 128.1 x 2, 127.9, 122.4, 115.0 x 2 (aromatics), 142.3 (Trz C-*C*-R), 126.1 (Trz C-*C*-H), 103.9 (C-1'), 101.5 (C-1), 81.6 (C-4), 75.9 (C-5'), 74.7 (C-3), 73.3 (C-3'), 73.1 (C-2), 72.6 (C-5), 70.6 (C-2'), 69.8 (PhCH_2), 68.4 (C-4'), 61.5 (Trz-CH_2OH), 60.7 (C-6'), 52.2 ($COCH_3$), 50.7 (C-6); ESI-HRMS: calcd. for $C_{30}H_{37}N_3NaO_{13}$ [M + Na]$^+$: 670.2219; found: 670.2222.

4.22. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(2-carboxymethyl-phenyl) ether (**16r**)*

Starting from 100 mg of **15r**, 66 mg of **16r** were obtained (92 % yield); $[\alpha]_D^{25}$ -12.3 (*c* 1, DMSO); mp 178-179 °C (ethanol); ^{1}H NMR (DMSO-d_6/D_2O, 500 MHz): δ 8.10 (1H, s, Trz-*H*), 7.60 (1H, m, aromatic), 7.49 (1H, m, aromatic), 7.32 (1H, m, aromatic), 7.27-7.21 (3H, m, aromatics), 7.15-7.13 (2H, m, aromatics), 7.00 (1H, m, aromatic), 5.22 (2H, m, Trz-CH_2OH), 5.00 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.5, H-6a), 4.53 (1H, dd, $J_{5,6b}$ 9.0 Hz, H-6b), 4.49, 4.30 (2H, 2d, *J* 12.1 Hz, CH_2Ph), 4.33 (1H, d, $J_{1',2'}$ 7.6 Hz, H-1'), 4.20 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.63 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.54-3.47 (3H, m, H-5', H-6'), 3.40 (1H, dd, $J_{2',3'}$ 9.5 Hz, H-2'), 3.36 (1H, t, $J_{3,4}$=$J_{4,5}$ 8.9, H-4), 3.34 (1H, dd, $J_{3',4'}$ 3.4 Hz H-3'), 3.30 (1H, t, $J_{2,3}$ 8.8 Hz, H-3), 3.08 (1H, dd, H-2); ^{13}C NMR (DMSO-d_6/D_2O, 126 MHz): δ 166.4 (CO_2CH_3), 157.3, 137.7, 133.9, 131.2, 128.5 x 2, 128.1 x 2, 127.9, 121.1, 120.8, 114.7 (aromatics), 142.9 (Trz C-*C*-R), 125.8 (Trz C-*C*-H), 104.0 (C-1'), 101.6 (C-1), 81.6 (C-4), 76.0 (C-5'), 74.8 (C-3), 73.3 (C-3'), 73.1 (C-2),

72.7 (C-5), 70.7 (C-2'), 70.0 (Ph*C*H_2), 68.4 (C-4'), 62.5 (Trz-*C*H_2OH), 60.8 (C-6'), 52.2 (CO*C*H_3), 50.7 (C-6); HRMS (ESI) m/z calcd. for $C_{30}H_{37}N_3NaO_{13}$ [M + Na]$^+$: 670.2219; found: 670.2230.

4.23. *{1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(4-carboxy-phenyl) ether (**16s**)*

Compound **16p** (40 mg) was suspended in 0.5 M NaOH (1 mL) and stirred at 70 °C for 2 h. Desalting was performed by passing through an Amberlite IR-120 (H) resin (35 mg, 90 % yield). $[\alpha]_D^{25}$ -12.7 (*c* 1, H_2O); ^{1}H NMR (D_2O/NaOH, 500 MHz) δ 8.02 (1H, s, Trz-*H*), 7.69-7.63 (2H, m, aromatic), 7.32-7.20 (3H, m, aromatic), 6.97-6.87 (4H, m, aromatic), 5.27 (2H, m, Trz-C*H*$_2$OH), 5.01 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.5, H-6a), 4.53 (1H, dd, $J_{5,6b}$ 9.5 Hz, H-6b), 4.47 (1H, d, $J_{1',2'}$ 6.7 Hz, H-1'), 4.27, 4.17 (2H, 2d, *J* 11.7 Hz, C*H*$_2$Ph), 4.13 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 3.87 (1H, d, $J_{3',4'}$ 2.6 Hz, H-4'), 3.77 (1H, ddd, $J_{4,5}$ 9.2 Hz, H-5), 3.74-3.68 (2H, m, H-6'), 3.65 (1H, m, H-5'), 3.60 (1H, dd, $J_{2',3'}$ 10.0 Hz, H-3'), 3.57 (1H, dd, H-2'), 3.55 (1H, t, $J_{3,4}$ 9.0 Hz, H-4), 3.49 (1H, t, $J_{2,3}$ 8.5 Hz, H-3), 3.24 (1H, dd, H-2); ^{13}C NMR (D_2O/NaOH, 126 MHz) δ 174.2 (*C*O_2H), 159.0, 135.4, 130.5 x 2, 128.4 x 2, 128.1 x 2, 128.0, 113.9 x 2 (aromatics), 142.6 (Trz C-*C*-R), 129.0 (Trz C-*C*-H), 102.4 (C-1'), 100.2 (C-1), 78.9 (C-4), 75.5 (C-5'), 74.4 (C-3), 73.0 (C-3'), 72.8 (C-2), 72.4 (C-5), 70.5 (C-2'), 70.2 (Ph*C*H_2), 68.6 (C-4'), 60.2 (Trz-*C*H_2OH), 60.8 (C-6'), 50.6 (C-6); HRMS (ESI) m/z calcd. for $C_{29}H_{35}N_3NaO_{13}$ [M + Na]$^+$: 656.2062; found: 656.2045.

*4.24. {1-[Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranosid-6-yl]-1H-1,2,3-triazol-4-yl}-methanol O-(2-carboxy-phenyl) ether (**16t**)*

Compound **16q** (40 mg) was suspended in 0.5 M NaOH (1 mL) and stirred at 70 °C for 2 h. Desalting was performed by passing through an Amberlite IR-120 (H) resin (34 mg, 88% yield). $[\alpha]_D^{25}$ -15.4 (*c* 1, H_2O); ^{1}H NMR (D_2O/NaOH, 500 MHz) δ 7.98 (1H, s, Trz-*H*), 7.37-7.29 (4H, m, aromatic), 7.20 (1H, m, aromatic), 7.11-7.06 (2H, m, aromatic), 7.03 (1H, m, aromatics), 6.91 (1H, m, aromatics), 5.28 (2H, m, Trz-C*H*$_2$OH), 4.97 (1H, dd, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 14.5, H-6a), 4.54 (1H, dd, $J_{5,6b}$ 9.2 Hz, H-6b), 4.49 (1H, d, $J_{1',2'}$ 7.5 Hz, H-1'), 4.43, 4.35 (2H, 2d, *J* 11.8 Hz, C*H*$_2$Ph), 4.24 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 3.89 (1H, d, $J_{3',4'}$ 3.3 Hz, H-4'), 3.77-3.69 (2H, m, H-6'), 3.69-3.65 (1H, m, H-5'), 3.63-3.51 (4H, m, H-2', H-3, H-3', H-4), 3.27 (1H, dd, $J_{2,3}$ 9.0 Hz, H-2); ^{13}C NMR (D_2O/NaOH, 126 MHz) δ 175.9 (*C*O_2H), 168.4, 153.7, 136.1, 130.0, 128.8 x 2, 128.6 x 2, 128.4, 128.3, 121.5, 114.2 (aromatics), 143.7 (Trz C-*C*-R), 125.9 (Trz C-*C*-H), 102.8 (C-1'), 100.8 (C-1), 79.4 (C-4), 75.8 (C-5'), 74.7 (C-3), 73.3 (C-3'), 73.0 (C-2), 72.8 (C-5), 71.2 (C-2'), 70.6 (Ph*C*H$_2$), 68.9 (C-4'), 61.8 (Trz-*C*H$_2$OH), 61.1 (C-6'), 50.9 (C-6); HRMS (ESI) m/z calcd. for $C_{29}H_{36}N_3O_{13}$ $[M + H]^+$: 634.2243; found: 634.2242.

*4.25. β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (lactosan, **18**).*

Starting from 50.0 mg of **3**, 27.6 mg of **18** were obtained (98 % yield). ^{1}H and ^{13}C-NMR spectra were in accordance with reported in literature [55].

*4.26. Benzyl β-D-galactopyranosyl-(1→4)-6-O-methanesulfonyl-β-D-glucopyranoside (**19**).*

Compound 5 (100 mg) was dissolved in CH_3OH (4 mL), H_2O (5 mL) and TEA (1 mL) and stirred overnight. Solvent was evaporated and the product dissolved in H_2O (2 mL) and desalted with an Amberlite MB-3A resin (2.9 mL wet) followed by a C-18 column. (53 mg, 80 % yield. $[\alpha]_D^{25}$ -12.0 (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 7.50-7.38 (5H, m, aromatics), 4.90, 4.81 (2H, 2d, *J* 11.7 Hz, C*H*$_2$Ph), 4.66 (1H, m, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 11.4 Hz, H-6a), 4.59 (1H, dd, $J_{5,6b}$ 4.2 Hz, H-6b), 4.61 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.44 (1H, d, $J_{1',2'}$ 7.8 Hz, H-1'), 3.93 (1H, m, $J_{3',4'}$ 3.4 Hz, H-4'), 3.84 (1H, m, $J_{4,5}$ 10.3 Hz, H-5), 3.81-3.75 (2H, m, H-6'), 3.75-3.69 (2H, m, H-4, H-5'), 3.67 (1H, dd, $J_{2',3'}$ 10.0 Hz, H-3'), 3.65 (1H, dd, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 8.6, H-3), 3.56 (1H, dd, H-2'), 3.38 (1H, dd, H-2), 3.22 (3H, d, C*H*$_3$); ^{13}C NMR (D_2O, 126 MHz) δ 136.6, 128.9 x 2, 128.7 x 2, 128.5 (aromatics), 103.0 (C-1'), 101.4 (C-1), 77.9 (C-4), 75.4 (C-5'), 74.2 (C-3), 72.7 (C-2), 72.5 (C-3'), 72.0 (Ph*C*H$_2$), 71.9 (C-5), 70.8 (C-2'), 68.5 x 2 (C-4', C-6'), 61.0 (C-6), 36.7 (C*H*$_3$); HRMS (ESI) m/z calcd. for $C_{20}H_{30}NaO_{13}S$ $[M + Na]^+$: 533.1299; found: 533.1313.

*4.27. Benzyl β-D-galactopyranosyl-(1→4)-6-azido-6-deoxy-β-D-glucopyranoside (**20**).*

Compound **6** (50 mg) was deacetylated as described for compound **5** (29.7 mg, 92%yield). $[\alpha]_D^{25}$ -16.0 (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 7.50-7.40

(5H, m, aromatics), 4.93, 4.77 (2H, 2d, *J* 11.6 Hz, C*H*$_2$Ph), 4.59 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 4.42 (1H, d, $J_{1',2'}$ 7.8 Hz, H-1'), 3.93 (1H, m, $J_{3',4'}$ 3.5 Hz, H-4'), 3.81-3.71 (5H, m, H-5', H-6, H-6'), 3.69-3.61 (2H, m, H-4, H-5), 3.93 (1H, dd, $J_{2',3'}$ 10.1 Hz, H-3'), 3.66 (1H, dd, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 8.9, H-3), 3.53 (1H, dd, H-2'), 3.38 (1H, dd, H-2); ^{13}C NMR (D_2O, 126 MHz) δ 136.4 128.8 x 2, 128.7 x 2, 128.5 (aromatics), 103.1 (C-1'), 101.0 (C-1), 79.3 (C-4), 75.4 (C-5'), 74.2 (C-3), 73.7 (C-5), 72.8 (C-2), 72.5 (C-3'), 71.6 (Ph*C*H$_2$), 70.9 (C-2'), 68.5 (C-4'), 61.0 (C-6'), 50.4 (C-6); HRMS (ESI) m/z calcd. for $C_{19}H_{27}N_3NaO_{10}$ $[M + Na]^+$: 480.1589; found: 480.1573.

4.28. *Benzyl β-D-galactopyranosyl-(1→4)-6-deoxy-β-D-glucopyranoside (**21**).*

Compound **14** (74 mg) was suspended in 5 mL of AcOH and warmed at 95 ºC until complete dissolution of the starting material. Then, 1.25 mL of water were added and the solution was kept at 95-100 ºC for an hour. The solvent was removed at reduced pressure and the remainder residue was dried in vacuum for several hours. Deprotection was performed with NaOMe as described above (43 mg, 95 % yield). $[\alpha]_D^{25}$ -22.0 (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 7.46, 7.40 (5H, m, aromatics), 4.91, 4.74 (2H, 2d, *J* 11.6 Hz, C*H*$_2$Ph), 4.54 (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 4.50 (1H, d, $J_{1',2'}$ 7.8 Hz, H-1'), 3.93 (1H, m, $J_{3',4'}$ 3.4 Hz, H-4'), 3.83-3.74 (2H, m, H-6'), 3.74-3.70 (1H, m, H-5'), 3.66 (1H, dd, $J_{2',3'}$ 9.9 Hz, H-3').3.62 (1H, m, $J_{4,5}$ 9.1 Hz $J_{5,6}$ 6.2 Hz, H-5), 3.55 (1H, dd, $J_{2',3'}$ 9.9, H-2'), 3.55 (1H, dd, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 9.1, H-3), 3.41 (1H, t, $J_{4,5}$ 9.1 Hz, H-4), 3.36 (1H, dd, H-2), 1.38 (3H, d, H-6); ^{13}C NMR (D_2O, 126 MHz) δ 136.5, 128.8 x 2, 128.7 x 2, 128.5 (aromatics), 103.1 (C-1'), 101.0 (C-1), 83.7 (C-4), 75.3 (C-

5'), 74.3 (C-3), 73.0 (C-2), 72.5 (C-3'), 71.6 (Ph*C*H_2), 71.0 (C-2'), 70.9 (C-5), 68.6 (C-4'), 61.0 (C-6'), 16.6 (C-6); HRMS (ESI) m/z calcd. for $C_{19}H_{29}O_{10}$ $[M + H]^+$: 417.1755; found: 417.1757

4.29. Enzymatic experiments

Lactose derivatives **16a**-**16t** and **17**-**21** and were incubated with recombinant TcTS (300 ng) in 20 mM Tris–HCl, pH 7.6 buffer, 30 mM NaCl, containing 1 mM 3'-sialyllactose (3'-SL, purchased from Elicityl, France) as a sialic acid donor, as described before [51]. The reaction mixtures were diluted with deionized water and analyzed by HPAEC. The percentage of sialylation was calculated by integration of all the sialylated species present. Control experiments without the addition of trans-sialidase were performed in parallel for each sample and chromatographed in the same conditions.

For the inhibition experiments compounds **16a**-**16t** and **17**-**21** (1 mM) were incubated in 20 mM Tris–HCl, 30 mM NaCl, pH 7 buffer (20 µl), containing 1 mM 3'-SL as donor, 1 mM LN (purchased from Elicityl, France), and recombinant TcTS (300 ng) for 15 min at room temperature. After dilution with deionized water, analysis by HPAEC-PAD was performed. Inhibition was calculated considering the decrease in the percentage of 3'-sialyl-*N*-acetyllactosamine (calculated on the total amount of sialylated compounds) in the presence of the inhibitor.

4.30. Matrix-assisted laser desortion/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

MS spectra were acquired in an Ultraflex II (Bruker Daltonics) MALDI-TOF-TOF, using dihydroxybenzoic acid (DHB) as matrix. The ions were detected in positive linear mode. MS^2 spectra for selected peaks were also performed.

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- Benzyl 6-azidolactoside was synthesized through the 4',6'-benzylidene derivative.
- A library of 6-triazoyl benzyl β-lactosides was synthesized by click chemistry.
- C-6 substituted benzyl β-lactosides were good alternative substrates for TcTS.
- Up to 70% of inhibition of sialylation of *N*-acetyllactosamine was obtained.
- Aromatic residues greatly increased the retention times of carbohydrates in HPAEC.