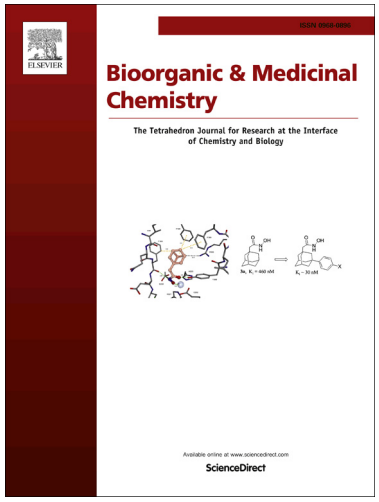
Accepted Manuscript



Synthesis of the *O*-linked hexasaccharide containing β-D-Gal*p*-(1→2)-D-Gal*f* in *Trypanosoma cruzi* mucins. Differences on sialylation by *trans*-sialidase of the two constituent hexasaccharides

Rosalía Agustí, M. Eugenia Giorgi, Verónica M. Mendoza, Gustavo A. Kashiwagi, Rosa M. de Lederkremer, Carola Gallo-Rodriguez

PII: S0968-0896(15)00080-2
DOI: http://dx.doi.org/10.1016/j.bmc.2015.01.056
Reference: BMC 12059

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 5 December 2014
Revised Date: 23 January 2015
Accepted Date: 30 January 2015

Please cite this article as: Agustí, R., Eugenia Giorgi, M., Mendoza, V.M., Kashiwagi, G.A., de Lederkremer, R.M., Gallo-Rodriguez, C., Synthesis of the *O*-linked hexasaccharide containing β-D-Gal*p*-(1→2)-D-Gal*f* in *Trypanosoma cruzi* mucins. Differences on sialylation by *trans*-sialidase of the two constituent hexasaccharides, *Bioorganic & Medicinal Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bmc.2015.01.056

Synthesis of the *O*-linked hexasaccharide containing β-D-Gal*p*-(1→2)-D-Gal*f* in *Trypanosoma cruzi* mucins. Differences on sialylation by *trans*-sialidase of the two constituent hexasaccharides

Rosalía Agustí,[+] *M. Eugenia Giorgi,*[+] *Verónica M. Mendoza, Gustavo A. Kashiwagi, Rosa M. de Lederkremer* and Carola Gallo-Rodriguez**

CIHIDECAR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina

[+]Both authors contributed equally to this work.

* Corresponding authors. Tel.: 54-11-4576-3346; fax: 54-11-4576-3352; e-mail: lederk@qo.fcen.uba.ar; cgallo@qo.fcen.uba.ar

Abstract

The hexasaccharide β-D-Gal*p*-(1→2)-[β-D-Gal*p*-(1→3)]-β-D-Gal*p*-(1→6)-[β-D-Gal*p*(1→2)-β-D-Gal*f*(1→4)]-D-GlcNAc (**10**) and its β-D-Gal*f*-(1→2)-β-D-Gal*f* containing isomer (**7**) are the largest carbohydrates in mucins of some strains of *T. cruzi*. The terminal β-D-Gal*p* units are sites of sialylation by the parasite *trans*-sialidase. Hexasaccharide **10** was chemically synthesized for the first time by a [3+3] nitrilium based convergent approach, using the trichloroacetimidate method of glycosylation. The ^{1}H NMR spectrum of its alditol was identical to the spectrum of the product released by β-elimination from the parasite mucin. The *trans*-sialylation reaction studied on the benzyl glycoside of **10** showed two monosialylated products whose relative abundance changed with time. On the other hand, only one product was produced by sialylation of the benzyl glycoside of **7**. A preparative synthesis of the latter and spectroscopic analysis of the product unequivocally established the sialylation site at the less hindered (1→3)-linked galactopyranose.

Keywords: *Trypanosoma cruzi*; mucins; trans-sialidase; glycosylation; galactofuranose; nitrilium; oligosaccharides.

1. Introduction

*Trypanosoma cruz*i, the etiologic agent of Chagas disease, differentiates during its life cycle as it travels from the insect vector to humans, undergoing biochemical and morphological changes.[1,2] Epimastigotes replicate in the midgut of a triatomine insect and transform in the intestine into non-proliferative metacyclic trypomastigotes that pass from the hindgut to the feces. The infective trypomastigotes enter the vertebrate host through a skin wound or mucosal membranes, invade cells and differentiate into amastigotes which, after several cycles of binary division, differentiate back into trypomastigotes. These non-replicative forms are released into circulation upon host-cell rupture and may then infect other cells or be taken by the insect vector on blood sucking. The ingested trypomastigotes transform into epimastigotes, closing the cycle. A dense glycocalix covers the surface of the parasite and its composition is characteristic of each differentiation stage.[3,4] Mucins are the most abundant of the surface glycoproteins and both, epimastigote and infective trypomastigote forms, have the same number of mucin molecules per surface area.[5] Their *O*-linked oligosaccharides, which account for up to 60% of the molecular mass, play the crucial role of being acceptors of the sialic acid that the parasite needs to build a negatively charged coat but is unable to biosynthesize. For the acquisition of sialic acid, *T. cruzi* expresses at its surface a trans-sialidase (TcTS), capable of transferring sialic acid residues from host glycoconjugates to the parasite mucins.[6-8] The transfer is specific and involves cleavage of an α(2→3)-linked sialic acid in the donor and formation of the same linkage with a terminal β-galactopyranosyl group in the acceptor substrate. The acceptor binding-site was studied with modified substrates and glycoside libraries showing the importance of substitution at the different galactose positions.[9] Galactose is a constituent of all mucin oligosaccharides, however, in epimastigotes, the monosaccharide may be present in the furanose and pyranose configurations.

By comparing structural data of mucins from different strains it follows that the *O*-linked oligosaccharides may be derived from two cores, β-D-Gal*p*(1→4)-GlcNAc or β-D-Gal*f*(1→4)-GlcNAc. These cores are further branched with various units of Gal*f* and/or Gal*p*. Galactofuranose was first reported in mucins of a sylvatic origin like the G strain, classified as *T. cruzi* I,[10,11] and found later in other strains belonging to the same group.[12] More recently, it was reported in the drug-resistant Colombiana strain, isolated from a chronic human case in Colombia.[13] The Tulahuen strain, now included in the new hybrid type VI[14] expresses mucins with substitution of GlcNAc by either Gal*p* or Gal*f*.[15] By a comparative kinetic study it was shown that the presence of Gal*f* does not interfere with the acceptance of sialic acid by the Gal*p* in the oligosaccharides.[16] The diminished virulence of the strains belonging to group I could be caused by the antigenicity of Gal*f*.[17] On the other hand, galactose only in the pyranose form was found in mucin oligosaccharides belonging to other strains[18-21] and some of these structures have been synthesized.[22]

In our laboratory we have undertaken the chemical synthesis of the mucin oligosaccharides, in particular of those containing Gal*f* (Figure 1).[23-27] Reduction with $NaBH_4$ gave the corresponding alditols that allowed the comparison with the oligosaccharide alditols obtained by reductive β-elimination from the natural mucins, confirming the proposed structures.

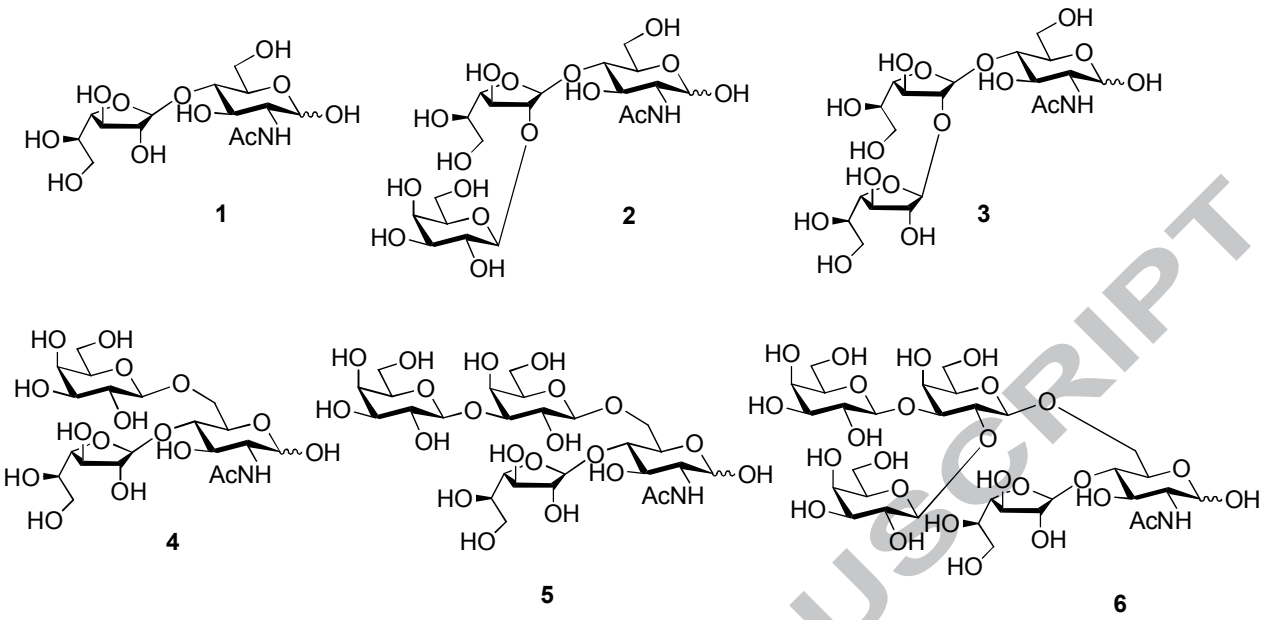
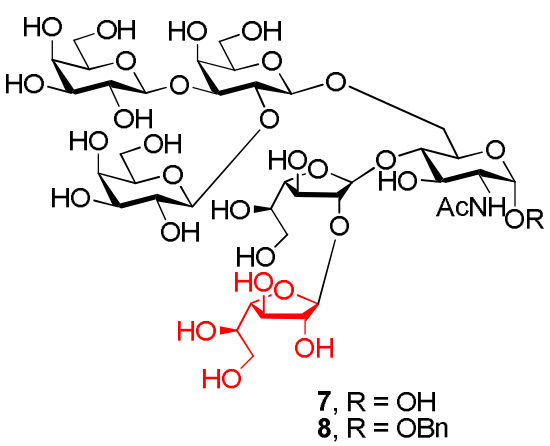
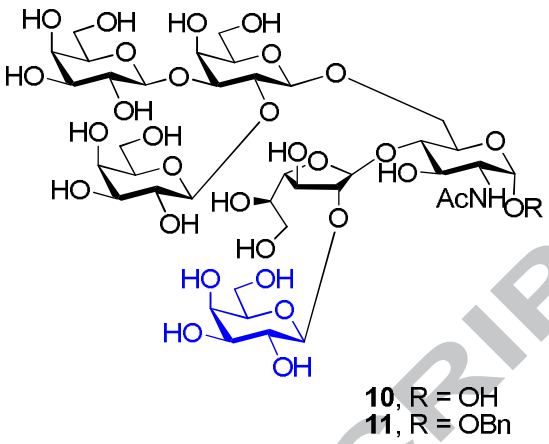


Figure 1. Structures of Gal*f*-containing mucin oligosaccharides from *T. cruzi* epimastigotes.

Hexasaccharides **7** and **10** (Figure 2) are the largest oligosaccharides obtained as alditols in *T. cruzi* epimastigote mucins by reductive β-elimination. [10-13,15] These isomeric structures differ in the galactose unit linked to the O-2 of the internal galactofuranose in the conserved β-D-Gal*f*(1→4)GlcNAc core. The external Gal*f* of the β-D-Gal*f*(1→2)-β-D-Gal*f*(1→4) unit present in **7** is replaced by a Gal*p* in **10**. We have recently reported the synthesis of the hexasaccharide **7**.[28] We describe now the synthesis of β-D-Gal*p*(1→2)-[β-D-Gal*p*(1→3)]-β-D-Gal*p*(1→6)[β-D-Gal*p*(1→2)-β-D-Gal*f*(1→4)]-D-GlcNAc (**10**) and the corresponding benzyl glycoside **11** and alditol 1**2** (Figure 2).





9, Gal*p*(β1→2)[Gal*p*(β1→3)]Gal*p*(β1→6)[Gal*f*(β1→2)Gal*f*(β1→4)]GlcNAcol

12, Gal*p*(β1→2)[Gal*p*(β1→3)]Gal*p*(β1→6)[Gal*p*(β1→2)Gal*f*(β1→4)]GlcNAcol

Figure 2. Structures of the largest oligosaccharides found in *T. cruzi* epimastigote mucins

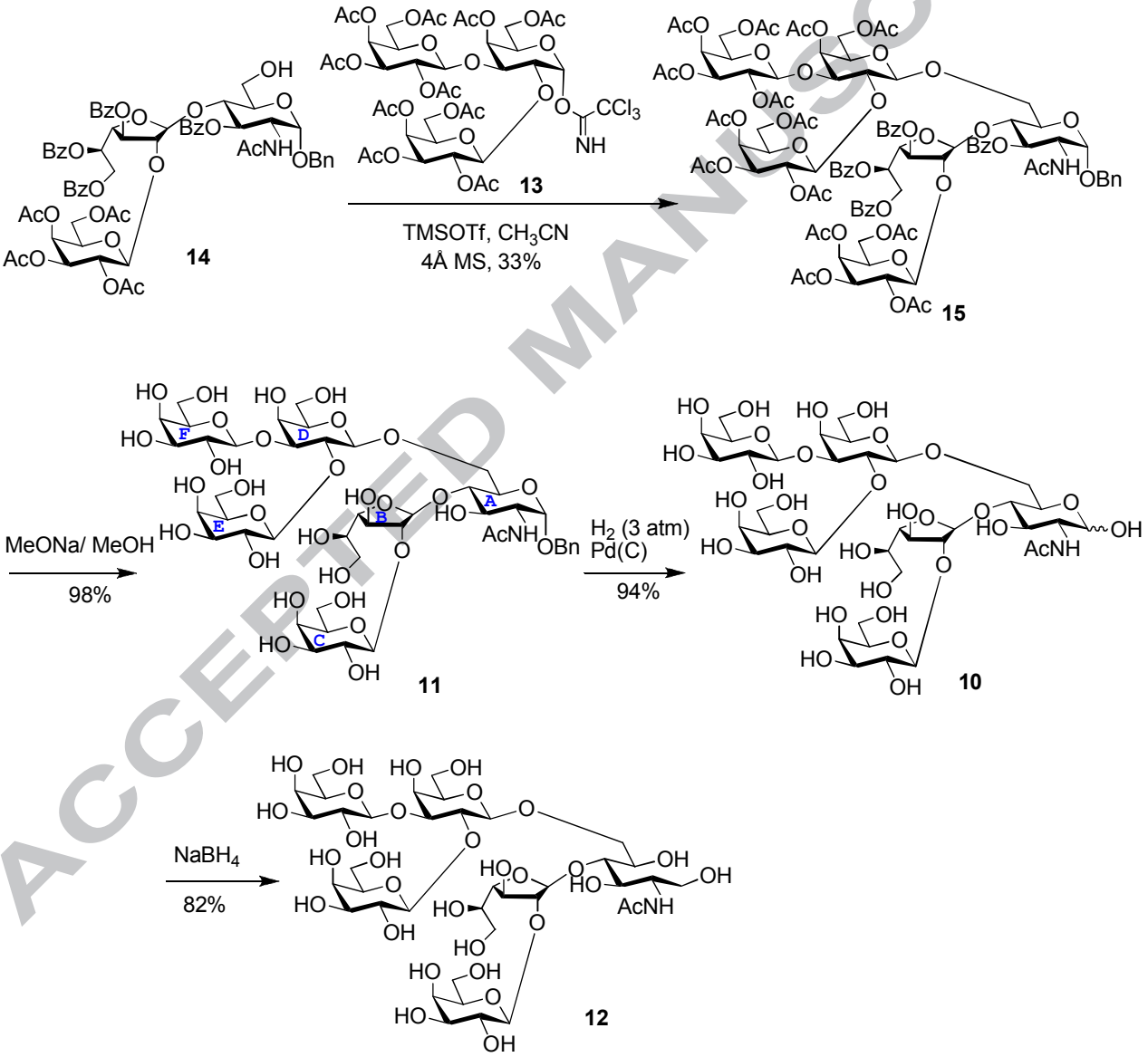
The benzyl hexasaccharide derivatives **8** and **11**, and their corresponding alditols **9** and **12** were analyzed as acceptors in the trans-sialidase reaction catalyzed by a recombinant TcTS in order to establish potential differences in specificity. Whereas already synthesized Gal*f*(1→2)containing hexasaccharide **7** presents two terminal Gal*p* residues, hexasaccharide **10** presents an extra terminal β-D-Gal*p* residue for possible sialylation. Kinetic studies were performed in order to determine the specificity and degree of sialylation.

2. Results and Discussion

2.1. Synthesis of hexasaccharide 10

Retrosynthetic analysis for hexasaccharide **10** indicated a [3+3] nitrilium based glycosylation between already synthesized Gal*p*-containing trisaccharide imidate **13**[26] and the trisaccharide acceptor **14**[27] which contains an internal Gal*f* (Scheme 1). The trichloroacetimidate method with acetonitrile as solvent, was used for selective β-glycosylation.[29,30] Due to the nitrile

effect[31-35] only the desired β-isomer was obtained, despite the absence of anchimeric participation in the donor **13**. The protected hexasaccharide **15** was obtained in 33% yield and unreacted acceptor **14** was also recovered. Interestingly, in the preparation of the Gal*f*(1→2)Gal*f* hexasaccharide **7,** similar reaction conditions gave higher yields (56 %).[28] When the terminal tetra-*O*-benzoyl-Gal*f* was replaced by a tetra-*O*-acetyl-Gal*p* in the trisaccharide acceptor **14**, the glycosylation yield diminished but the β-stereochemistry was maintained.



Scheme 1. Synthesis of hexasaccharide **10** and its benzyl glycoside **11** and alditol **12**

The ^{1}H and ^{13}C NMR spectra assignments for **15** were performed on the basis of the COSY and HSQC experiments. In the ^{13}C NMR spectrum, the signals corresponding to the six anomeric carbons could be easily identified. Thus, the signal for the new β-D-Gal*p*(1→6) linkage appeared at δ 102.3 ppm, more deshielded than the ones for the anomeric carbons of the three terminal β-D-Gal*p* (δ 100.0, 99.9 and 99.3 ppm). The furanosic anomeric carbon in β-configuration appeared even more deshielded (δ 105.9 ppm), as expected. Comparing with the ^{13}C NMR spectrum of the peracetylated benzyl glycoside of the related pentasaccharide **6**,[26] substitution at C-2 of the Gal*f* caused a 1.2 ppm shift to a higher field. On the other hand, C-1 of α-GlcNAc was shown at δ 96.1 ppm, as expected. The anomeric β-configuration of the new glycosidic linkage was confirmed by the coupling constant of $J_{1,2}$ 7.6 Hz for the Gal*p*(1→6) H-1 signal at δ 4.66 in the ^{1}H NMR spectrum. This signal correlated (HSQC) with C-1 at 102.3 ppm. The next step was the deacylation of **15** with sodium methoxide to give benzyl glycoside **11** in 98 % yield. ^{13}C and ^{1}H NMR spectra assignments for **11** were performed based on HSQC, COSY and ROESY experiments and by comparison with the shifts for the benzyl hexasaccharide **8** and the related benzyl glycoside of pentasaccharide **6**. In the anomeric region of the ^{13}C NMR spectrum of **11**, the most deshielded carbon at δ 107.5 corresponded to C-1^{B} of the β-Gal*f*, whereas at δ 96. 6 appeared de C-1^{A} of GlcNAc. The C-1 of the four Gal*p* units appeared at δ 104.7 (C-1^{F} Gal*p*(1→3))**,** 103.4 (C-1^{E} Gal*p*(1→2)Gal*p*), 102.4 and 102.3 (C-1^{D} Gal*p*(1→6) and C-1^{C} Gal*p*(1→2)-Gal*f*)**.** In the ^{1}H-NMR spectrum four β-Gal*p* anomeric carbons were identified by their typical *J* values (7-8 ppm) and unambiguously assigned by bi-dimensional spectroscopy. Two doublets at δ 5.54 ($J_{1,2}$ = 2Hz) and δ 4.96 ($J_{1,2}$ = 3.5Hz) were assigned to the β-Gal*f* and α-GlcNAc respectively. Each terminal Gal*p* unit could be assigned based on the ROESY experiment. The signal of H-1^{C} of the Gal*p*(β1→2)linked to Gal*f* at δ 4.60 correlated with H-2^{B} (δ 4.38) of the Gal*f* unit. The H-1^{E} of the

Gal*p*(β1→2) linked to Gal*p* at δ 4.83 showed a key correlation with H-2^D of internal Gal*p* (δ 3.96), whereas the H-1^F of the Gal*p*β(1→3) (δ 4.67) correlated with H-3^D of the internal Gal*p* D (δ 4.02).

The free hexasaccharide **10** was obtained in 94% yield by hydrogenolysis of **11** with H_2 and Pd(C). The NMR spectra indicated that **10** was an anomeric mixture with a preponderance of the α-anomer. In the ^{13}C-NMR spectrum of **10** the signals for β and α GlcNAc appeared at δ 95.9 and 91.4 ppm respectively.

Reduction of **10** with $NaBH_4$ gave alditol 1**2** (82%). Comparison of its ^{1}H and ^{13}C NMR spectra confirm the structures proposed for the hexasaccharide alditol obtained by reductive β-elimination of *T. cruzi* mucins.[10,11]

2.2. Kinetic properties of the hexasaccharides in the TcTS reaction

Compounds **11** and **12** present three terminal β-Gal*p* units for possible sialylation by TcTS whereas in the benzyl glycoside **8** and alditol **9** of the isomeric hexasaccharide **7**[28] one of the terminal Gal*p* is replaced by a Gal*f* and, consequently, have one less site for sialylation. The four hexasaccharide derivatives **8**, **9**, **11** and **12** were assayed as acceptor substrates for TcTS using conditions previously described for incubation with the donor 3´siallyllactose (SL).[26] The progress of sialic acid transfer was followed by HPAEC-PAD under three different conditions (see Experimental).

Incubation with TcTS and SL of benzyl glycoside **8,** containing two external Gal*p* units**,** showed only one sialylated species, **S-8** (Table 1). This behaviour was in agreement with previous results obtained for the benzyl glycoside of pentasaccharide **6**[26] and for benzyl 2,3-di-*O*-(β-D-Gal*p*)-β-D-Gal*p*.[36] Monosialylation ocurred only at the less hindered (1→3) linked Gal*p* unit with

no evidence of disialylated products.[26,36] 2,3-di-*O*-(β-D-Gal*p*)-β-D-Gal*p* is the terminal constituent of both hexasaccharides and pentasaccharide **6** (Figure 1 and 2)**.** On the other hand, benzyl glycoside **11**, with three terminal Gal*p* showed two monosialylated compounds, **S-11a** and **S-11b**, and a trace of another compound, **S_2-11**, that would correspond to a disialylated derivative (Table 1). Besides, the replacement of an external Gal*p* by a Gal*f* caused a significant increase in the retention times as previously observed for other Gal*f*-containing oligosaccharides[16,37] (Table 1). The same experiment performed on the alditols gave similar results. Gal*f*(1→2)Gal*f*-containing compound **9** produced only one sialylated species **S-9**, whereas two monosialylated compounds (**S-12a** and **S-12b**) and traces of a disialylated product (**S_2-12**) were observed in the case of **12**. Accordingly, radiolabeled mono and disialylated species have been detected by *trans*-sialylation of alditols released by reductive β-elimination from epimastigote and metacyclic trypomastigote mucins (G strain).[10]

Table 1. K_M and retention times of compounds **8**, **9**, **11** and **12** and their sialylated derivatives.

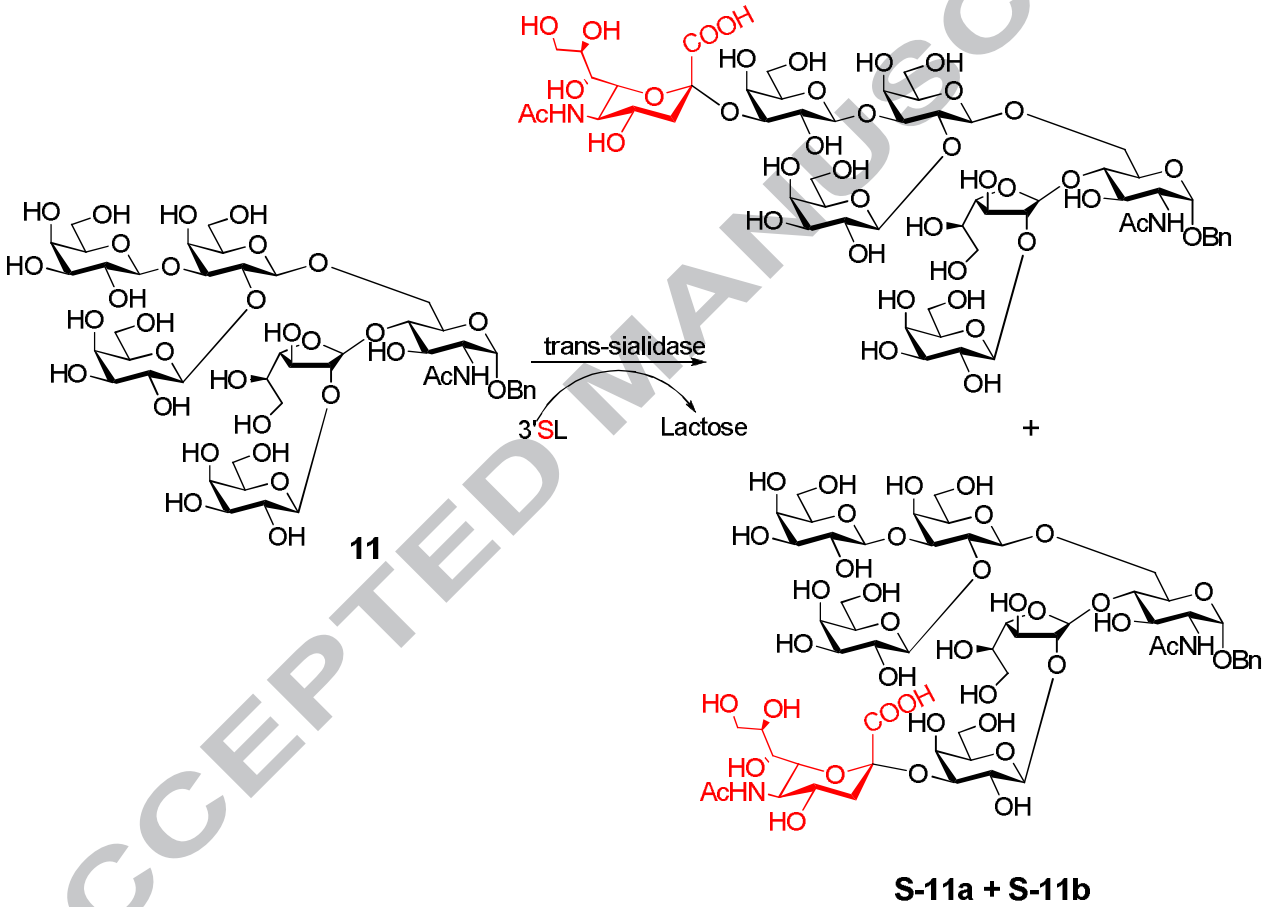
Compd	Retention time*			Sialylated Compound	Retention time*			K_M (mM)
	Cond. 1	Cond. 2	Cond. 3		Cond. 1	Cond. 2	Cond. 3	
8	0.23	0.75	0.70	**S-8**	2.73	1.33	1.45	0.12 ± 0.01
9	0.52	0.81	0.70	**S-9**	2.91	1.31	1.13	0.32 ± 0.21
11	0.18	0.38	0.28	**S-11a**	0.68	0.89	0.80	
				S-11b	0.74	0.91	0.85	0.40 ± 0.01
				S_2-11	2.02	1.49	1.59	
12	0.19	0.38	0.30	**S-12a**	0.71	0.94	0.81	
				S-12b	0.78	0.96	0.85	0.46 ± 0.06
				S_2-12	1.89	1.49	1.54	

*Relative to SL retention time (7.8-8.3 min for Condition 1; 13.6-14.3 min for Condition 2 and 12-12.5 min for Condition 3).

The apparent K_M values for each acceptor were estimated in order to quantify their relative affinity to the enzyme (Table 1). The values obtained were similar, supporting previous results

showing that the presence of a Gal*f* residue in the acceptor substrate does not interfere with the sialylation reaction.[16]

Hexasaccharide **10** may be formally seen as the combination of two trisaccharides: 2,3-di-*O*-(β-D-Gal*p*)-β-D-Gal*p* and Gal*p*(β1→2)Gal*f*(β1→4)GlcNAc (**2**), both good acceptors of sialic acid.[16] Thus, we could foreseen the formation of two monosialylated products, resulting from sialylation of either of the Gal*p* acceptor residues of the constituent trisaccharides (Scheme 2).



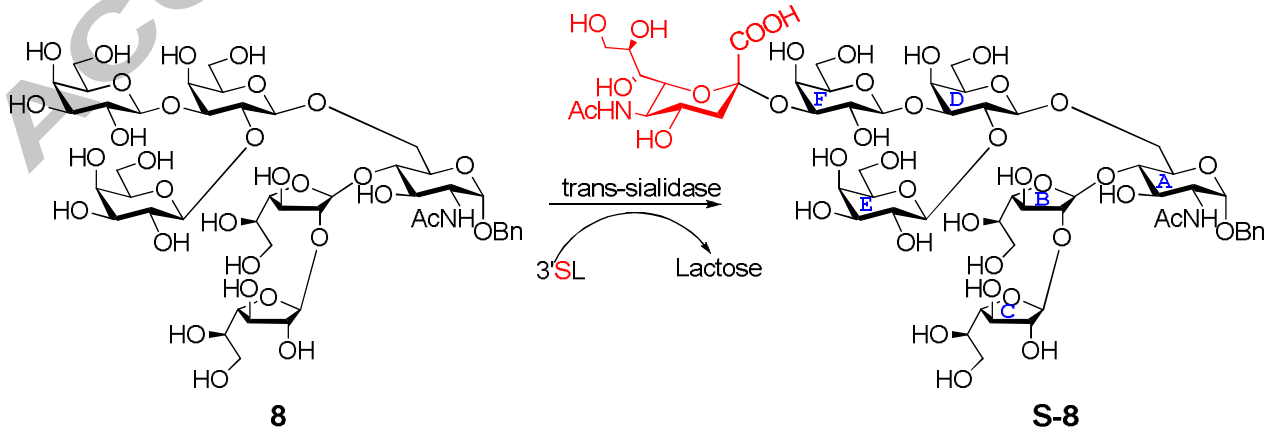
Scheme 2. Sialylation of compound **11** by TcTS.

In order to investigate the kinetic of the sialylation, the reaction was studied for the benzyl glycoside **11** using different ratios of the donor SL to the acceptor and different times of incubation. With a 1:1 ratio of SL to **11** and after 15 min incubation with TcTS one main monosialylated product (R_t 10.1 min, **S-11a**) was detected (data not shown). Increasing the amount

of SL (2:1 ratio) resulted in the appearance of the expected **S-11a** and two minor peaks of R_t 10.6 and 20.1 min that would correspond to the other monosialylated (**S-11b**) and the disialylated compound (**S_2-11**), respectively (Figure 3). Increasing the incubation times from 15 to 120 min resulted in an inversion of the proportions of the monosialylated derivatives (**S-11a** and **S-11b**) with no increase in the amount of disialylated compound (**S_2-11**). At the same time a higher consumption of SL and production of sialic acid was observed. Based on our previous results[26,36] and the studies described below on *trans*-sialylation of **8**, we assume that only the external β-Gal*p*(1→3) and β-Gal*p*(1→2)Gal*f* linked units could be sialylated (Scheme 2). However, we cannot predict, at the present time, which of the two Gal*p* residues is sialylated first or, if **S-11b** is formed at the expense of **S-11a** acting as sialic acid donor. The same experiment performed on alditol **12** showed that the relative proportions of the monosialylated derivatives did not change with time (data not shown). Introduction of a second sialic acid in the same molecule by TcTS is not as favorable as monosialylation.

2.3. Selective sialylation of hexasaccharide 8

With the aim of confirming the only site of sialylation in hexasaccharide **8**, a preparative sialylation reaction was performed (Scheme 3).



Scheme 3. Site of sialylation of hexasaccharide **8** by TcTS

The α-benzyl glycoside **8** was selected because the hydrophobic aglycon allowed an easier isolation by anion exchange chromatography of the sialylated derivatives from the remaining sialyllactose used as donor. On the other hand, the natural hexasaccharide is α-linked to threonine or serine in the mucins. The reaction was monitored by HPAEC and no evidence of the presence of another monosialylate or disialylated compound was observed (Figure 4). The reaction product was purified using an AG1X2 (acetate form) resin column. After elution of neutral sugars with water, the acidic components were eluted with 200 mM pyridinium acetate buffer, pH 5.0. The sialyl derivative **S-8** was characterized on the basis of NMR spectroscopy.

2.3.1. Characterization of S-8

The expected molecular mass of the *trans*-sialylation product was confirmed by ESI -TOF positive mass spectrometry. The peak at 1435.4804 was assigned to $[M+Na]^+$. Peaks corresponding to $[M+Na\text{-}Gal]^+$, $[M\text{-}Gal\text{-}OBn]^+$ and $[M+Na\text{-}Gal\text{-}sialic\ acid]^+$ were observed at 1273.4354, 1144.3876 and 982.3339, respectively. In the ^1H NMR spectrum of the sialylated oligosaccharide, the presence of the diagnostic signals for the sialyl residue corresponding to the deoxy group were observed at δ 1.81 ppm (H-3a) and δ 2.76 ppm (H-3e) (Figure 5). Integration of these signals confirmed that only one sialic acid unit has been introduced. A new singlet corresponding to the NAc group of neuraminic acid appeared at δ 2.03 ppm.

The six anomeric protons were distinctly shown with the two β-Gal*f* appearing as singlets at δ 5.47 and 5.24 ppm. The shifts were compared with those for the hexasaccharide substrate **8**.[28] As a consequence of sialylation the signal for the anomeric proton of the β-Gal*p*(1→3) was shifted to

lower fields (δ 4.71ppm) with respect to the signal in the original hexasaccharide (δ 4.66 ppm). Two dimensional NMR experiments (COSY, TOCSY, HSQC and NOESY) allowed the unequivocal determination of the site of sialylation. The characteristic doublet of doublets of H-3^F signal of the sialylated Gal*p* was shifted from δ 3.68-3.59 in **8** to δ 4.09 ppm in **S-8** ($J_{2,3}$ = 8 Hz and $J_{3,4}$ = 3.4 Hz). The signal for the H-2^F (TOCSY spectrum) that correlates with H-3^F was also shifted downfield (δ 3.64 ppm) and correlated with H-1^F at δ 4.71 ppm assigned to the Gal*p*-β(1→3) linked unit (F). The H-2^D signal (δ 3.85) of the internal Gal*p* (unit D) was determined by a correlation with H-1^D (δ 4.54) in the COSY experiment. As NOESY correlation between H-3^D and H-1^F was overlapped by the HDO signal, we looked for the NOESY correlation between H-2^D and H-1^E which indicated that Gal*p*(1→2) (unit E) was not sialylated. In this way, we confirmed that the Gal*p*-β(1→3) unit (F) was sialylated as in the case of benzyl 2,3-di-O-(β-D-Gal*p*)-β-D-Gal*p*[36] and the benzyl glycoside of pentasaccharide **6**.[26]

3. Conclusion

The β-D-Gal*p*-(1-2)-β-D-Gal*f* containing hexasaccharide **10** was chemically synthesized for the first time, by a convergent approach. Analysis by NMR spectroscopy of its alditol **12** and comparison with the data described for the natural product[11] confirmed the structure of the oligosaccharide present in the *T. cruzi* mucins. Benzyl glycoside **11** has three external Gal*p* plausible of being sialylated, however, studies on the behavior on sialylation using the TcTS enzyme showed only two monosialylated products whose relative abundance changed with time. Enzymatic synthesis and spectroscopical analysis of **S-8** unequivocally established the sialylation site of the hexasaccharide **8** at the less hindered (1→3)-linked galactopyranose.

Further studies to determine which Gal*p* is sialylated first in **11** could contribute to understand if a modulation in sialylation is operating through mucin oligosaccharides. Also, the Gal*f* containing hexasaccharides could be useful for the characterization of the unknown glycosyl transferases involved in the construction of the *T. cruzi* mucins.

4. Experimental

4.1. General

TLC was performed on 0.2 mm silica gel 60 F254 aluminium supported plates. Detection was effected by exposure to UV light or by spraying with 10% (v/v) H_2SO_4 in EtOH and charring. Column chromatography was performed on silica gel 60 (230–400 mesh). NMR spectra were recorded with a Bruker AVANCE II 500 spectrometer at 500 MHz (^{1}H) and 125.8 MHz(^{13}C). Chemical shifts are given relative to the signal of internal acetone standard at δ 2.22 and 30.89 ppm for ^{1}H NMR and ^{13}C NMR spectra, respectively, when recorded in D_2O. ^{1}H and ^{13}C assignments were supported by 2D COSY, TOCSY, NOESY and HSQC experiments. High resolution mass spectra (HRMS) were recorded on a BRUKER micrOTOF-Q II spectrometer.

For the sialylation experiments a recombinant TcTS expressed in *Escherichia coli* was kindly provided by the group of A. C. C. Frasch from Universidad Nacional General San Martin (Buenos Aires, Argentina). Sialyllactose was obtained from bovine colostrum by an adaptation of a reported method.[38] Analysis by HPAEC-PAD was performed using a Dionex ICS 3000 HPLC system equipped with a pulse amperometric detector. The following programs were used: Condition 1, a CarboPac PA-100 ion exchange analytical column (4 ×250 mm) equipped with a guard column PA-100 (4 × 50 mm) and 60 mM NaAcO in 100 mM NaOH at a flow rate of 1.0 mL/min and 25 °C; Condition 2, a CarboPac PA-20 ion exchange analytical column (3 ×150 mm)

equipped with a guard column PA-20 (3 × 30 mm) and a lineal gradient over 30 min from 20 to 200 mM NaAcO in 100 mM NaOH at a flow rate of 0.5 mL/min and 25 °C; Condition 3, a CarboPac PA-100 ion exchange analytical column (4 ×250 mm) equipped with a guard column PA-100 (4 × 50 mm) and a lineal gradient over 60 min from 50 to 300 mM NaAcO in 100 mM NaOH at a flow rate of 0.9 mL/min and 25 °C

4.2. Synthesis

4.2.1. Benzyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→2)-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)]-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (15)

To a flask containing recently purified and dried benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside[27] (**14**, 114 mg, 0.093 mmol), and activated 4Å powdered molecular sieves, a solution of *O*-[2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-di-*O*-acetyl-α-D-galactopyranosyl] trichloroacetimidate[26] (**13**, 122 mg, 0.114 mmol, 1.2 equiv) in freshly distilled anhydrous CH_3CN (5 mL) was added, and the suspension was cooled to −20 °C under an argon atmosphere. After 15 min of vigorous stirring, TMSOTf (7 μL, 0.039 mmol, 0.4 equiv) was added and the stirring continued for 24 h at −18 °C and then for additional 26 h more at 5 °C. After cooling the mixture to 0 ºC, the reaction was stopped by the addition of Et_3N (5.5 μL, 0.41 equiv), filtered and the molecular sieves were washed with CH_3CN (2 x 3 mL). The filtrate was concentrated under reduced pressure at room temperature and the residue was purified by column chromatography (10:17 hexane-EtOAc).

The first fraction gave unreacted acceptor **14** (71 mg, 62%). The next fraction afforded 65 mg of hexasaccharide derivative **15** (33%) as a foamy solid: R_f 0.25 (1:1.7 hexane-EtOAc, double development), $[\alpha]_D$ +1.9º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.04 – 7.24 (m, 25H, arom.), 6.20 (d, 1H, *J* = 9.6 Hz, N*H*), 5.62 (dd, 1H, *J* = 10.6, 9.4 Hz, H-3 GlcNAc), 5.51 (bs, 1H, H-1 Gal*f*), 5.40–5.33 (m, 6H, H-5 Gal*f*, H-3 Gal*f*, 4 H-4 of 4 Gal*p* units), 5.22 (dd, 1H, *J* = 10.3, 8.0 Hz, H-2 Gal*p* β(1→3)[†]), 5.15 (dd, 1H, *J* = 10.6, 3.4 Hz, H-3 Gal*p* β(1→2)-Gal*p* [†]), 5.12 (dd, 1H, *J* = 10.3, 8.1 Hz, H-2 Gal*p*-β(1→2)-Gal*f* [†]), 5.08 (dd, 1H, *J* = 10.6, 7.6 Hz, H-2 Gal*p*-β(1→2)-Gal*p*[†]), 5.02 (dd, 1H, *J* = 10.3, 3.5 Hz, H-3 Gal*p*-β(1→3)[†]), 5.01 (d, 1H, *J* = 8.0 Hz, H-1 Gal*p*-β(1→3)[†]), 4.99 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3 Gal*p*-β(1→2)-Gal*f* [†]), 4.98 (d, 1H, *J* = 3.4 Hz, H-1 GlcNAc), 4.94 (d, 1H, *J* = 8.1 Hz, H-1 Gal*p*-β(1→2)-Gal*f* [†]), 4.87 (d, 1H, *J* = 7.6 Hz, H-1 Gal*p*-β(1→2)-Gal*p* [†]); 4.84, 4.56 (2d, 2H, *J* = 11.7 Hz, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 7.6 Hz, H-1 Gal*p*-β(1→6)), 4.48–4.42 (m, 3H, H-2 GlcNAc, H-4 Gal*f*, H-6a Gal*f*), 4.36–4.23 (m, 5H), 4.22 (bs, 1H, H-2 Gal*f*), 4.20–4.09 (m, 3H), 4.08 (dd, 1H, *J* = 9.1, 3.8 Hz, H-3 Gal*p*-β(1→6)), 4.05–3.96 (m, 6H), 3.91 (dd, 1H, *J* = 9.1, 7.6 Hz, H-2 Gal*p*-β(1→6)), 3.91–3.79 (m, 3H), 2.19, 2.16, 2.09, 2.07 (4s, 12H, C*H*$_3$CO); 2.04, 2.03 (2s, 9H, C*H*$_3$CO); 2.02, 2.01, 1.99 (3s, 9H, C*H*$_3$CO), 1.97 (s, 6H, C*H*$_3$CO), 1.95, 1.92 (2s, 6H, C*H*$_3$CO), 1.80 (s, 6H, C*H*$_3$CO). †The assignments could be interchanged; ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.4, 170.3 x 2, 170.2, 170.1 x 2, 170.0, 169.9, 169.8, 169.6, 169.5, 168.9 (CH$_3$*C*O); 167.0 (CH$_3$*C*ONH); 165.8, 165.5, 165.2 (Ph*C*O); 136.7–128.3 (C-aromatics); 105.9 (C-1 Gal*f*), 102.3 (C-1 Gal*p*-β(1→6)), 100.0, 99.9, 99.3 (C-1 Gal*p*-β(1→2)-Gal*p*, C-1 Gal*p*-β(1→2)-Gal*f*, C-1 Gal*p*-β(1→3)), 96.2 (C-1 GlcNAc), 87.0 (C-2 Gal*f*), 80.0 (C-4 Gal*f*), 79.0 (C-2 Gal*p*-β(1→6)), 76.8 (C-3 Gal*f*), 75.7 (C-4 GlcNAc), 75.3 (C-3 Gal*p*-β(1→6)); 71.8, 71.2, 70.8, 70.7, 70.6 x 2, 70.5, 70.2, 70.1; 69.9 and 69.8 (C-2 Gal*p*-β(1→2), C-2 Gal*p*-β(1→3), *C*H$_2$Ph), 69.4 (C-6 GlcNAc), 68.7, 68.2, 66.9 x 2, 66.8; 62.7 (C-6 Gal*f*); 61.8, 60.6

x 2, 60.2 (4 C-6 of 4 Gal*p* units), 52.2 (C-2 GlcNAc), 22.9 (*C*H_3CONH); 20.9 x 2, 20.7, 20.6 x 4, 20.5 x 4, 20.4 (*C*H_3CO); HRMS (ESI) m/z calcd for $C_{101}H_{115}NNaO_{49}$ $[M+Na]^+$: 2148.6436. Found: 2148.6451.

4.2.2. Benzyl β-D-galactopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→2)-β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranoside (11)

To a cooled (0 ºC) solution of **15** (31 mg, 0.015 mmol) in anhydrous methanol (1 mL), was added a solution 2.6 M $NaOCH_3$-CH_3OH (200 μL) and the mixture was stirred for 1h at 0 °C followed by 2 h at rt. The solution was purified by cationic exchange chromatography column (Amberlite IR-120 plus resin 200 mesh, H^+ form) eluting with 9:1 CH_3OH-H_2O. The eluate was concentrated under reduced pressure and the methyl benzoate was co-evaporated with water (5 x 1 mL). Further purification through a C8 cartridge, elution with water followed by concentration at 30 ºC gave 16 mg of **11** (98%), as an amorphous white solid: R_f 0.35 (7:1:1 n-propanol-EtOH-H_2O); $[\alpha]_D$ +29.7º (*c* 1,H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 7.48 – 7.38 (m, 5H, aromatics), 5.54 (d, 1H, *J* = 2.0 Hz, H-1^B), 4.96 (d, 1H, *J* = 3.5 Hz, H-1^A), 4.83 (d, 1H, *J* = 7.7 Hz, H-1^E); 4.74, 4.57 (2d, 2H, *J* = 12.2 Hz, C*H*$_2$Ph), 4.67 (d, 1H, *J* = 7.4 Hz, H-1^F), 4.64 (d, 1H, *J* = 7.9 Hz, H-1^D), 4.60 (d, 1H, *J* = 7.9 Hz, H-1^C), 4.38 (dd, 1H, *J* = 4.3, 2.0 Hz, H-2^B), 4.26 (dd, 1H, *J* = 7.4, 4.3 Hz, H-3^B), 4.20 (d, 1H, *J* = 3.2 Hz, H-4^D), 4.13 (dd, 1H, *J* = 10.0, 1.0 Hz, H-6a^A), 4.10 (dd, 1H, *J* = 7.5, 3.5 Hz, H-4^B), 4.02–3.57 (m, 31H), 1.96 (s, 3H, C*H*$_3$CONH); ^{13}C NMR(D_2O, 125.8 MHz): δ 174.9 (CH_3*C*ONH); 137.6-128.9 (C-aromatics), 107.5 (C-1^B), 104.7 (C-1^F), 103.4 (C-1^E); 102.4, 102.3 (C-1^D and C-1^C), 96.6 (C-1^A), 87.6 (C-2^B), 83.7 (C-3^D), 82.9 (C-4^B), 78.6 (C-4^A), 76.1 (C-2^D); 76.0, 75.9, 75.7 x 2, 75.1, 73.6, 73.4, 73.2, 72.1, 71.8, 71.5, 70.9, 70.7 (*C*H_2Ph); 70.3, 69.9,

69.8, 69.5; 69.3 (C-4^D), 69.1; 68.4 (C-6^A), 63.3 (C-6^B); 62.0, 61.8, 61.6, 61.4 (C-6^D, C-6^E, C-6^F, C-6^C), 54.3 (C-2^A), 22.4 (*C*H_3CONH). HRMS (ESI) m/z calcd for $C_{45}H_{71}NNaO_{31}$ $[M+Na]^+$: 1144.3908. Found: 1144.3890.

4.2.3. β-D-Galactopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→2)-β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α,β-D-glucopyranose (10)

To a solution of **11** (15 mg, 0.013 mmol) in 9:1 CH_3OH-H_2O (2 mL), 10% Pd(C) (16 mg) were added and the suspension was hydrogenated for 22 h at 40 psi (3 atm) at rt when a tlc showed no**11** left. The catalyst was filtered and the filtrate was concentrated at 25 ºC, affording **10** (13 mg, 94 %) as a hygroscopic amorphous solid: R_f 0. 21 (7:2:2 n-Propanol–EtOH–H_2O), $[\alpha]_D$ +0.5º (*c* 1,H_2O);^{1}H NMR (D_2O, 500 MHz): δ anomeric region and diagnostic signals: 5.55 (m, 1H, H-1 Gal*f* α and β anomers), 5.21 (d, 0.75H, *J* = 3.4 Hz; H-1 α-GlcNAc), 4.83 (d; 0.25H, *J* = 7.7 Hz; H-1 β-GlcNAc), 4.73 – 4.58 (m; 4H; 4 H-1 Gal*p* α anomer; 4 H-1 Gal*p* β anomer); 4.40 (dd; 0.75H; *J* = 4.1, 1.9 Hz; H-2 Gal*f* α anomer), 4.38 (dd; 0.25H; *J* = 3.9, 1.7 Hz; H-2 Gal*f* β anomer), 2.05 (s; 3H; C*H*$_3$CONH). ^{13}C NMR (D_2O, 50.3 MHz): δ anomeric region and diagnostic signals: 175.6, 175.3 (CH_3*C*ONH α and β anomers), 107.6 (C-1 Gal*f* α anomer), 107.5 C-1 Gal*f* β anomer);104.7, 103.6, 102.4, 102.3 (4 C-1 Gal*p*); 95.9 (C-1 β-GlcNAc), 91.4 (C-1 α-GlcNAc), 57.5 (C-2 β-GlcNAc), 54.9 (C-2 α-GlcNAc), 23.0, 22.7 (*C*H_3CONH α and β anomers). HRMS (ESI) m/z calcd for $C_{38}H_{65}NNaO_{31}$ $[M+Na]^+$: 1054.3433. Found: 1054.3455.

4.2.4. β-D-Galactopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→2)-β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-D-glucitol (12) [10]

To a cooled solution (0ºC) of **10** (11 mg, 0.011 mmol) in 9:1 methanol-water (2 mL), sodium borohydride (30 mg, 0.79 mmol) was added and the suspension was gently stirred at rt for 16 h, when TLC analysis showed disappearance of the starting compound. The solution was decationized by passing through a column of Amberlite IR-120 plus, the resin was washed with water and the combined solutions were concentrated to dryness. The residue was co-evaporated with methanol (4 x 1 mL), dissolved in deionized water and the solution was filtered through a C8 cartridge. Evaporation of the filtrate under reduced pressure gave 9 mg of **12** (82 %) as an amorphous solid: $[\alpha]_D$ -1.1º (*c* 0.6, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 5.53 (bs, 1H, H-1 Gal*f*), 4.85 (d, 1H, *J* = 7.8 Hz), 4.66 (d, 1H, *J* = 7.6 Hz), 4.63 (d, 1H, *J* = 7.9 Hz, H-1 Gal*p*-β(1→6)), 4.59 (d, 1H, *J* = 7.8 Hz), 4.43 (d, 1H, *J* = 3.1 Hz, H-2 Gal*f*), 4.25 (dd, 1H, *J* = 3.1, 6.4 Hz, H-3 Gal*f*), 4.20 (d, 1H, *J* = 3.0 Hz, H-4 Gal*p*-β(1→6)), 4.19 - 4.12 (m, 3H, H-4 Gal*f*, H-2 GlcNAc, H-6a GlcNAc), 4.06 (m, 1H, H-5 GlcNAc), 4.01 (dd, 1H, *J* = 9.7, 3.0 Hz, H-3 Gal*p*-β(1→6)), 3.96 - 3.54 (m, 30H), 2.05 (s, 3H, C*H*$_3$CONH); ^{13}C NMR (D_2O, 125.8 MHz): δ 175.1 (CH_3*C*ONH), 107.8 (C-1 Gal*f*); 104.7, 103.4, 102.4, 102.1; 88.1 (C-2 Gal*f*), 83.9 (C-4 Gal*f*), 83.5 (C-3 Gal*p*-β(1→6)); 78.1, 76.5 (C-2 Gal*p*-β(1→6)), 76.4 (C-3 Gal*f*); 76.0, 75.9, 75.8, 75.3, 73.5 x 2, 73.2, 72.2, 71.8, 71.5, 71.3; 71.1 (C-6 GlcNAc); 70.3, 69.7, 69.5, 69.4, 69.2, 69.0; 63.5 (C-6 Gal*f*); 61.8; 61.7 x 2, 61.5 x 2 (C-1 GlcNAcol, 4 C-6 Gal*p* units); 53.2 (C-2 GlcNAcol); 22.8 (*C*H_3CONH). HRMS (ESI) m/z calcd for $C_{38}H_{67}NNaO_{31}$ $[M+Na]^+$: 1056.3589. Found: 1056.3572.

4.2.5. Benzyl 5-*N*-acetyl-α-D-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→2)-β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranoside (S-8)

Benzyl β-D-galactopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→2)-β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranoside[28] (**8**, 6 mg) and 3´-sialyllactose (6.5 mg) were incubated with 0.022 mg of recombinant TcTS in 0.5 mL of 20 mM Tris buffer pH 7.4 containing 30 mM NaCl for 14 h at 25 °C. The synthesis was performed in triplicate and each reaction analyzed by HPAEC. The three incubation mixtures were combined and the sialylated compound **S-8** was purified by passing through an anion exchange resin (AG1X2, acetate form, BioRad, 1.2 x 15 cm). Neutral compounds, namely benzyl hexasaccharide **8** and lactose were eluted with H_2O and sialylated compounds with 200 mM pyridinium acetate buffer pH 5. Fractions (1.5 mL) were collected and analyzed by HPAEC. The remaining sialyllactose was eluted with the first 80 mL of 200 mM pyridinium acetate buffer. Further elution with the same buffer afforded sialylated compound **S-8**. The pooled fractions were concentrated under vacuum at rt, redissolved in water and lyophilized to afford 2.7 mg of **S-8** as an amorphous white powder. ^{1}H NMR (D_2O, 500 MHz): δ 7.46-7.38 (m, 5H, aromatics), 5.48 (d, 1H, *J* = 1.2 Hz, H-1^B), 5.24 (d, 1H, *J* =1.5 Hz, H-1^C), 4.96 (d, 1H, *J* = 3.5 Hz, H-1^A), 4.85 (d, 1H, *J* = 7.7 Hz, H-1^E); 4.74, 4.57 (2d, 2H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.71 (d, 1H, *J* = 8.0 Hz, H-1^F), 4.54 (d, 1H, *J* = 7.7 Hz, H-1^D), 4.27–4.23 (m, 2H, H-2^B, H-3^B), 4.20–4.19 (m, 2 H, H-6a^A, H-4^D), 4.14 (dd, 1H, *J* = 1.7, 3.5 Hz, H-2^C), 4.12-4.09 (m, 2H, H-3^C, H-4^D), 4.08 (dd, 1 H, *J* = 8.0, 3.2 Hz, H-3^F), 4.01 (dd, 1H, *J* = 6.5, 4.0 Hz, H-4^C), 4.03-3.56 (m, 33 H), 2.77 (dd, 1H, *J* = 12.4, 4.6 Hz, H-3e Neu5Ac), 2.03 (s, 3 H, C*H*$_3$CONH Neu5Ac), 1.95 (s, 3 H, C*H*$_3$CONH), 1.81 (t, 1 H, *J* = 12.1 Hz, H-3a Neu5Ac); ^{13}C NMR (D_2O): δ 175.7, 174.8

(CH_3CONH), 129.4 (aromatic), 107.2 (C-1^B), 106.4 (C-1^C), 104.8 (C-1^F), 103.6 (C-1^E), 102.7 (C-1^D), 96.6 (C-1^A), 86.0 (C-2^B), 84.2 (C-4^C), 83.9 (C-3^D), 84.4 (C-4^B), 82.4 (C-2^C), 78.5 (C-4^A), 77.6 (C-3^C), 76.5 (C-3^F), 76.3, 76.2, 75.6, 75.5, 75.3, 73.7, 73.5, 72.7, 72.4, 72.3, 71.5, 71.2, 70.8 (CH_2Ph), 70.5, 70.4, 70.0, 69.7, 69.3, 69.1, 69.0, 68.7, 68.3, 63.5, 63.4, 63.4, 62.0, 61.7, 61.6; 54.4 (C-2^A), 52.4 (C-5 Neu5Ac), 40.4 (C-3 Neu5Ac), 22.8 (CH_3CONH, Neu5Ac), 22.4 (CH_3CONH).

HRMS (ESI) Calcd for $C_{56}H_{88}N_2NaO_{39}$ $[M+Na]^+$: 1435.4856, found: 1435.4804.

Acknowledgments

We thank the group of A.C.C Frasch from Universidad Nacional General San Martin, Argentina, for the kind gift of trans-sialidase from *Trypanosoma cruzi.* This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica, Universidad de Buenos Aires and CONICET. R. M. de Lederkremer, Rosalía Agusti and C. Gallo-Rodriguez are research members of CONICET.

Supplementary data

NMR spectra for compounds **15**, **11, 10, 12** and **S-8**.

References

1. Brener, Z. *Ann. Ver. Microbiol*. **1973**, *27*, 347.
2. Moncayo, A. *Mem. Inst. Oswaldo Cruz*. **2003**, *98*, 577.
3. Lederkremer, R. M.; Agustí, R. *Adv. Carbohydr. Chem. Biochem*. **2009**, *62*, 31.

4. Buscaglia, C. A.; Campo, V. A.; Frasch, A. C. C.; Di Noia, J. M. *Nat. Rev. Microbiol.* **2006**, *4*, 229.

5. Pereira-Chioccola, V. L.; Acosta-Serrano, A.; Correira de Almeida, I.; Ferguson, M. A. J.; Souto-Padron, T.; Rodrigues, M. M.; Travassos, L. R.; Schenkman, S. *J. Cell Sci*. **2000**, *113*, 1299.

6. Schenkman, S.; Jiang, M. S.; Hart, G. W.; Nussenzweig, V. *Cell* **1991**, *65*, 1117.

7. Frasch, A. C. C. *Parasitol. Today* **2000**, *16*, 282.

8. Ferrero-Garcia, M. A.; Trombetta, S. E.; Sanchez, D. O.; Reglero, A.; Frasch, A. C. C.; Parodi, A. J. *Eur. J. Biochem*. **1993**, *213*, 765.

9. Harrison, J. A.; Kartha, K. P. R.; Fournier, E. J. L.; Lowary, T. L.; Malet, C.; Nilsson, U. J.; Hindsgaul, O.; Schenkman, S.; Naismith, J. H.; Field, R. A. *Org. Biomol. Chem.*, **2011**, *9*, 1653–1660

10. Acosta-Serrano, A.; Schenkman, S.; Yoshida, N.; Mehlert, A.; Richardson, J. M.; Ferguson, M. A. J. *J. Biol. Chem.* **1995**, *270*, 27244.

11. Previato, J. O.; Jones, C.; Gonçalves, L. P. B.; Wait, R.; Travassos, L. R.; Mendonça-Previato, L. *Biochem. J.* **1994***, 301,* 151.

12. Agrellos, O. A.; Jones, C.; Todeschini, A. R.; Previato, J. O.; Mendonça-Previato, L. *Mol. Biochem. Parasitol.* **2003**, *126*, 93.

13. Todeschini, A. R.; de Almeida, E. G.; Agrellos, O. A.; Jones, C.; Previato, J. O.; Mendonca-Previato, L. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 270.

14. Zingales, B.; Andrade, S. G.; Briones, M. R.; Campbell; D. A.; Chiari, E.; Fernandes, O.; Guhl, F.; Lages-Silva, E.; Macedo, A. M.; Machado, C. R.; Miles, M. A; Romanha, A. J.; Sturm, N. R.; Tibayrenc, M.; Schijman, A. G. *Mem. Inst. Oswaldo Cruz.* **2009**, *104*, 1051.

15. Jones, C.; Todeschini, A. R.; Agrellos, O. A.; Previato, J. O.; Mendonça-Previato, L. *Biochemistry* **2004**, *43*, 11889.

16. Agustí, R.; Giorgi, M. E.; Mendoza, V. M.; Gallo-Rodriguez, C.; Lederkremer, R. M. *Bioorg. Med. Chem.* **2007**, *15*, 2611.

17. de Arruda, M. V.; Colli, W.; Zingales, B. *Eur. J. Biochem.* **1989**, *182*, 413.

18. Previato, J. O.; Jones, C.; Gonçalves, P. B.; Wait, R.; Travassos, L. R.; Parodi, A. J.; Mendonça-Previato, L. *J. Biol. Chem.* **1995**, *270*, 7241.

19. Salto, M. L.; Gallo-Rodriguez, C.; Lima, C.; Lederkremer, R. M. *Anal. Biochem.* **2000**, *279*, 79.

20. Pollevick, G. D.; Di Noia, J. M.; Salto, M. L.; Lima, C.; Leguizamon, M. S.; Lederkremer, R. M.; Frasch, A. C. C. *J. Biol. Chem.* **2000**, *275*, 27671.

21. Todeschini, A. R.; da Silveira, E. X.; Jones, C.; Wait, R.; Previato, J. O.; Mendonça-Previato, L. *Glycobiology* **2001**, *11*, 47.

22. van Well, R. M.; Collet, B., Y. M.; Field, R. A. *Synlett* **2008**, 2175.

23. Gallo-Rodriguez, C.; Varela, O.; Lederkremer, R. M. *J. Org. Chem.* **1996**, *61*, 1886.

24. Gallo-Rodriguez, C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* **1998**, *305*, 163.

25. Gallo-Rodriguez, C.; Gil-Libarona, M. A.; Mendoza, V. M.; Lederkremer, R. M. *Tetrahedron,* **2002,** *58*, 9373.

26. Mendoza, V. M.; Agusti, R.; Gallo-Rodriguez, C.; Lederkremer, R. M. *Carbohydr. Res.* **2006**, *341*, 1488.

27. Mendoza, V. M.; Kashiwagi, G. A.; Lederkremer, R. M.; Gallo-Rodriguez, C. *Carbohydr. Res.* **2010**, *345*, 385.

28. Kashiwagi, G. A.; Mendoza, V. M.; Lederkremer, R. M.; Gallo-Rodriguez, C. *Org. Biomol. Chem.* **2012**, *10*, 6322.

29. Schmidt, R. R., Jung, K.-H. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker, **1997**; pp 283.

30. Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694.

31. Pougny, J.-R.; Sinay, P. *Tetrahedron Lett.* **1976**, 4073.

32. Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans.* I, **1990**, 747.

33. Bohé, L.; Crich, D. *C. R. Chimie* **2011**, *14*, 3.

34. Schmidt, R. R.; Zhu, X. Glycosyl Trichloroacetimidates. In *Glycoscience*. Fraser-Reid, B; Tatsuta, K; Thiem, J., Eds.; Springer-Verlag Berlin Heidelberg, 2008, pp. 451.

35. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

36. Agustí, R.; Mendoza, V. M.; Gallo-Rodriguez, C.; Lederkremer, R. M. *Tetrahedron-Asymmetr*. **2005**, *16*, 541.

37. Salto, M. L.; Gallo-Rodriguez, C.; Lima, C.; Lederkremer, R. M. *Anal. Biochem*. **2000,** *279*, 79.

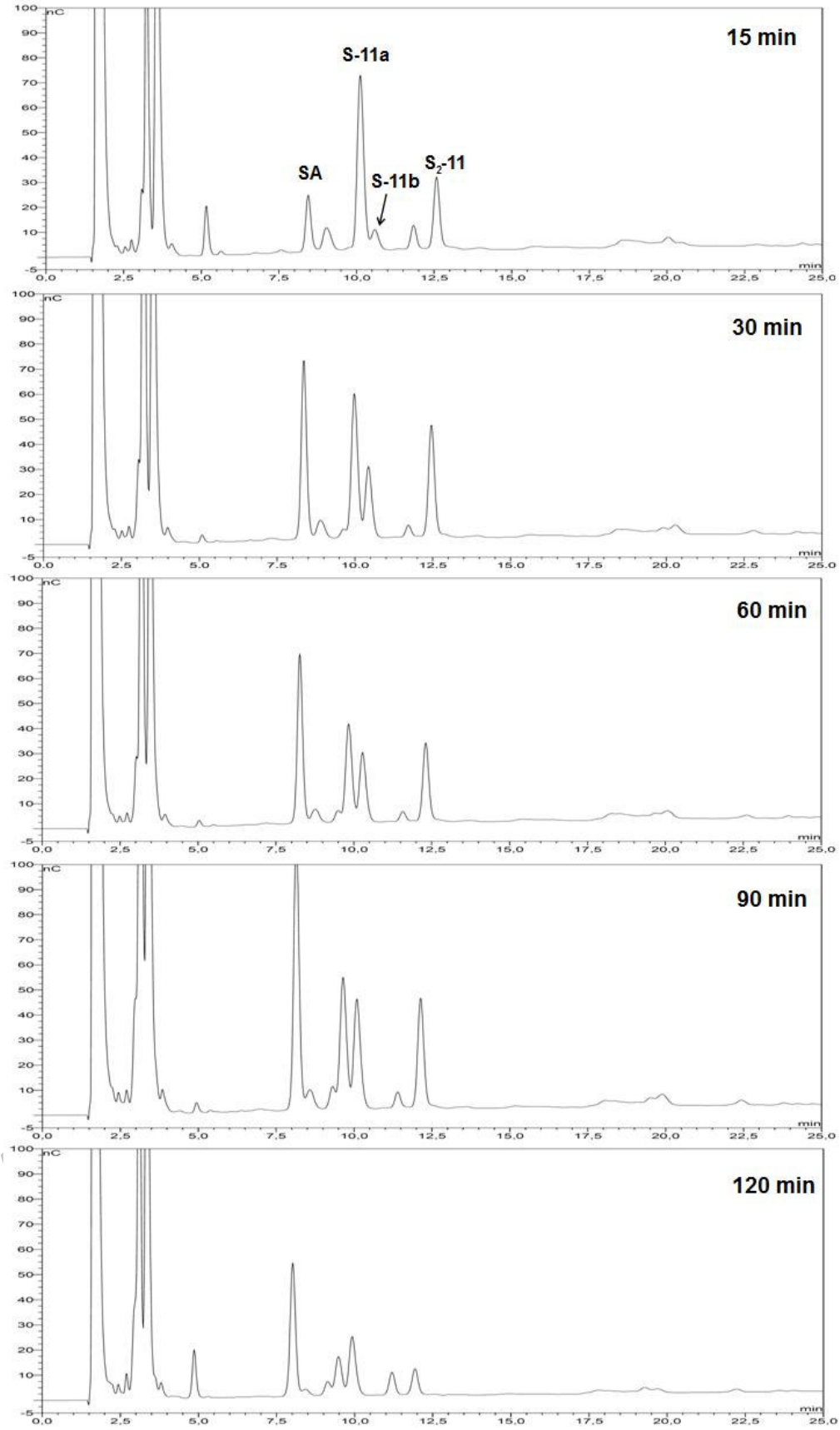
38. Veh, R. W; Michalski, J.; Corfield, A. P.; Sander-Wewer, M.; Gies, D.; Schauer, R. *J Chromatogr A*. **1981**, *212*, 313.

Legends to figures

Figure 3- Analysis of sialylation of benzyl hexasaccharide **11** with time. Compound **11** (1 mM) was incubated with SL (2 mM) and TcTS for 15, 30, 60, 90 and 120 min at room temperature. The incubation mixtures were analyzed by HPAEC under condition 3. SL, sialyllactose; SA, sialic acid, S-**11a** and **S-11b**, monosialylated compound **11**; S_2-**11**, disialylated compound **11**.

Figure 4- Sialic acid transfer by TcTS to the benzyl hexasaccharide **8**. Compound **8** (1 mM) was incubated with SL (1 mM) in the absence (A) or presence (B) of TcTS for 15 min at room temperature. The incubation mixtures were analyzed by HPAEC under condition 3. L, lactose; SL, sialyllactose; S-**8**, sialylated compound **8**.

Figure 5- ^{1}H-NMR spectrum of sialylated compound **8** (D_2O, 500 MHz)


15 min
S-11a
SA
S-11b
S2-11
30 min
60 min
90 min
120 min
nC
min