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Synthesis of β-D-Galf-(1–3)-D-GlcNAc by the Trichloroacetamidate Method and of β-D-Galf-(1–6)-D-GlcNAc by $SnCl_4$-Promoted Glycosylation

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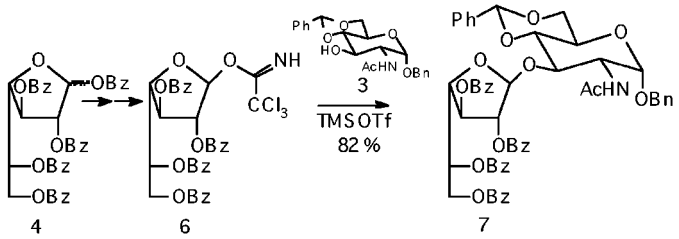
ABSTRACT



In a continuation of our studies on the characterization of the glycoproteins of *T. cruzi* new galactofuranosyl disaccharides were synthesized. β-D-Galf-(1–3)-D-GlcNAc (1) was prepared by employing the trichloroacetamidate procedure for the glycosylation step. The mild conditions of this reaction are appropriate for condensation of 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl trichloroacetamidate (6) with acid-labile benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (3). On the other hand, tin(IV) chloride promoted condensation of benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (11) with penta-O-benzoyl-α,β-D-galactofuranose (4) gave the derivative of β-D-Galf-(1–6)-D-GlcNAc (2) in 78% yield.

In connection with our studies on the structure of *O*-linked oligosaccharides in the mucins of *T. cruzi* we synthesized the new disaccharides β-D-Gal*f*-(1−3)-D-GlcNAc (**1**) and β-D-Gal*f*-(1−6)-D-GlcNAc (**2**). These, together with β-D-Gal*f*-(1−4)-D-GlcNAc, obtained from mucins[1] and synthesized in our laboratory,[2] are the possible disaccharides Gal*f*-GlcNAc, necessary for unequivocal identification of the unit found in the mucins of *T. cruzi*. It is interesting that the presence of galactofuranose is dependent on the protozoan strain; for example, in the Y strain the *O*-linked oligosaccharides contain only galactopyranose, whereas Gal*f* and Gal*p* units are present in the G strain.[1,3]

Glycopyranosides have been extensively used for glycosylation by the trichloroacetamidate method.[4] A recent report[5] on the use of this method for the preparation of some galactofuranosyl disaccharides, as methyl glycosides, prompts us to report our results.

We have extensively employed $SnCl_4$-promoted glycosidation to link galactofuranosyl units to many acceptors.[2,6]

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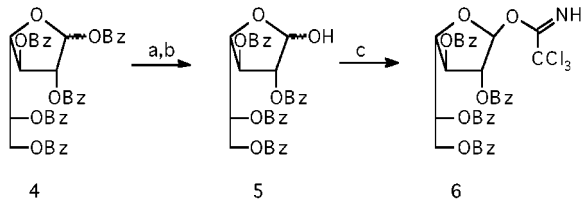
This method has the advantage of using a peracylated galactofuranose donor which can be obtained in a one-step reaction from D-galactose.[7] However, this facile procedure fails when acid labile acceptors have to be glycosylated.

For the synthesis of disaccharide **1**, a derivative of GlcNAc with the free OH-3 was required. 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**3**) was easily prepared as described.[7] Because of the lability of the benzylidene group to acidic media, the trichloroacetamidate method was employed to build the furanosic linkage. Thus, penta-*O*-benzoyl-α,*β*-D-galactofuranose[7] (**4**) was treated with 32% HBr in glacial AcOH and the resulting galactofuranosyl bromide was further hydrolyzed with the assistance of $AgCO_3$,[8] to give 2,3,5,6-tetra-*O*-benzoyl-α,*β*-D-galactofuranose (**5**) in 90% yield.

It is known that the use of a strong base such as DBU, in the synthesis of galactopyranosyl trichloroacetamidates, leads to the more stable α-anomer.[4] However, treatment of **5** with DBU and Cl_3CCN at 0 °C yielded 2,3,5,6-tetra-*O*-benzoyl-*β*-D-galactofuranosyl trichloroacetamidate (**6**) as a very reactive syrup in 85% yield (Scheme 1).[9] This compound

Scheme 1[a]



[a]Legend: (a) 32% HBr in glacial AcOH, 2 h, room temperature; (b) acetone−H_2O, Ag_2CO_3, 40 °C, 40 min, 90%; (c) Cl_3CCN, DBU, 0 °C, 85%.

has to be stored under an argon atmosphere at −20 °C. In the ^{1}H NMR, the signal for H-1 appears at low fields (δ 6.71) with $J_{1,2} < 1$ indicating the *β*-configuration (a trans relationship between H-1 and H-2) for trichloroacetamidate **6**. The ^{13}C NMR spectrum showed signals at 102.9 (C-1), 84.6 (C-4), and 80.8 (C-2), confirming that the *β*-D-galactofuranosyl trichloroacetamidate was formed (Table 1).

Condensation of **6** with **3** proceeded smoothly, by employing TMSOTf as catalyst (Scheme 2), with *β*-stereoselective formation of benzyl 2,3,5,6-tetra-*O*-benzoyl-*β*-D-galactofuranosyl-(1−3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**7**) in 82% yield.[10] Due to a *β*-furanosyl linkage, the ^{1}H NMR spectrum of **7** showed $J_{1',2'} < 1$ Hz and, the C-1′ resonance in the ^{13}C NMR appeared at 105.9 ppm. The resonance of C-3 was shifted downfield to 77.5 ppm, as expected for the glycosidation of OH-3 (Table 1). Hydrolysis of the benzylidene group of **7** was performed with aqueous acetic acid at 85 °C, conditions which did not affect the labile furanosyl linkage. Crystalline benzyl 2,3,5,6-tetra-*O*-benzoyl-*β*-D-galactofuranosyl-(1−3)-2-acetamido-2-deoxy-α-D-glucopyranoside (**8**; mp 196 °C) was obtained in 82% yield. O-Debenzoylation of **8** with sodium methoxide afforded crystalline benzyl glycoside **9** (mp 193−195 °C) in 98% yield. Hydrogenolysis of the benzyl group of **9** by treatment with ammonium formate−10% Pd/C in hot methanol afforded free *β*-D-Gal*f*-(1−3)-D-GlcNAc (**1**) in 98% yield as a crystalline solid (mp 170−172 °C). The anomeric region of the ^{13}C NMR spectrum showed the resonances of C-1′ (109.1 and 109.3 ppm) and C-1 of GlcNAc (91.9 and 95.4 for the α- and *β*-anomers, respectively) with an α:*β* ratio of 3:2, as indicated by the integrals of the anomeric region in the proton NMR. Sodium borohydride reduction of **1** yielded *β*-D-galactofuranosyl-(1−3)-2-acetamido-2-deoxy-D-glucitol (**10**) in 97% yield.

On the other hand, disaccharide **2** could be obtained by the easy $SnCl_4$-promoted glycosylation. As precursor of the reducing end, benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**11**) was selected.[7] Condensation of acid-stable **11** with 1.1 equiv of **4** resulted in a regio- and

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(9) **Procedure for the Preparation of 6.** To a solution of **5** (700 mg, 1.17 mmol) in dry CH_2Cl_2 (13 mL) was added trichloroacetonitrile (0.58 mL, 5.8 mmol). After the mixture was cooled at 0 °C, DBU (0.05 mL, 0.33 mmol) was added; this solution was stirred for 30 min and concentrated in vacuo. The crude product was purified on a short silica gel column to afford 740 mg of trichloroacetamidate **6** as a syrup (85%, R_f 0.65, 10:1 toluene−EtOAc) which was stored at −20 °C under an argon atmosphere: $[\alpha]_D$ −6.6 (c = 2, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.73 (s, N*H*), 8.1−7.1 (m, 20H), 6.71 (s, 1H, H-1), 5.93 (m, 1H, H−5), 5.79 (d, 1H, J = 4.4 Hz, H-3), 5.77 (s, 1H, H-2), 4.87 (t, 1H, J = 4.0 Hz, H-4), 4.82 (dd, 1H, J = 12.1, 5.1 Hz, H-6a), 4.75 (dd, 1H, J = 12.1, 6.6 Hz, H-6b).

(10) **Procedure for the Preparation of 7**. To a solution of acetamidate **6** (700 mg, 0.94 mmol) in dry Cl_2CH_2 (30 mL) and CH_3CN (1 mL) were added anhydrous benzyl 2-acetamido−4,6-*O*-benzylidene-2-deoxy−α-D-glucopyranoside (**3**; 360 mg, 0.90 mmol) and 4 Å powder molecular sieves. The suspension was vigorously stirred for 5 min at room temperature under Ar and then cooled to −20 °C. TMSOTf was added (150 μL, 0.83 mmol) and the stirring continued for 1 h. After the mixture was warmed to room temperature, solid $NaHCO_3$ (200 mg) was added with vigorous stirring and the suspension was filtered over Celite. The filtrate was diluted with Cl_2CH_2 (150 mL), washed with water (3 × 100 mL), dried ($MgSO_4$), filtered, and concentrated. Purification of the residue by silica gel column chromatography (10:1 toluene-EtOAc) yielded **7** (720 mg, 82%) as a foamy solid. Unreacted **3** was also recovered (52 mg, 14%). Compound **7**: R_f 0.56, 2:1 toluene−EtOAc; $[\alpha]_D$ +32.6° (c = 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.1−7.1 (m, 30H), 5.86 (dt, 1H, J = 8.8, 2.9 Hz, H-5′), 5.80 (d, 1H, J = 9.5 Hz, N*H*), 5.48 (d, 1H, J = 4.8 Hz, H-3′), 5.41 , 5.40 (2s, 2H, H-1′, PhC*H*), 5.33 (br s, 1H, H-2′), 4.93 (d, 1H, J = 3.7 Hz, H-1), 4.77, 4.51 (2d, 2H, J = 11.7 Hz, PhCH_2), 4.62 (dd, 1H, J = 4.8, 2.9 Hz, H-4′), 4.51 (m, 1H, H-2), 4.45 (t, 1H, J = 8.8 Hz, H−6′a), 4.26 (dd, 1H, J = 10.2, 4.4 Hz, H−6a), 4.09 (t, 1H, J = 9.7 Hz, H-3), 3.96 (m, 1H, H-5), 3.90 (dd, 1H, J = 8.8, 2.9 Hz, H-6′b), 3.73 (t, 1H, J = 10.2 Hz, H-6b), 3.67 (t, 1H, J = 9.1 Hz, H-4), 1.98 (s, 3H, CH_3). A 2D-COSY ^{1}H NMR correlation spectrum allowed the assigment of the ^{1}H signals. Anal. Calcd for $C_{56}H_{51}NO_{15}$: C, 68.77; H, 5.26. Found: C, 68.76; H, 5.22.

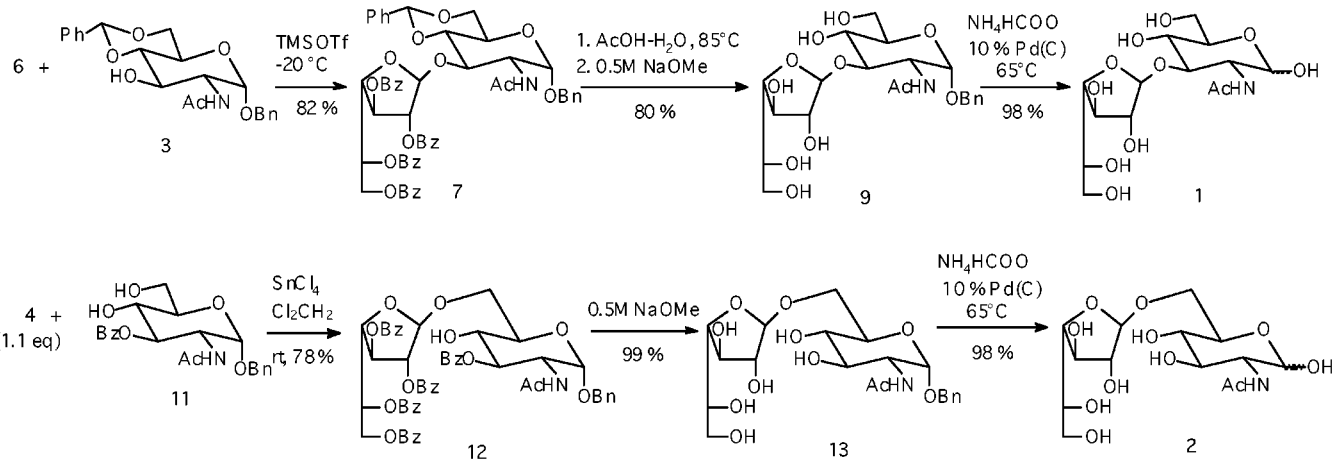
(11) **Procedure for the Preparation of 12.** To an externally cooled (0 °C) solution of 1,2,3,5,6-penta-*O*-benzoyl-α,*β*-D-galactofuranose[7] (**4**; 0.65 g, 0.92 mmol) in dry Cl_2CH_2 (14 mL) was added tin(IV) chloride (0.11 mL, 0.93 mmol). After 15 min of stirring at 0 °C, a solution of **11** (0.35 g, 0.84 mmol) in dry CH_3CN (1 mL) was slowly added, and stirring was continued for 15 h at room temperature. The mixture was diluted with Cl_2CH_2 (40 mL) and poured into saturated aqueous $NaHCO_3$ with vigorous stirring. The aqueous layer was extracted with Cl_2CH_2 (2 × 50 mL), and the combined organic solutions were washed with water until pH 7, dried ($MgSO_4$), filtered, and concentrated. The resulting syrup was purified by column chromatography (9:1 and then 6:1 toluene−EtOAc). First, benzyl 4,6-di-*O*-(2,3,5,6-tetra-*O*-benzoyl-*β*-D-galactofuranosyl)-2-acetamido-3-*O*-benzoyl-2-deoxy−α-D-glucopyranoside (R_f 0.35, 4:1 toluene−EtOAc; 0.06 g, 4%) was eluted. Next, a fraction from the column (R_f 0.23, 4:1 toluene−EtOAc) afforded **12** (0.65 g, 78%), with the same spectroscopic and physical properties as already described.

Table 1. ^{13}C NMR (50.3 MHz) Chemical Shifts for Compounds **1**, **2**, **5**−**10**, **13**, and **14**

compd		C-1	C-2[a]	C-3	C-4	C-5	C-6	CH_2Ph	$CHPh$
1[b]	GlcNAc	91.9 (α), 95.4 (β)	54.1	79.6	69.4	72.4	61.7		
	Gal*f*	109.1, 109.3	82.0	77.3	83.4	71.3	63.7		
2[b]	GlcNAc	91.8 (α), 95.9 (β)	54.9	71.6*	71.2	71.5*	68.0		
	Gal*f*	108.9	81.9	77.7	83.7	71.7*	63.7		
5[c]	Gal*f*	101.0 (β), 96.0 (α)	81.7	77.7	82.7	70.6	63.2		
6[c]	Gal*f*	102.9	80.8	77.0	84.6	70.1	63.4		
7[c]	GlcNAc	97.8	52.9	73.6	80.5	64.2	68.9	70.4#	102.0
	Gal*f*	105.9	82.0**	77.5	82.4**	70.2#	63.4		
8[c]	GlcNAc	96.9	52.2	81.2	70.4*	71.6	62.6#	69.9*	
	Gal*f*	108.8	83.6**	76.6	84.0**	70.3*	62.5#		
9[b]	GlcNAc	97.0	53.7	79.7	70.4*	72.9	61.4	69.2*	
	Gal*f*	109.0	82.0	77.3	83.5	71.3	63.7		
10[b]	GlcNAc-ol	61.3	54.0	77.2	71.0*	71.8*	63.6#		
	Gal*f*	109.5	82.1	77.2	83.9	71.4*	63.7#		
13[b]	GlcNAc	96.8	54.5	71.7*	70.9#	72.0*	67.6	70.7#	
	Gal*f*	108.8	81.9	77.6	83.6	71.7*	63.7		
14[b]	GlcNAc-ol	61.8	54.6	70.8*	70.0*	71.7*	69.3*		
	Gal*f*	108.7	81.9	77.6	83.8	71.7*	63.7		

[a] Signals marked *, #, and ** indicate that they may be interchanged. [b] Recorded in D_2O. [c] Recorded in $CDCl_3$.

Scheme 2



stereoselective glycosylation of the primary OH-6 of **11** to afford benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1−6)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**12**) in 78% yield.[11] This compound was previously obtained as a minor product in the synthesis of benzyl 4,6-di-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside and presented the same spectroscopic and physical data.[7] Further *O*-debenzoylation of **12** with methanolic sodium methoxide gave benzyl β-D-galactofuranosyl-(1−6)-2-acetamido-2-deoxy-α-D-glucopyranoside (**13**; mp 107−108 °C) in 99% yield. Crystalline β-D-Gal*f*-(1−6)-D-GlcNAc (**2**; mp 159−160 °C) was obtained in 98% yield by treatment of **13** with ammonium formate−10% Pd/C in hot methanol. Sodium borohydride reduction of **2** gave the corresponding alditol **14** as a hygroscopic syrup in 99% yield.

The trichloroacetamidate method was recently employed to obtain galactofuranosyl disaccharides as methyl glycosides[5] which are not amenable to hydrolysis, leading to the free sugar, without cleavage of the galactofuranosyl linkage. We have now described the preparation of two free galactofuranosyl disaccharides employing two different procedures for the glycosylation step, depending on the substituents in the acceptor. Moreover, the glycosyl alditols obtained by $NaBH_4$ reduction could be useful for comparison with alditols liberated by reductive β-elimination from mucins of *T. cruzi*.[1]

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Supporting Information Available: Full characterization and proton spectra for compounds **1/2/5/8**−**10/13/14** and a detailed description of experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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