First Synthesis of β-D-Gal*f*(1−4)GlcNAc, a Structural Unit Attached *O*-Glycosidically in Glycoproteins of *Trypanosoma cruzi*

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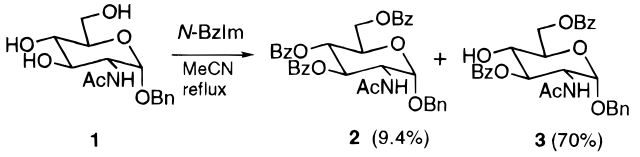
Introduction

The glycobiology of galactofuranose is a topic of increasing interest. This monosaccharide is a constituent of polysaccharides and glycoconjugates from infectious bacteria,[1,2] protozoa,[3,4] and fungi,[5−7] while the mammal host does not biosynthesize glycoconjugates containing Gal*f*. In particular, galactofuranose is a component of glycoinositol phospholipids, with anchor characteristics, isolated from *Trypanosoma cruzi*[8,9] and *Leishmania* species.[10,11] Also, recently, *O*-linked oligosaccharides of different size, all attached by the unit β-D-Gal*f*(1−4)-GlcNAc, have been released by β-elimination from glycoproteins of *T.* cruzi.[4] This is a unique type of linkage, and the presence of Gal*f* could be related to the antigenicity of the glycoprotein.[12] The disaccharide is further branched by different numbers of β-Gal*p* units turning the sugar chain in an acceptor of sialic acid, in a *trans*-sialidase reaction previously characterized in *T.* cruzi.[13,14] Interestingly, the presence of β-D-Gal*f* units in the sialic acid acceptor glycoproteins of *T.* cruzi epimastigote forms is dependent on the strain. Thus, in the Y strain the *O*-linked oligosaccharides do not contain β-galactofuranosyl units.[15]

The synthetic galactofuranose-containing oligosaccharides could be useful for the identification of strains of *T.* cruzi as well as for studies on the inhibition of the biosynthesis of these unique *O*-linked chains. Our laboratory has been involved in the synthesis of galactofuranose disaccharides[16,17] and in the characterization of galactofuranose-containing glycoconjugates from *T.* cruzi.[8,9] This, together with the fact that the synthesis of β-D-Gal*f*(1−4)-D-GlcNAc (**8**) was not previously reported, prompted us to synthesize the disaccharide. The corresponding alditol β-D-Gal*f*(1−4)-D-GlcNAc-ol (**9**) was also prepared, and its ^{1}H and ^{13}C NMR chemical shifts were compared with the data reported for the oligosaccharide alditols obtained by reductive β-elimination of the *T.* cruzi glycoproteins.[4]

Results and Discussion

For the synthesis of the target disaccharide **8** a partially protected derivative of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) having HO-4 free was required. Such an intermediate (**3**) was successfully prepared by selective benzoylation of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[18] (**1**). Acylation with different reagents, under a variety of conditions, of pyranosides having the *gluco* configuration have indicated[19] a lower reactivity for HO-4. In our laboratory, *N*-benzoylimidazole has been successfully employed for the regioselective benzoylation of all the HO-groups, except HO-4, of methyl α-D-glucopyranoside.[20] For this reason, compound **1** was benzoylated with *N*-benzoylimidazole (2.4 mol per mol of **1**) in refluxing acetonitrile. The resulting mixture was chromatographed to give the desired 3,6-di-*O*-benzoyl derivative **3** in ∼70% yield. The perbenzoate **2** was isolated in low yield (9.4%). The structure of **3** was established by comparison of its spectral data with those for **2**. Thus, the H-4 signal in **2** appeared shifted downfield in ∼1.9 ppm, with respect to the same signal in **3**, and the signals for H-3 and H-5 were shifted downfield less than 0.5 ppm, indicating that HO-4 was the free hydroxyl group of **3**. This result was confirmed by the ^{13}C NMR spectra of **2** and **3** (Table 1). As observed for other partially benzoylated derivatives of sugars[21] the benzoylation of a given hydroxyl group causes a small displacement for the α-carbon atom but a larger upfield shift for the β-carbon. Thus, the C-4 signal of **2** (identified by a heteronuclear 2D-COSY experiment) appears 0.4 ppm downfield with respect to the C-4 signal of **3**, whereas the resonances of C-3 and C-5 in the spectrum of **2** were shifted upfield (2.9 and 1.5 ppm, respectively) compared to the same signals in **3**. These displacements were expected to occur on benzoylation of the free hydroxyl group of **3**, if such a group was located on C-4.



1 **2** (9.4%) **3** (70%)

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Table 1. ^{13}C NMR (50.3 MHz) Chemical Shifts for Compounds 1−3 and 5−10

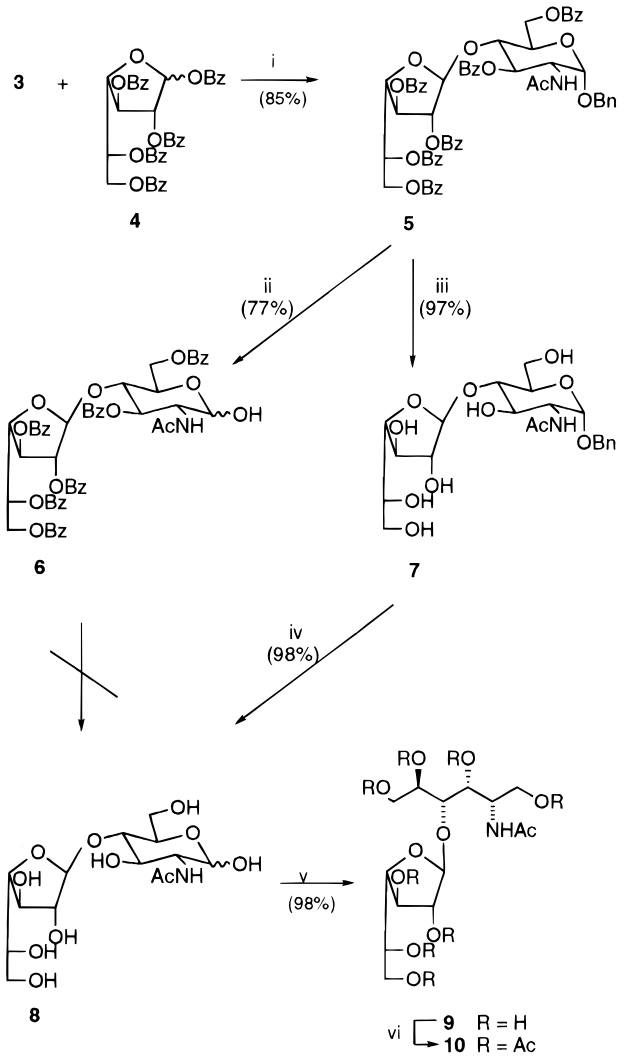
	δ^c (ppm)						
compd	C-1	C-2	C-3	C-4	C-5	C-6	CH_2Ph
1[a]	96.7	54.5	71.7	70.9*	72.9	61.4	70.5*
2[b]	96.7	52.3	71.7	69.3	68.4	62.9	70.2
3[b]	96.8	51.6	74.6	68.9	69.9	63.4	70.7
5[b] GlcNAc	96.7	52.2	72.3	74.7	69.5	62.7	70.0
Gal*f*	107.0	82.3	77.1	82.7	70.1	63.4	
6[b] GlcNAc	91.7	52.6	72.1	74.8	69.0	62.7	
Gal*f*	106.9	82.2	77.1	82.7	70.1	63.4	
7[a] GlcNAc	96.6	54.5	71.8*	77.0	71.3*	60.8	70.2
Gal*f*	108.7	81.9	78.3	83.6	70.6*	63.6	
8[a] GlcNAc	91.5 (α); 95.8 (β)	54.9	71.3*	77.0	71.3*	60.9	
Gal*f*	108.6	81.9	78.5	83.5	70.0*	63.6	
9[a] GlcNAc-ol	61.8	53.6	69.4	78.8	72.1*	62.9	
Gal*f*	109.0	82.1	77.2	83.8	71.4*	63.8	
10[b] GlcNAc-ol	61.6	47.9	70.4#	75.4*	69.5#	62.3	
Gal*f*	106.9	80.9	75.9*	81.6	69.8#	62.7	

[a] Recorded in D_2O. [b] Recorded in $CDCl_3$. [c] Signals marked with * or # may be interchanged.

The condensation of **3** with penta-*O*-benzoyl-D-galactofuranose[22] (**4**) was performed employing tin(IV) chloride as catalyst (Scheme 1). In previous work from this laboratory,[16,17] we have demonstrated the utility of this Lewis acid to activate the anomeric center of peracylated glycofuranose derivatives, leading to the formation of a glycosidic linkage having the 1,2-*trans* configuration. The high stereoselectivity for the glycosylation may be attributed to the participation of the C-2 substituent to stabilize the positive charge on C-1, generated by the remotion of the anomeric substituent by the Lewis acid. The resulting acyloxonium ion (or an orthoester intermediate[23]) blocks one of the sides of C-1 and induces the attack of the HO-donor moiety from the unblocked side, leading to the 1,2-*trans* glycoside. This procedure has the additional advantage that the precursor of the nonreducing end of the disaccharide is a per-*O*-acylated furanose and not a rather unstable glycofuranosyl halide,[24] usually employed as glycosyl donor. As expected, the tin(IV) chloride-catalyzed condensation of **3** and **4** afforded stereoselectively the disaccharide **5** in 85% yield. The anomeric configuration for the furanose unit was established as β by the small value for $J_{1',2'}$ (<1 Hz), indicative of a *trans* relationship between H-1 and H-2. Also, the C-1′ resonance appeared at low field, with a δ value (107.0 ppm) similar to that observed for the anomeric carbon of β-galactofuranosides,[16,17] confirming the β-configuration for C-1′.

In order to remove the benzyl group on C-1 of **5**, hydrogenolysis was attempted under a variety of conditions, but in all the cases, the reaction was extremely slow. The best results were obtained when palladium hydroxide followed by 10% Pd/C were employed as catalysts and the hydrogenation was performed at atmospheric pressure for 10 days. Regardless, only partial hydrogenolysis occurred, compound **6** was isolated by column chromatography in 77% yield, and unreacted **5** (11%) was also recovered. Furthermore, the alkaline *O*-debenzoylation of **6** was unsuccessful, as several decomposition products were detected. Alternatively, *O*-debenzoylation of **5** with sodium methoxide in methanol afforded the crystalline disaccharide glycoside **7** in 97% yield. As observed for **5**, hydrogenation of **7** also led to incomplete reaction. However, heterogeneous catalytic transfer hydrogenolysis[25] provided a highly efficient means for the removal of the *O*-benzyl group.

Scheme 1[a]



[a] Reagents: (i) $SnCl_4$, Cl_2CH_2, 20 h, 0 °C to rt; (ii) H_2 (1 atm), catalyst, 10 days; (iii) NaOMe, MeOH; (iv) NH_4CO_2H, 10% Pd/C, MeOH, reflux, 1 h; (v) $NaBH_4$, 9:1 MeOH−H_2O, 16 h; (vi) Ac_2O, C_5H_5N.

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Treatment of **7** with ammonium formate and 10% Pd/C, in refluxing methanol for 1 h, afforded syrupy **8** in 98% yield. Compound **8** was obtained in crystalline form by slow crystallization from methanol. The disaccharide **8** constitutes the intact linkage unit between the threonine or serine residues of the protein and oligosaccharides of Gal*p* in the 38/43 kDa glycoproteins of *T*. cruzi. The ^{13}C NMR spectrum of **8** showed the resonance for the anomeric carbons of Gal*f* (108.6 ppm) and GlcNAc (91.5 ppm and 95.8 ppm, for the α and β anomers, respectively).

Sodium borohydride reduction of **8** afforded the crystalline alditol Gal*f*-β-(1−4)GlcNAc-ol (**9**), which showed in its ^{13}C NMR spectrum the diagnostic signals (C-anomeric 109.0 ppm, C-1 and C-4 of GlcNAc-ol 61.8 and 78.8) almost identical to those reported for the "disaccharide alditol" isolated from the glycoprotein of *T*. cruzi.[4] Thus, the present study confirms by synthesis the structure of the naturally occurring and biologically interesting disaccharide Gal*f*-β-(1−4)-GlcNAc (**8**). As the synthetic route here described led to **8** in a high overall yield (56.5%), a considerable amount of the compound was available for the complete determination of its physical and spectral properties.

Experimental Section

General. The following solvent systems were used for analytical thin-layer chromatography (TLC): (A) 2:1 toluene−EtOAc, (B) 1:1 toluene−EtOAc, (C) 7:1:1 nPrOH−EtOH−H_2O. TLC was performed on 0.2 mm silica gel 60 F254 (Merck) aluminum-supported plates. Detection was effected by exposure to UV light or by spraying with 5% (v/v) sulfuric acid in EtOH and charring. Column chromatography was performed on silica gel 60 (230−400 mesh, Merck).

Benzyl 2-Acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (3). To a suspension of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[18] (**1**, 1.5 g, 4.8 mmol) in dry acetonitrile (38 mL) was added a solution of *N*-benzoylimidazole (2 g, 11.5 mmol) in dry acetonitrile (10 mL). The mixture was heated at the reflux temperature for 17 h. After cooling, water was added (1 mL) and the solution was stirred for 0.5 h. The solvent was evaporated in vacuo, and the syrupy residue was washed with cold water (15 mL, twice) and then dissolved in dichloromethane (100 mL). The solution was extracted with 5% aqueous HCl, brine, saturated aqueous $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography (2:1 hexane−EtOAc). The fastest migrating component (R_f 0.48, solvent A) was identified as benzyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**2**, 0.28 g, 9.4%) which after recrystallization from methanol gave: mp 159−160 °C; $[\alpha]_D$ +61° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.15−7.25 (m, 20H), 5.91 (d, 1H, *J* = 9.2 Hz, N*H*), 5.81−5.71 (m, 2H), 5.10 (d, 1H, *J* = 3.4 Hz), 4.85 (d, 1H, *J* = 11.7 Hz), 4.64−4.62 (m, 5H), 1.85 (s, 3H). Anal. Calcd for $C_{36}H_{33}NO_9$: C, 69.32; H, 5.34; N, 2.25. Found: C, 69.01; H, 5.21; N, 2.16.

The next fraction from the column (R_f 0.35, solvent A) afforded compound **3** (1.70 g, 70.3%) as a foamy solid: $[\alpha]_D$ +120° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.15−7.25 (m, 15H), 5.85 (d, 1H, *J* = 9.6 Hz, N*H*), 5.39 (dd, 1H, *J* = 10.7, 9.1 Hz), 4.98 (d, 1H, *J* = 3.6 Hz), 4.78 (d, 1H, *J* = 11.8 Hz), 4.75 (dd, 1H, *J* = 12.2, 4.2 Hz), 4.54 (d, 1H, *J* = 11.8 Hz), 4.50 (dd, 1H, *J* = 12.2, 2.1 Hz), 4.47 (ddd, 1H, *J* = 10.7, 9.6, 3.6 Hz), 4.07 (ddd, 1H, *J* = 9.8, 4.2, 2.1 Hz), 3.85 (ddd, 1H, *J* = 9.8, 9.1, 4.4 Hz), 3.38 (d, 1H, *J* = 4.4 Hz, O*H*), 1.79 (s, 3H). Anal. Calcd for $C_{29}H_{29}NO_8$: C, 67.03; H, 5.63; N, 2.70. Found: C, 67.08; H, 5.56; N, 2.47.

Benzyl 2-Acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-α-D-glucopyranoside (5). To an externally cooled (0 °C) solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose (**4**, 0.61 g, 0.87 mmol) in dry dichloromethane (5 mL) was added tin(IV) chloride (0.11 mL, 0.93 mmol). After 10 min of stirring at 0 °C, a solution of **3** (0.30 g, 0.578 mmol) in dry dichloromethane (5 mL) was slowly added, and the stirring was continued for 20 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 × 30 mL), and the combined organic solutions were washed with water until pH 7, dried ($MgSO_4$), filtered, and concentrated. TLC monitoring of the residue showed a main product having R_f 0.50 (solvent A). This product was isolated by column chromatography (9:1 toluene−EtOAc and then 5:1 toluene−EtOAc), affording compound **5** (0.54 g, 85%) as a foamy solid: $[\alpha]_D$ +63° (*c* 0.9, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.15−7.10 (m, 35H), 5.80 (d, 1H, *J* = 9.6 Hz, N*H*), 5.70−5.60 (m, 2H), 5.60 (d, 1H, *J* = 4.8 Hz), 5.46 (s, 1H), 5.36 (d, 1H, *J* = 0.9 Hz), 5.01 (d, 1H, *J* = 3.7 Hz), 4.87 (d, 1H, *J* = 13.1 Hz), 4.81 (d, 1H, *J* = 11.9 Hz), 4.64 (d, 1H, *J* = 13.1 Hz), 4.58 (d, 1H, *J* = 11.9 Hz), 4.70−4.45 (m,1H), 4.40−4.20 (m, 5H), 1.76 (s, 3H). Anal. Calcd for $C_{63}H_{55}NO_{17}$: C, 68.91; H, 5.05; N, 1.28. Found: C, 69.00; H, 5.19; N, 1.32.

2-Acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-α,β-D-glucopyranose (6). A suspension of 10% $Pd(OH)_2/C$ (0.10 g) in EtOAc (2 mL) was hydrogenated at 15 psi (1 atm) for 1 h, and then a solution of compound **5** (0.70 g, 0.64 mmol) in EtOAc (7 mL) was added. The mixture was hydrogenated for 3 days at atmospheric pressure. The catalyst was filtered, and 10% Pd/C (0.10 g) was added to the filtrate. Further hydrogenation (1 atm) for 7 additional days afforded, after filtration and concentration, a syrup which was purified by column chromatography (1:1 toluene−EtOAc). The starting material **5** (0.08 g, 11%) was first eluted (R_f 0.75, solvent B). The next fraction from the column afforded compound **6** (0.497 g, 77%) as an α,β mixture (R_f 0.31 and 0.19, solvent B) that was recrystallized from 9:1 EtOH−water: mp 199−202 °C, $[\alpha]_D$ +46.3° (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{56}H_{49}NO_{17}$: C, 66.73; H, 4.90; N, 1.39. Found: C, 66.98; H, 5.17; N, 1.23.

Benzyl 2-Acetamido-2-deoxy-4-*O*-β-D-galactofuranosyl-α-D-glucopyranoside (7). To a suspension of **5** (1.7 g, 1.55 mmol) in anhydrous methanol cooled at 0 °C was added 0.55 M sodium methoxide in methanol (18.6 mL). After being stirred for 1 h at 0 °C, the reaction mixture was warmed to room temperature. The stirring was maintained for an additional hour, and then water (1 mL) was added. The solution was passed through a column (1.5 cm × 6 cm) containing BioRad AG 50W-X12 (H^+) resin. The solvent was removed under vacuum, and the remaining methyl benzoate was eliminated by five successive coevaporations with water to afford **7** as a crystalline white solid (0.71 g, 97%), R_f 0.68, solvent C. After recrystallization from 2:1 EtOAc−methanol it had: mp 196.5−197.5 °C; $[\alpha]_D$ +58° (*c* 1, H_2O); ^1H NMR (200 MHz, D_2O) anomeric region δ 5.03 (bs, 1H), 4.88 (d, 1H, *J* = 2.8 Hz). Anal. Calcd for $C_{21}H_{31}NO_{11}\cdot H_2O$: C, 51.32; H, 6.77; N, 2.85. Found: C, 51.77; H, 7.17; N, 2.90.

2-Acetamido-2-deoxy-4-*O*-β-D-galactofuranosyl-α,β-D-glucopyranose (8). To a solution of **6** (0.30 g, 0.63 mmol) in methanol (30 mL) were added 10% Pd/C (60 mg) and ammonium formate (0.10 g, 1.59 mmol). The mixture was heated under reflux for 1 h in a water bath and then filtered and concentrated. The resulting syrup was dried in vacuo at 50 °C for 10 min, affording an amorphous solid, which was dissolved in MeOH (2 mL) and passed through a column of BioRad AG 501-X8 mixed resin. Evaporation of the solvent under vacuum gave pure compound **8** (0.253 g, 98%) which slowly crystallized from MeOH: mp 191−192 °C; $[\alpha]_D$ −48° (*c* 1, H_2O); R_f 0.52 and 0.47 (solvent C) for the α and β anomers, respectively; ^1H NMR (200 MHz, D_2O) anomeric region δ 5.15 (d, *J* = 2.8 Hz, α anomer), 5.05 (bs, 1H). The signal for the β anomer of GlcNAc was overlapped with that of DHO. Anal. Calcd for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57; N, 3.65. Found: C, 44.00; H, 6.45; N, 3.57.

2-Acetamido-2-deoxy-4-*O*-β-D-galactofuranosylglucitol (9). To a solution of **8** (91.5 mg, 0.24 mmol) in 9:1 methanol−water (10 mL) was added sodium borohydride (90.2 mg, 2.34 mmol), and the mixture was stirred overnight at room temperature. The solution was decationized by elution through a column of BioRad AG 50W-X12 (H^+ form) resin. The solvent was evaporated, and the boric acid was eliminated by five successive coevaporations with methanol and finally by ion-exchange column chromatography on BioRad AG 501-X8 resin. Evaporation of the solvent

afforded compound **9** (92 mg, 100%) as a chromatographically homogeneous (R_f 0.34, solvent C) colorless syrup, which slowly crystallized from methanol: mp 161−162 °C; $[\alpha]_D$ −42° (*c* 0.8, H_2O); ^{1}H NMR (200 MHz, D_2O) anomeric region δ 5.21 (bs, 1H). Anal. Calcd for $C_{14}H_{27}NO_{11}$: C, 43.63; H, 7.06; N, 3.63. Found: C, 43.90; H, 7.01; N, 3.77.

Compound **9** was conventionally acetylated to give syrupy **10**, whose ^{1}H- and ^{13}C-NMR spectra were recorded: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.86 (d, 1H, J = 9.5 Hz, N*H*), 5.33 (dt, 1H, J = 6.8, 4.4 Hz), 5.16 (bs, 1H), 5.18−5.01 (m, 4H), 4.63 (m, 1H), 4.51 (dd, 1H, J = 12.5, 3.2 Hz), 4.44−3.94 (m, 8H), 2.08−1.95 (9 s, Ac).

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