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One-pot synthesis of β-D-Gal*f*(1 → 4)[β-D-Gal*p*(1 → 6)]-D-GlcNAc, a 'core' trisaccharide linked *O*-glycosidically in glycoproteins of *Trypanosoma cruzi* [1]

Carola Gallo-Rodriguez, Oscar Varela, Rosa M. de Lederkremer *

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

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Abstract

Tin(IV) chloride-promoted condensation of benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**4**) with penta-*O*-benzoyl-β-D-galactopyranose (**6**) gave the derivative of β-D-Gal*p*-(1 → 6)-α-D-GlcNAc **7** in 80% yield. This was glycosylated with penta-*O*-benzoyl-α,β-D-galactofuranose (**5**), employing the same catalyst, to afford the protected benzyl per-*O*-benzoyl-β-D-Gal*f*(1 → 4)[β-D-Gal*p*(1 → 6)]D-GlcNAc **10** in 41% yield. Alternatively, compound **10** was obtained directly in a one-pot reaction from **4**, by sequential addition of **6** and **5** (34% yield). β-Glycosidic linkages were diastereoselectively formed. De-*O*-benzoylation of **10**, followed by heterogeneous catalytic transfer hydrogenolysis of the benzyl group afforded the free trisaccharide β-D-Gal*f*(1 → 4)[β-D-Gal*p*(1 → 6)]-D-GlcNAc (**14**) in 98% yield from **10**. Sodium borohydride reduction of **14** gave the corresponding alditol, whose spectral data were identical to those reported for the alditol obtained from the 38–43 kDa cell-surface glycoprotein of *Trypanosoma cruzi*. © 1998 Elsevier Science Ltd

Keywords: Galactofuranose; Trisaccharide; *Trypanosoma cruzi*

1. Introduction

Glycoproteins containing a novel class of *O*-linked oligosaccharide chains have been characterized in the protozoan parasite *Trypanosoma cruzi*, the agent of Chagas' disease [1,2], and have been defined as mucin-like glycoproteins [3].

Oligosaccharides, which are the major acceptors of sialic acid in a tran-sialidase reaction [4,5] are linked to threonine and/or serine through GlcNAc rather than GalNAc units which are commonly found in vertebrate mucins. Most of the *O*-linked GlcNAc residues are substituted with one to five galactosyl units. The presence of β-D-Gal*f* units is dependent on the protozoan strain, for example, in the Y strain the *O*-linked oligosaccharides contain only galactopyranose [6]. Both Gal*f* and Gal*p* units are present in the oligosaccharides of the G strain [1] and all of them

* Corresponding author.

[1] Dedicated to Professor Eduardo G. Gros on the occasion of his 65th birthday.

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contain the β-D-Gal*f*(1 → 4)GlcNAc structure, recently synthesized in our laboratory [7]. Further substitution occurs with β-D-Gal*p*, which turns the sugar chain into an acceptor of sialic acid. This group of oligosaccharides contain the common structure β-D-Gal*f*(1 → 4)[β-D-Gal*p*(1 → 6)]-D-GlcNAc. The presence of β-D-Gal*f* units in *T. cruzi* glycoproteins represents an important site for antibody recognition [8,9].

Synthetic galactofuranose-containing oligosaccharides should be useful for the identification of strains of *T. cruzi*, as well as for studies on inhibition of the biosynthesis of these unique *O*-linked chains. This, together with the fact that the synthesis of β-D-Gal*f*(1 → 4)[β-D-Gal*p*(1 → 6)]-D-GlcNAc has not been previously reported, prompted us to synthesize the trisaccharide as well as the corresponding alditol, which has been previously isolated by reductive β-elimination of *T. cruzi* glycoproteins [1,2].

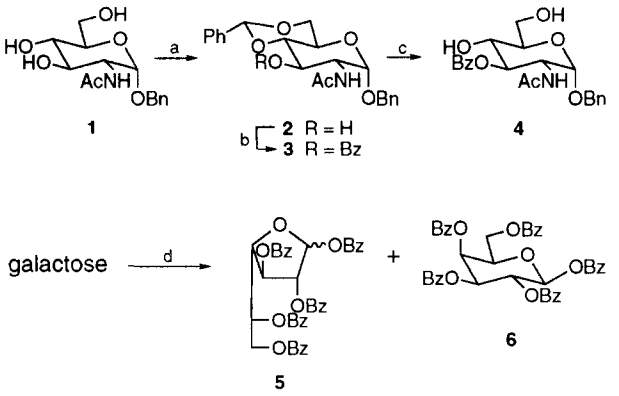
2. Results and discussion

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**) was obtained as already reported [10]. The key glycosyl acceptor intermediate, benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**4**) was readily prepared by a procedure (Scheme 1) which improved the overall yield (68%) in comparison with that described previously [10] (36%). Thus, Evans benzylidenation [11] of **1** with α,α-dimethoxytoluene in DMF, containing a trace of *p*-toluenesulfonic acid, afforded **2** in 88% yield. Benzoylation of **2** gave benzoate **3** in 95% yield. On removal of the benzyli-

Table 1
^{13}C NMR (50.3 MHz) chemical shifts for compounds **2–4**, **6–9**, and **10–15**

Compound	δ (ppm)							
	C-1	C-2	C-3	C-4	C-5	C-6	CH_2Ph	CHPh
2[a] GlcNAc	97.3	54.6	68.5*	82.0	63.1	67.7	69.3*	101.5
3[b] GlcNAc	97.5	52.7	70.8*	79.3	63.4	68.9	70.2*	101.6
4[b] GlcNAc	96.9	51.9	75.0	69.3*	72.1	62.0	70.0*	
6[b] Gal*p*	93.1	68.8	71.6	68.0	72.5	61.8		
7[b] GlcNAc	96.4	51.3	75.4	69.4*	69.8*	68.1	70.9*	
Gal*p*	102.2	69.4*	71.5*	69.4*	71.5*	62.0		
8[c] GlcNAc	96.8	54.5	71.7*	70.7	72.0*	69.2**	70.7	
Gal*p*	104.3	71.7*	73.6	69.6**	76.0	61.9		
9[c] GlcNAc[d]	91.8(α), 95.9(β)	54.9	71.3	71.5*	72.9*	69.5		
Gal*p*	104.2	71.6*	73.6	70.8	76.0	61.9		
10[b] GlcNAc	96.4	52.0	69.9*	75.9	71.4**	67.4***	69.9*	
Gal*p*	101.7	69.5*	71.5**	68.1***	72.6**	61.9		
Gal*f*	107.0	80.9	76.4	82.5	69.7*	62.9		
11[c] GlcNAc	97.0	52.6	72.6	73.9	70.4*	70.0*	70.4*	
Gal*f*	106.4	82.2	77.0	82.8	70.4*	63.5		
Gal*f*	105.3	81.3	77.1	82.5	70.2*	63.9		
12[c] GlcNAc	96.8	51.8	75.1	69.3	71.2	67.0	69.8	
Gal*f*	106.5	81.2	77.3	82.5	70.2	63.5		
13[b] GlcNAc	96.7	54.4	69.6*	78.0	70.3*	68.3*	70.8*	
Gal*p*	104.1	71.5*	73.6	70.6*	76.0	61.9		
Gal*f*	108.5	81.9	76.9	83.5	71.4*	63.7		
14[c] GlcNAc[d]	91.6(α), 95.9(β)	54.8	69.5*	78.1	70.1*	68.6*		
Gal*p*	104.0	71.4*	73.5	70.1*	76.0	61.8		
Gal*f*	108.5	81.7	76.8	83.5	71.4*	63.6		
15[c] GlcNAc	61.6	53.5	69.1	78.6	70.6	71.2		
Gal*p*	104.1	71.7	73.5	69.5	76.0	61.9		
Gal*f*	108.9	82.1	77.1	83.5	71.4	63.8		

[a]Recorded in Me_2SO-d_6.
[b]Recorded in $CDCl_3$.
[c]Recorded in deuterium oxide.
[d]Data for the α anomer.
Signals marked with *, ** or *** may be interchanged.

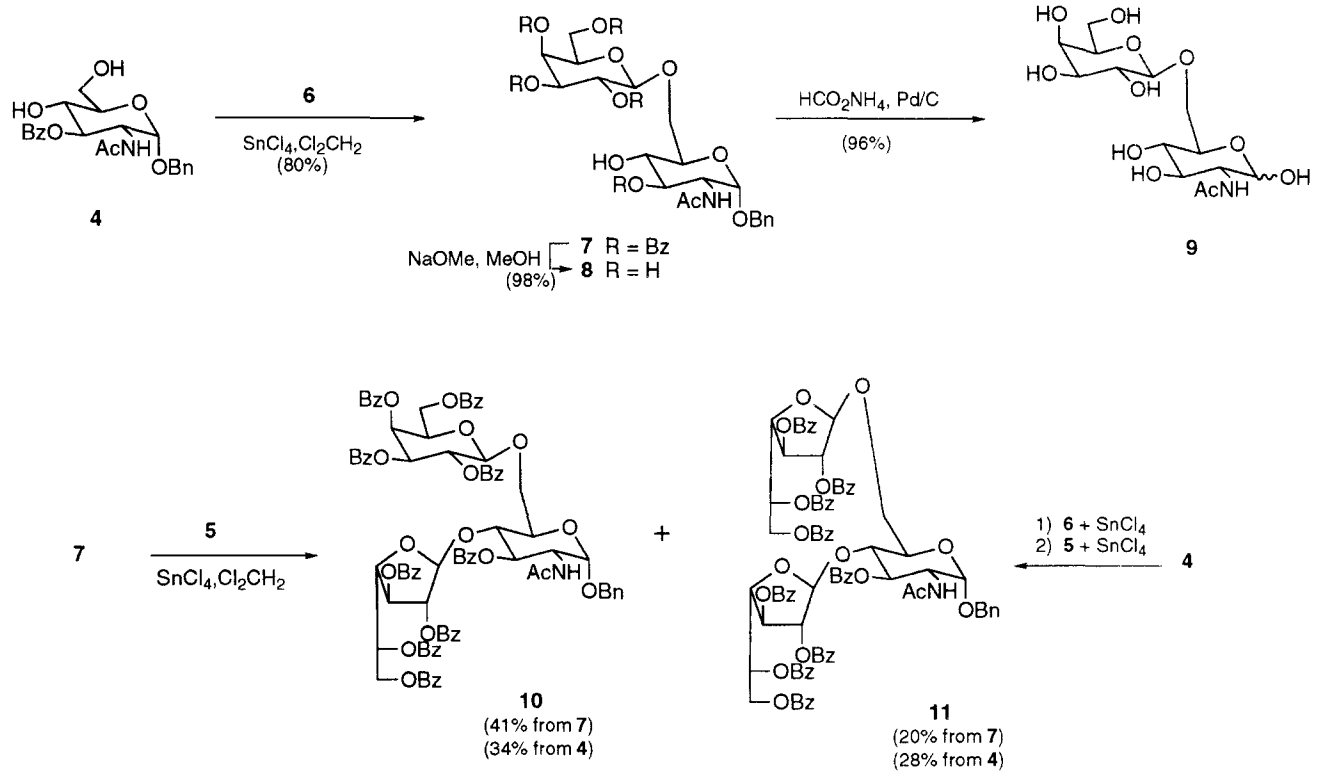


Scheme 1. (a) α,α-dimethoxytoluene, *p*-toluenesulfonic acid, DMF, 60 °C, 88%; (b) benzoyl chloride, C_5H_5N, 95%; (c) AcOH–water, 100 °C, 81%; (d) benzoyl chloride, C_5H_5N, 100 °C.

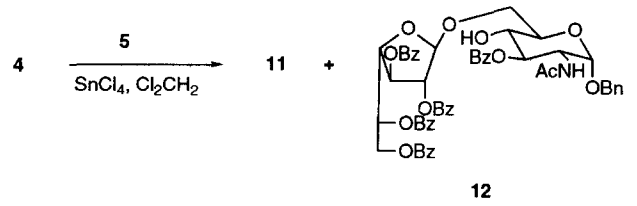
dene group with aqueous acetic acid, **4** was obtained in 81% yield.

The two galactosyl donors were obtained by high-temperature benzoylation of D-galactose. Penta-*O*-benzoyl-α,β-D-galactofuranose (**5**) crystallized from EtOH [12] and from the mother liquors crystalline penta-*O*-benzoyl-β-D-galactopyranose (**6**, Scheme 1), not previously described, was obtained. The ^{13}C NMR spectrum showed the C-1 resonance at 93.1 ppm, but no signals in the region where C-4 and C-2 of benzoylated Gal*f* are observed (75–85 ppm). Furthermore, the large $J_{1,2}$ value (8.3 Hz) in its ^1H NMR spectrum, indicated a β-pyranosyl configuration for the anomeric center. The signals in the ^{13}C NMR spectrum (Table 1) were assigned by comparison with the corresponding acetylated compound [13].

We have employed tin(IV) chloride to promote the condensation of peracylated galactofuranose with alcohols or sugar derivatives having a free OH-group [7,14,15]. This reaction leads to stereoselective formation of 1,2-*trans* glycosides, as the acyloxy substituent at C-2 can participate anchimerically via an acyloxonium ion [16]. The tin(IV) chloride-catalyzed glycosylation proved to be successful when furanoses act as glycosyl donors and when selectively protected aldonolactones were the glycosyl acceptors [14,15]. Excellent yields were obtained since only C-1 of the furanosyl unit can be activated by the Lewis acid. This glycosylation procedure gave poor yields when partially protected monosaccharides were used as precursors of the reducing end. However, we found [7] that the anomeric linkage of benzyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside was stable under the conditions employed for tin(IV)



Scheme 2.



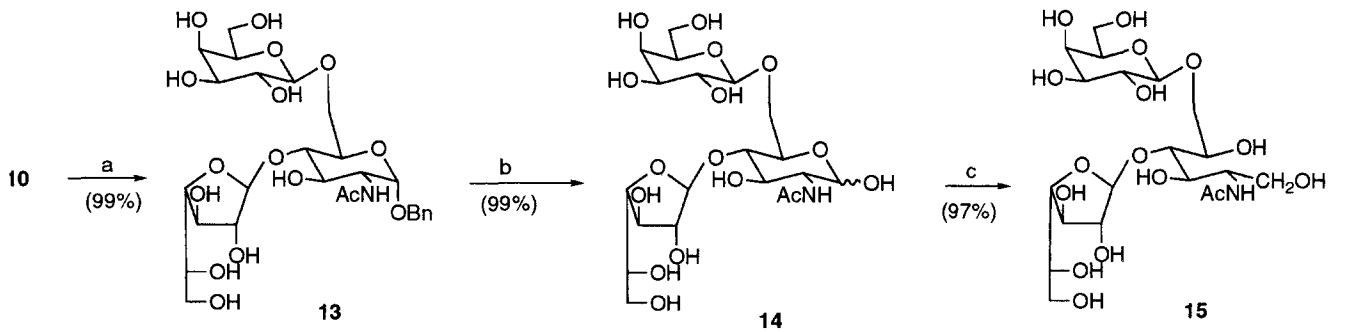
Scheme 3.

chloride-promoted glycosylation of penta-*O*-benzoyl-D-galactofuranose (**5**). Activation of the anomeric center by removal of the C-1 substituent is more easily accomplished in a furanosyl than in a pyranosyl derivative. Considering the stability of a benzylated 2-acetamido-2-deoxy-α-D-glucopyranosyl derivative, we studied the tin(IV) chloride-catalyzed condensation of acceptor **4** by the per-*O*-benzoyl pyranosyl derivative **6** (Scheme 2). The reaction was highly regio- and stereoselective, affording disaccharide **7** in 80% yield. The large $J_{1',2'}$ (7.8 Hz) value indicated a β-configuration for the Gal*p* anomeric center. The ^{13}C NMR spectrum of **7** (Table 1) confirmed that OH-6 had been regioselectively glycosylated, because of the large downfield shift [13] of the C-6 resonance (~ 6 ppm) with respect to that of **4**. The structure of **7** was confirmed by conversion into the known β-D-Gal*p*(1 → 6)-D-GlcNAc (**9**). *O*-Debenzoylation of **7** with sodium methoxide afforded crystalline benzyl glycoside **8**, in 98% yield. The *O*-benzyl group of **8** was efficiently removed by heterogeneous catalytic hydrogenolysis [17], with ammonium formate and 10% Pd/C in hot methanol. The resulting free disaccharide **9** gave the same optical rotation as that previously reported [18]. However, in our case **9** was obtained crystalline, and its ^{1}H and ^{13}C NMR were also recorded. The latter gave rise to a single signal for C-1 of Gal*p*, but those of the reducing end consisted of two signals at 91.8 and 95.5 ppm, corresponding to the α and β-anomers [13], respectively (ratio ~ 2:1). A similar composition was also indicated by the ^{1}H NMR spectrum, according to signals at 5.12 ($J_{1,2}$ 3.4 Hz, α-anomer) and 4.37 ppm ($J_{1,2}$ 7.4 Hz, β-anomer).

The high regioselectivity in favor of glycosylation of OH-6 of the diol **4** should be expected, since the primary OH-6 is less hindered than the OH-4 group. Interestingly, we also found that OH-4 could not be glycosylated even when **4** or **7** were treated with an excess of **6** in the presence of stannic chloride. However, glycosylation of the OH-4 group of **7** occurred with an excess of penta-*O*-benzoyl-D-galactofuranose (**5**), and the trisaccharide derivative **10** was obtained as a foamy solid in 41% yield. The ^{13}C NMR spectrum of **10** contained, in the C-1 region resonances due to α-GlcNAc (97.0 ppm), β-Gal*p* (101.7 ppm), and β-Gal*f* (107.0 ppm). The *J* values for H-1 of each monosaccharide unit also confirmed the anomeric configurations: 4.96 ($J_{1,2}$ 3.6 Hz), 5.13 ($J_{1',2'}$ 7.7 Hz) and 5.08 ppm ($J_{1'',2''} < 1$ Hz).

In the condensation of **7** with **5**, a byproduct (**11**) was obtained in addition to **10**. Its ^{13}C NMR showed a C-1 signal of GlcNAc at 97.0 ppm and two signals (106.4 and 105.3 ppm), indicating the presence of two Gal*f* moieties. Compound **11** was thus characterized as the product of transglycosylation, namely the per-*O*-benzoylated derivative of β-D-Gal*f*(1 → 4)[β-D-Gal*f*(1 → 6)]-D-GlcNAc.

The structure of **11** was confirmed by a direct and independent synthesis (Scheme 3). Glycosylation of **4** with **5** in the presence of tin(IV) chloride, gave **11** as the major product (63% yield,) and the monoglycosylated derivative of **4** as byproduct (**12**, 27% yield). The ^{13}C NMR spectrum of **12** showed a signal at 106.5 ppm corresponding to C-1 of the Gal*f* unit and a downfield shift (5 ppm) for the C-6 signal with respect to the same signal in **4**, indicating that glycosylation took place at OH-6. The structure of **12** was then established as benzyl 2-acetamido-3-*O*-benzoyl-



Scheme 4. (a) NaOMe, MeOH; (b) HCO_2NH_4, 10% Pd/C, MeOH, 65 °C, 1 h; (c) $NaBH_4$, MeOH.

2-deoxy-6-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-α-D-glucopyranoside.

As the two-step conversion of **4** into **10** involved the same catalyst and reaction conditions, and that no glycosylation of the OH-4 group of **7** by **6** took place, suggested that the process could be simplified by employing a one-pot reaction. Accordingly, **4** was treated with penta-*O*-benzoyl-β-D-galactopyranose (**6**) in the presence of tin(IV) chloride, until TLC monitoring showed complete reaction of **4**. Following addition of a solution of penta-*O*-benzoyl-α,β-D-galactofuranose (**5**) and tin(IV) chloride in CH_2Cl_2, the expected trisaccharide **10** was formed and isolated in a yield (34%) comparable to that of the previous synthesis. The one-pot procedure provided a direct way to obtain the protected trisaccharide **10** from readily available and stable peracylated glycosyl donors, avoiding isolation and purification of the intermediate disaccharide **7**.

O-Debenzoylation of **10** with sodium methoxide afforded the crystalline trisaccharide benzyl glycoside **13** in 99% yield (Scheme 4). Hydrogenolysis of the benzyl group of **13** with ammonium formate–10% Pd/C in hot methanol led to glassy **14**, in 99% yield. The ^{13}C NMR spectrum of **14** contained C-1 resonances of Gal*f* (108.5 ppm), Gal*p* (104.0 ppm) and GlcNAc (91.6 and 95.9 ppm for the α and β anomers, respectively). The trisaccharide **14** constitutes a new core structure in oligosaccharides linked to threonine or serine residues of proteins [19].

Sodium borohydride reduction of **14** afforded the glassy alditol β-D-Gal*f*(1 → 4)[β-D-Gal*p*(1 → 6)]-D-GlcNAc-ol (**15**) in 97% yield. The ^1H and ^{13}C NMR spectra of synthetic **15** were identical to those reported for the disubstituted alditol liberated by reductive β-elimination from mucins of the G strain of *T. cruzi* [1].

3. Experimental

General methods.—Melting points were determined with a Thomas–Hoover apparatus. Optical rotations were measured with a Perkin–Elmer 343 polarimeter. NMR spectra were recorded with a Bruker AC 200 spectrometer at 200 MHz (^1H) and 50.3 MHz (^{13}C). Chemical shifts were tentatively assigned by comparison with the spectra of related compounds. Analytical TLC was performed on 0.2 mm Silica Gel 60F254 (Merck) aluminium supported plates. Detection was effected by exposure to UV light or spraying with 5% (v/v) sulfuric acid in EtOH and heat charring. Column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck).

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (2).—A suspension of dry benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside [10] (**1**, 4 g, 12.9 mmol), α,α-dimethoxytoluene (1.93 mL, 12.9 mmol), *p*-toluenesulfonic acid (6.5 mg) in anhydrous DMF (10 mL) was placed in an evaporator and rotated under vacuum at 60 °C for 2.5 h. DMF was distilled off by raising the temperature to 100 °C, affording a solid which was macerated with 2% NaHCO3 (20 mL) at room temperature and then heated at 100 °C. The mixture was cooled to 0 °C, filtered and washed with water, to afford **2** (4.5 g, 88%) as a crystalline solid: mp 260–262 °C (EtOH), lit. 260–261 °C from pyridine–H_2O [10]; $[\alpha]_D$ +91° (*c* 1, pyridine); ^1H NMR ($CDCl_3$): δ 7.52–7.36 (m, 10 H), 5.82 (d, 1 H, *J* 9.3 Hz, N*H*), 5.58 (s, 1 H, PhC*H*), 4.95 (d, 1 H, *J* 3.8 Hz, H-1), 4.77, 4.51 (d, 2 H, *J* 11.8 Hz, PhCH_2), 4.31–4.19 (m, 2 H), 4.01–3.57 (m, 4 H), 3.02 (bs, 1 H, O*H*), 2.00 (s, 3 H, CH_3).

Benzyl 2-acetamido-3-O-benzoyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**3**)).—To a suspension of **2** (2.0 g, 5.0 mmol) in pyridine (17 mL), benzoyl chloride (1.16 mL, 10 mmol) was slowly added. After stirring at room temperature for 3 h, the solution was poured into ice-water (100 mL) affording a white precipitate which was washed with water five times. The solid was dissolved in CH_2Cl_2 (100 mL) and was extracted with saturated aqueous $NaHCO_3$ (2 × 100 mL), water (2 × 100 mL), dried ($MgSO_4$) and filtered. Further evaporation of the solvent afforded **3** (2.4 g, 95%), which from EtOH gave: mp 233–234 °C, lit. 218–220 °C from pyridine–H_2O [10]; $[\alpha]_D$ +30° (*c* 1, pyridine); ^1H NMR ($CDCl_3$): δ 8.03 (d, 2 H, *J* 7.3 Hz), 7.60–7.28 (m, 13 H), 5.90 (d, 1 H, *J* 9.2 Hz, N*H*), 5.64 (t, 1 H, *J* 9.3 Hz, H-3), 5.58 (s, 1 H, PhC*H*), 5.01 (d, 1 H, *J* 3.5 Hz, H-1), 4.80 (d, 1 H, *J* 11.8 Hz, PhCH_2), 4.56 (m, 2 H, PhCH_2, H-2), 4.30 (dd, 1 H, *J* 4.3, 10.1 Hz, H-6), 4.04 (dt, 1 H, *J* 4.3, 9.3 Hz, H-5), 3.91 (t, 1 H, *J* 9.3 Hz, H-4), 3.83 (t, 1 H, *J* 10.1 Hz, H-6′), 1.82 (s, 3 H, CH_3).

Benzyl 2-acetamido-3-O-benzoyl-2-deoxy-α-D-glucopyranoside (**4**).—To a stirred solution of **3** (1.5 g, 3.0 mmol) in HOAc (40 mL) at 100 °C, water (25 mL) was slowly added and heating continued for 1 h. The mixture was cooled and concentrated, and the residue subjected to successive dissolution and evaporation of water (4 × 10 mL) and then of toluene

(2 × 10 mL) to afford a syrup which from toluene gave **4** (1.0 g, 81%): mp 113–115 °C, lit. 95–97 °C from benzene [10]; $[\alpha]_D$ +75° (*c* 1, pyridine); ^{1}H NMR ($CDCl_3$): δ 8.03 (d, 2 H, *J* 8.7 Hz), 7.59–7.31 (m, 8 H), 5.90 (d, 1 H, *J* 9.6 Hz, N*H*), 5.64 (dd, 1 H, *J* 9.0, 10.7 Hz, H-3), 4.95 (d, 1 H, *J* 3.6 Hz, H-1), 4.74, 4.50 (d, 2 H, *J* 11.8 Hz, PhCH_2), 4.42 (ddd, 1 H, *J* 3.6, 9.6, 10.7 Hz, H-2), 3.98 (t, 1 H, *J* 9.0 Hz, H-4), 3.85–3.79 (m, 3 H), 3.41 (bs, 1 H, O*H*), 2.30 (bs, 1 H, O*H*), 1.78 (s, 3 H, CH_3).

*1,2,3,5,6-Penta-*O*-benzoyl-α,β-*D*-galactofuranose* (**5**) *and 1,2,3,4,6-penta-*O*-benzoyl-β-*D*-galactopyranose* (**6**).—D-Galactose (5 g, 0.028 mol) in pyridine (70 mL) was heated with the exclusion of moisture in a boiling-water bath for 2 h. The mixture was cooled to 60 °C and benzoyl chloride (20 mL, 0.17 mol) was slowly added. After stirring at 60 °C for 1.5 h, water (10 mL) was added, and the stirring continued for 0.5 h at room temperature. The solution was slowly poured into ice-water (500 g) with stirring, affording an amorphous solid. The liquors were decanted and the remaining solid was washed five times with water. The solid was dissolved in boiling EtOH (800 mL) and slow crystallization took place. After two days, the crystals of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose (**5**, 6.4 g, 34%, R_f 0.60 and 0.54, 9:1 toluene–EtOAc) were obtained. The mother liquors were kept at room temperature for 10 days and the second crop of crystals was filtered. The compound was characterized as 1,2,3,4,6-penta-*O*-benzoyl-β-D-galactopyranose (**6**, 7.2 g, 37%; R_f 0.58, 9:1 toluene–EtOAc). It gave: mp 129–131 °C (EtOH); $[\alpha]_D$ +89.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.15–7.15 (m, 25 H), 6.33 (d, 1 H, *J* 8.3 Hz, H-1), 6.12 (dd, 1 H, *J* 10.2, 8.3 Hz, H-2), 6.11 (d, 1 H, *J* 3.4 Hz, H-4), 5.82 (dd, 1 H, *J* 10.2, 3.4 Hz, H-3), 4.69 (dd, 1 H, *J* 9.4, 6.0 Hz, H-6), 4.52–4.68 (m, 1 H, H-5), 4.47 (dd, 1 H, *J* 9.4, 5.1 Hz, H-6′). Anal. Calcd for $C_{41}H_{32}NO_{11}$: C, 70.28; H, 4.57. Found: C, 70.15; H, 4.70.

*Benzyl (2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1 → 6)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-*D*-glucopyranoside* (**7**).—To a cooled (0 °C) solution of 1,2,3,4,6-penta-*O*-benzoyl-β-D-galactopyranose (**6**, 1.21 g, 1.73 mmol) in dry CH_2Cl_2 (12 mL), tin(IV) chloride (0.25 mL, 2.14 mmol) was added. After 15 min stirring at 0 °C, benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**4**, 0.60 g, 1.42 mmol), in a 1:1 mixture of dry CH_2Cl_2–acetonitrile (12 mL), was slowly added and the solution stirred at room temperature for 16 h. The mixture was diluted with CH_2Cl_2 (75 mL) and poured into saturated aqueous $NaHCO_3$ (30 mL) with vigorous stirring. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic solutions washed with water until the washing liquids reached pH 7, dried ($MgSO_4$), filtered and concentrated. The resulting amorphous solid crystallized from EtOH (10 mL) affording **7** (1.0 g, 70% yield). From the mother liquors an additional crop of crystals (0.15 g, 80% overall yield) was obtained after column chromatography (9:1 toluene–EtOAc and then 5:1 toluene–EtOAc). It gave: mp 207–208 °C (EtOH); $[\alpha]_D$ +106° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.05–7.00 (m, 30 H), 5.92 (d, 1 H, *J* 3.2 Hz, H-4′), 5.78 (dd, 1 H, *J* 10.3, 7.8 Hz, H-2′), 5.71 (d, 1 H, *J* 9.5 Hz, N*H*), 5.54 (dd, 1 H, *J* 10.3, 3.2 Hz, H-3′), 5.14 (dd, 1 H, *J* 10.4, 9.3 Hz, H-3), 4.86 (d, 1 H, *J* 7.8 Hz, H-1′), 4.71 (d, 1 H, *J* 3.6 Hz, H-1), 4.60 (dd, 1 H, *J* 10.6, 5.8 Hz), 4.51, 4.16 (d, 2 H, *J* 11.8 Hz, PhCH_2), 4.40–4.20 (m, 3 H), 3.88–3.70 (m, 2 H), 3.63 (dd, 1 H, *J* 9.2 Hz), 2.85 (bs, 1 H, O*H*), 1.66 (s, 3 H, CH_3). Anal. Calcd for $C_{56}H_{51}NO_{16}$: C, 67.65; H, 5.14; N. 1.41. Found: C, 67.43; H, 5.19; N, 1.44.

*Benzyl β-*D*-galactopyranosyl-(1 → 6)-2-acetamido-2-deoxy-α-*D*-glucopyranoside* (**8**).—To a suspension of **7** (0.88 g, 0.89 mmol) in anhydrous MeOH (25 mL) cooled at 0 °C, 0.5 M NaOMe in MeOH (10.6 mL) was added. After stirring at 0 °C for 0.5 h and at room temperature for 1.5 h, water (2 mL) was added. The solution was passed through a column (1.5 × 6 cm) containing BioRad AG 50W-X12 (H^+) resin. The solvent was evaporated and the remaining methyl benzoate eliminated by five successive coevaporations with water, to afford **8** (0.41 g, 98%). From EtOH it had: mp 234–235 °C; $[\alpha]_D$ +117° (*c* 1, H_2O); ^{1}H NMR (D_2O): H-1 region δ 4.88 (d, 1 H, *J* 3.0 Hz, GlcNAc), 4.39 (d, 1 H, *J* 7.1 Hz Gal*p*). Anal. Calcd for $C_{21}H_{31}NO_{11}$: C, 53:27; H, 6.60; N, 2.96. Found: C, 53.08; H, 6.62; N, 3.25.

*β-*D*-Galactopyranosyl-(1 → 6)-2-acetamido-2-deoxy-*D*-glucopyranose* (**9**).—To a suspension of **8** (150 mg, 0.317 mmol) in MeOH (15 mL), 10% Pd/C (60 mg) and ammonium formate (60 mg, 0.951 mmol) were added. The mixture was heated in a 65 °C water bath for 1 h, filtered and concentrated. The resulting syrup was dissolved in MeOH (2 mL) and passed through a column of BioRad AG 501-X8 mixed resin. Evaporation of the solvent gave pure **9** (116 mg, 96%) which slowly crystallized from MeOH–EtOH: mp 144–148 °C; $[\alpha]_D$ −30.4° (*c* 1, H_2O), lit. −30.9° (*c* 1, H_2O) [18]; ^{1}H NMR (D_2O): H-1 region

δ 5.12 (d, 0.7 H, *J* 3.4 Hz, α-GlcNAc), 4.37 (d, 0.3 H, *J* 7.4 Hz, β-GlcNAc), 4.34 (d, 1 H, *J* 7.4 Hz, Gal *p*).

*Benzyl (2,3,5,6-tetra-*O*-benzoyl-β-*D*-galactofuranosyl)-(1 → 4)-[2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl-(1 → 6)]-2-acetamido-3-*O*-benzoyl-2-deoxy-α-*D*-glucopyranoside* (**10**).—*Method a.* To a cooled (0 °C) solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose (**5**, 0.63 g, 0.90 mmol) in dry CH_2Cl_2 (6 mL), tin(IV) chloride (0.13 mL, 1.08 mmol) was added. After 15 min stirring at 0 °C, **7** (0.70 g, 0.705 mmol) in 2:1 CH_2Cl_2–acetonitrile (6 mL) was slowly added. The mixture was stirred at room temperature for 16 h, additional amounts of tin(IV) chloride (0.13 mL, 1.08 mmol) and **5** (0.8 g, 1.1 mmol) in acetonitrile (10 mL) added and stirring continued for another 21 h. The mixture was processed as described above (Section 3.7), and the resulting syrup purified by column chromatography (9:1 toluene–EtOAc, then 5:1 toluene–EtOAc). The foamy product (R_f 0.35; 4:1 toluene–EtOAc) was identified as 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 (**11**, 0.2 g, 20% yield). It gave: $[\alpha]_D$ +37° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.25–6.90 (m, 50 H), 6.11 (m, 1 H), 5.85–5.58 (m, 6 H), 5.46 (s, 1 H), 5.43 (d, 1 H, *J* 1.2 Hz), 5.27 (s, 1 H), 4.99 (d, 1 H, *J* 3.7 Hz), 4.93–4.73 (m, 3 H), 4.76, 4.52 (d, 2 H, *J* 11.8 Hz, PhCH_2), 4.60–4.10 (m, 8 H), 1.80 (s, 3 H, CH_3). Anal. Calcd for $C_{90}H_{77}NO_{25}$: C, 68.73; H, 4.94; N, 0.89. Found: C, 68.86; H, 5.22; N, 0.99.

The next fraction from the column (R_f 0.19; 4:1 toluene–EtOAc) afforded **10** (0.44 g, 41%) as a foamy solid: $[\alpha]_D$ + 56.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.15–7.00 (m, 50 H), 6.03 (d, 1 H, *J* 3.2 Hz, H-4′), 5.88 (dd, 1 H, *J* 7.7, 10.3 Hz, H-2′), 5.70 (d, 1 H, *J* 9.8 Hz, N*H*), 5.66–5.63 (m, 2 H), 5.56 (dd, 1 H, *J* 10.7, 8.7 Hz, H-3), 5.33 (m, 1 H, H-5″), 5.30 (d, 1 H, *J* 1 Hz, H-2″), 5.13 (d, 1 H, *J* 7.7 Hz, H-1′), 5.08 (s, 1 H, H-1″), 4.93 (d, 1 H, *J* 3.6 Hz, H-1), 4.74 (dd, 1 H, *J* 9.9, 4.3 Hz), 4.70, 4.39 (d, 2 H, *J* 12.0 Hz, PhCH_2), 4.50–4.28 (m, 5 H), 4.19–3.99 (m. 4 H), 3.92 (t, 1 H, *J* 9.9 Hz, H-4), 1.76 (s, 3 H, CH_3). Anal. Calcd for $C_{90}H_{77}NO_{25}$: C, 68.73; H, 4.94; N, 0.89. Found: C, 68.70; H, 5.20; N, 0.88.

Method b. To a cooled (0 °C) solution of 1,2,3,4,6-penta-*O*-benzoyl-β-D-galactopyranose (**6**, 0.746 g, 1.07 mmol) in dry CH_2Cl_2 (7 mL), tin(IV) chloride (0.15 mL, 1.28 mmol) was added. After 15 min stirring at 0 °C, a solution of **4** (0.385 g, 0.93 mmol) in dry CH_2Cl_2 (2 mL) and acetonitrile (3 mL) was slowly added, and the solution stirred at room temperature (~ 18 h). When no remaining **4** was detected by TLC (R_f 0.33, 1:3 toluene–EtOAc), a solution of **5** (1.12 g, 1.6 mmol) in dry CH_2Cl_2 (6 mL) and tin(IV) chloride (0.224 mL, 1.92 mmoL) were added successively. After 24 h stirring at room temperature, the mixture was treated as previously described. Column chromatography of the mixture afforded **11** (0.41 g, 27.8%) and **10** (0.5 g, 34%). Unreacted **7** (0.15 g, 16%) was also recovered.

*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* (**11**).—To a cooled (0 °C) solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose (**5**, 1.61 g, 2.3 mmol) in dry CH_2Cl_2 (14 mL), tin(IV) chloride (0.32 mL, 2.8 mmol) was added. After 15 min stirring at 0 °C, **4** (0.40 g, 0.96 mmol) in acetonitrile (6 mL) was slowly added. The mixture was stirred at room temperature for 27 h and processed as already described above. The resulting syrup was purified by column chromatography (9:1 toluene–EtOAc, then 6:1 toluene–EtOAc). Compound **11** (R_f 0.35; 4:1 toluene–EtOAc) eluted first (0.96 g, 63%) and showed identical physical and spectroscopic properties as those described above.

Concentration of the next fraction from the column (R_f 0.23; 4:1 toluene–EtOAc) afforded benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-6-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-α-D-glucopyranoside (**12**, 0.25 g, 27%) as a foamy solid: $[\alpha]_D$ +44.1° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.15–7.80 (m, 10 H), 7.65–7.15 (m, 20 H), 6.06 (m, 1 H, H-5′), 5.81 (d, 1 H, *J* 9.6 Hz, N*H*), 5.70 (d, 1 H, *J* 5.4 Hz, H-3′), 5.51 (d, 1 H, *J* 1.3 Hz, H-2′), 5.40 (s, 1 H, H-1′), 5.38 (m, H-3), 4.96 (d, 1 H, *J* 3.7 Hz, H-1), 4.85–4.71 (m, 4 H), 4.53–4.42 (m, 2 H), 4.12–3.85 (m, 4 H), 3.12 (bs, 1 H, O*H*), 1.82 (s, 3 H, CH_3). Anal. Calcd for $C_{56}H_{51}NO_{16}$: C, 67.65; H, 5.17. Found: C, 67.86; H, 5.33.

Benzyl β-D-galactofuranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 → 6)]-2-acetamido-2-deoxy-α-D-glucopyranoside (**13**).—To a suspension of **10** (0.40 g, 0.25 mmol) in anhydrous MeOH at 0 °C, 0.5 M NaOMe in MeOH (5.5 mL) was added. After stirring for 1.5 h at room temperature, water (1 mL) was added, and the solution passed through a column (1.5 cm × 6 cm) containing BioRad AG 50W-X12 (H^+) resin. The solvent was evaporated and the remaining methyl benzoate eliminated by five successive co-evaporations with water, to afford **13** as a white solid (0.16 g, 99%), R_f 0.65 (7:1:2 nPrOH–EtOH–H_2O).

Crystallization from MeOH gave: mp 142–144 °C; $[\alpha]_D$ +48.5° (*c* 1, H_2O); ^1H NMR (D_2O): H-1 region δ 5.07 (bs, 1 H, Gal*f*), 4.85 (d, 1 H, *J* 2.1 Hz, GlcNAc), 4.33 (d, 1 H, *J* 7.2 Hz, Gal*p*). Anal. Calcd for $C_{27}H_{41}NO_{16} \cdot 1H_2O$: C, 49.61; H, 6.63; N, 2.14. Found: C, 49.84; H, 6.45; N, 2.26.

β-D-galactofuranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 → 6)]-2-acetamido-2-deoxy-D-glucopyranose (**14**).—To a suspension of **13** (85 mg, 0.134 mmol) in MeOH (8 mL), 10% Pd/C (30 mg) and ammonium formate (25 mg, 0.40 mmol) were added. The mixture was heated in a 65 °C water bath for 20 min, then filtered and concentrated. The residue was dissolved in MeOH (2 mL) and passed through a column containing BioRad AG 501-X8 mixed resin. Evaporation of the solvent under vacuum gave the α,β mixture of **14** (72 mg, 99%) as a hygroscopic colorless glass: R_f 0.48 and 0.38 (7:1:2 nPrOH–EtOH–H_2O) for the α and β anomers; $[\alpha]_D$ −20° (*c* 1, H_2O); ^1H NMR (D_2O): H-1 region δ 5.14 (d, 0.6 H, *J* 2.9 Hz, α-GlcNAc), 5.09 (s, 1 H, Gal*f*), 4.37 (d, 0.4 H, *J* 7.4 Hz, β-GlcNAc), 4.36 (d, 1 H, *J* 7.2 Hz, Gal*p*). Anal. Calcd for $C_{20}H_{35}NO_{16}$: C, 44.02; H, 6.47. Found: C, 44.16; H, 6.76.

β-D-galactofuranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 → 6)]-2-acetamido-2-deoxy-D-glucitol (**15**).—To a solution of **14** (70 mg, 0.128 mmol) in MeOH (7 mL), $NaBH_4$ (50 mg, 1.3 mmol) was added, and the mixture 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 boric acid eliminated by five successive evaporations with MeOH, and finally, by ion-exchange column chromatography on BioRad AG 501-X8 resin. Evaporation of the solvent afforded **15** (68 mg, 97%) as a homogeneous glass: R_f 0.40, 7:1:2 nPrOH–EtOH–H_2O; $[\alpha]_D$ −17° (*c* 1, H_2O); ^1H NMR (D_2O): H-1 δ 5.14 (s, 1 H, Gal*f*), 4.36 (d, 1 H, *J* 7.3 Hz, Gal*p*). Anal. Calcd for $C_{20}H_{35}NO_{16} \cdot H_2O$ C, 42.48; H, 6.95. Found: C, 42.15; H, 7.06.

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