Synthesis of 2,3-di-*O*-(β-D-Gal*p*)-D-Gal*p*, a synthon for the mucin oligosaccharides of *Trypanosoma cruzi*

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Dedicated to Prof. Roberto Rossi on his 60th Anniversary and Prof. Edmundo Rúveda on his 70th Anniversary

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Abstract

The synthesis of an anomeric free derivative of 2,3-di-*O*-(β-D-Gal*p*)-D-Gal*p* as a synthon for the mucin oligosaccharides of *Trypanosoma cruzi* is described. The title compound was synthesized *via* two approaches. The first one involved 2,3-di-*O*-glycosylation of 5,6-*O*-isopropylidene-D-galactono-1,4-lactone by *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate. The second approach was based on glycosylation of a convenient derivative of galactopyranose. The synthesized trisaccharide is the acceptor unit of sialic acid in the *trans*-sialidase reaction that takes place in *T. cruzi.*

Keywords: Galactose trisaccharide, mucins, *Trypanosoma cruzi*, D-galactono-1,4-lactone, glycosylation, trichloroacetimidate

Introduction

The mucin-like glycoproteins are major components in the surface of *Trypanosoma cruzi*, the agent of American trypanosomiasis.[1] The oligosaccharides in the mucins are *O*-linked to the protein *via* GlcNAc which is further substituted with galactose. Most of the Gal residues are present as β-Gal*p* although in some strains a β-Gal*f* residue is also present.[2,3] The terminal β-Gal*p* residues are acceptors of sialic acid in the *trans*-sialidase reaction, involved in the invasion of the host cells.[4,5] In the past few years we have been working on the synthesis of galactofuranose-containing oligosaccharides, components of the mucins of the G-strain.[6-8] From the same strain a penta- and a hexasaccharide (Figure 1) have been obtained as alditols by reductive β-elimination of the mucins.[3] The trisaccharide 2,3-di-*O*-(β-D-galactopyranosyl)-D-galactose is a common unit in the larger oligosaccharides, and a site for sialylation. It has been

also recently characterized in the Dm28C strain.[9] The same trisaccharide, present as a triterpene glycoside in a sponge of the genus *Erylus*, has been characterized by two dimensional NMR spectroscopy.[10] A 3-(methoxycarbonyl)propyl α-glycoside of the trisaccharide has been obtained as a by-product in a photochemistry study.[11]

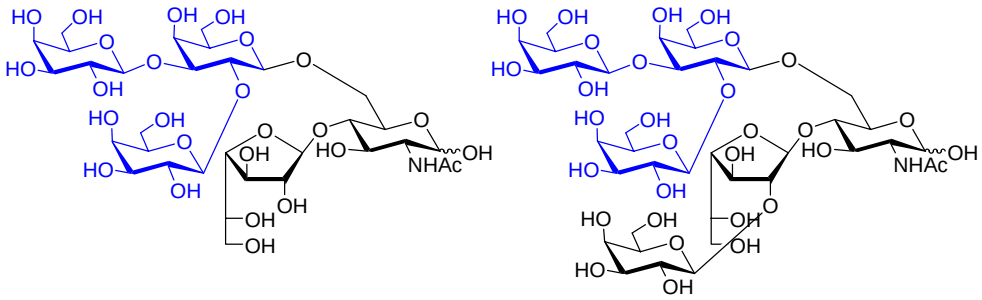


Figure 1. Structure of the penta and hexasaccharide in mucins of *T. cruzi* (G strain). The trisaccharide synthon is shown in blue.

In this paper we describe the synthesis of 4,6-di-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactopyranose (**8**) as a synthon for the construction of the penta and hexasaccharide of the mucins of the G[2,3] and Dm28C[9] strains. The free trisaccharide **14** obtained in this study, will be evaluated as sialic acid acceptor.

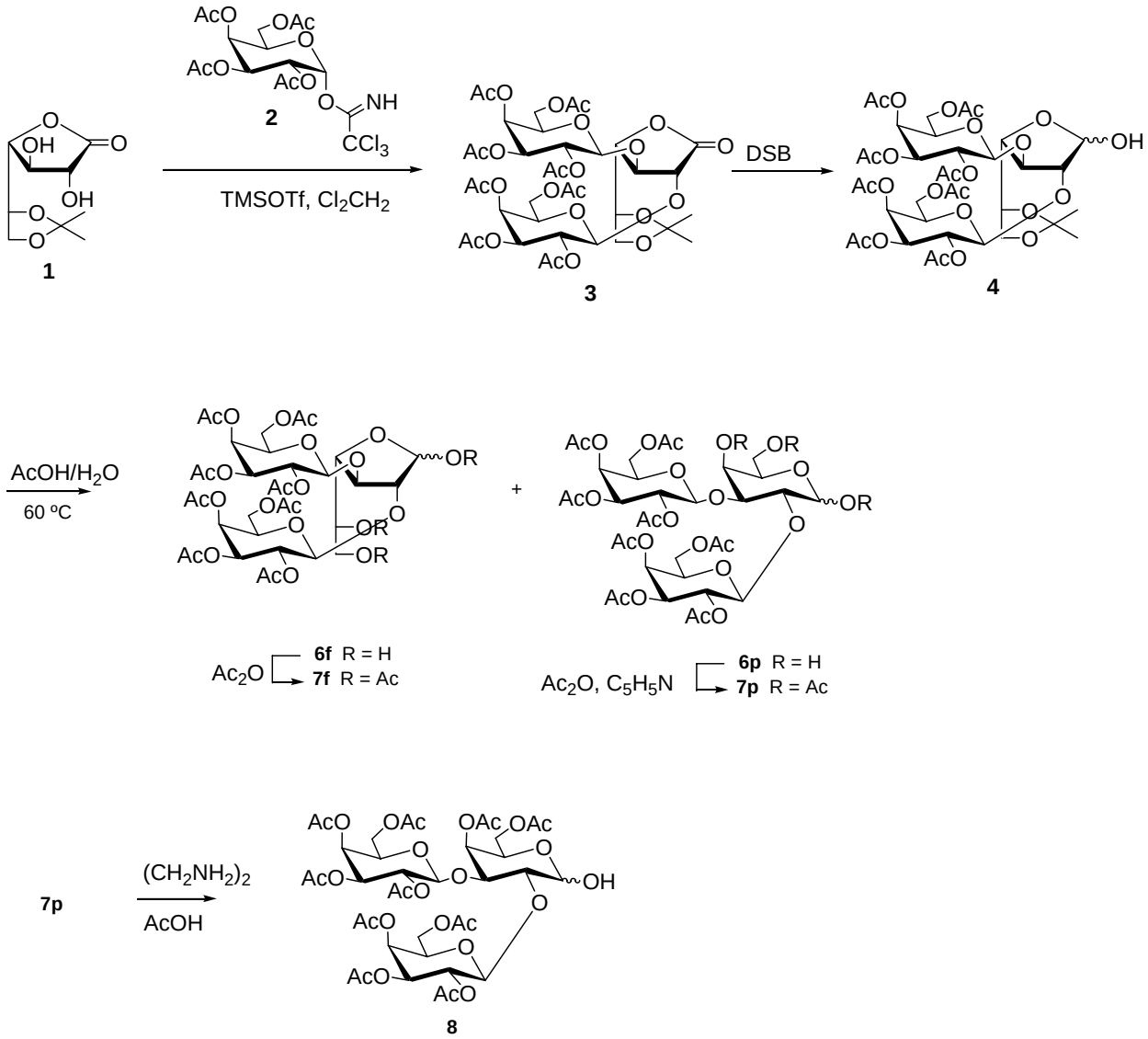
Results and Discussion

For the synthesis of trisaccharide **8**, two approaches were evaluated. The first attempt involved the aldonolactone approach. Aldonolactones are selectively substituted and are good precursors for the reducing end. Recently, we synthesized a β-D-Gal*p*-(1-3)-D-Gal*p* fragment using D-galactono-1,4-lactone as precursor for the Gal*p* unit, taking advantage of the facility to obtain a 3-OH free crystalline derivative from this lactone.[8] In the present case, for the synthesis of the target trisaccharide **8**, 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (**1**)[12] was employed as acceptor, and the trichloroacetimidate method of glycosylation[13] was used (Scheme 1). Thus, glycosylation with 2.4 equivalents of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate (**2**)[14] with a catalytic amount of TMSOTf gave 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-5,6-*O*-isopropylidene-D-galactono-1,4-lactone (**3**) with 82 % yield. The ^{13}C NMR spectrum showed the two resonances of the anomeric carbons at 101.0 and 99.4 ppm. The β-pyranosic configurations were confirmed by the ^{1}H NMR spectrum as indicated by the two doublets (J = 7.9 and 7.8 Hz) centered at 4.73 and 4.72 ppm for H-1′ and H-1″.

With the lactonic trisaccharide **3** in hand, the next steps were to reduce the lactonic function and to isomerize the furanosic reducing end to the pyranosic configuration. Thus, reduction of **3** with diisoamylborane[15] afforded the trisaccharide derivative **4** purified by column chromatography in 57 % yield, together with 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-

galactopyranosyl)-D-galactose (**6**, 14 %), and its precursor 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactono-1,4-lactone (**5**, 15 %). The steric hindrance due to substitution in positions–2,3 of the furanosic ring in compounds **3** and **4** must be responsible for the lability of the isopropylidene group giving **5** and **6**. In previous work from our laboratory isopropylidene lactone derivatives showed to be stable.[8,16]

Compound **4** was hydrolyzed to **6** by heating at 60 °C with aqueous acetic acid. The ^{13}C NMR spectrum of **6** showed the resonances for the reducing end anomeric carbons at 95.5 (C-1α *furanosic*), 94.8 (C-1β *pyranosic*), 92.5 ppm (C-1α *pyranosic*). The signal for C-1β furanosic was overlapped at ~100 ppm with the signals for the anomeric carbons of the β-Gal*p* substituents. The anomeric composition could not be estimated from the ^{1}H NMR spectrum because of the high superposition of signals.



Scheme 1. Synthesis of **8** by the aldonolactone approach.

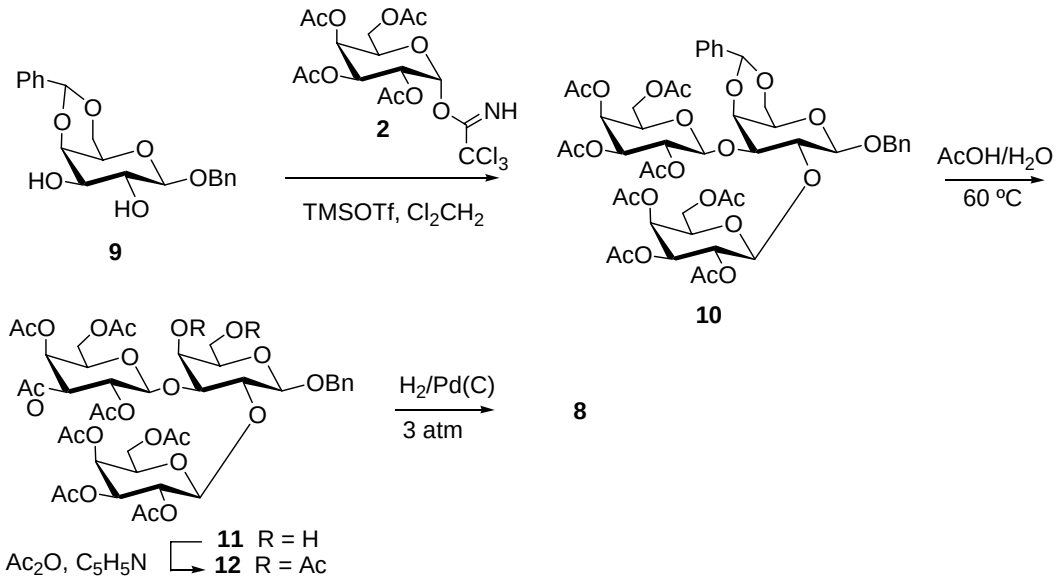
In order to obtain mainly the pyranosic configuration at the reducing end, acetylation of **6** was performed in pyridine at low temperature. The crude product was a mixture of β-furanosic:α-furanosic:β-pyranosic:α-pyranosic acetates in 34:20:6:40 ratio as shown by integration of the anomeric protons in the ^{1}H NMR spectrum: δ 6.36 (d, *J* = 3.8 Hz, H-1α *pyranosic*), 6.31 (bs, H-1β *furanosic*), 6.17 (d, *J* = 4.2 Hz, H-1α *furanosic*), 5.63 (d, *J* = 7.6 Hz, H-1β *pyranosic*). The poor isomerization to the pyranosic configuration could be related to the high steric hindrance caused by the 2,3-di-*O*-Gal*p* substitutions. In fact, acetylation of an analogous derivative of β-D-Gal*p*-(1-3)-D-Gal afforded the pyranosic acetate form in 80 % yield.[8]

The last step for the synthesis of synthon **8** was the hydrolysis of the anomeric acetate which was accomplished by treatment of **7p** with ethylenediamine-acetic acid (Kováč′s procedure).[17] Compound **8** was obtained in the α-anomeric configuration as shown in the ^{1}H NMR spectrum.

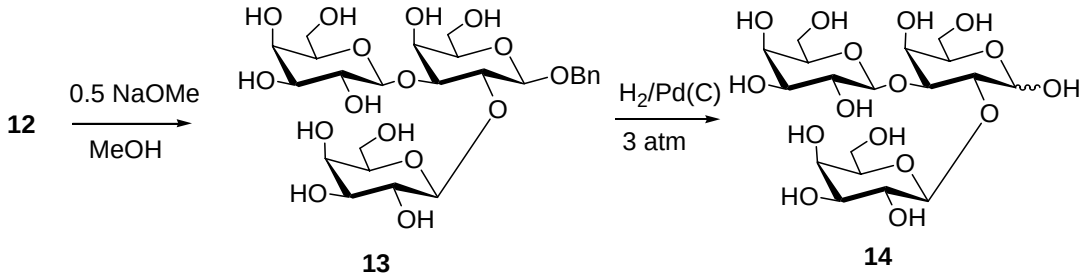
The low proportion obtained (46 %) of the pyranosic trisaccharide **7p** and its difficult purification led us to look for another route. As starting material benzyl 4,6-di-*O*-benzylidene-β-D-galactopyranoside[18] (**9**), obtained in three steps from penta-*O*-acetyl-α,β-D-galactose, was employed. The use of a benzyl glycoside provides the access to the anomeric free trisaccharide necessary for further condensation by the trichloroacetimidate method. Thus, glycosylation of **9** with 2.4 equivalents of trichloroacetimidate **2** gave trisaccharide benzyl 4,6-*O*-benzylidene-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**10**) in 59 % yield (Scheme 2). In the ^{13}C NMR spectrum the three β-galactopyranosic anomeric carbons resonate at 100.7, 100.3, and 99.7 ppm. On the other hand, the ^{1}H NMR spectrum showed three doublets at 4.92, 4.85 and 4.56 ppm with coupling constants of 7.7, 8.0 and 7.7 Hz respectively, that confirmed the β-pyranosic assignments. Glycosylation of compound **9** by the Köenigs-Knorr reaction was described.[19] In that case, the two possible disaccharide derivatives were obtained, by 2-*O* or 3-*O*-mono-substitution with a Gal*p* residue. Di-*O*-substitution that would afford compound **10** was not reported.

Further hydrolysis of the benzylidene of **10**, followed by acetylation of the product gave benzyl 4,6-di-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**12**) in 68% yield after the two steps. Hydrogenolysis of the benzyl glycoside of **12** gave the anomeric free trisaccharide **8,** in this case in the β-configuration**.** Isomerization of the anomeric carbon in a chloroformic solution for a week gave a 3:1 β/α mixture.

Finally, the free trisaccharide 2,3-di-*O*-β-D-galactopyranosyl-β-D-galactose (**14**) was synthesized (Scheme 3). Thus, treatment of **12** with sodium methoxide gave crystalline benzyl 2,3-di-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**13**) in 90 % yield. Hydrogenolysis of **13** with H_2/Pd (C) gave the crystalline trisaccharide **14** as a mixture of the four anomers β-furanosic:α-furanosic:β-pyranosic:α-pyranosic in a 12:5:25:58 ratio.



Scheme 2



Scheme 3

In conclusion, in this work we synthesized the trisaccharide synthon, precursor of the penta and hexasaccharide constituents of the mucins from *T. cruzi* (Figure 1) *via* the glycosylaldonolactone approach that included a Gal*p* isomerization and by a classical approach. The free trisaccharide 2,3-di-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**14)** was also synthesized as a useful tool for studying its acceptor properties in the *trans*-sialidase reaction.

Experimental Section

General Procedures. 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 10 % (v/v) sulfuric acid in EtOH and charring. Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. NMR

spectra were recorded with a Bruker AC 200 spectrometer at 200 MHz (^{1}H) and 50.3 MHz (^{13}C) or with a Bruker AM 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). Homo and heteronuclear correlation spectroscopy experiments were performed when indicated. The two branching Gal*p* were indistinctly numbered with prime or double prime.

5,6-*O*-Isopropylidene-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactono-1,4-lactone (3). To a flask containing recently purified and dried 5,6-*O*-isopropylidene-D-galactono-1,4-lactone[12] (**1**, 0.55 g, 2.56 mmol) and activated 4 Å powdered molecular sieves, was added a solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate[14] (**2**, 3.00 g, 6.10 mmol) in freshly distilled anhydrous CH_2Cl_2 (50 mL) and the suspension was cooled to –20 º C. After 15 min of vigorous stirring, TMSOTf (184 µl, 1.01 mmol) was slowly added and the stirring continued for 1 h until TLC examination showed consumption of imidate **2**. The reaction was quenched by addition of triethylamine (132 µl, 1.01 mmol) and the mixture was allowed to reach room temperature and then filtered over Celite. The filtrate was concentrated and the residue was purified by column chromatography (7:3 toluene-EtOAc) to give 1.85 g of foamy solid that crystallized from 3:1 hexane-isopropanol.The product was characterized as **3** (82 %): R_f 0.55 (1:2 toluene-EtOAc), $[\alpha]_D$ –16.6 º (*c* 1, $CHCl_3$); mp 102-105 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.44 (dd, 1H, *J* = 3.4, 1.3 Hz, H-4″), 5.42 (dd, 1H, *J* = 3.4, 1.1 Hz, H-4′), 5.22 (dd, 1H, *J* = 7.8, 10.3 Hz, H-2″), 5.18 (dd, 1H, *J* = 7.9, 10.4 Hz, H-2′), 5.09 (dd, 1H, *J* = 10.4, 3.4 Hz, H-3′), 5.04 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3″), 4.79 (dd, 1H, *J* = 7.7, 6.6 Hz, H-3), 4.73 (d, 1H, *J* = 7.9 Hz, H-1′), 4.72 (d, 1H, *J* = 7.8 Hz, H-1″), 4.60 (d, 1H, *J* = 7.7 Hz, H-2), 4.40 (ddd, 1H, *J* = 2.3, 6.6, 6.8 Hz, H-5), 4.25 (dd, 1H, *J* = 6.1, 11.5 Hz, H-6″a), 4.25 (dd, 1H, *J* = 6.6, 2.3 Hz, H-4), 4.17 (dd, 1H, *J* = 6.8, 11.3 Hz, H-6′a), 4.16 (dd, 1H, *J* = 6.8, 11.5 Hz, H-6″b), 4.12 (dd, 1H, *J* = 6.3, 11.3 Hz, H-6′b), 4.10 (dd, 1H, *J* = 6.8, 8.7 Hz, H-6a), 3.98 (dd, 1H, *J* = 6.6, 8.7 Hz, H-6b), 3.98 (m, 1H, H-5′), 3.92 (m, 1H, H-5″), 2.17, 2.16, 2.13, 2.12, 2.07, 2.06, 2.00, 1.99 (8s, 24H, C*H*$_3$CO), 1.41, 1.38 (2s, 6H, (C*H*$_3$)$_2$C); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3, 170.2, 170.1, 170.0, 169.9, 169.7, 169.5 (CH$_3$*C*O), 168.3 (C-1), 110.3 ((CH$_3$)$_2$*C*), 101.0 (C-1″), 99.4 (C-1′), 79.8 (C-3), 79.1 (C-2), 78.5 (C-4), 73.6 (C-5), 71.3 (C-5″), 70.9 (C-5′), 70.4 (C-3′, C-3″), 68.7 (C-2′), 68.5 (C-2″), 66.8, 66.7 (C-4′, C-4″), 65.1 (C-6), 61.3, 61.1 (C-6′, C-6″), 25.9, 25.7 ((*C*H$_3$)$_2$C), 20.8, 20.7, 20.6, 20.5 (*C*H$_3$CO). The assignments were supported by homo and heteronuclear correlation spectroscopy experiments.

Anal. Calcd for $C_{37}H_{50}O_{24}$.: C, 50.57; H, 5.73 . Found: C, 50.38; H, 5.68.

5,6-*O*-Isopropylidene-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactofuranose (4). A freshly solution of bis(2-butyl-3-methyl)borane in anhydrous THF was prepared from 2,2-dimethylbutene (2.8 ml, 23.9 mmol) and 3.4 M BH_3 in THF (3.5 ml, 11.95 mmol) cooled at 0 °C. This solution was cannula-added to a flask containing dried **3** (1.75 g, 1.99 mmol) under an argon atmosphere. After 3 h, dissolution was total and the stirring continued for additional 45 h until TLC examination showed consumption of lactone **3**. The reaction was quenched with water, and then 30 % H_2O_2 maintaining the pH 6-8 with 2.5M KOH as already described.[15]

After addition of water (15 mL), the mixture was extracted with CH_2Cl_2 (3 x 80 mL). The organic phase was washed with water, dried (Na_2SO_4), and concentrated. Boric acid was removed by careful co-evaporation of the syrup with methanol at room temperature. The residue was purified by column chromatography (1:1 toluene-EtOAc). Compound **4** (1.0 g, 57 %) eluted first (R_f 0.5, 1:3 toluene-EtOAc) as an amorphous solid, that crystallized from 9:1 hexane-isopropanol: mp 95-100 ºC, $[\alpha]_D$ –14.5º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (δ of the β anomer are listed, only few signals of the α anomer are shown for integration purposes) 5.46 (dd, 0.75H, *J* = 2.5, 4.3 Hz, H-1β), 5.41 (dd, 0.75H, *J* = 3.6, 1.1 Hz, H-4″), 5.38 (dd, 0.75H, *J* = 3.4, 1.3 Hz, H-4′), 5.22 (dd, 0.75H, *J* = 7.9, 10.4 Hz, H-2″), 5.15 (dd, 0.75H, *J* = 7.9, 10.4 Hz, H-2′), 5.06 (dd, 0.75H, *J* = 10.4, 3.4 Hz, H-3′), 5.05 (dd, 0.75H, *J* = 10.4, 3.6 Hz, H-3″), 4.60, 459 (2d, 1.5H, *J* = 7.9 Hz, H-1′ and H-1″), 4.61 (d, 0.25H, *J* = 7.9 Hz, H-1′ or H-1″ from α anomer), 4.57 (d, 0.25H, *J* = 7.7 Hz, H-1′ or H-1″ from α anomer), 4.23-4.11 (m), 4.03 (dd, 0.75H, *J* = 2.5, 4.5 Hz), 4.00 (dd, 0.75H, *J* = 6.7, 8.7 Hz), 3.97-3.92 (m, 1.5H), 3.87 (dd, 0.75H, *J* = 6.7, 8.7 Hz), 3.55 (d, 0.25H, *J* = 6.0 Hz, OH-α), 3.21 (d, 0.75H, *J* = 4.3 Hz, OH-β), 2.17, 2.16, 2.11, 2.08, 2.07, 1.99, 1.98 (8s, C*H*$_3$CO), 1.43, 1.37 (2s, 4.5H, (C*H*$_3$)$_2$C), 1.46, 1.39 (2s, 1.5H, (C*H*$_3$)$_2$C from α anomer). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ for the β anomer: 170.4, 170.2, 170.1, 170.0, 169.5, 169.2 (CH$_3$*C*O), 109.6 ((CH$_3$)$_2$*C*), 100.6, 100.5, 99.7 (C-1′, C-1″, C-1β), 94.5 (C-1α), 89.4 (C-2), 81.3 ,80.2 (C-3, C-4), 75.4, 71.0, 70.7, 70.6, 70.4; 68.9, 68.8 (C-2′, C-2″); 66.9 (C-4′, C-4″), 65.2 (C-6), 61.7, 61.1 (C-6′, C-6″), 26.2, 25.3 ((*C*H$_3$)$_2$C), 20.8, 20.7, 20.6, 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{37}H_{52}O_{24}$: C, 50.45; H, 5.95. Found: C, 50.41, H, 6.04.

Next fraction of the column (R_f 0.3, 1:3 toluene-EtOAc) afforded 0.20 g of a foamy compound which was characterized as 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactono-1,4-lactone (**5**) (15 %): $[\alpha]_D$ +0.6º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.42 (dd, 1H, *J* = 2.5, 1.5 Hz, H-4′or H-4″), 5.41 (dd, 1H, *J* = 2.4, 1.3 Hz, H-4′or H-4″), 5.20 (dd, 1H, *J* = 7.6, 10.2 Hz, H-2″), 5.18 (dd, 1H, *J* = 7.9, 10.4 Hz, H-2′), 5.07 (dd, 1H, *J* = 10.4, 3.4 Hz, H-3′), 5.05 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3″), 4.90 (dd, 1H, *J* = 8.3, 7.1 Hz, H-3), 4.78 (d, 1H, *J* = 7.6 Hz, H-1″), 4.73 (d, 1H, *J* = 7.9 Hz, H-1′), 4.63 (d, 1H, *J* = 8.3 Hz, H-2), 4.33 (dd, 1H, *J* = 7.1, 2.2 Hz, H-4), 4.21-4.13 (m, 5H), 4.05-4.01 (m, 1H), 3.98, 3.92 (2m, 2H, H-5′and H-5″), 3.82-3.71 (m, 2H, H-6a,6b), 3.03 (d, 1H, *J* = 7.6 Hz, OH), 2.18, 2.17, 2.13, 2.12, 2.09, 2.08, 2.00, 1.99 (8s, 24H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7, 170.6, 170.1, 170.0, 169.9, 169.7, 169.6 (CH$_3$*C*O), 168.9 (C-1), 101.4, 99.0 (C-1′, C-1″), 80.1, 79.9, 78.6 (C-4, C-3, C-2), 71.5, 71.3, 70.4 (x2), 68.8, 68.7, 67.1, 67.0, 63.4 (C-6), 61.8, 61.7 (C-6′, C-6″), 20.8, 20.7, 20.6, 20.5 (*C*H$_3$CO).

Last fraction of the column (R_f 0.1, 1:3 toluene-EtOAc) gave 0.24 g of 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactose (**6**) (14 %) as a foamy solid that crystallized from 8:2 hexane-EtOAc, as a mixture of anomers (R_f 0.28, 0.18 and 0.11, EtOAc): mp 115-125 º C, $[\alpha]_D$ –3.7 º (*c* 1, $CHCl_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ anomeric region 101.5, 101.1, 101.0, 100.8, 100.7, 100.6, 100.5 (C-1′and C-1″ of each anomer, and C-1β *furanosic*), 95.5 (C-1α *furanosic*), 94.8 (C-1β *pyranosic*), 92.5 (C-1α *pyranosic*).

Anal. Calcd for $C_{34}H_{48}O_{24}$: C, 48.57; H, 5.75. Found: C, 48.12; H, 5.84.

2,3-Di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactose (6). To a solution of 5,6-*O*-isopropylidene-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactofuranose (**4**, 0.73 g, 0.83 mmol) in acetic acid (4.2 mL) warmed at 60º C, was added H_2O (1.5 mL) slowly with stirring. After 30 min, the mixture was cooled, concentrated under vacuum and the acetic acid was eliminated by successive coevaporations with water and then, toluene to give 0.69 g of **6** (98 %) with the same properties as described above.

1,5,6-Tri-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactofuranose (7f) and 1,4,6-tri- *O*-acetyl - 2,3-di- *O* - (2,3,4,6- tetra –*O* –acetyl -β- D - galactopyranosyl)-D-galactopyranose (7p). To a stirred solution of 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactose (**6**, 0.69 g, 0.82 mmol) in dry pyridine (3.8 ml) at -15°C, acetic anhydride (3 ml) was added dropwise over 45 min, and the mixture was stirred for 13 h at –18 °C. After 6h at 5 °C, TLC monitoring of the crude reaction mixture showed two main spots of R*f* 0.58, 0.53 (toluene-EtOAc 1:3). The mixture was cooled to 0 ºC, MeOH (5 ml) was added and the stirring continued for 30 min. The solution was diluted with CH_2Cl_2 (150 ml), and washed with 10 % HCl (2 x 40 ml), water (40 ml), saturated aqueous $NaHCO_3$ (40 ml) and water (80 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated to give 0.72 g of a foamy solid (91%). ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) showed the anomeric signals with the following integration δ 6.36 (d, 0.4 H, *J* = 3.8 Hz, H-1α *pyranosic*), 6.31 (bs, 0.34H, H-1β *furanosic*), 6.17 (d, 0.20H, *J* = 4.2 Hz, H-1α *furanosic*), 5.63 (d, 0.06H, *J* = 7.6 Hz, H-1β *pyranosic*). The crude mixture was partially separated by column chromatography (9:2 toluene-EtOAc) to give a first fraction of 0.33 g which contained mainly the α and β furanosic products **7f** (R*f* 0.58, 42 %), ^{1}H NMR ($CDCl_3$, 200 MHz): δ anomeric region 6.31 (bs, 0.6H, H-1β*f*), 6.17 (d, 0.4H, *J* = 4.2 Hz, H-1α*f*). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ anomeric region 101.5, 101.3, 100.1, 99.7, 99.4 (C-1′, C-1″ of each anomer and C-1β*f*), 93.0 (C-1α*f*).

A second fraction (R*f* 0.53) was characterized as the pyranosic product **7p** (0.29 g, 29 %) that crystallized from 2:1 hexane-ether. Compound **7p** gave: mp 103-107 º C, $[\alpha]_D$ +19.2º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.36 (d, 1H, *J* = 3.8 Hz, H-1), 5.45 (d, 1H, *J* = 3.3 Hz, H-4), 5.35 (d, 1H, *J* = 3.4 Hz, H-4′), 5.33 (d, 1H, *J* = 3.6, H-4″), 5.14 (dd, 1H, *J* = 7.7, 10.3 Hz, H-2′), 5.08 (dd, 1H, *J* = 7.5, 10.5 Hz, H-2″), 4.99 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3′), 4.97 (dd, 1H, *J* = 10.5, 3.6 Hz, H-3″), 4.63 (d, 1H, *J* = 7.5 Hz, H-1″), 4.57 (d, 1H, *J* = 7.7 Hz, H-1′), 4.23 (dd, 1H, *J* = 6.0, 11.2 Hz), 4.15-3.87 (m, 10H), 2.18, 2.16, 2.15, 2.14, 2.12, 2.08, 2.07, 2.06, 2.04, 1.98, 1.97 (11s, 33H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 170.5, 170.4, 170.3, 170.1, 170.0, 169.9, 169.6, 169.5, 168.9, 168.7 (CH$_3$*C*O), 101.4, 100.4 (C-1′, C-1″), 91.3 (C-1), 75.3, 72.0, 70.8, 70.7, 70.6, 70.2, 69.8, 69.7, 69.6, 68.6; 66.8, 66.7 (C-4′, C-4″); 62.1 (C-6), 61.1, 60.9 (C-6′, C-6″), 21.0, 20.9 , 20.7, 20.6 , 20.5 (*C*H$_3$CO).

Anal. Calcd for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.49; H, 5.70.

4,6-Di-*O*-Acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactopyranose (8) from 1,4,6-tri-O-acetyl-2,3-di-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-galactopyranose (7p). To a cooled solution (0 º C) of glacial acetic acid (13 μl) in THF (2.9 ml)

ethylenediamine (13 μl, 0.19 mmol) was added with stirring. After 10 min, 1,4,6-tri-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-galactopyranose (7p, 124.5 mg, 0.129 mmol) was added and the solution was stirred for 20 h at room temperature. The mixture was diluted with CH_2Cl_2 (50 ml), washed with water (10 ml), 10% HCl (10 ml), water (30 ml); saturated aqueous $NaHCO_3$ (20 ml), and water (2 x 30 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated. Purification of the residue by column chromatography (3:7 toluene-EtOAc) gave 98 mg of 8 in the α-configuration at the free anomeric carbon, as an amorphous syrup that crystallized from 10:2:1 hexane-ether-isopropanol (80 %): R*f* 0.35 (3:7 toluene-EtOAc), mp 115-118 º C. Isomerization in a chloroformic solution for a week gave a 9:1 α/β mixture: $[\alpha]_D$ +11.4 º (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (δ for the α anomer are listed, only the H-2 signal of the β anomer is shown for integration purposes) 5.43 (dd, 0.9H, *J* = 3.6, 1.5 Hz), 5.38 (m, 2H), 5.34 (dd, 0.9H, *J* = 3.4, 1.3), 5.24 (dd, 0.9H, *J* = 7.9, 10.6 Hz, H-2′), 5.08 (dd, 0.9H, *J* = 7.5, 10.6 Hz, H-2″), 5.02 (dd, 0.9H, *J* = 10.6, 3.4 Hz), 5.00 (dd, 0.9H, *J* = 10.6, 3.6 Hz), 4.65 (d, 0.9H, *J* = 7.5 Hz, H-1″), 4.59 (d, 0.9H, *J* = 7.9 Hz, H-1′), 4.37 (m, 0.9H, H-5), 4.23 (dd, 0.9H, *J* = 9.7, 3.6 Hz, H-3), 4.22 (dd, 0.9H, *J* = 7.0, 11.5 Hz), 4.18 (dd, 0.9H, *J* = 7.0, 11.3 Hz), 4.14 (dd, 0.9H, *J* = 7.0, 11.3 Hz), 4.12 (dd, 0.9H, *J* = 5.4, 11.7 Hz), 4.09 (dd, 0.9H, *J* = 5.9, 11.5 Hz), 4.02 (dd, 0.9H, *J* = 7.2, 11.5 Hz), 3.93 (m, 0.9H, H-5′ or H-5″), 3.90 (dd, 0.9H, *J* = 9.7, 3.2 Hz, H-2), 3.87 (m, 0.9H, H-5′ or H-5″), 3.68 (dd, 0.1H, *J* = 7.5, 9.5 Hz, H-2 β anomer), 2.18, 2.17, 2.13, 2.12, 2.08, 2.07, 2.06, 1.99, 1.97 (10s, C*H*$_3$CO);^{13}C NMR ($CDCl_3$, 125 MHz): δ of α anomer 170.6, 170.5, 170.3, 170.1, 169.9, 169.7, 169.6, 168.9 (CH_3*C*O), 101.7, 100.2 (C-1′, C-1″), 95.5 (C-1 β anomer), 92.4 (C-1α anomer), 78.3, 71.4, 71.2, 70.8, 70.7, 70.3, 70.2, 69.7, 69.3, 67.2, 67.0, 66.9; 62.4 (C-6), 61.3, 61.0 (C-6′, C-6″), 21.0, 20.9, 20.8, 20.6, 20.5, 20.4 (*C*H_3CO).

Anal. Calcd $C_{38}H_{52}O_{26}$: C, 49.35; H, 5.67. Found: C, 49.17; H, 5.76.

From benzyl 4,6-di-O-acetyl-2,3-di-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (12). To a solution of **12** (200 mg, 0.197 mmol) in EtOAc (4.5 mL), was added 10% Pd/C Deguzza type E101 NE/W (100 mg), and the suspension was hydrogenated 2 h at 1.5 atm and 33 º C. The catalyst was filtered and the filtrate was concentrated to give 178 mg of compound **8** (98 %) in the β-configuration at the free anomeric carbon. Isomerization of the compound in a chloroformic solution for a week gave a 3:1 β/α mixture: $[\alpha]_D$ +6.7 º (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (δ for the β anomer are listed, only the H-5 signal of the α anomer is shown for integration purposes) 5.41 (dd, 0.75H, *J* = 3.8, 0.9 Hz, H-4), 5.38 (dd, 0.75H, *J* = 3.5, 1.1 Hz, H-4″), 5.36 (dd, 0.75H, *J* = 3.4, 1.1 Hz, H-4′), 5.26 (dd, 0.75H, *J* = 7.9, 10.5 Hz, H-2′), 5.09 (dd, 0.75H, *J* = 7.5, 10.7 Hz, H-2″), 5.00 (dd, 0.75H, *J* = 10.5, 3.4 Hz, H-3′), 4.99 (dd, 0.75H, *J* = 10.7, 3.5 Hz, H-3″), 4.67 (dd, 0.75H, *J* = 4.3, 7.5 Hz, H-1), 4.65 (d, 0.75H, *J* = 7.5 Hz, H-1″), 4.63 (d, 0.75H, *J* = 7.9 Hz, H-1′), 4.43 (d, 0.75H, *J* = 4.3 Hz, OH-), 4.37 (m, 0.25H, H-5 α anomer), 4.20-4.12 (m), 4.05 (dd, 0.75H, *J* = 7.2, 11.7 Hz), 3.98 (m, 0.75H), 3.89 (dd, 0.75H, *J* = 9.5, 3.8 Hz, H-3), 3.85 (m, 0.75H), 3.80 (m, 0.75H), 3.68 (dd, 0.75H, *J* = 7.5, 9.5 Hz, H-2), 2.18, 2.17, 2.14, 2.13, 2.10, 2.09, 2.07, 2.06, 1.99, 1.98 (10s, 30H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 170.4, 170.3, 170.2, 170.1, 170.0, 169.8, 169.4, 169.3, 169.1

(CH_3CO), 100.9, 100.5 (C-1′, C-1″), 95.5 (C-1 β), 92.4 (C-1α), 80.5, 76.1 (C-2, C-3), 71.4 (x2), 70.9, 70.6, 70.3, 69.7 (x2), 69.2, 66.9; 62.4 (C-6); 61.3, 61.2 (C-6′, C-6″), 20.9, 20.8, 20.7, 20.6, 20.5, 20.4 (CH_3CO).

Benzyl 4,6-*O*–benzylidene-2,3-di-*O*-(2,3,4,6-tetra-*O*–acetyl -β- D - galactopyranosyl) -β-D - galactopyranoside (10). A suspension of dried benzyl 4,6 – *O* – benzylidene - β - *D* - galactopyranoside[18] (**9**, 0.97 g, 2.71 mmol), *O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactopyranosyl) tricloroacetimidate[14] (**2**, 3.34 g, 6.78 mmol), activated 4 Å powdered molecular sieves in anhydrous CH_2Cl_2 (130 mL) was stirred under an argon atmosphere at – 15 º C for 15 min and TMSOTf (0.4 equiv, 196 µl) was slowly added. After stirring for 1 h, TLC examination showed consumption of **9** (R*f* 0.15, toluene-EtOAc 1:2). Triethylamine (151 µl) was slowly added and the mixture was allowed to reach room temperature. The suspension was filtered, the solid washed with CH_2Cl_2 and the filtrate was concentrated under vacuum. Purification of the residue by silica gel column chromatography (3:2 toluene-EtOAc) yielded 1.65 g of pure **10** (59 %, R*f* 0.45, toluene-EtOAc 1:2) which crystallized from isopropanol: mp 115-117 º C, $[\alpha]_D$ – 17.2 º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.55-7.25 (m, 10 H), 5.53 (s, 1H, PhC*H*), 5.37(dd, 1H, *J* = 3.4, 1.1 Hz, H-4′), 5.29 (dd, 1H, *J* = 3.4, 1.1 Hz, H-4″), 5.21 (dd, 1H, *J* = 7.7, 10.5 Hz, H-2″), 5.15 (dd, 1H, *J* = 8.0, 10.3 Hz, H-2′), 5.09 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3′), 5.04, 4.60 (2d, 2H, *J* = 11.6 Hz, PhCH_2), 4.93 (dd, 1H, *J* = 10.5, 3.4 Hz, H-3″), 4.92 (d, 1H, *J* = 7.7 Hz, H-1″), 4.85 (d, 1H, *J* = 8.0 Hz, H-1′), 4.56 (d, 1H, *J* = 7.7 Hz, H-1), 4.35 (dd, 1H, *J* = 1.6, 12.3 Hz, H-6a), 4.26 (dd, 1H, *J* = 3.7, 0.9 Hz, H-4), 4.19 (dd, 1H, *J* = 7.7, 9.7 Hz, H-2), 4.18 (dd, 1H, *J* = 6.7, 11.2 Hz, H-6′a), 4.14 (dd, 1H, *J* = 6.4, 11.2 Hz, H-6′b), 4.09 (dd, 1H, *J* = 7.5, 11.2 Hz, H-6″a), 4.06 (dd, 1H, *J* = 1.7, 12.3 Hz, H-6b), 3.97 (dd, H, *J* = 6.2, 11.2 Hz, H-6″b), 3.91 (m, 2H, H-5′, H-3), 3.63 (ddd, 1H, *J* = 1.1, 6.4, 7.4 Hz, H-5″), 3.40 (m, 1H, H-5), 2.16, 2.14, 2.04, 2.03, 1.97, 1.96 (6s, 24H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 170.2, 170.1, 170.0, 169.6, 169.3 (CO), 137.7, 137.5, 128.7, 128.3, 127.9, 127.6, 127.3, 126.1 (arom.), 101.0 (PhCH), 100.7, 100.3 (C-1′, C-1″), 99.7 (C-1), 77.6, 76.3 (C-2, C-3), 75.6, 71.0, 70.9, 70.8, 70.6, 70.4 (PhCH_2), 70.2, 69.6, 68.9 (C-6), 67.2, 66.9, 66.3; 61.3, 60.9 (C-6′, C-6″); 20.9, 20.6, 20.5 (CH_3CO).

Anal. Calcd. $C_{48}H_{58}O_{24}$: C, 56.58; H, 5.74. Found: C, 56.40; H, 5.78.

Benzyl 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (11). To a solution of benzyl 4,6-*O*–benzylidene-2,3– di –*O*-2,3,4,6 – tetra–*O* –acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**10**, 0.71 g, 0.69 mmol) in acetic acid (5 mL) warmed at 80 º C, H_2O (2.5 mL) was slowly added with stirring until turbidity. After 1 h, the mixture was cooled and extracted with CH_2Cl_2 (3 x 100 ml). The organic phase was washed with saturated aqueous $NaHCO_3$ (2 x 80 ml), water (2 x 100 ml), dried ($MgSO_4$) and evaporated. The crude product was purified by recrystallization from a mixture of hexane-EtOAc-toluene (80:15:5) to give 0.49 g of **11** (75%)**:** mp 112-115 º C, $[\alpha]_D$ -25.2º (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.4-7.29 (m, 5 H), 5.38 (d, 1H, *J* = 3.4 Hz, H-4′), 5.31 (d, 1H, *J* = 3.5Hz, H-4″), 5.20 (dd, 1H, *J* = 7.8, 10.3 Hz, H-2″), 5.16 (dd, 1H, *J* = 8.0, 10.5 Hz, H-2′), 5.11 (dd, 1H, *J* = 10.5, 3.4 Hz, H-3′), 4.99, 4.65 (2d, 2H, *J* = 11.9 Hz, PhCH_2), 4.98 (dd, 1H, *J* = 10.3, 3.5 Hz, H-3″), 4.87 (d, 1H, *J* = 8.0 Hz, H-1′), 4.81 (d, 1H, *J* = 7.8 Hz, H-1″), 4.52 (d, 1H, *J* = 7.8 Hz, H-1), 4.16-3.94 (m, 9H), 3.82 (dd, 1H, *J* = 9.1,

3.5 Hz, H-3), 3.80 (m. 1H), 3.67, 3.51 (2m, 2H), 2.84 (bs, 1H, OH), 2.18, 2.14, 2.11, 2.07, 2.06, 2.00, 1.99, 1.97 (8s, 24H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.4, 170.2, 170.1, 170.0, 169.9, 169.8, 169.4 (CH$_3$*C*O), 137.0, 128.3, 127.5, 127.4 (arom.), 100.9, 100.3, 99.4 (C-1, C-1′, C-1″), 81.1 (C-2), 76.3 (C-3), 73.7, 71.2, 70.9, 70.8 (Ph*C*H$_2$), 70.6, 70.5, 70.1, 69.6, 68.5, 67.1 (x2); 62.3, 61.5, 61.0 (C-6, C-6′, C-6″); 21.0, 20.6, 20.5 (*C*H$_3$CO).

Benzyl 4,6-di-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (12). To a stirred solution of benzyl 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**11**, 0.73 g, 0.785 mmol) in dry pyridine (3.7 mL) cooled to 0 °C, acetic anhydride was slowly added (3.7 ml) and the solution was stirred at 5 °C for 16 hs. The mixture was cooled to 0 º C, MeOH (5 ml) was added and the stirring continued for 30 min. The solution was diluted with CH_2Cl_2 (200 ml) and washed with 10 % HCl, water, saturated aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), filtered and concentrated. Purification by chromatography on a silica gel short column (hexane-EtOAc 2:3) gave 0.75 g of **12** as a foamy solid (90 % yield) that crystallized from 5:1 hexane-ether. R*f* 0.43 (1:1 toluene-EtOAc), mp 94-97 º C, $[\alpha]_D$ –15.3 º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.41-7.30 (m, 5 H), 5.38 (m, 1H, H-4), 5.34 (dd, 1H, *J* = 3.1, 1.3 Hz, H-4′), 5.31 (dd, 1H, *J* = 3.5, 1.3 Hz, H-4″), 5.17 (dd, 1H, *J* = 7.8, 10.5 Hz, H-2″), 5.10 (dd, 1H, *J* = 7.0, 10.4 Hz, H-2′), 5.09 (dd, 1H, *J* = 10.4, 3.1 Hz, H-3′), 5.00, 4.62 (2d, 2H, *J* = 11.8 Hz, PhC*H*$_2$), 4.92 (dd, 1H, *J* = 10.5, 3.5 Hz, H-3″), 4.84 (d, 1H, *J* = 7.8 Hz, H-1″), 4.76 (d, 1H, *J* = 7.0 Hz, H-1′), 4.55 (d, 1H, *J* = 7.5 Hz, H-1), 4.16 (dd, 1H, *J* = 5.7, 11.7 Hz, H-6a), 4.14 (d, 2H, *J* = 6.7 Hz, H-6′a, H-6′b), 4.10 (dd, 1H, *J* = 7.7, 11.2 Hz, H-6″a), 4.09 (dd, 1H, *J* = 7.0, 11.7 Hz, H-6b), 4.01 (dd, H, *J* = 6.0, 11.2 Hz, H-6″b), 3.91 (m, 2H, H-2, H-3), 3.89 (dt, 1H, *J* = 1.3, 6.7 Hz, H-5′), 3.76 (ddd, 1H, *J* = 1.1, 5.7, 7.0 Hz, H-5), 3.62 (m, 1H, *J* = 1.3, 6.0, 7.7 Hz, H-5″), 2.17, 2.15, 2.10, 2.08, 2.07, 2.05, 2.00, 1.97 (8s, 30H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.5, 170.3, 170.3, 170.1, 170.1, 170.0, 169.9, 169.8, 169.4, 169.1 (CH$_3$*C*O), 137.0, 128.4, 127.9, 127.4, (arom.), 100.8 (C-1), 99.9 (C-1′), 99.7 (C-1″), 77.8 (C-2), 76.3 (C-3), 71.1, 71.0 (C-3′, C-5″), 70.9 (Ph*C*H$_2$), 70.8 (C-5′), 70.6 (C-3′, C-5″), 69.9 (C-2″), 69.7 (C-2′), 68.8 (C-4), 67.0 (C-4′), 66.9 (C-4″), 62.2 (C-6); 61.0, 60.9 (C-6′, C-6″); 20.9, 20.7, 20.6, 20.5 (*C*H$_3$CO). The assignments were supported by homo and heteronuclear correlation spectroscopy experiments.

Anal. Calcd for $C_{45}H_{58}O_{26}$: C, 53.25; H, 5.76. Found: C, 53.25; H, 5.82.

Benzyl 2,3-di-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (13). To a flask containing benzyl 4,6-di-*O*-acetyl-2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**12,** 295 mg, 0.291 mmol) was added 3 ml of a cooled 0.4 M sodium methoxide solution. After 45 min of stirring at 0 °C, a precipitate was formed and TLC examination showed only a more polar compound than **12**. Water was added and the resulting solution was decationized by elution through a column of Amberlite IR 120 H^+ resin. Evaporation of the solution gave 0.171 g of a white crystalline solid (99 % yield) which was recrystallized from methanol-water: R*f* 0.35 (7:1:1 n-propanol-ethanol-water), mp 286-290 º C, $[\alpha]_D$ –0.5 º (*c* 1, water); ^{1}H NMR (D_2O, 500 MHz): δ 7.46-7.33 (m, 5H, arom.), 4.93, 4.72 (2d, 2H, *J* = 11.7 Hz, PhC*H*$_2$); 4.71, (d, 1H, *J* = 8.0 Hz, H-1″); 4.59 (d, 2H, *J* = 7.3, Hz, H-1, H-1′); 4.15 (d, 1H, *J* = 2.4

Hz, H-4); 3.90 (m, 2H, H-2, H-3); 3.86 (d, 1H, *J* = 3.3 Hz, H-4′ or 4″); 3.84 (d, 1H, *J* = 3.3 Hz, H-4′ or H-4″); 3.76-3.64 (m, 5H); 3.63-3.49 (m, 7H); 3.46 (dd, 1H, *J* = 8.0, 10.0 Hz, H-2′ or H-2″); ^{13}C NMR (D_2O, 125 MHz): δ 137.4, 129.2, 128.9 (arom.), 104.3, 103.7 (C-1′, C-1″), 101.3 (C-1), 82.9 (C-2), 77.5 (C-3), 75.7, 75.6, 75.2, 73.4, 73.3 (C-3′, C-3″, C-5, C-5′, C-5″), 72.2, 71.7 (C-2′, C-2″), 71.8 (Ph*C*H$_2$); 69.3 (C-4); 69.2, 69.1 (C-4′, C-4″); 61.5 61.4, 61.2 (C-6, C-6′, C-6″). The assignments were supported by heteronuclear correlation spectroscopy experiments.
Anal. Calcd for $C_{25}H_{38}O_{16}.1½H_2O$: C, 48.31; H, 6.65. Found: C, 48.25; H, 6.71.

2,3-Di-*O*-β-D-galactopyranosyl-β-D-galactose (14). To a solution of benzyl 2,3-di-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**13**, 92.6 mg, 0.155 mmol) in water (4 mL), was added 10% Pd/C Deguzza type E101 NE/W (80 mg), and the suspension was hydrogenated for 3 h at 1.5 atm. The catalyst was filtered and the solution was passed through a C-8 reverse phase cartridge and eluted with water. The solution was concentrated at r.t. to give 75.4 mg of a glassy solid (**14**, 96 %) that crystallized upon addition of methanol: R*f* 0.3 (7:1:2 n-propanol-ethanol-water), mp 175-180 º C, $[\alpha]_D$ +33.2 º (*c* 1, water). Analysis of the spectra showed that **14** was a mixture of the four configurations at the reducing end: ^{1}H NMR (D_2O, 500 MHz): δ anomeric region 5.41 (d, 0.12H, *J* = 1.0 Hz, H-1β *furanosic*); 5.38, (d, 0.58H, *J* = 3.8 Hz, H-1α *pyranosic*); 5.33 (d, 0.05H, *J* = 4.5 Hz, H-1α *furanosic*); 4.73 (d, 0.25H, *J* = 8.00 Hz, H-1β *pyranosic*); 4.63 (d, 0.58H, *J* = 7.8 Hz, H-1′or H-1″); 4.62 (d, 0.25H, *J* = 7.8 Hz, H-1′ or H-1″), 4.59 (d, 0.25H, *J* = 7.6 Hz, H-1′or H-1″), 4.57 (d, 0.58H, *J* = 7.8 Hz, H-1′or H-1″); ^{13}C NMR (D_2O, 125 MHz): δ anomeric region 104.7, 104.6, 103.5, 103.4, 102.9, 102.6 (C-1′, C-1″), 101.1 (C-1β *furanosic*), 95.7 (C-1β *pyranosic*), 95.6 (C-1α *furanosic*), 92.6 (C-1α *pyranosic*).
Anal. Calcd for $C_{18}H_{32}O_{16}.H_2O$: C, 41.38; H, 6.56. Found: C, 41.36; H, 6.50.

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