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Synthesis of β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc, a tetrasaccharide component of mucins of *Trypanosoma cruzi*

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Abstract—The synthesis of free β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc and the corresponding alditol which has been previously isolated by reductive β-elimination of *Trypanosoma cruzi* glycoproteins are described. A convergent route was envisioned by condensing an acceptor derivative of β-D-Gal*f*-(1→4)-D-GlcNAc with a donor derivative of β-D-Gal*p*-(1→3)-D-Gal*p*. The trichloroacetimidate method, as well as $SnCl_4$-promoted condensation were utilized for the introduction of the galactofuranosyl unit. On the other hand, the glycosyl-aldonolactone approach, followed by reduction of the lactone with diisoamylborane, and further isomerization to the galactopyranose configuration gave the donor derivative, which was condensed by the trichloroacetimidate method. Moreover, a synthon for the introduction of the β-D-Gal*p*-(1→3)-D-Gal*f* unit is described.

1. Introduction

The mucins of *Trypanosoma cruzi* have novel *O*-linked oligosaccharides[1–5] which are the major acceptors of sialic acid in a *trans*-sialidase reaction.[6,7] The oligosaccharides are linked to the protein via GlcNAc which is further substituted with galactose. This sugar could be in the pyranose configuration or both, galactofuranose (Gal*f*) and galactopyranose (Gal*p*) units may be present, depending on the strain. The isomeric oligosaccharide alditols obtained by reductive β-elimination of the glycoprotein can be easily differentiated by high-pH anion exchange chromatography.[8] All of the oligosaccharides of the G strain of *T. cruzi* contain the β-D-Gal*f*-(1→4)-D-GlcNAc structure previously synthesized in our laboratory.[9] Further substitution with 1–4 units of β-D-Gal*p* turns the sugar chain into an acceptor of sialic acid, which may be transferred from the host glycoconjugates. Thus, the surface mucins of the parasite seem to be involved in the invasion of host cells.[10] The core trisaccharide β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc has been also synthesized.[11]

Only the mucins from the G strain contain β-D-Gal*f*. The structures of the *O*-oligosaccharides of different *T. cruzi* strains may be related to the infectivity of the parasite.[12] Thus, synthetic galactofuranose-containing oligosaccharides should be useful for identification of strains of *T. cruzi*, as well as for studies on the biosynthesis of these unique O-linked chains.

Here, we report for the first time, the synthesis of free β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc and of the corresponding alditol which has been previously isolated by reductive β-elimination of the *T. cruzi* mucins and characterized by NMR spectroscopy.[1,2]

2. Results and discussion

We have previously synthesized the trisaccharide β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc, with a moderate yield by introduction of the Gal*f* unit into the 4-position of the GlcNAc in the disaccharide β-D-Gal*p*-(1→6)-D-GlcNAc. To overcome the steric effect caused by the Gal*p* linked to C-6, we have now first introduced the Gal*f* at the 4-OH of the GlcNAc. In summary, to accomplish the synthesis of the target tetrasaccharide β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc (**1**) a convergent route was envisioned by condensing an acceptor derivative of β-D-Gal*f*-(1→4)-D-GlcNAc with a donor derivative of β-D-Gal*p*-(1→3)-D-Gal*p*.

2.1. Synthesis of the acceptor derivative of β-D-Gal*f*-(1→4)-D-GlcNAc (Scheme 1)

The benzyl glycoside of GlcNAc (**2**) was employed as

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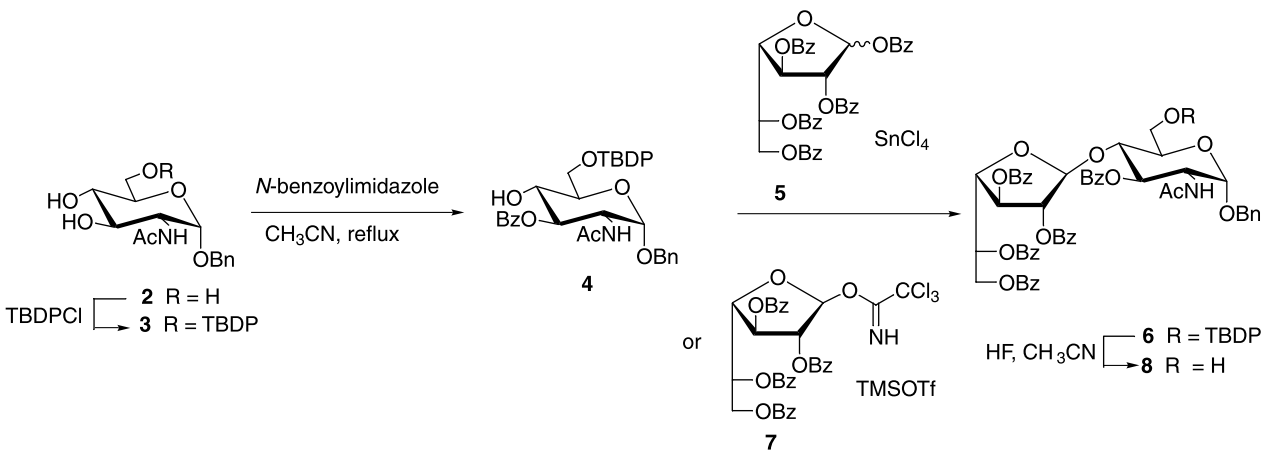


Scheme 1.

starting material in order to get the free target tetrasaccharide. It should be noted that the free sugar would lead to the corresponding alditol by $NaBH_4$ reduction, thus, it could also be obtained radiolabeled. A free OH-4 derivative of GlcNAc, with chemically different protecting groups at OH-6 and OH-3, was required. *tert*-Butyldiphenylsilyl was chosen for protection of OH-6 because it would resist the strong acidic conditions of the $SnCl_4$ galactofuranosyl glycosylation step. Thus, treatment of **2** with 1.2 equiv. of *tert*-butyldiphenylchlorosilane gave the silyl derivative **3** with 92% yield. This compound reacted with 1.1 equiv. of *N*-benzoylimidazole in refluxing acetonitrile to obtain, regioselectively, the 3-*O*-benzoyl derivative **4** in 65% yield. It was previously reported that acylation of pyranosides having the *gluco* configuration showed a lower reactivity for HO-4.[13] In agreement, benzoylation of **2** with *N*-benzoylimidazole afforded the 3,6-di-*O*-benzoyl derivative in 17 h.[9] In the present case, longer reaction time was needed probably due to the bulkier silane protecting group. In the ^{1}H NMR spectrum of **4**, the signal of H-3 (δ 5.36 ppm) appeared 1.7 ppm shifted downfield compared to the same signal in compound **3**, confirming the regioselectivity of the benzoylation. Signals were assigned by comparison with the spectra of the analogous benzyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside.[9]

With the GlcNAc acceptor **4** in hand, construction of the β-D-Gal*f*-(1→4)-D-GlcNAc linkage was attemped. We have extensively employed the $SnCl_4$-promoted glycosylation for construction of the furanosidic linkage from the donor penta-*O*-benzoyl-α,β-D-galactofuranose, easily obtained in one step from galactose.[11] In fact, reaction of **4** with penta-*O*-benzoyl-α,β-D-galactofuranose **5** gave the corresponding disaccharide **6** with 46% yield and high diatereoselectivity. The low yield of this reaction may be also attributed to the bulky silane. The signal at 105.6 ppm for C-1′ in the ^{13}C NMR spectrum showed the β-furanoic configuration,[14] and it was in agreement with H-1′ and H-2′ resonances, in the ^{1}H NMR spectrum, that appeared as singlets at 5.75 and 5.31 ppm, respectively.[9,11,15] The moderate yield, long reaction time and the difficulty in the purification of this compound prompted us to evaluate the trichloroacetimidate method, that we have used before, for the construction of this linkage.[15,16] In this case, the trichloroacetimidate donor[15] is obtained from **5**. Thus, condensation of *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactofuranoside) trichloroacetimidate **7** with **4** in the presence of TMSOTf as catalyst gave disaccharide **6** in 1.5 h, which was easily purified, with 77% yield.

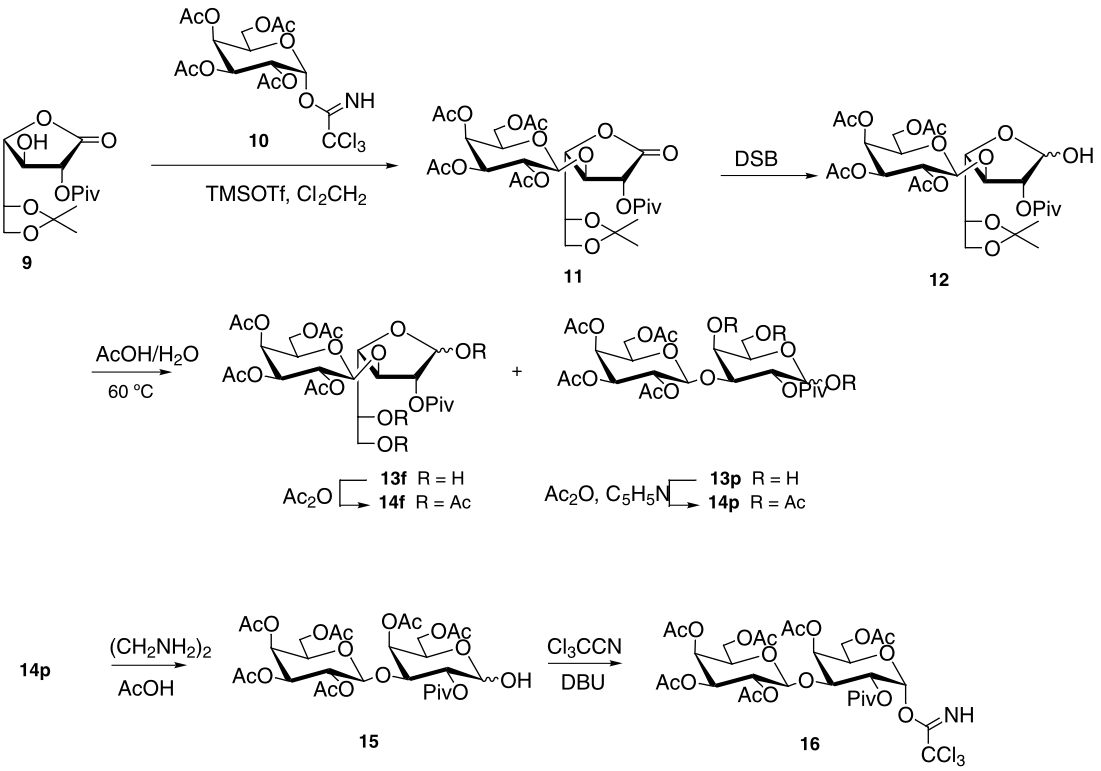
Removal of the silyl group by tetra-*N*-butylammonium fluoride in THF gave compound **8** with the OH-6 free in only 31% yield, with a significant formation of by-products probably due to acyl migration. When 5% HF (48 wt% in water) in acetonitrile was employed, compound **8** was obtained with 89% yield. In spite of the acid liability of the galactofuranosidic linkage, no hydrolysis was observed during the overnight reaction.

2.2. Synthesis of the donor derivative of β-D-Gal*p*-(1→3)-D-Gal*p*

For the synthesis of the β-D-Gal*p*-(1→3)-D-Gal*p* fragment, D-galactono-1,4-lactone was selected as the precursor of the reducing Gal*p* unit (Scheme 2). We have previously described the synthesis of 5,6-*O*-isopropylidene-2-*O*-pivaloyl-D-galactono-1,4-lactone (**9**), a convenient derivative for O-3 glycosidation. Compound **9** is obtained crystalline in 45% yield from D-galactono-1,4-lactone.[16] Condensation of **9** with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate (**10**)[17] in the presence of a catalytic amount of TMSOTf gave diastereoselectively the lactonic disaccharide **11** in 84% yield. The ^{13}C NMR spectrum showed the resonance of C-1′ at 100.9 ppm. Moreover, the coupling constant of H-1′ (4.59 ppm, J=7.8 Hz) also indicated the β-pyranosidic configuration.

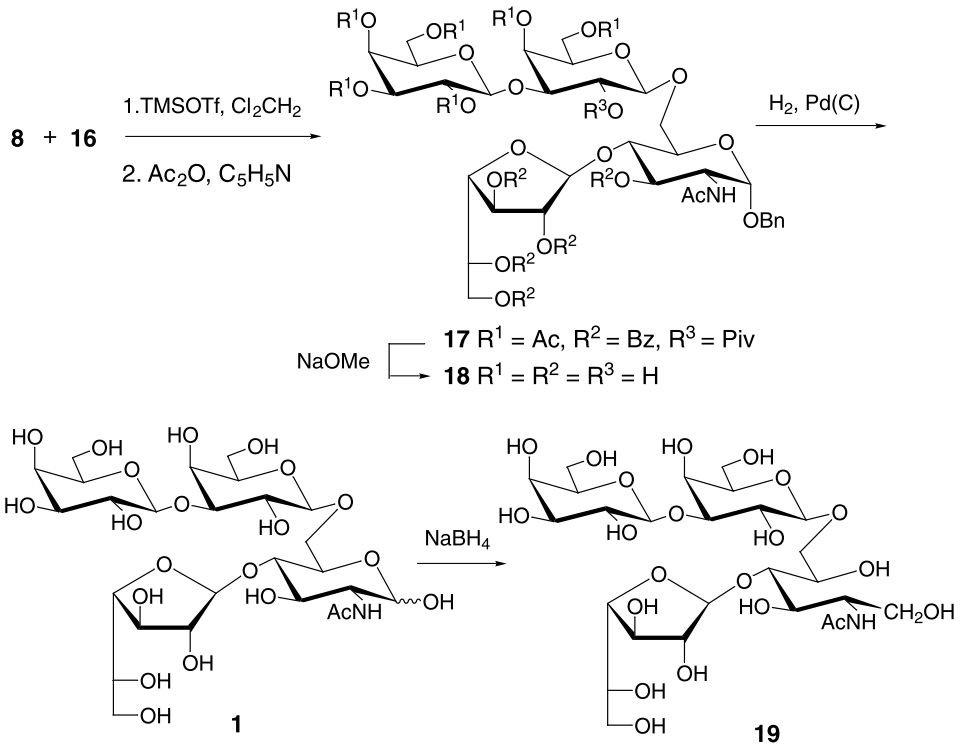
Reduction of the lactonic function of **11** with diisoamylborane gave **12** in 92% yield as an α/β anomeric mixture. It should be noted that compound **12** is also a synthon for the introduction of β-D-Gal*p*-(1→3)-D-Gal*f*, internal unit present in a *Streptococcus pneumoniae* capsular polysaccharide.[18]

In order to isomerize the Gal*f* reducing end of **12** to the corresponding galactopyranose, hydrolysis of the isopropylidene group was accomplished. Thus, treatment of **12** with aqueous acetic acid gave 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-*O*-pivaloyl-D-galactose **13** in 94%



Scheme 2.

yield. Acetylation of **13** gave the mixture of the four anomers **14**, β-furanosic/α-furanosic/β-pyranosic/α-pyranosic in 12:8:33:47 ratio as indicated by integration of the anomeric protons in the ^{1}H NMR spectrum. For synthetic purpose, a very short column chromatography afforded a mixture that contained mainly **14**-αp and **14**-βp. Mild deprotection of the anomeric acetates with ethylenediamine–acetic acid in THF[19] afforded 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-acetyl-2-*O*-pivaloyl-D-galactopyranose **15** in 75% yield.



Scheme 3.

2.3. Glycosylation and further deprotection to afford 1

Glycosylation of acceptor **8** with **15** was performed by the trichloroacetimidate method. Thus, the anomeric free precursor of β-D-Galp-(1→3)-D-Galp **15** was reacted with trichloroacetonitrile and DBU to yield, after purification by column chromatography, the corresponding α-trichloroacetimidate **16** in 88% yield (Scheme 2). Glycosylation of **16** with 1 equiv. of β-D-Gal*f*-(1→4)-D-GlcNAc **8** gave the tetrasaccharide derivative **17** that comigrated with the starting material **8** and the reducing disaccharide **15**, which is a by product of this reaction (Scheme 3). For that reason, acetylation of the crude product was performed, and compound **17** could be easily purified by column chromatography in 67% yield. ^{13}C NMR spectrum showed resonances at 106.8, 101.3, 100.9 and 95.9 ppm that corresponded to the four anomeric carbons of Gal*f*, both Galp and GlcNAc units. The ^{1}H NMR spectrum was also in agreement with the tetrasaccharide structure.

With tetrasaccharide **17** in hand, the usual deprotection steps were performed. Treatment of **17** with 0.5 M sodium methoxide gave the benzyl glycoside **18** in 85% yield. Further hydrogenolysis of the anomeric benzyl group with H_2/Pd gave the free pure tetrasaccharide β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-GlcNAc (**1**) as an amorphous solid. Reduction of **1** with $NaBH_4$ gave the corresponding alditol **19**. The chemical shifts in the ^{1}H and ^{13}C NMR spectra of **19** were in agreement with those reported for the alditol obtained by reductive β-elimination from mucins of *T. cruzi*.[1,2]

3. Experimental

3.1. General

TLC was performed on 0.2 mm silica gel 60 F254 (Merck) aluminium 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). The assignments were supported by homonuclear COSY and/or DEPT experiments.

3.1.1. Benzyl 2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (3). To a solution of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside **2**[20] (1.5 g, 4.8 mmol) in anhydrous DMF (15 mL), imidazole (0.82 g, 12.0 mmol) and *t*-butyldiphenylchlorosilane (1.70 mL, 6.5 mmol) were added under an argon atmosphere. After stirring at rt for 2 h, the reaction mixture was diluted with CH_2Cl_2 (250 mL), and then washed with H_2O (6×150 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to give a residue which was purified by silica gel column chromatography (97:3 CH_2Cl_2–methanol) to yield 2.44 g of an amorphous solid which was identified as benzyl 2-acetamido-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (**3**, 92%): R_f 0.19 (4:1 EtOAc–hexane), $[\alpha]_D$=55.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.80–7.20 (m, 15H), 5.88 (d, 1H, *J*=8.6 Hz, N*H*), 4.89 (d, 1H, *J*=3.9 Hz, H-1), 4.74, 4.44 (2d, 2H, *J*=11.8 Hz, PhCHH), 4.08 (ddd, 1H, *J*=3.9, 8.6, 10.2 Hz, H-2), 3.92 (dd, 1H, *J*=3.7, 10.9 Hz, H-6), 3.87 (dd, 1H, *J*=5.0, 10.9 Hz, H-6′), 3.76–3.68 (m, 2H, H-5, H-3), 3.60 (t, 1H, *J*=9.3 Hz, H-4), 3.45, 3.03 (2bs, 2H, 2O*H*), 2.02 (s, 3H, NHCH_3), 1.08 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 171.8 (CO), 137.0–127.7 (aromatic), 96.4 (C-1), 74.1 (C-3), 72.6 (C-4), 71.6 (C-5), 69.2 (PhCH_2), 64.2 (C-6), 53.6 (C-2), 26.8 ((CH_3)$_3$C), 23.2 (CH_3CONH), 19.2 ((CH$_3$)$_3$*C*). Anal. calcd for $C_{31}H_{39}NO_6Si\cdot 1/2H_2O$: C, 66.64; H, 7.22. Found C 66.73, H 7.47.

3.1.2. Benzyl 2-acetamido-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (4). To a solution of compound **3** (2.2 g, 4.0 mmol) in anhydrous acetonitrile (45 mL), was added a solution of freshly prepared *N*-benzoylimidazole[9] (0.76 g, 4.4 mmol) in anhydrous acetonitrile (4 mL). The mixture was heated at the reflux temperature with exclusion of moisture for 48 h. After cooling to rt, water (1 mL) was added and stirring continued for 30 min. The solvent was removed under vacuum, and the residue was dissolved in CH_2Cl_2 (200 mL). The solution was extracted with 5% HCl, H_2O, saturated aqueous $NaHCO_3$ and H_2O until it reached pH 7. The organic layer was dried ($MgSO_4$), filtered and concentrated. The resulting syrup was purified by silica gel column chromatography (4:1 toluene–EtOAc) to afford compound **4** (1.7 g, 65%) as an amorphous solid: Further elution from the column with ethyl acetate gave unreacted starting material **3** (0.47 g, 21%). Compound **4** gave: R_f 0.88 (Cl_2CH_2–metanol 95:5), $[\alpha]_D$=48.1° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.04 (d, 2H, *J*=8.2 Hz), 7.70–7.25 (m, 18H), 5.76 (d, 1H, *J*=9.3 Hz, N*H*), 5.36 (dd, 1H, *J*=10.2, 9.8 Hz, H-3), 4.94 (d, 1H, *J*=3.7 Hz, H-1), 4.71, 4.46 (2d, 2H, *J*=11.6 Hz, PhC*HH*), 4.44 (ddd, 1H, *J*=3.7, 9.3, 10.2 Hz, H-2), 3.96–3.88 (m, 3H, H-4, H-5, H-6), 3.84 (dd, 1H, *J*=4.8, 9.6 Hz, H-6′), 3.50 (1bs, 1H, O*H*), 1.81 (s, 3H, NHCH_3), 1.08 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 169.9, 167.8 (CO), 137.7–125.2 (aromatic), 96.4 (C-1), 75.0 (C-3), 71.8, 70.4 (C-4, C-5), 69.3 (PhCH_2), 64.2 (C-6), 51.6 (C-2), 26.8 ((CH_3)$_3$C), 22.9 (CH_3CONH), 19.2 ((CH$_3$)$_3$*C*). Anal. calcd for $C_{38}H_{43}NO_7Si\cdot H_2O$: C, 67.93; H, 6.76. Found: C 67.97, H 6.86.

3.1.3. Benzyl (2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (6). Method (a): To an externally cooled (0°C) solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose[11] (**5**, 0.127 g, 0.18 mmol) in dry Cl_2CH_2 (2 mL), tin(IV) chloride (21 μL, 0.18 mmol) was added. After 15 min of stirring at 0°C, a solution of **4** (0.10 g, 0.153 mmol) in dry Cl_2CH_2 (2 mL) was slowly added, and stirring was continued for 36 h at room temperature. The mixture was diluted with Cl_2CH_2 (40 mL) and poured into saturated aq. $NaHCO_3$ 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 (10:1 toluene–EtOAc) to yield

87 mg of compound **6** as an amorphous solid (46% yield). Further elution from the column gave 13 mg of starting material **4**. Compound **6** gave: R_f 0.38 (4:1 toluene–EtOAc); $[\alpha]_D$=29.9° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.05–7.15 (m, 40H), 5.77 (d, 1H, *J*=9.6 Hz, N*H*), 5.75 (bs, 1H, H-1′), 5.70 (m, 1H, H-5′), 5.62 (dd, 1H, *J*=10.5, 10.0 Hz, H-3), 5.55 (d, 1H, *J*=4.1 Hz, H-3′), 5.31 (bs, 1H, H-2′), 4.94 (d, 1H, *J*=3.9 Hz, H-1), 4.63, 4.45 (2d, 2H, *J*=11.8 Hz, PhC*HH*), 4.52 (ddd, 1H, *J*=3.7, 9.6, 10.5 Hz, H-2), 4.47 (t, 1H, *J*=9.3 Hz, H-4), 4.39–4.35 (m, 2H, H-6′$_a$, H-6′$_b$), 4.20 (dd, 1H, *J*=3.8, 4.1 Hz, H-4′), 4.10 (dd, 1H, *J*=3.2, 12.1 Hz, H-6$_a$), 3.88–3.86 (m, 2H, H-5, H-6$_b$), 1.77 (s, 3H, NHC*H*$_3$), 1.02 (s, 9H, C(C*H*$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 169.8, 166.8, 165.8, 165.4 (CO), 137.7–125.2 (aromatic), 105.6 (C-1′), 96.4 (C-1), 82.8, 82.2 (C-2′, C-4′), 77.6 (C-3′), 72.4, 71.9, 71.8 (C-3,4,5), 70.3 (C-5′), 69.7 (Ph*C*H$_2$), 63.8 (C-6′), 62.4 (C-6), 52.4 (C-2), 26.7 ((*C*H$_3$)$_3$C), 23.0 (CH_3CONH), 19.2 ((CH$_3$)$_3$*C*). Anal. calcd for $C_{72}H_{69}NO_{16}Si$: C, 70.17; H, 5.64. Found: C, 69.93; H, 5.92.

Method (b): A suspension of dried **4** (0.92 g, 1.40 mmol), *O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-trichloroacetimidate[15] (**7**, 1.1 g, 1.5 mmol), activated 4 Å powdered molecular sieves in anhydrous Cl_2CH_2 (25 mL) was stirred under an argon atmosphere at room temperature for 1 h. The mixture was cooled to −12°C, TMSOTf (0.90 mL, 0.5 mmol) was slowly added and the stirring continued for 1.5 h. The suspension was filtered, diluted with CH_2Cl_2 (200 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL) and then with H_2O until it reached pH 7 (2×150 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under vacuum. Purification of the residue by silica gel column chromatography (10:1 toluene–EtOAc) yielded 1.3 g of pure **6** (77%) which presented the same physical properties as described above.

3.1.4. Benzyl (2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-(1→4)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (8). To a solution of 5% HF (48 wt% in water) in acetonitrile (30 mL) was added benzyl (2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-(1→4)-2-acetamido-3-*O*-benzoyl-6-*O*-*t*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (**6**, 1.1 g, 0.89 mmol), and the mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with saturated aqueous $NaHCO_3$ and H_2O (100 mL) until pH 7, dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by silica gel column chromatography (5:3 toluene–EtOAc) to give 0.81 g of an amorphous solid which was identified as **8** (92%), R_f 0.35 (1:1 toluene–EtOAc), $[\alpha]_D$=44.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.10–7.10 (m, 30H), 5.75 (d, 1H, *J*=9.5 Hz, N*H*), 5.63 (t, 1H, *J*=10.9 Hz, H-3), 5.65–5.55 (m, 2H, H-5′, H-3′), 5.47 (bs, 1H, H-1′), 5.28 (bs, 1H, H-2′), 5.00 (d, 1H, *J*=3.7 Hz, H-1), 4.76, 4.54 (2d, 2H, *J*=12.0 Hz, PhC*HH*), 4.49 (ddd, 1H, *J*=3.7, 9.5, 10.6 Hz, H-2), 4.40–3.90 (m, 7H) H-4, H-6′$_a$, H-6′$_b$, H-4′, H-5, H-6$_a$, H-6$_b$), 1.76 (s, 3H, NHC*H*$_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 169.9, 166.9, 166.1, 165.9, 165.5 (CO), 136.8–128.2 (aromatic), 106.8 (C-1′), 96.9 (C-1), 82.8, 82.1 (C-2′, C-4′), 77.2 (C-3′), 73.8, 72.3, 71.6 (C-3,4,5), 70.2, 70.1 (C-5′, Ph*C*H$_2$), 63.5 (C-6′), 60.9 (C-6), 52.4 (C-2), 23.0 (CH_3CONH). Anal. calcd for $C_{56}H_{51}NO_{16}\cdot H_2O$: C, 66.45; H, 5.28. Found: C 66.44, H 5.43.

3.1.5. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-5,6-*O*-isopropylidene-2-*O*-pivaloyl-D-galactono-1,4-lactone (11). A suspension of dried 5,6-*O*-isopropylidene-2-*O*-pivaloyl-D-galactono-1,4-lactone[16] (**9**, 2.0 g, 6.6 mmol), *O*-(2,3,4,6-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate[17] (**10**, 4.2 g, 8.5 mmol), activated 4 Å powdered molecular sieves in freshly distilled anhydrous Cl_2CH_2 (35 mL) was vigorously stirred at room temperature. After 1 h, the suspension was cooled to −20°C, TMSOTf (0.31 mL, 1.70 mmol) was slowly added and the stirring continued for 3 h. After 20 h at −20°C, the mixture was neutralized by addition of *N*,*N*-diisopropylethylamine, and filtered over Celite. The filtrate was diluted with Cl_2CH_2, washed with H_2O until it reached pH 7, dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue (5:1 toluene–EtOAc) gave 3.5 g of an amorphous solid which was characterized as **11** (84%): R_f 0.57 (1:1 toluene–EtOAc), $[\alpha]_D$=−27.5° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.49 (d, 1H, *J*=6.0 Hz, H-2), 5.39 (d, 1H, *J*=3.3 Hz, H-4′), 5.18 (dd, 1H, *J*=10.4, 7.8 Hz, H-2′), 4.97 (dd, 1H, *J*=10.4, 3.3 Hz; H-3′), 4.59, (d, 1H, *J*=7.8 Hz, H-1′), 4.54–4.51 (m, 1H), 4.43–4.33 (m, 2H), 4.15–3.87, (m, 5H), 2.15, 2.06, 1.98 (3s, 12H, C*H*$_3$CO), 1.41, 1.38 (2s, 6H, (C*H*$_3$)$_2$C), 1.28 (s, 9H, (C*H*$_3$)$_3$CCO); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 176.7 ((CH$_3$)$_3$C*C*O), 170.1, 170.0, 169.4, 168.7 (CH$_3$*C*O), 110.2 ((CH$_3$)$_2$*C*), 100.9 (C-1′), 79.2 (C-4), 73.6 (C-5), 73.0 (C-3), 71.0 (C-2), 70.5 (C-5′, C-3′), 68.3 (C-2′), 66.8 (C-4′), 65.0 (C-6), 61.1 (C-6′), 38.7, 26.9 ((*C*H$_3$)$_3$CCO), 25.8, 25.6 ((*C*H$_3$)$_2$C), 20.5 (*C*H$_3$CO). Anal. calcd for $C_{28}H_{40}O_{16}$: C, 53.16; H, 6.37. Found: C 53.03, H 6.81.

3.1.6. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-5,6-*O*-isopropylidene-2-*O*-pivaloyl-D-galactofuranose (12). A freshly prepared solution of bis(2-butyl-3-methyl)borane (20.5 mmol) in anhydrous THF (5.5 mL) cooled to 0°C was added to a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-5,6-*O*-isopropylidene-2-*O*-pivaloyl-D-galactono-1,4-lactone (**11**, 2.6 g, 4.1 mmol) in anhydrous THF (8 mL) and the solution was stirred for 16 h at room temperature under an argon atmosphere and quenched with water, and then 30% H_2O_2 maintaining the pH 6–8 with 2.5 M KOH as already described.[21] After addition of water (30 mL), the mixture was extracted with Cl_2CH_2 (3×100 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 crude product was purified by a short column chromatography (5:2 toluene–EtOAc) to yield pure **12** as an amorphous solid (92% yield). R_f 0.45 (1:1 toluene–ethyl acetate). $[\alpha]_D$=−23.8° (*c* 1, $CHCl_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 177.6 ((CH$_3$)$_3$C*C*O), 170.2, 169.9 (CH$_3$*C*O), 109.9 ((CH$_3$)$_2$*C*), 100.8 (C-1′), 99.7 (C-1β), 94.5 (C-1α), 83.6 (C-4β), 82.5* (C-2β), 81.4* (C-3β), 80.9, 78.8, 78.7, 75.8, 70.9, 70.7, 68.5 (C-2′), 67.0 (C-4′), 66.8, 65.3 (C-6), 61.2 (C-6′), 38.8, 38.6 ((CH$_3$)$_3$*C*CO), 27.0, 26.9 ((*C*H$_3$)$_3$CCO), 26.2, 26.1, 25.6, 25.2 ((*C*H$_3$)$_2$C), 20.5 (*C*H$_3$CO). Anal. calcd for $C_{28}H_{42}O_{16}$: C, 52.99; H, 6.67. Found: C 53.26, H 6.78.

3.1.7. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-*O*-pivaloyl-D-galactose (13). To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-5,6-*O*-isopropylidene-2-*O*-pivaloyl-D-galactofuranose (**12**, 0.50 g, 0.79 mmol) in acetic acid (4 mL) warmed at 60°C, was added H_2O (1 mL) slowly and with stirring. After 50 min of stirring, the mixture was cooled, concentrated under vacuum and the acetic acid was eliminated by successive co-evaporations with toluene. The resulting syrup was purified by column chromatography (1:1 toluene–ethyl acetate) to yield 0.44 g of **13** as an amorphous solid (94%) consisting of a mixture of anomers: R_f 0.48, 0.43 and 0.32 (ethyl acetate). $[\alpha]_D$=20.6° (*c* 1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ anomeric region 101.3, 101.0, 100.6 (C-1′), 100.2 (C-1β*f*), 96.0 (C-1α*f*), 93.7 (C-1β*p*), 90.1 (C-1α*p*). Anal. calcd for $C_{25}H_{38}O_{16}$: C, 50.50; H, 6.44. Found: C 50.60, H 6.54.

3.1.8. Peracetylation of 13. 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,5,6-tri-*O*-acetyl-2-*O*-pivaloyl-β-D-galactofuranose (14*f*β); 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-β-D-galactopyranose (14*p*β), and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-α-D-galactopyranose (14*p*α). To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-*O*-pivaloyl-D-galactose (**13**, 0.373 g, 0.63 mmol) in dry pyridine (3 mL) at 0°C, acetic anhydride was slowly added (3 mL). After 20 h at 5°C, the mixture was cooled to 0°C, MeOH (3 mL) was added and the stirring continued for 30 min. Methyl acetate was evaporated and the mixture was slowly poured into ice-water (200 g) affording an amorphous solid which was washed with H_2O and dissolved in Cl_2CH_2 (30 mL). The organic layer was washed with H_2O (2×30 mL), dried ($MgSO_4$), filtered and concentrated. TLC monitoring of the crude mixture of the reaction showed three spots of R_f 0.42, 0.53, 0.59 (1:1 toluene–EtOAc). The mixture was partially separated by chromatography on a silica gel column (9:2 toluene–EtOAc) affording 63.5 mg (14%) of the product of R_f 0.59, 28 mg (6%) of a mixture of products of R_f 0.59 and 0.53, 106 mg (23%) of the product of R_f 0.53, 61 mg (13%) of a mixture of products of R_f 0.53 and 0.42; and lastly 137 mg (30%) of compound of R_f 0.42 (86% overall yield).

The product of R_f 0.59 was an amorphous solid identified as 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,5,6-tri-*O*-acetyl-2-*O*-pivaloyl-β-D-galactofuranose (**14*f*β**), $[\alpha]_D$=−26.8° (*c* 1, $CHCl_3$), ^1H NMR ($CDCl_3$, 200 MHz): δ 6.13 (s, 1H, H-1), 5.40 (m, 1H, H-5), 5.39 (d, 1H, *J*=3.3 Hz, H-4′), 5.17 (dd, 1H, *J*=7.7, 10.4 Hz, H-2′), 5.04 (dd, 1H, *J*=3.3, 10.4 Hz, H-3′), 4.95 (d, 1H, *J*=1.1 Hz, H-2), 4.69 (d, 1H, *J*=7.7 Hz, H-1′), 4.38–3.91 (m, 7H), 2.14, 2.09, 2.06, 2.04, 2.01, 1.97 (6s, 21H, CH_3CO), 1.22 (s, 9H, $(CH_3)_3CCO$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 177.3 ($(CH_3)_3CCO$), 170.2, 169.9, 169.3 (CH_3CO), 101.0 (C-1′), 99.2 (C-1), 83.5 (C-4), 82.8 (C-3), 81.7 (C-2), 70.9, 70.7, 69.6, 68.6, 66.9, 63.0 (C-6′), 61.0 (C-6), 38.7 ($(CH_3)_3CCO$), 26.9 ($(CH_3)_3CCO$), 20.9, 20.8, 20.6 (CH_3CO). Anal. calcd for $C_{31}H_{44}O_{19}$: C, 51.67; H, 6.15. Found: C, 51.68, H, 6.24.

The product of R_f 0.53 was actually a mixture, which after recrystallization from EtOH–H_2O gave 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-β-D-galactopyranose (**14β*p***): mp 81–83°C (EtOH–H_2O), $[\alpha]_D$=9.6° (*c* 1, $CHCl_3$), ^1H NMR ($CDCl_3$, 200 MHz): δ 5,65 (d, 1H, *J*=8.4 Hz, H-1), 5.44 (d, 1H, *J*=3.4 Hz, H-4′), 5.37 (d, 1H, *J*=3.6 Hz, H-4), 5.33 (dd, 1H, *J*=8.4, 9,5 Hz, H-2), 5.08 (dd, 1H, *J*=7.8, 10.6 Hz, H-2′), 4.87 (dd, 1H, *J*=3.4, 10.6 Hz, H-3′), 4.57 (d, 1H, *J*=7.8 Hz, H-1′), 4.26–3.75 (m, 7H), 2.18, 2.08, 2.06, 2.02, 1.96 (5s, 21H, CH_3CO), 1.23 (s, 9H, $(CH_3)_3CCO$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 176.3 ($(CH_3)_3CCO$), 170.3, 169.8, 169.2, 168.9 (CH_3CO), 101.0 (C-1′), 92.0 (C-1), 74.6 (C-3), 72.4, 70.9, 70.5, 70.0, 68.9, 68.4, 66.7, 61.8* (C-6′), 61.0* (C-6), 38.8 ($(CH_3)_3CCO$), 27.1 ($(CH_3)_3CCO$), 20,6, 20.5 (CH_3CO). Anal. calcd for $C_{31}H_{44}O_{19}$: C 51.67, H, 6.15. Found: C 51.52, H 6.03.

The last fraction, of R_f 0.42, crystallized from EtOH and was characterized as 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-α-D-galactopyranose (**14α*p***): mp 179–181°C, $[\alpha]_D$=+48.5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 6.33 (d, 1H, *J*=3.8 Hz, H-1), 5.50 (d, 1H, *J*=3.4 Hz, H-4), 5.37 (dd, 1H, *J*=3.4, 0.9 Hz, H-4′), 5.26 (dd, 1H, *J*=10.4, 3.8 Hz, H-2), 5.08 (dd, 1H, *J*=10.6, 7.7 Hz, H-2′), 4.90 (dd, 1H, *J*=10.6, 3.4 Hz, H-3′), 4.66 (d, 1H, *J*=7.7 Hz, H-1′), 4.35–3.84 (m, 7H), 2.18, 2.17, 2.14, 2.06, 1.97, 1.96 (6s, 21H, CH_3CO), 1.20 (s, 9H, $(CH_3)_3CCO$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 176.9 ($(CH_3)_3CCO$), 170.3, 170.0, 169.8, 168.9, 168.6 (CH_3CO), 100.6 (C-1′), 89.2 (C-1), 70.8, 70.6, 70.4, (C-3, C-5′, C-3′), 69.3, 69.2, 68.7, 66.5 (C-4′), 61.9 (C-6), 60.6 (C-6′), 38.7 ($(CH_3)_3CCO$), 27.1 ($(CH_3)_3CCO$), 20.8, 20.6, 20.5, 20.4 (CH_3CO). Anal. calcd for $C_{31}H_{44}O_{19}$: C, 51.67; H, 6.15. Found: C 51.69, H 6.13.

3.1.9. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-pivaloyl-α,β-D-galactopyranose (15). To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-1,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-α,β-D-galactopyranose (**14αβ*p***, 0.50 g, 0.69 mmol) in THF (16 mL) cooled to 0°C, glacial acetic acid (57 mL, 0.99 mmol) and ethylenediamine (57 mL, 0.85 mmol) were sequentially added and the mixture was stirred for 32 h at room temperature. After quenching the reaction by addition of H_2O, the mixture was extracted with Cl_2CH_2 (2×30 mL). The combined organic phases were washed sequentially with HCl 5%, H_2O, saturated aqueous $NaHCO_3$ and H_2O until pH 7, dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product (3:1 toluene–EtOAc and then 2.5:1) gave 352 mg (75% yield) of an amorphous solid identified as **15**: R_f 0.35 (1:1 toluene–EtOAc), $[\alpha]_D$=33.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 5,47 (d, 1H, *J*=3.3 Hz, H-4), 5.41 (d, 1H, *J*=3.7 Hz, H-1, α anomer), 5.36 (d, 1H, *J*=3.0 Hz, H-4′), 5.00–5.15 (m, 2H), 4.91 (dd, 1H, *J*=10.6, 3.3 Hz, H-3′), 4.71 (d, 1H, *J*=8.0 Hz, H-1′), 2.17, 2.12, 2.14, 2.06, 1.97, 1.96 (6s, 18H, CH_3CO), 1.26 (s, 9H, $(CH_3)_3CCO$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 177.6 ($(CH_3)_3CCO$), 170.6, 170.4, 170.1, 169.0 (CH_3CO), 100.6 (C-1′), 96.0 (C-1β), 90.3 (C-1α), 71.0, 70.7, 70.6, 70.0, 68.9, 66.8, 62.4 (C-6′), 60.9 (C-6), 38.7 ($(CH_3)_3C$), 27.2 ($(CH_3)_3C$), 20.7, 20.6, 20.5 (CH_3CO). Anal. calcd for $C_{29}H_{42}NO_{18}$: C, 51.33; H, 6.24. Found: C 51.03, H 6.35.

3.1.10. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-pivaloyl-β-D-galactopyranosyl-(1→6)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→4)]-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (17). To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-pivaloyl-α,β-D-galactopyranose (**15**, 313 mg, 0.46 mmol), trichloroacetonitrile (0.23 mL, 2.31 mmol) in anhydrous Cl_2CH_2 (5 mL) cooled to 0°C, DBU (0.027 mL, 0.185 mmol) was slowly added. After 45 min, the solution was concentrated under reduced pressure and the residue was purified by column chromatography (3:1:0.3 toluene–EtOAc–TEA) to give 334 mg of syrupy *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-5,6-di-*O*-acetyl-2-*O*-pivaloyl-α-D-galactopyranosyl) trichloroacetimidate (**16**, 88%): R_f 0.63 (1:1 toluene–EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.69 (s, 1H, NH), 6.55 (d, 1H, *J*=3.7 Hz, H-1), 5.57 (dd, 1H, *J*=3.7, 1 Hz, H-4), 5.30–5.40 (m, 2H), 5.10 (dd, 1H, *J*=10.6, 7.7 Hz, H-2′), 4.91 (dd, 1H, *J*=10.6, 3.7 Hz, H-3′), 4.70 (d, 1H, *J*=7.7 Hz, H-1′), 4.45–4.10 (m, 5H), 4.00 (dd, 1H, *J*=7.0, 11.3 Hz), 3.86 (t, 1H, *J*=7 Hz), 2.18, 2.16, 2.07, 2.04, 1.99, 1.97 (6s, 18H, CH_3CO), 1.21 (s, 9H, $(CH_3)_3CCO$; ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 177.2, 170.4, 170.1, 169.9, 169.0 (CO), 160.5 (CN), 100.5 (C-1′), 93.4 (C-1), 71.0, 70.8, 70.5, 69.8, 69.4, 68.8, 66.8, 62.0* (C-6′), 61.2* (C-6), 38.8 ($(CH_3)_3C$), 27.2 ($(CH_3)_3C$), 20.7, 20.5 (CH_3CO).

Compound **16** may be stored in a freezer (−20°C) for several weeks without appreciable decomposition.

A suspension of **16** (334 mg, 0.406 mmol), benzyl (2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-(1→4)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**8**, 400 mg, 0.406 mmol), activated 4 Å powdered molecular sieves in anhydrous Cl_2CH_2 (10 mL) was vigorously stirred under an argon atmosphere at rt for 30 min. The reaction mixture was cooled to −12°C and TMSOTf (29 mL; 0.162 mmol) was slowly added and the stirring continued for 1 h. The reaction was quenched by addition of TEA (23 mL, 0.162 mmol), filtered and concentrated. In order to improve separation from remaining compound **8** and by-product **15**, the mixture was acetylated. The crude product was dissolved in dry pyridine (2 mL), cooled to 0°C, and then acetic anhydride (2 mL) was added, and stirring continued at room temperature for 1 h. The mixture was quenched by addition of MeOH (2 mL) and then diluted with Cl_2CH_2. The organic solution was sequentially washed with 10% HCl (2×40 mL), H_2O (40 mL), saturated aqueous $NaHCO_3$ (2×40 mL) and H_2O until it reached pH 7, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (5:2 toluene–EtOAc) to give 0.45 g of an amorphous and hygroscopic solid identified as **17** (67%): R_f 0.39 (1:1 toluene–EtOAc), $[\alpha]_D$=+21.1° (*c* 1, Cl_3CH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.02, 7.94, 7.90, 7.86, 7.80 (5d, 10H, *J*=7.3 Hz, arom), 7.60–7.22 (m, 20H, arom), 5.76 (d, 1H, *J*=9.6 Hz, N*H*), 5.67–5.56 (m, 3H, H-3, H-3′, H-5′), 5.36 (d, 1H, *J*=3.9 Hz, H-4″), 5.35 (d, 1H, *J*=4.3 Hz, H-4‴), 5.28 (bs, 1H, H-2′), 5.25 (bs, 1H, H-1′), 5.25 (dd, 1H, *J*=9.8, 8.2 Hz, H-2″), 5.05 (dd, 1H, *J*=10.6, 7.8 Hz, H-2‴), 4.92 (d, 1H, *J*=3.6 Hz, H-1), 4.86 (dd, 1H, *J*=10.6, 3.6 Hz, H-3‴), 4.81, 4.49 (2d, 2H, *J*=11.9 Hz, PhC*HH*), 4.53 (d, 1H, *J*=7.7 Hz, H-1‴), 4.50 (d, 1H, *J*=8.2 Hz, H-1″), 4.52 (ddd, 1H, *J*=3.6, 9.6, 10.7 Hz, H-2), 4.26–4.32 (m, 3H), 4.21 (dd, 1H, *J*=6.0, 11.2 Hz, 1H), 4.18–4.11 (m, 4H), 4.00 (dd, 1H, *J*=6.6, 11.4 Hz), 3.9 (t, 1H, *J*=9.6 Hz, H-4), 3.86–3.77 (m, 4H), 2.16, 2.09, 2.04, 2.00 (4s, 12H, CH_3CO), 1.95 (s, 6H, CH_3CO), 1.76 (s, 3H, CH_3CONH), 1.21 (s, 3H, $C(CH_3)_3$); ^{13}C NMR (Cl_3CD, 125 MHz): δ 175.9, 170.4, 170.1, 170.0, 169.9, 169.3, 166.7, 165.8, 165.6, 165.5, 165.4 (CO), 136.9–128.0 (arom.), 106.8 (C-1′), 101.3, 100.9 (C-1″, C-1‴), 95.9 (C-1), 82.3, 81.9 (C-4′, C-2′), 76.8, 75.7, 74.8, 72.3, 71.2, 70.9, 70.6, 70.4, 70.2, 70.0, 69.3 ($PhCH_2$), 69.0, 68.2, 68.0 (C-6), 66.6, 63.3 (C-6′), 61.6, 60.8 (C-6″, 6‴), 52.0 (C-2), 38.6 ($(CH_3)_3C$), 27.2 ($(CH_3)_3C$), 23.0 (CH_3CONH), 20.6, 20.5 (CH_3CO). Anal. calcd for $C_{85}H_{91}NO_{33}\cdot 1/2H_2O$: C, 61.35; H, 5.57. Found: C 61.20, H 5.23.

3.1.11. Benzyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranoside (18). To a suspension of **17** (400 mg, 0.24 mmol) in anhydrous MeOH (6 mL) cooled at 0°C, 1 M NaOMe in MeOH (6 mL, 6 mmol) was added. After stirring for 3.5 h at room temperature and 16 h at 5°C, the solution was passed through a column (1.5×6 cm) containing Amberlite IR 120 plus resin. After concentration under vacuum, the residue was dissolved in H_2O, and the solution was washed with ether (10 mL) and 1:1 EtAcO–*n*-hexane (2×10 mL), and concentrated to afford **18** (0.162 g, 0.203 mmol, 85%) which slowly crystallized by addition of methanol. Mp 158–160°C (methanol). R_f 0.47 (7:1:2 *n*-propanol–ethanol–water), $[\alpha]_D$=38.2° (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 7.39–7.34 (m, 5H), 5.10 (d, 1H, *J*=2 Hz, H-1′), 4.88 (d, 1H, *J*=3.5 Hz, H-1), 4.70, 4.51 (2d, 2H, *J*=12.0 Hz, PhC*HH*), 4.57 (d, 1H, *J*=7.6 Hz, H-1″), 4.42 (d, 1H, *J*=7.8 Hz, H-1‴), 4.13 (d, 1H, *J*=3.4 Hz), 4.09 (dd, 1H, *J*=11.5, 2.0 Hz), 4.02–4.05 (m, 3H), 3.91 (ddd, 1H, *J*=2.0, 9.9, 3.6 Hz), 3.87–3.53 (m, 18H), 1.90 (s, 3H, CH_3CONH); ^{13}C NMR (D_2O, 500 MHz): δ 174.8 (CO), 137.5, 129.3, 129.1, 129.0 (arom.), 108.2 (C-1′), 104.9, 103.5 (C-1″, C-1‴), 96.4 (C-1), 83.2, 82.5 (C-4′, C-2′), 81.6, 77.8, 76.6, 75.6, 75.3, 73.1, 71.6, 71.1, 70.5 ($PhCH_2$), 70.4, 70.3, 70.0, 69.2, 69.1, 68.0 (C-6), 63.3 (C-6′), 61.5 (C-6″,6‴), 54.1 (C-2), 22.3 (CH_3CONH). Anal. calcd for $C_{33}H_{51}NO_{21}$: C 49.68; H 6.44. Found: C 49.74, H 6.46.

3.1.12. β-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranose (1). A suspension of benzyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucopyranoside (**18**, 80 mg, 0.10 mmol) in a mixture of 9:1 methanol–water (3.5 mL) and 10% Pd/C Deguzza type E101 NE/W (Aldrich) (200 mg), was hydrogenated for 8 h at 45 psi (3 atm). The catalyst was filtered over Celite, and the filtrate was concentrated. After dissolving the residue in water, the resulting solution was passed through a C8 cartridge. Evaporation of the solvent afforded compound **1** (60 mg, 85%) as a glassy solid. R_f 0.27 and 0.22 (7:1:2 *n*-propanol–ethanol–water), $[\alpha]_D$=−29.8° (*c* 1, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ anomeric region 5.16 (d, 0.7H, *J*=3.15 Hz, H-1α GlcNAc), 5.12 (bs, 1 Hz, H-1′ Gal*f*), 4.58 (d, 1H, *J*=7.5 Hz, H-1″), 4.45 (d, 0.3H, *J*=7.7 Hz, H-1β GlcNAc), 4.44 (d, 1H, *J*=7.8 Hz, H-1‴); ^{13}C NMR (D_2O,

500 MHz): δ 175.3, 175.0 (CO), 108.3 (C-1′), 104.9, 103.5 (C-1″, C-1‴), 95.6 (C-1β), 91.3 (C-1α), 83.2, 82.5 (C-4′, C-2′), 81.6, 77.9, 76.6, 75.6, 75.4, 73.1, 71.6, 71.1, 70.4, 70.3, 69.9, 69.8, 69.2, 68.3 (C-6), 63.3 (C-6′), 61.5 (C-6″,6‴), 54.5 (C-2), 22.5 (*C*H_3CONH). Anal. calcd for $C_{26}H_{45}NO_{21}$: C, 44.13; H, 6.41. Found: C 43.85, H 6.49.

3.1.13. β-D-Galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-glucitol (19). To a solution of **1** (30 mg, 0.042 mmol) in 9:1 methanol–water (2 mL), sodium borohydride (90.2 mg, 2.34 mmol) was added, and the mixture was stirred at room temperature for 7 h. The solution was neutralized by elution through a column of Amberlite IR-120 plus, and concentrated to dryness. The residue was co-evaporated with methanol (4×1 mL), dissolved in water and the solution filtrated through C8 cartridge. Evaporation of the residue and further treatment with a mixture of acetone–methanol, gave 27 mg of **19** (90%) as an amorphous white solid. R_f 0.23, (7:1:2 *n*-propanol–ethanol–water), $[\alpha]_D=-10.5°$ (*c* 1, H_2O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ anomeric region 5.15 (d, 1H, *J*=2.4 Hz, Gal*f*), 4.56 (d, 1H, *J*=7.6 Hz, β-Gal*p*), 4.42 (d, 1H, *J*=7.9 Hz, β-Gal*p*); ^{13}C NMR (D_2O, 500 MHz): δ 175.0 (CO), 108.6 (C-1′), 104.9, 103.5 (C-1″, C-1‴), 83.2, 82.5 (C-4′, C-2′), 81.8, 78.3, 76.7, 75.6, 75.4, 73.1, 71.6, 71.1, 70.9 (C-6), 70.6, 70.3, 69.2, 69.0, 68.8, 63.4 (C-6′), 61.5, 61.3 (C-6″, 6‴, C-1), 53.3 (C-2), 22.7 (CH_3CONH). Anal. calcd for $C_{26}H_{47}NO_{21}$: C 44.00; H 6.68. Found: C 43.99, H 6.80.

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