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Synthesis of the 2″-Hydroxy, 4″-Deoxy and 4″-Epi Analogues of β-D-GalNAc-(1→3)-α-D-Gal-(1→4)-β-D-Gal, the Terminal Trisaccharide of Globotetraose

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Radical deoxygenation of methyl 3,6-di-*O*-benzoyl-2-deoxy-4-*O*-imidazol-1-yl-thiocarbonyloxy-2-phthalimido-β-D-glucopyranoside **5** gave methyl 3,6-di-*O*-benzoyl-2,4-dideoxy-2-phthalimido-β-D-glucopyranoside **6**, which was converted into the corresponding methyl thioglycoside donor **9**. Methylsulfenyl trifluoromethanesulfonate-promoted glycosylation of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside **10**, followed by removal of protecting groups gave the 4″-deoxy analogue **12** of the terminal trisaccharide of globotetraose. Silver trifluoromethanesulfonate-promoted glycosylation of the same disaccharide alcohol **10** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl bromide **13** and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **16**, followed by deblocking, gave the 2″-hydroxy and 4″-epi analogues **15** and **18**, respectively.

Antigens of the globo series of glycolipids, such as globotetraosyl ceramide (Fig. 1), are recognized *in vivo* by antibodies of the P blood-group system and by various bacterial proteins such as the pilus-associated PapG adhesin protein of uropathogenic *Escherichia coli*,[1] verotoxin from *E. coli*,[2] Shiga toxin from *Shigella dysenteriae*,[3] and the adhesin from *Streptococcus suis*.[4] Furthermore, glycolipids of the globo series have been suggested to be important as tumor-associated antigens on Burkitt lymphoma cells,[5] human teratocarcinoma cells,[6] as well as other tumor cells,[7] and are also enriched in body fluids of patients suffering from Fabry's disease.[8]

A detailed analysis of the molecular recognition of galabiose by the PapG adhesion protein of *E. coli* pili[9] has been reported by us, based on inhibition of hemagglutination by a large collection of galabiose analogues.[10] Galabiosides with hydrophobic aglycones (instead of a β-D-glucose unit as in the natural globo glycolipids) showed improved inhibitory power and it was also indicated that the *N*-acetyl-β-D-galactosamine unit was important for binding to the PapG adhesin. It has also been shown that different strains of uropathogenic *E. coli*, having different types of G adhesins (I, II, and III), recognize different sugar epitopes on globo series glycolipids.[11] The binding epitope of the type III G adhesins seems to involve the *N*-acetyl-β-D-galactosamine residue.

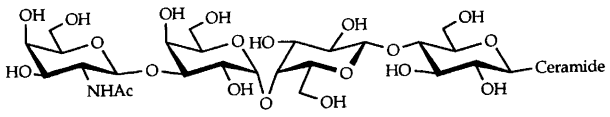

Fig. 1.

We have recently described the synthesis of the Forssman pentasaccharide and all the corresponding di-, tri-, and tetra-saccharide fragments,[12] suitable for studying the binding epitopes of the different G adhesins in various bioassays. Furthermore, the synthesis of the 2″-, 3″-, 4″-, and 6″-deoxy-globotriosides (including their neoglycolipids) has recently been reported.[13] The present paper describes the synthesis of the 2″-hydroxy, 4″-deoxy and 4″-epi analogues of the terminal trisaccharide of globotetraose (**12**, **15**, **18**), of use for studying the interaction of the G adhesins with the GalNAc-β residue more in detail.

Our synthetic method involved β-glycosylation of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside **10**,[12] with three different glycosyl donors, followed by removal of protecting groups. Two of the donors 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl bromide **13**[14] and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **16**, were readily available, while the third, methyl 3,6-di-*O*-benzoyl-2,4-dideoxy-2-phthalimido-1-thio-β-D-glucopyranoside **9**, had to be synthesized from

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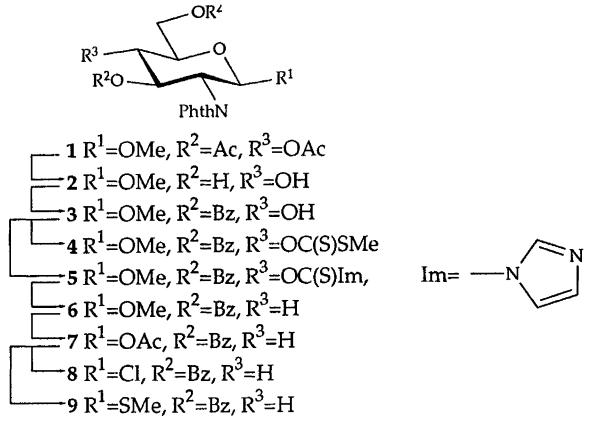
glucosamine hydrochloride. During the synthesis of **9** we needed an anomeric protecting group which was stable towards a wide range of reaction conditions and then selectively transformed into a good leaving group (halide or thioglycoside) prior to glycosylation of the galabioside **10**. The 2-(trimethylsilyl)ethyl group[15] is usually the group of choice to meet these requirements, but in the case of 2-deoxy-2-phthalimidoglycosides the more readily accessible β-methyl glycoside can be used, since acetolysis of methyl 2-deoxy-2-phthalimido-β-D-glycosides normally proceeds in very high yield.[16]

Results and discussion

The synthesis of the deoxygenated glucosamine donor **9** was performed as follows (Scheme 1). Zemplén deacetylation of the fully acetylated phthalimido derivative **1**,[17] followed by regioselective benzoylation with benzoyl chloride in pyridine at −45 °C gave the 3,6-dibenzoate **3** in 62% yield. An attempt to convert the alcohol **3** into the corresponding methylthio(thiocarbonyl) derivative **4** with sodium hydride–carbon disulfide–methyl iodide in tetrahydrofuran[18] resulted in a rather low yield (31%) of **4**, probably because of the instability of the benzoyl groups under these conditions. On the other hand, thiocarbonylation under milder conditions, using thiocarbonyldiimidazole,[18] gave a quantitative yield of the crude imidazol-1-yl(thiocarbonyl) derivative **5**. Crude **5** was deoxygenated immediately with tributyltin hydride–2,2′-azoisobutyronitrile in refluxing toluene[18] to give the deoxygenated glucosamine analogue **6** (87% from compound **3**). Conversion of **6** into suitable glycosyl donors was performed via acetolysis of the methyl aglycone with sulfuric acid in acetic acid–acetic anhydride[16] to give the crude 1-*O*-acetate **7** (98%, α/β 28:72). The acetolysis of the deoxygenated compound **6** was complete within 2 h, in contrast with the parent glucosamine derivative **1**, which, upon treatment under the same conditions, was converted into the 1-*O*-acetate in 24 h. Treatment of **7** with dichloromethyl methyl ether and boron trifluoride–diethyl ether in dichloromethane[19] furnished the corresponding β-chloride **8** in quantitative yield. An efficient synthesis and use of the corresponding benzylated α/β-chloride has recently been described by Kaine *et al.*[20]



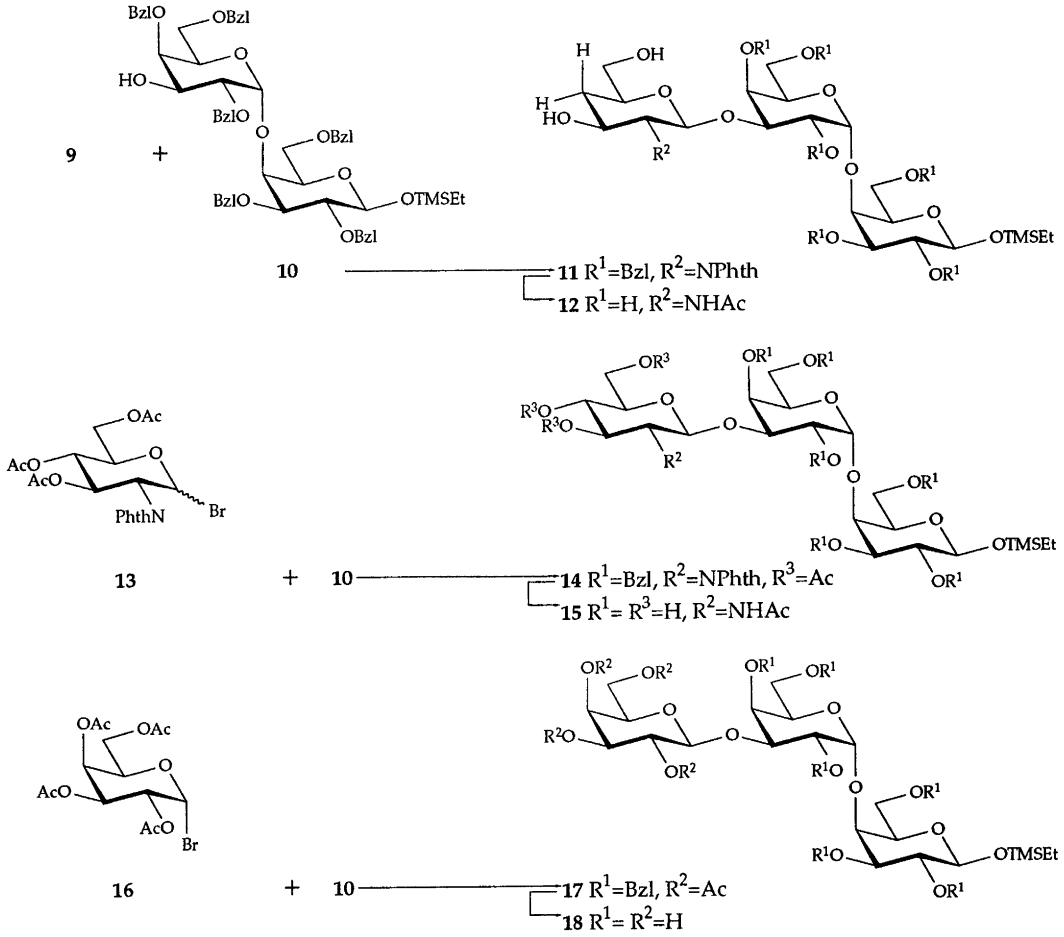
Scheme 1.

Attempts to glycosylate 2-trimethylsilylethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside **10**[12] with the chloride **8** and silver trifluoromethanesulfonate in dichloromethane failed. Only trace amounts of the desired trisaccharide were formed together with a substantial amount of glycal by HCl-elimination from **8** according to TLC analysis. However, it has been shown[21] that glycal formation, using sialic acid-derived thioglycosyl donors, can be suppressed in nitrile solvents at low temperature. Thus, compound **7** was transformed into the corresponding methyl thioglycoside **9** with methylthiotrimethylsilane and trimethylsilyl trifluoromethanesulfonate[22] in dichloromethane in 76% yield. Methylsulfenyl trifluoromethanesulfonate[23]-promoted glycosylation of **10** (1.5 equiv.) with the thioglycoside **9** in dichloromethane–acetonitrile at −78 °C, followed by deacetylation to simplify purification, gave the trisaccharide **11** (39%) from **9** (Scheme 2). This result implies that the glycal formation from 2-deoxy-2-phthalimido glycosyl donors can be decreased by performing glycosylations at low temperature with a nitrile as the solvent. Whether the choice of donor and/or promoter is important for the outcome of the reaction remains to be investigated. Hydrogenolysis of the benzyl ethers and hydrazinolysis of the phthalimido group, followed by *N*-acetylation, afforded the 4″-deoxy trisaccharide **12**.

The syntheses of the saccharides **15** and **18** were more straightforward, using the well known glycosyl donors **13**[14] and **16**. Silver trifluoromethanesulfonate-promoted glycosylation of the galabioside alcohol **10** with the bromide **13** (α:β 2:3) gave the trisaccharide **14** (38%). The moderate yield was again due to glycal formation by HBr elimination from the bromide **13**. Hydrogenolysis of the benzyl groups, hydrazinolysis, and acetylation in acetic anhydride–pyridine, followed by *O*-deacetylation gave the 4″-epi derivative **15** (72%). Glycosylation of **10** with the acetyl(bromo)galactose **16** using silver trifluoromethanesulfonate as the promotor in dichloromethane, gave the trigalactoside derivative **17** (65%). Hydrogenolysis and deacetylation then furnished the trisaccharide **18**.

Experimental

General methods. Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Varian XL-300 spectrometer. Chemical shifts are given in ppm relative to the signal for Me_4Si. 1,4-Dioxane was used as an internal reference (67.4 ppm) in ^{13}C NMR experiments in D_2O. The FAB-MS spectra were recorded with a JEOL SX102 spectrometer. Rotary evaporation was carried out with bath temperatures at or below



Scheme 2.

40 °C. Anhydrous Na_2SO_4 was used as a drying agent for the organic extracts in the work-up procedures. TLC was performed on Kieselgel 60 F_{254} plates (Merck). Column chromatography was performed using SiO_2 (Matrex LC-gel; 60 A, 35-70 MY, Grace). Compounds **1**,[17] **10**[12] and **13**[14] were prepared as described. Compound **16** is commercially available.

*Methyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**).* Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **1**[17] (10.0 g, 22.3 mmol) was treated with methanolic sodium methoxide (103 ml, 0.06 M) for 16 h, then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give a quantitative yield of **2**. A sample was crystallised from heptane–EtOAc, m.p. 159–162 °C, $[\alpha]^{25}_D$ +75° (*c* 1.2, $CHCl_3$). ^1H NMR (CD_3OD): δ 5.08 (d, 1 H, *J* 8.5 Hz, H-1), 4.23 (dd, 1 H, *J* 8.4, 10.7 Hz, H-3), 3.96 (dd, 1 H, *J* 8.5, 10.7 Hz, H-2), 3.94 (dd, 1 H, *J* 2.0, 11.9 Hz, H-6), 3.75 (dd, 1 H, *J* 5.3, 11.9 Hz, H-6), 3.42 (s, 3 H, OMe); *m/z* calc. for $C_{18}H_{26}NO_{10}$ (*M* + H + glycerol); 416.1552; found 416.1557. *Anal.* $C_{15}H_{17}NO_7$: C, 55.7; H, 5.3; N, 4.3. Found C, 54.5; H, 5.5; N, 3.5.

*Methyl 3,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**3**).* To a solution of compound **2** (7.21 g, 22.3 mmol) in pyridine (100 ml) was added benzoyl chloride (5.9 ml, 50.8 mmol) in pyridine (5 ml) at −45 °C. After 2 h, MeOH (0.2 ml) was added and the temperature was allowed to rise to ambient. Concentration of the reaction mixture followed by column chromatography (heptane–EtOAc 2:1) gave **3** (7.60 g, 62%), $[\alpha]^{25}_D$ +75° (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.91 (dd, 1 H, *J* 8.0, 10.8 Hz, H-3), 5.38 (d, 1 H, *J* 8.4 Hz, H-1), 4.81 (dd, 1 H, *J* 3.9, 12.0 Hz, H-6), 4.69 (dd, 1 H, *J* 1.9, 12.0 Hz, H-6), 4.43 (dd, 1 H, *J* 8.4, 10.8 Hz, H-2), 4.00–3.82 (m, 2 H, H-4, H-5), 3.48 (s, 3 H, OMe). *Anal.* $C_{29}H_{25}NO_9$: C, 65.5; H, 4.7; N, 2.6. Found C, 64.8; H, 4.7; N, 2.4.

*Methyl 3,6-di-O-benzoyl-2,4-dideoxy-2-phthalimido-β-D-xylo-hexopyranoside (**6**).* Compound **3** (1.00 g, 1.90 mmol), *N,N*-thiocarbonyldiimidazole (1.54 g, 8.64 mmol) and dry 1,2-dimethoxyethane (40 ml) were refluxed overnight, concentrated, diluted with CH_2Cl_2, washed with aqueous HCl (1 M) and then saturated aqueous $NaHCO_3$, dried, and concentrated. The residue

was passed through a silica gel column (heptane–EtOAc 1:2) to give a quantitative yield of crude **5**. The residue was dissolved in dry toluene (40 ml) and Bu_3SnH (1.25 ml, 4.7 mmol) in dry toluene (3 ml) was added over 20 min followed by 2,2′-azoisobutyronitrile (catalytic amount) under N_2. The mixture was refluxed overnight and then concentrated. Column chromatography of the residue (heptane–EtOAc 3:1) gave **6** (846 mg, 87%), $[\alpha]_D^{25}$ +61° (*c* 0.8, $CDCl_3$). ^1H NMR ($CDCl_3$): δ 5.88 (dt, 1 H, *J* 5.2, 10.8 Hz, H-3), 5.33 (d, 1 H, *J* 8.4 Hz, H-1), 4.54 (dd, 1 H, *J* 5.8, 11.5 Hz, H-6), 4.47 (dd, 1 H, *J* 4.4, 11.5 Hz, H-6), 4.39 (dd, 1 H, *J* 8.4, 10.8 Hz, H-2), 4.17 (m, 1 H, H-5), 3.47 (s, 3 H, OMe), 2.53 (br dd, 1 H, *J* 5.3, 11.9 Hz, H-4eq), 1.83 (q, 1 H, *J* 11.7 Hz, H-4ax). *Anal.* $C_{29}H_{25}NO_8$: C, H, N.

*1-O-Acetyl-3,6-di-O-benzoyl-2,4-dideoxy-2-phthalimido-α/β-D-xylo-hexopyranose (**7**).* To a solution of compound **6** (823.2 mg, 1.60 mmol) in Ac_2O (5.5 ml) and AcOH (1.1 ml) was added H_2SO_4 in AcOH (10% v/v, 0.41 ml). The mixture was stirred for 2 h, then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and water, dried, and concentrated to give **7** (852.6 mg, 98%, α:β 28:72). Crude **7** was used in the synthesis of **8** and **9** without further purification. **7**β had ^1H NMR ($CDCl_3$): δ 6.58 (d, 1 H, *J* 8.9 Hz, H-1), 6.03 (dt, 1 H, *J* 5.2, 10.7 Hz, H-3), 4.54 (dd, 1 H, *J* 8.9, 10.7 Hz, H-2), 4.48 (m, 2 H, H-6), 4.31 (m, 1 H, H-5), 2.56 (br ddd, 1 H, *J* 2.0, 5.2, 12.9 Hz, H-4eq), 2.01 (s, 3 H, OAc), 1.90 (br q, 1 H, *J* 12.2 Hz, H-4ax). **7**α had ^1H NMR ($CDCl_3$): δ 6.70 (dt, 1 H, *J* 5.1, 11.4 Hz, H-3), 6.42 (d, 1 H, *J* 3.2 Hz, H-1), 4.54 (dd, 1 H, *J* 3.2, 11.4 Hz, H-2), 4.48 (m, 2 H, H-6), 4.31 (m, 1 H, H-5), 2.72 (br ddd, 1 H, *J* 2.0, 5.2, 12.9 Hz, H-4eq), 2.09 (s, 3 H, OAc), 1.88 (br q, 1 H, *J* 12.3 Hz, H-4ax).

*3,6-Di-O-benzoyl-2,4-dideoxy-2-phthalimido-β-D-xylo-hexopyranosyl chloride (**8**).* To a solution of crude **7** (70.1 mg, 129 μmol) in dry $CHCl_3$ (500 μl) was added dichloromethyl methyl ether (89 μl, 1.0 mol) and boron trifluoride–diethyl ether (64 μl, 387 μmol) at 0°C. After 4 h, the reaction mixture was diluted with CH_2Cl_2, washed with cold aqueous saturated $NaHCO_3$ and cold water, dried (Na_2SO_4), and concentrated to give a quantitative yield of **8**. Crude **8** was used immediately in an unsuccessful attempt to glycosylate **10**. ^1H NMR ($CDCl_3$): δ 6.27 (d, 1 H, *J* 9.3 Hz, H-1), 5.91 (dt, 1 H, *J* 5.2, 10.5 Hz, H-3), 4.62 (dd, 1 H, *J* 9.3, 10.5 Hz, H-2), 4.51 (m, 2 H, H-6), 4.28 (m, 1 H, H-5), 2.58 (ddd, 1 H, *J* 2.0, 5.2, 12.8 Hz, H-4eq), 1.97 (br q, 1 H, *J* 12.8 Hz, H-4ax).

*Methyl 3,6-di-O-benzoyl-2,4-dideoxy-2-phthalimido-1-thio-β-D-xylo-hexopyranoside (**9**).* To a solution of compound **7** (134.3 mg, 0.248 mmol) in dry CH_2Cl_2 (2 ml) was added methylthio(trimethyl)silane (139 μl, 0.98 mmol) and trimethylsilyl trifluoromethanesulfonate (56 μl, 0.289 mmol) under N_2. After 6 h, the mixture was quenched with diisopropylethylamine (0.3 ml), stirred for 5 min, diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and water, dried, and concentrated. Column chromatography (heptane–EtOAc 2:1) of the residue gave **9** (99.5 mg, 76%), $[\alpha]_D^{25}$ +64° (*c* 1.2, $CDCl_3$). ^1H NMR ($CDCl_3$): δ 5.98 (dt, 1 H, *J* 5.2, 10.6 Hz, H-3), 5.46 (d, 1 H, *J* 10.4 Hz, H-1), 4.53 (dd, 1 H, *J* 5.7, 11.7 Hz, H-6), 4.52 (t, 1 H, *J* 10.5 Hz, H-2), 4.47 (dd, 1 H, *J* 4.3, 11.7 Hz, H-6), 4.22 (m, 1 H, H-5), 2.57 (ddd, 1 H, *J* 2.0, 5.3, 12.7 Hz, H-4eq), 2.21 (s, 3 H, SMe), 1.83 (br q, 1 H, *J* 11.7 Hz, H-4ax). *Anal.* $C_{29}H_{25}NO_7S$: C, H, N.

*2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzyl-4-O-[2,4,6-tri-O-benzyl-3-O-(2,4-dideoxy-2-phthalimido-β-D-xylo-hexopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranoside (**11**).* A mixture of **9** (305 mg, 0.573 mmol), 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside **10**[12] (848 mg, 0.862 mmol), and molecular sieves (1 g, 3 Å) in dry CH_2Cl_2–acetonitrile (1:1, 20 ml) was stirred under N_2 for 1 h. Silver trifluoromethanesulfonate (200 mg, 0.778 mmol) was added, the mixture was cooled to −78°C, and methanesulfenyl bromide[23] (153 μl, 0.575 mmol) in 1,2-dichloroethane (1 ml) was added. After 23 h diisopropylamine (1 ml) was added. The reaction mixture was stirred for 1 h before the temperature was allowed to rise to ambient, then filtered through Celite, and concentrated. Column chromatography (heptane–EtOAc, 5:1→3:1 gradient) of the residue gave an impure trisaccharide (420 mg). The trisaccharide was de-*O*-benzylated in methanolic sodium methoxide (2.6 ml, 0.6 M) overnight and the mixture was neutralised with Amberlite IR-120 (H^+) resin, filtered and concentrated. Column chromatography (toluene–EtOH 30:1) gave pure **11** (282 mg, 39%), $[\alpha]_D^{25}$ +14° (*c* 1.5, $CDCl_3$). ^1H NMR ($CHCl_3$): δ 5.54 (d, 1 H, *J* 8.2 Hz, H-1″), 4.81 (d, 1 H, *J* 3.7 Hz, H-1′), 4.30 (d, 1 H, *J* 7.6 Hz, H-1), 2.12 (br dd, 1 H, *J* 5.2, 12.7 Hz, H-4eq), 1.63 (br q, 1 H, *J* 12.2 Hz, H-4ax), 1.13 (m, 2 H, CH_2Si), 0.03 (s, 9 H, $SiMe_3$). *Anal.* $C_{73}H_{83}NO_{16}Si$: C, 69.7, H, 6.7; N, 1.1. Found C, 70.2; H, 6.8; N, 1.1.

*2-(Trimethylsilyl)ethyl 4-O-[3-O-(2,4-dideoxy-2-acetamido-β-D-xylo-hexopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranoside (**12**).* Compound **11** was hydrogenated over Pd-on-charcoal (10%, 244 mg) in acetic acid (4 ml) for 1.5 h, the mixture was filtered through Celite, and concentrated. The residue was dissolved in EtOH (5.2 ml), hydrazine hydrate (0.42 ml) was added, and the mixture was refluxed for 2 h, then concentrated and co-concentrated with toluene twice to remove the excess of hydrazine hydrate. The residue was *N*-acetylated with Ac_2O (4.2 ml) in water (2.8 ml)–methanol (14 ml) for 1 h, then concentrated. Column chromatography (CH_2Cl_2–MeOH–H_2O, 30:10:1→10:4:1 gradient) of the residue gave **12** (88.7 mg, 68%), $[\alpha]_D^{25}$ +78° (*c* 0.58, H_2O). ^1H NMR ($CDCl_3$): δ 4.90 (d, 1 H, *J* 3.7 Hz, H-1′), 4.57 (d, 1 H, *J* 8.4 Hz, H-1″), 4.45 (d, 1 H, *J* 7.8 Hz, H-1), 4.36 (br t, 1 H, *J* 6.5 Hz, H-5′), 4.22 (br d, 1 H, *J* 2.0 Hz,

H-4′), 3.57 (dd, 1 H, *J* 8.4, 10.1 Hz, H-2″), 3.49 (dd, 1 H, *J* 7.8, 10.1 Hz, H-2), 2.00 (s, 3 H, Ac), 1.97 (br dd, 1 H, *J* 5.1, 11 Hz, H-4eq), 1.44 (br q, 1 H, *J* 11.3 Hz, H-4ax), 0.98 (m, 2 H, CH_2Si), 0.00 (s, 9 H, $SiMe_3$).

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranoside (**14**).* A mixture of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside **10**[12] (578.8 mg, 0.589 mmol), molecular sieves (0.5 g, 4 Å), silver trifluoromethanesulfonate (314 mg, 1.24 mmol), and tetramethylurea (149 µl, 1.24 mmol) in dry CH_2Cl_2 (16.5 ml) was stirred under nitrogen for 30 min, cooled to −70°C, and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl bromide **13**[14] (587 mg, 1.18 mmol) in dry CH_2Cl_2 (1.3 ml) was added, and the temperature was allowed to rise to ambient. After 20 h, the mixture was cooled to 0°C and additional silver trifluoromethanesulfonate (280 mg, 1.11 mmol) and bromide **13** (47 mg, 0.1 mmol) were added. After 23 h, the reaction mixture was filtered through Celite and concentrated. Column chromatography of the residue (heptane–EtOAc, 5:1→4:1 gradient) gave **14** (312.6 mg, 38%), $[\alpha]_D^{25}$ +24° (*c* 1, $CHCl_3$), and unchanged alcohol **10** (315.5 mg, 54%). ^{1}H NMR ($CDCl_3$): δ 5.90 (dd, 1 H, *J* 9.0, 10.6 Hz, H-3″), 5.75 (d, 1 H, *J* 8.3 Hz, H-1″), 5.16 (br t 1 H, *J* 9.6 Hz, H-4″), 4.78 (d, 1 H, *J* 3.6 Hz, H-1′), 4.43 (dd, 1 H, *J* 8.3, 10.6 Hz, H-2″), 4.31 (d, 1 H, *J* 7.6 Hz, H-1), 2.07, 1.95, 1.85 (3 s, 3 H each, Ac), 1.10 (m, 2 H, CH_2Si), 0.05 (s, 9 H, $SiMe_3$). *Anal.* $C_{79}H_{89}NO_{20}Si$: C, 67.7; H, 6.4; N, 1.0. Found C, 67.2; H, 6.4; N, 0.9.

*2-(Trimethylsilyl)ethyl 4-*O*-[3-*O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranoside (**15**).* Compound **14** (305.5 mg, 0.218 mmol) was hydrogenated over Pd-on-charcoal (10%, 200 mg) in AcOH (4 ml) for 19 h, filtered through Celite and concentrated. The residue was dissolved in EtOH (5.5 ml), hydrazine hydrate (0.22 ml) was added, and the mixture was refluxed for 1 h. The solution was concentrated and then co-concentrated with EtOH three times to remove the excess of hydrazine hydrate. The residue was acetylated in Ac_2O (3 ml) and pyridine (3 ml) overnight, then concentrated. Column chromatography (toluene–EtOH, 19:1) removed most of the UV-active impurities emanating from the hydrazinolysis. The residue was de-*O*-acetylated in methanolic sodium methoxide (10 ml, 0.05 M) for 2 h, neutralised with Duolite (H^+) resin, filtered, and concentrated. Column chromatography (CH_2Cl_2–MeOH, 2:1) of the residue gave **15** (101.4 mg, 72%), $[\alpha]_D^{25}$ +62° (*c* 0.62, H_2O). ^{1}H NMR (D_2O): δ 4.90 (d, 1 H, *J* 3.4 Hz, H-1′), 4.66 (d, 1 H, *J* 8.3 Hz, H-1″), 4.44 (d, 1 H, *J* 7.8 Hz, H-1), 4.36 (br t, 1 H, *J* 6.5 Hz, H-5″), 4.22 (br d, 1 H, *J* 2.3 Hz, H-4′), 2.00 (s, 3 H, Ac), 0.99 (m, 2 H, CH_2Si), −0.01 (s, 9 H, $SiMe_3$). ^{13}C NMR (D_2O): δ 175.6, 103.7, 103.1, 101.1, 80.1, 77.6, 76.5, 75.7, 74.5, 73.3, 71.8, 71.1, 70.6, 69.6, 68.9, 68.4, 61.33, 61.26, 60.7, 56.5, 23.0, 18.6, −1.50.

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranoside (**17**).* A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **16** (523.4 mg, 1.27 mmol), 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside **10**[12] (625.8 mg, 0.636 mmol) and molecular sieves (0.5 g, 4 Å) in dry CH_2Cl_2 (18 ml) was stirred under nitrogen for 30 min, cooled to −30°C. Silver trifluoromethanesulfonate (339 mg, 1.34 mmol) and tetramethylurea (161 µl, 1.34 mmol) were added, and the temperature was allowed to rise to ambient. After 4 h, the mixture was cooled to −30°C and additional silver trifluoromethanesulfonate (113 mg, 0.45 mmol) and tetramethylurea (54 µl, 0.45 mmol) were added. After 5 h, the reaction mixture was filtered through Celite and concentrated. Column chromatography of the residue (heptane–EtOAc, 5:1→2:1 gradient) gave **17** (539.3 mg, 65%), $[\alpha]_D^{25}$ +24° (*c* 1.1, $CHCl_3$), and unchanged alcohol **10** (192.1 mg, 31%). ^{1}H NMR ($CDCl_3$): δ 5.44 (br d, 1 H, *J* 3.2 Hz, H-4″), 5.34 (dd, 1 H, *J* 7.9, 10.3 Hz, H-2″), 5.06 (dd 1 H, *J* 3.5, 10.3 Hz, H-3″), 5.01 (d, 1 H, *J* 7.9 Hz, H-1″), 4.97 (d, 1 H, *J* 3.2 Hz, H-1′), 4.31 (d, 1 H, *J* 7.8 Hz, H-1), 2.12, 1.98, 1.95, 1.78 (4 s, 3 H each, Ac), 1.10 (br dd, 2 H, *J* 8.1, 9.4 Hz, CH_2Si), 0.02 (s, 9 H, $SiMe_3$). *Anal.* $C_{73}H_{88}O_{20}Si$: C, 66.8; H, 6.8. Found C, 66.1; H, 6.7.

*2-(Trimethylsilyl)ethyl 4-*O*-[3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranoside (**18**).* Compound **17** (511.4 mg, 0.389 mmol) was hydrogenated (1 atm) over Pd-on-charcoal (10%, 350 mg) in AcOH (7 ml) for 22 h, filtered through Celite and concentrated. The residue was deacetylated in methanolic sodium methoxide (10.7 ml, 0.12 M) for 3 h, neutralised with Duolite (H^+) resin, filtered and concentrated. Column chromatography (CH_2Cl_2–MeOH–H_2O 65:35:5) of the residue gave **18** (202.4 mg, 86%), $[\alpha]_D^{25}$ +66° (*c* 1, H_2O). ^{1}H NMR (D_2O): δ 4.96 (d, 1 H, *J* 3.7 Hz, H-1′), 4.58 (d, 1 H, *J* 7.6 Hz, H-1″), 4.44 (d, 1 H, *J* 7.7 Hz, H-1), 4.36 (br t, 1 H, *J* 6.5 Hz, H-5′), 4.27 (br d, 1 H, *J* 2.4 Hz, H-4′), 0.99 (m, 2 H, CH_2Si), −0.01 (s, 9 H, $SiMe_3$). ^{13}C NMR (D_2O): δ 105.2, 103.1, 100.9, 79.9, 77.9, 75.8, 75.7, 73.4, 71.9, 71.8, 71.2, 69.7, 69.4, 69.0, 68.6, 61.8, 61.3, 60.8, 18.6, −1.52.

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