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Synthesis of deoxy and acylamino derivatives of lactose and use of these for probing the active site of *Neisseria meningitidis* *N*-acetylglucosaminyltransferase

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Abstract—Derivatives of lactose with the galactose ring substituents replaced by deoxy or acylamino functions were prepared. The 2′-, 3′-, 4′- and 6′-deoxy, 3′-acetamido and 3′-benzamido derivatives of phenyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (phenyl β-lactoside) were synthesized from disaccharide or monosaccharide precursors. The derivatives were tested as substrates for the *N*-acetylglucosaminyltransferase from *Neisseria meningitidis*, which uses lactosyl derivatives as acceptors and UDP-GlcNAc as the donor in a β-(1→3) glycosylation reaction. The 6′-deoxy derivative was nearly threefold as active as phenyl β-lactoside, whereas the 2′- and 4′-deoxy derivatives were less active. The other derivatives were inactive, as expected.

Keywords: *Neisseria meningitidis*; Glycosyltransferase; Acceptor specificity; Lactose; Deoxygenated

1. Introduction

Glycosyltransferases are important enzymes, both from a biological and chemical standpoint. Biologically, they are essential components in the post-translational machinery of the cell that decorates proteins and other molecules with carbohydrate structures. In bacteria, carbohydrates are often the dominating cell surface structures, and the extent and type of glycosylation is crucial for bacterial virulence and antigenicity. Chemically, glycosyltransferases can be used as preparative tools in laboratory synthesis of biologically active carbohydrates. These enzymes are able to form, in one step, specific glycosidic linkages with high stereo- and regioselectivity. Here, glycosyltransferases of bacterial origin are particularly useful, because of their relative ease of cloning/overexpression as compared to transferases from eukaryotic sources. We have previously[1–3] prepared, studied and used a bacterial *N*-acetylglucosaminyltransferase (from *Neisseria meningitidis*), which utilizes galactosyl derivatives as the acceptors and UDP-GlcNAc as the donor to form a β-D-GlcNAc-(1→3)-D-Gal glycosidic linkage. By examining[1] different substrates for the enzyme, it was found that both UDP-GlcNAc and UDP-GalNAc could be used as donors, but none of the other common nucleotide sugars were substrates. On the acceptor side, the enzyme could glycosylate a variety of terminal α- or β-pyranose derivatives of the D-*galacto* configuration, and *p*-nitrophenyl-lactoside was the best acceptor in these experiments. Pyranose derivatives with D-*gluco*, D-*manno*, or 2-acetamido-2-deoxy-D-*galacto* configuration were not glycosylated to any measurable extent.

To expand the above studies on the acceptor side, we have now prepared deoxy and acylamino derivatives of phenyl β-lactoside. The details of the chemical syntheses of these compounds are reported here, together with experiments in which the derivatives were used as acceptors in enzymatic reactions.

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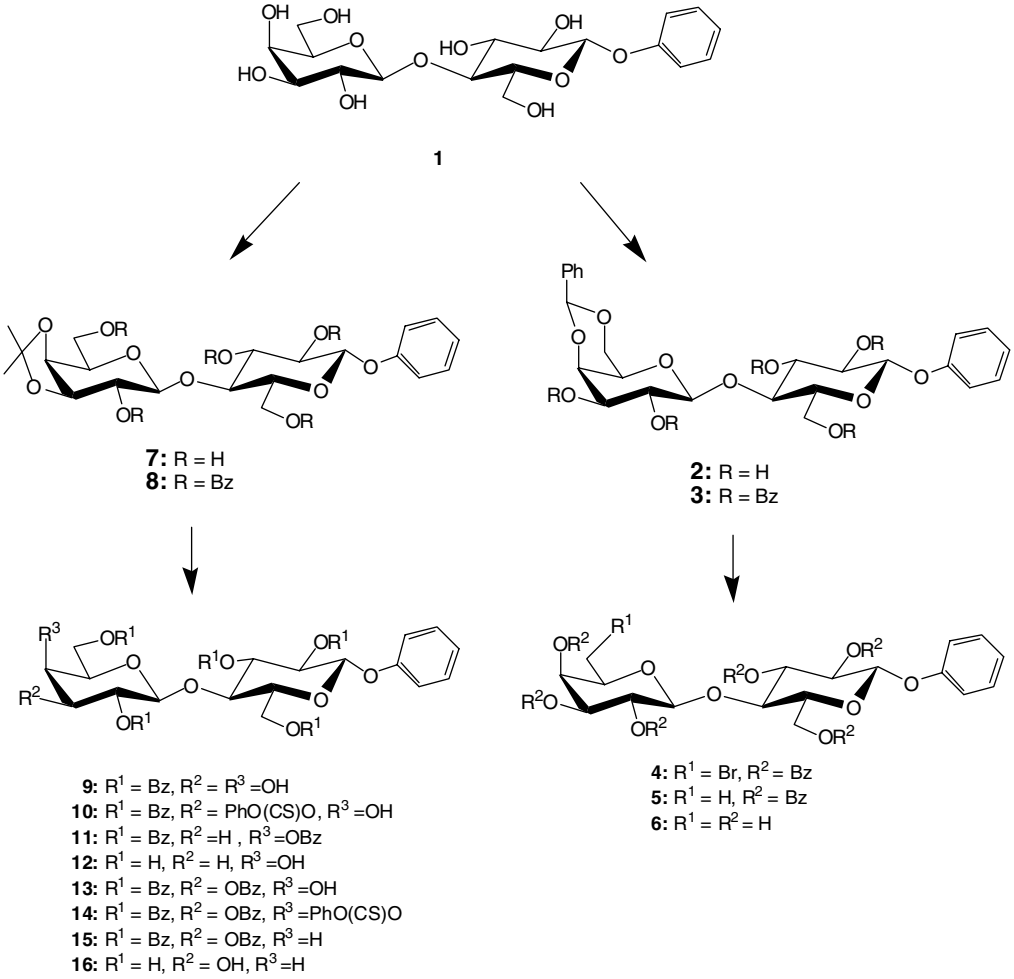
2. Results and discussion

Phenyl β-lactoside was chosen as the parent compound. It had a similar acceptor activity as *p*-nitrophenyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (*p*-nitrophenyllactoside), which was the best acceptor found in the earlier studies.[1] The phenyl derivative is, however, better suited to go through various synthetic transformations than the *p*-nitrophenyl derivative, because it is stable to conditions such as radical deoxygenations, LAH reductions and catalytic hydrogenations. The phenyl group also has good UV-absorbing and lipophilic properties, which are advantageous in some enzyme assays.

The deoxygenated phenyl glycosides **6**, **12**, **16** and **24** were synthesized using synthetic approaches different from those previously reported for the synthesis of various deoxy derivatives of methyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (methyl β-lactoside).[4,5] In the case of **12**, **16** and **24**, the 3′, 4′ and 2′ hydroxy functions, respectively, were removed by tributyltin-mediated radical reduction of thiocarbonyl derivatives. In the case of compound **6**, the 6′-hydroxy function was removed by NBS-opening[6] of a benzylidene compound followed by catalytic hydrogenation. The 3′-acylamino functions of compounds **33** and **34** were introduced by oxidation of the selectively protected 3′-OH compound **27**, followed by a sequence of oximation, LAH reduction, acylation and debenzylation.

The following synthetic steps were carried out: lactose was peracetylated with acetic anhydride and sodium acetate, and the resulting octaacetate was reacted with phenol and boron trifluoride etherate[7] to give per-*O*-acetylated phenyl β-lactoside, which was deacetylated with sodium methoxide to give phenyl β-lactoside[8] (**1**) in 59% yield from lactose (Scheme 1). This method of preparation of **1** has, to our knowledge, not previously been described. It was found to be inexpensive, convenient and easy to scale up. Treatment of **1** with benzaldehyde dimethyl acetal under acidic conditions gave the 4′,6′-benzylidene acetal **2**, which was directly benzoylated with benzoyl chloride in pyridine to give **3** (77% yield from **1**). Treatment of **3** with *N*-bromosuccinimide gave the expected 6′-bromo derivative **4** (29%), which



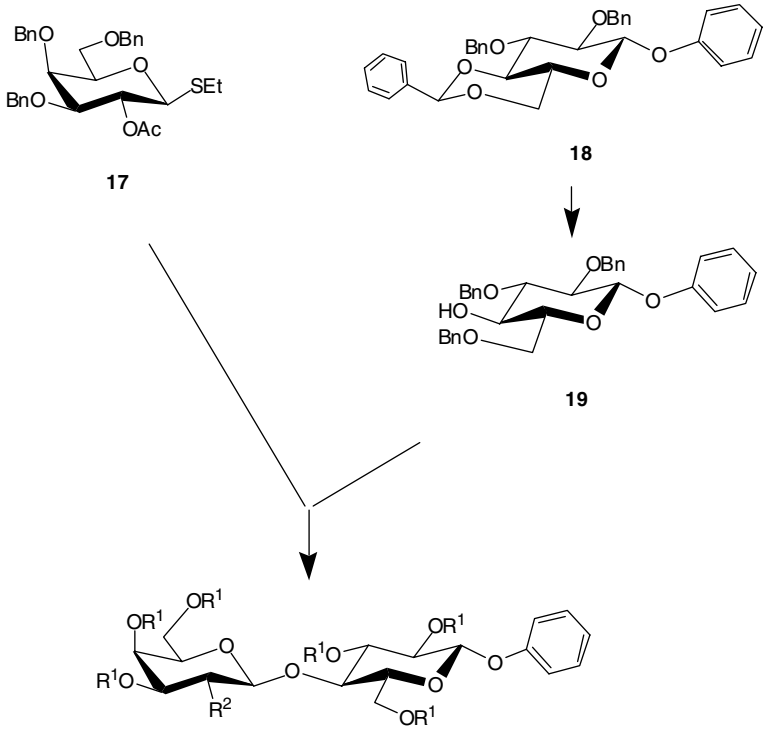
Scheme 1.

was hydrogenated (Pd/C) and debenzoylated (sodium methoxide) to give the first target compound, the 6′-deoxy derivative **6**, in 40% yield. Although none of the steps were yield optimized, this synthetic pathway produced sufficient material for biological experiments (Scheme 1).

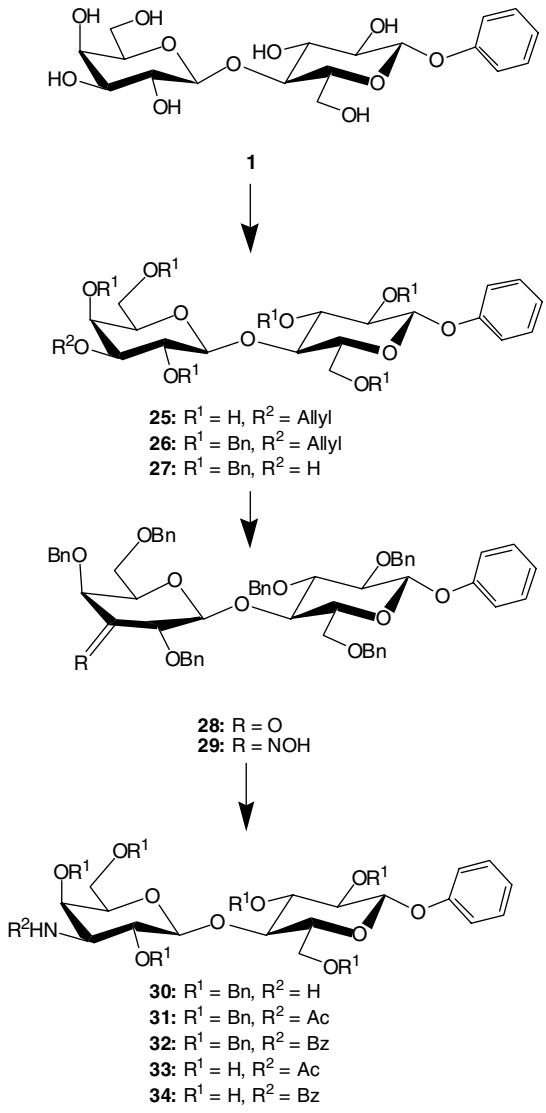
For the synthesis of the 3′- and 4′-deoxy derivatives **12** and **16**, (Scheme 2) phenyl β-lactoside (**1**) was converted to the 3′,4′-diol **9** by a sequence involving acetonation (2,2-dimethoxypropane/acetone/DMF) followed by benzoylation (benzoyl chloride in pyridine) to give **8** (63%). Hydrolysis (aqueous acetic acid) of **8** gave **9** (89%). Selective 3′-acylation of the diol **9** with phenylchlorothionoformate followed by tributyltin-mediated radical deoxygenation gave **11** in low (7.6%) but sufficient yield. That deoxygenation had indeed occurred at the 3′-position was evident from the ^{1}H NMR coupling pattern and high-field chemical shift of the (two) 3′-protons. The ^{1}H COSY spectrum also showed a distinct signal for the 4′-OH proton, with a clear scalar coupling to H-4′, confirming that the 4′-OH group was still intact. Debenzoylation (sodium methoxide) of **11** gave the second target compound, the 3′-deoxy derivative **12** (88%).

Monobenzoylation of the 3′,4′-diol **9** with benzoyl chloride in pyridine gave **13** (60%), the position of benzoylation being evident from the (low field) ^{1}H NMR chemical shift of H-3′. Reaction of **13** with phenylchlorothionoformate (to give **14**) followed by tributyltin-mediated radical deoxygenation gave **15** (76%), which was debenzoylated (sodium methoxide) to give the third target compound, the 4′-deoxy derivative **16** (90%) (Scheme 2).

The fourth target compound, the 2′-deoxy derivative **24**, was synthesized from monosaccharide derivatives, because it was considered[4] that isolation of the 2′-position in a lactosyl disaccharide would be more complicated than with the 3′-, 4′- and 6′-positions. Thus, phenyl 4,6-*O*-benzylidene-β-D-glucopyranoside[9] was treated with benzyl bromide/DMF and then sodium cyanoborohydride/HCl to give the 2,3,6-tribenzyl derivative **19**[10] in 37% yield (Scheme 3). Thioglycoside **17**[11] was then used to glycosylate **19** (DMTST promotion), and the resulting disaccharide **20** (obtained in 70% yield) was deacetylated with sodium methoxide to give **21** (98% yield). Attempted treatment of **21** with phenylchlorothionoformate failed for unknown reasons, therefore the methyl xanthate derivative **22** was prepared instead by treatment of **21** first with carbon disulfide/NaH and then methyl iodide. Tributyltin-mediated radical deoxygenation of **22** gave **23** (56% yield from **21**). Finally,



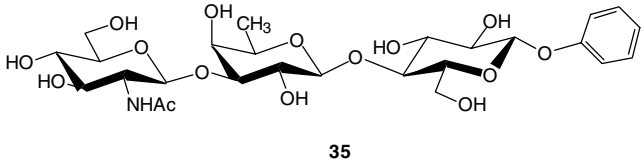
Scheme 2.



Scheme 3.

catalytic hydrogenation (Pd/C) produced the 2′-deoxy derivative **24** in 94% yield (Scheme 3).

For the synthesis of the 3′-acylamino derivatives, phenyl β-lactoside (**1**) was selectively 3′-allylated with allyl bromide/dibutyltin oxide to give **25** (47% yield), which was benzylated (benzyl bromide/NaH, 63% yield) and deallylated (81% yield) to afford the 3′-OH compound **27** (Scheme 4). Oxidation (oxalyl chloride/DMSO) of **27** gave the 3′-keto derivative **28**, which was treated with hydroxylamine and pyridine to give the oxime **29**.



Scheme 4.

Table 1. Acceptor activity of synthesized lactose derivatives

Acceptor	% Activity
Lac-OPh (**1**)	100
2′-Deoxy (**24**)	45
3′-Deoxy (**12**)	3
4′-Deoxy (**16**)	15
6′-Deoxy (**6**)	289
3′-NHAc (**33**)	1
3′-NHBz (**34**)	0

The '% activity' numbers represent relative radioactive counts measured by the enzymatic assay. Phenyl β-lactoside (**1**) was set to 100%.

Reduction of **29** with lithium aluminium hydride gave the equatorial (according to ^{1}H NMR coupling constants after acylation) 3′-amino derivative **30** as the only detectable epimer. In situ acylation of **30** with either acetic anhydride/pyridine or benzoyl chloride/pyridine gave compounds **31** (38% yield) and **32** (61% yield), catalytic hydrogenation of which gave the 3′-acylamino derivatives **33** (67% yield) and **34** (78% yield) (Scheme 4).

The synthesized deoxy and acylamino derivatives were tested, using a radioactivity-based assay, as acceptors in an enzymatic reaction with the *N. meningitidis N*-acetylglucosaminyltransferase using UDP-GlcNAc as the donor. The results are summarized in Table 1. As expected, the 3′-deoxy (**12**) and 3′-acylamino (**33** and **34**) derivatives did not function as acceptors, as they do not carry a 3′-OH group (in a future investigation, these compounds will be studied as inhibitors of this enzyme). However, the 2′-, 4′- and the 6′-deoxy derivatives (**24**, **16** and **6**) were acceptors. The 2′ (**24**) and 4′ derivatives (**16**) were less active than the phenyl β-lactoside reference (45% and 15% yield, respectively), and the 6′-deoxy derivative (**6**) was nearly threefold as active. To ensure that the 6′-deoxy derivative **6** was glycosylated in the normal way, a preparative enzymatic reaction was performed. The product was isolated and characterized as the expected β-(1→3) linked trisaccharide **35**. Although these data are very preliminary, they reconfirm the earlier findings[1] that the *N. meningitidis N*-acetylglucosaminyltransferase displays a relatively broad specificity towards both acceptor and donor substrates, while maintaining strict product specificity. Both properties are valuable for an enzyme that is to be used in a preparative manner. Further experiments are in progress to investigate the inhibitory capacity of the synthesized derivatives and the 3-dimensional details of the enzyme–substrate interaction.

3. Experimental

3.1. General methods

Concentrations were performed at reduced pressure (bath temperature <40 °C). NMR spectra were recorded

at 303 or 323 K with a Bruker DRX 400 spectrometer using $CDCl_3$ (internal $CHCl_3$, δ_H 7.26 ppm at 303 K), D_2O (internal HOD δ_H 4.69 ppm, internal acetone δ_C 30.70 ppm at 303 K) or CD_3OD (internal CH_3OH, δ_H 3.31 ppm, δ_C 47.6 ppm at 303 K). Some spectra were recorded in D_2O at 323 K, and for these spectra the δ_H of the internal HOD was set to 4.50 ppm and the δ_C for internal acetone was set to 30.55 ppm. Only selected NMR data are reported. Assignments were corroborated by appropriate 2-D experiments. Spectral data for **6**, **12**, **16**, **24**, **33**, **34**, **35** are given in tabular form, with proton coupling constants in parenthesis. The ES-TOF HRMS spectra were recorded in the positive ion mode with a Micromass LCT instrument, using acetonitrile/water as solvent and tetradecylammonium ion as reference mass (*m*/*z* 578.6604). TLC was performed on Silica Gel F_{254} (Merck, Darmstadt, Germany) with detection by UV-light and by staining with 5% H_2SO_4 in ethanol or 0.5% ninhydrin in butanol. Column chromatography was performed on Matrex silica gel 60 Å (35–70 μm, Amicon). Isolute cartridges (C-18 EC) were from International Sorbent Technology, Mid Glamorgan, UK. Molecular sieves (powdered 4 Å) were dried at 280 °C/0.5 Torr overnight. Dry CH_2Cl_2 was prepared by distillation from P_2O_5. Dimethyl(thiomethyl)sulfonium triflate (DMTST) was prepared essentially as previously described,[12] and was stored under dry nitrogen at −20 °C. Recombinant *N. meningitidis* β-(1→3)-*N*-acetylglucosaminyltransferase (GlcNAc-T) was prepared as described,[1] the unit definition was based on *p*-nitrophenyl β-lactoside as the substrate.

3.2. Phenyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (phenyl β-lactoside) (1)

A mixture of anhydrous sodium acetate (13.5 g, 165 mmol) and Ac_2O (270 mL, 2.86 mol) was refluxed while lactose (13.5 g, 39.4 mmol) was added in portions. The mixture was then refluxed for 3 h, cooled to 40–50 °C and poured into ice water (2 L) during vigorous stirring. After further stirring overnight at rt, the precipitated solid was filtered off and dried to produce crude β-lactose octaacetate, which was recrystallized from CH_3OH to give pure material (19.3 g, 28.4 mmol, 72%). This material was mixed with phenol (16 g, 170 mmol), 4 Å molecular sieves (13 g) and dry CH_2Cl_2 (110 mL) and stirred while boron trifluoride etherate (4.0 mL, 31.6 mmol) was added. After stirring overnight the mixture was filtered and the filtrate was evaporated. Crystallization of the residue from diethyl ether gave 16.8 g, 23.6 mmol (83%) of relatively pure phenyl 2,3,6,2′3′4′6′-hepta-*O*-acetyl-β-lactoside, which was taken up in methanolic sodium methoxide (0.05 M, 150 mL) and further stirred overnight at rt. Neutralization with Dowex-50 (H^+), filtration, addition of a few drops of aqueous ammonia and evaporation gave **1** (9.8 g, 23.4 mmol, the total yield from lactose was 59%) as a colourless solid, which was more than 95% pure according to its NMR spectrum. The physical constants were as reported.[8]

3.3. Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (3)

A solution of **1** (7.85 g, 18.8 mmol) in dry *N*,*N*-dimethylformamide (20 mL) containing benzaldehyde dimethylacetal (6.0 mL, 40.0 mmol) and *p*-toluenesulfonic acid (100 mg, 526 mmol) was rotated on a rotary evaporator at 50 °C and 200 mbar for 6 h, after which time no more change was detected by TLC. The product was precipitated by addition of diethyl ether–EtOAc (99:1), which yielded crude solid **2** (8.43 g, 16.6 mmol). This material was dissolved in dry pyridine (50 mL) and the solution was cooled in ice while benzoyl chloride (30 mL, 258 mmol) was added in portions. After 3 h at rt, water (0.3 mL) was added, and, after 30 min, the mixture was partitioned between CH_2Cl_2 and water, and the organic layer was washed with 2 M aq H_2SO_4 and 1 M aq $NaHCO_3$. Drying ($MgSO_4$) of the organic layer and concentration gave a syrup, which was crystallized from CH_3OH to give **3** as a colourless solid (14.7 g, 14.3 mmol, 76%), $[\alpha]_D$ +96 (*c* 0.5, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 5.91 (t, 1H, J = 9.2 Hz, H-3), 5.81 (dd, 1H, J = 7.9, 10.3 Hz, H-2′), 5.61 (dd, 1H, J = 7.7, 8.8 Hz, H-2), 5.30 (s, 1H, PhCH), 5.26 (d, 1H, J = 7.7 Hz, H-1), 5.19 (dd, 1H, J = 3.5, 10.3 Hz, H-3′), 4.88 (d, 1H, J = 7.9 Hz, H-1′), 4.64 (dd, 1H, J = 2.0, 11.6 Hz, H-6a), 4.39 (dd, 1H, J = 5.5, 11.8 Hz, H-6b), 4.33 (dd, 1H, J = 0.5, 3.3 Hz, H-4′), 4.29 (dd, 1H, J = 8.8, 9.4 Hz, H-4), 4.04 (m, 1H, H-5), 3.78 (dd, 1H, J = 1.0, 12.0 Hz, H-6a′), 3.61 (dd, J = 1.0, 12.0 Hz, H-6b′), 3.04 (m, H-5′). HRMS: Calcd for $C_{60}H_{54}O_{16}N$: 1044.3443. Found: 1044.3451 ($M+NH_4^+$).

3.4. Phenyl 4-*O*-(6-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside (6)

A mixture of **3** (1.50 g, 1.46 mmol) and carbon tetrachloride (30 mL) was stirred and heated to 70 °C, then 1,2-dichloroethane (15 mL) was added, followed by barium carbonate (4 g) and, with continued heating (85 °C) and stirring for another 3 h, *N*-bromosuccinimide (0.36 g, 2.02 mmol) was added in portions. The mixture was filtered and concentrated. The residue was purified by column chromatography (toluene/EtOAc, 12:1), giving **4** as a syrup (0.4 g, 362 μmol, 25%); $[\alpha]_D$ +69 (*c* 0.5, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 5.85 (t, 1H, J = 9.2 Hz, H-3), 5.73 (dd, 1H, J = 7.9, 9.4 Hz, H-2′), 5.69 (dd, 1H, J = 7.9, 10.1 Hz, H-2), 5.35 (dd, 1H, J = 3.3, 10.3 Hz, H-3′), 5.24 (d, 1H, J = 7.9 Hz, H-1), 4.88 (d, 1H, J = 7.9 Hz, H-1′), 4.66 (dd, 1H, J = 1.4,

Table 2. NMR data for **6** (D_2O, 303 K)

	H-1 (*J*) C-1	H-2 (*J*) C-2	H-3 (*J*) C-3	H-4 (*J*) C-4	H-5 (*J*) C-5	H-6 (*J*) C-6	H-6′ (*J*) C-6	C	CH_o (*J*)	CH_m (*J*)	CH_p (*J*)
Glc	5.13 (7.8) 100.02	3.59 72.71	3.71 74.23	3.67 78.65	3.74 74.89	3.94 59.97	3.79 59.97				
Gal	4.40 (7.8) 103.01	3.48 70.72	3.64 72.76	3.73 71.23	3.80 71.07	1.25 (6.4) 15.40	1.25 (6.4) 15.40				
OPh								156.55	7.12 116.30	7.38 130.00	7.13 123.14

12.1 Hz, H-6a), 4.46 (dd, 1H, J = 5.3, 12.1 Hz, H-6b), 4.34 (t, 1H, J = 9.6 Hz, H-4), 3.98 (m, 1H, H-5), 3.76 (m, 1H, H-5′), 2.82 (dd, 1H, J = 6.2, 10.5 Hz, H-6a′), 2.62 (dd, 1H, J = 7.0, 10.5 Hz, H-6b′). A solution of this material (0.20 g, 181 μmol) in CH_3OH/EtOAc (3:2, 3.0 mL) containing 4 Å molecular sieves (1.3 g) and palladium on carbon (10%, 0.53 g) was stirred under a hydrogen atmosphere for 48 h. The mixture was filtered and evaporated and the residue was purified by column chromatography (toluene/EtOAc, 12:1) to give **5** (0.12 g, 117 μmol). This material was mixed with methanolic sodium methoxide (0.1 M, 8 mL) and heated with stirring to 70 °C for 30 min, after which time TLC detected no more change. After cooling, the mixture was neutralized with Dowex-50 (H^+), filtered and evaporated to give a residue, which was partitioned between EtOAc and water (5:5 mL). The aqueous layer was washed with EtOAc and lyophilized. The residue was dissolved in water (2 mL) and the solution was slowly passed through a C-18 Isolute cartridge (0.5 g, pre-conditioned with first 5 mL of CH_3OH, then 20 mL of water). The cartridge was washed with water (5 mL) and then the desired material was eluted with 10–50% CH_3OH to give **6** (0.03 g, 74.5 μmol, 41% from **4**) as a colourless solid, $[\alpha]_D$ −33 (*c* 0.5, CH_3OH). HRMS: Calcd for $C_{10}H_{26}O_{10}Na$: 425.1424. Found: 425.1422 ($M+Na^+$). The NMR data for **6** are provided in Table 2.

3.5. Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (8)

A mixture of **1** (4.0 g, 9.56 mmol), *N*,*N*-dimethylformamide (7.0 mL), acetone (220 mL) 2,2-dimethoxypropane (7.0 mL, 56.9 mmol) and concentrated H_2SO_4 (0.01 mL) was heated at reflux until TLC indicated no more change (1 h). After cooling to rt, the precipitate was collected, washed with cold acetone and dried to give crude **7** (3.0 g, 6.54 mmol). This material was taken up in dry pyridine (60 mL) and the solution was cooled in ice while benzoyl chloride (7.5 mL, 64.6 mmol) was added in portions. After 3 h at rt, water (0.2 mL) was added, and, after 30 min, the mixture was partitioned between CH_2Cl_2 and water and the organic layer was washed with 2 M aq H_2SO_4 and 1 M aq $NaHCO_3$. Drying of the organic layer ($MgSO_4$) and concentration gave a syrup, which was purified by column chromatography to give **8** (5.86 g, 5.99 mmol, 63%), $[\alpha]_D$ +59 (*c* 0.5, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.80 (dd, J = 9.0, 9.4 Hz, H-3), 5.69 (dd, J = 7.7, 9.4 Hz, H-2), 5.24 (d, 1H, J = 7.7 Hz, H-1), 5.17 (t, 1H, 7.8 Hz, H-2′), 4.65 (dd, 1H, J = 2.0, 12.1 Hz, H-6a), 4.63 (d, 1H, J = 7.6 Hz, H-1′), 4.49 (dd, 1H, J = 5.7, 12.1 Hz, H-6b), 3.72 (dd, 1H, J = 7.5, 11.4 Hz, H-6b′), 1.54 (s, 3H, C–CH_3), 1.27 (s, 3H, C–CH_3). HRMS: Calcd for $C_{56}H_{54}O_{16}N$: 996.3443. Found: 996.3427 ($M+NH_4^+$).

3.6. Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (9)

A solution of compound **8** (5.86 g, 5.99 mmol) in aq 85% acetic acid (100 mL) was stirred at 95 °C for 1 h, then concentrated and extensively co-concentrated with toluene. The remaining solid was triturated with CH_3OH and dried to give **9** (4.98 g, 5.30 mmol, 89%), $[\alpha]_D$ +54 (*c* 0.5, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.77 (t, J = 9.2 Hz, H-3), 5.70 (dd, 1H, J = 7.7, 9.0 Hz, H-2), 5.24 (dd, 1H, H-2′), 5.22 (d, 1H, J = 7.7 Hz, H-1), 4.63–4.69 (m, 2H, H-1′, H-6a), 4.53 (dd, 1H, J = 5.9, 11.8 Hz, H-6b), 4.21 (t, 1H, J = 9.2 Hz, H-4). HRMS: Calcd for $C_{53}H_{50}O_{16}N$: 956.3130. Found: 956.3132 ($M+NH_4^+$).

3.7. Phenyl 4-*O*-(3-deoxy-β-D-*xylo*-hexopyranosyl)-β-D-glucopyranoside (12)

A solution of compound **9** (4.0 g, 4.26 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (2.28 g, 18.7 mmol) in dry CH_2Cl_2 (150 mL) was stirred and cooled to 0 °C while phenylchlorothionoformate (0.86 mL, 6.2 mmol) was added. The solution was stirred at rt until TLC indicated no more change (4 h), then diluted with CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$ solution and brine, before the organic layer was dried (Na_2SO_4) and concentrated. This material was directly taken up in dry benzene (200 mL), the solution was blanketed

Table 3. NMR data for **12** (D_2O, 303 K)

	H-1 (*J*) C-1	H-2 (*J*) C-2	H-3_{ax} (*J*) C-3	H-3_{eq} (*J*) C-3	H-4 (*J*) C-4	H-5 (*J*) C-5	H-6 (*J*) C-6	H-6′ (*J*) C-6	C	CH_o (*J*)	CH_m (*J*)	CH_p (*J*)
Glc	5.12 (7.9) 100.01	3.59 72.70	3.73 74.25		3.75 78.13	3.73 74.97	3.94 60.02	3.79 60.02				
Gal	4.43 (7.9) 104.94	3.72 65.59	1.70 (13.7, 11.8, 3.1) 37.05	2.18 (13.7, 3.2, 5.0) 37.05	3.98 65.69	3.72 78.71	3.70 61.28	3.70 61.28				
OPh									156.53	7.11 116.62	7.37 (7.8) 129.99	7.11 123.40

with nitrogen, tributyltin hydride (5.95 mL, 22.1 mmol) was added, the solution was heated to 80 °C and azoisobutyronitrile (20 mg) was added. After 1 h, the mixture was concentrated and the residue was diluted with acetonitrile (250 mL) and washed several times with petroleum ether. The acetonitrile layer was concentrated and the residue was redissolved in CH_2Cl_2. The solution was washed with 0.1 M aqueous HCl, aq $NaHCO_3$ solution, and brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography to give pure **11** (0.30 g, 325 μmol, 7.6%) as a syrup ^{1}H NMR data ($CDCl_3$): δ 5.81 (t, J = 9.2 Hz, H-3), 5.73 (dd, 1H, J = 7.7, 9.5 Hz, H-2), 5.27 (d, 1H, J = 7.7 Hz, H-1), 5.23 (m, 1H, H-2′), 4.75 (dd, 1H, J = 2.0, 12.0 Hz, H-6a), 4.71 (d, 1H, J = 7.9 Hz, H-1′), 4.59 (dd, 1H, J = 6.1, 12.0 Hz, H-6b), 4.26 (t, 1H, J = 9.7 Hz, H-4), 2.49 (ddd, 1H, J = 3.1, 5.5, 13.6 Hz, H-3a′), 2.24 (d, 1H, J = 8.1 Hz, 4′-OH), 1.66 (ddd, 1H, J = 3.1, 11.2, 13.6 Hz, H-3b′). A solution of **11** (0.21 g, 228 μmol) in methanolic sodium methoxide (0.05 M, 11 mL) was stirred at rt overnight, then neutralized with Dowex-50 (H+) and concentrated. The residue was partitioned between EtOAc and water. The aqueous layer was washed with EtOAc, partially concentrated on a rotary evaporator and finally lyophilized. The residue was dissolved in water (5 mL) and the solution was slowly passed through a C-18 Isolute cartridge (2 g, pre-conditioned with first 20 mL of CH_3OH, then 100 mL of water). The cartridge was washed with water (20 mL) and then the desired material was eluted with 10–50% CH_3OH to give **12** (80 mg, 199 μmol, 87% from **11**), $[\alpha]_D$ −56 (c 0.5, CH_3OH); HRMS: Calcd for $C_{18}H_{26}O_{10}Na$: 425.1424. Found: 425.1426 ($M+Na^+$). The NMR data for **12** are provided in Table 3.

3.8. Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (13)

A solution of compound **9** (200 mg, 0.213 mmol) in dry pyridine (4 mL) was stirred and cooled to 0 °C while benzoyl chloride (0.025 mL, 0.213 mmol) was added. After 3 h at rt, water (0.050 mL) was added, and then, after 30 min, the mixture was partitioned between CH_2Cl_2 and water. The organic layer was washed with 1 M aq HCl and 1 M aq $NaHCO_3$, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (toluene/EtOAc, 4:1) to give **13** (134 mg, 0.128 mmol, 60%), $[\alpha]_D$ +30 (c 0.5, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.85 (dd, 1H, J = 9.3 Hz, H-3), 5.76 (dd, 1H, J = 8.1, 10.5 Hz, H-2′), 5.71 (dd, 1H, J = 7.8, 9.3 Hz, H-2), 5.25 (d, 1H, J = 7.8 Hz, H-1), 5.18 (dd, 1H, J = 3.4, 10.5 Hz, H-3′), 4.82 (d, 1H, J = 6.1 Hz, H-1′), 4.63 (dd, 1H, J = 2.2, 12.0 Hz, H-6a), 4.49 (dd, 1H, J = 5.9, 12.0 Hz, H-6b), 4.26 (dd, 1H, J = 9.5 Hz, H-4), 4.03 (ddd, 1H, J = 2.2, 5.9, 9.5 Hz, H-5). HRMS: Calcd for $C_{60}H_{54}O_{17}N$: 1060.3392. Found: 1060.3392 ($M+NH_4^+$).

3.9. Phenyl 4-*O*-(4-deoxy-β-D-*xylo*-hexopyranosyl)-β-D-glucopyranoside (16)

A solution of **13** (550 mg, 527 μmol) and 4-(*N*,*N*-dimethylamino)pyridine (250 mg, 2.05 mmol) in dry CH_2Cl_2 (25 mL) was stirred and cooled to 0 °C while phenylchlorothionoformate (0.110 mL, 793 μmol) was added. The solution was stirred overnight at rt, then diluted with CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$ solution and brine, dried (Na_2SO_4) and evaporated to give crude **14**. This material was directly taken up in dry benzene (20 mL), the solution was blanketed with nitrogen, tributyltin hydride (0.83 mL, 3.09 mmol) was added, the solution was heated to 80 °C and azoisobutyronitrile (15 mg, 91.5 μmol) was added. After 1 h, the mixture was concentrated and the residue was diluted with acetonitrile (30 mL) and washed several times with petroleum ether. The acetonitrile layer was concentrated and the residue was redissolved in CH_2Cl_2. The solution was washed with 0.1 M aq HCl, aq $NaHCO_3$ solution, and brine, dried (Na_2SO_4), concentrated and purified by column chromatography to afford **15** (0.41 g, 399 μmol, 76%), ^{1}H NMR data ($CDCl_3$): δ 5.82 (t, 1H, J = 9.2 Hz, H-3), 5.67 (dd, 1H, J = 7.7,

Table 4. NMR data for **16** (D_2O, 303 K)

	H-1 (*J*) C-1	H-2 (*J*) C-2	H-3 (*J*) C-3	$H\text{-}4_{ax}$ (*J*) C-4	$H\text{-}4_{eq}$ (*J*) C-4	H-5 (*J*) C-5	H-6 (*J*) C-6	H-6′ (*J*) C-6	C	CH_o (*J*)	CH_m (*J*)	CH_p (*J*)
Glc	5.10 (7.8) 100.00	3.58 72.75	3.70 74.28	3.70 78.80		3.71 75.00	3.94 (12.0) 60.04	3.79 (4.2, 12.0) 60.04				
Gal	4.41 (7.8) 103.10	3.19 (7.8, 9.1) 74.93	3.73 70.17	1.41 (12.7, 12.0) 33.99	1.93 (4.8, 12.7) 33.99	3.66 72.90	3.69 63.78	3.57 63.78				
Oph										7.11	7.36 (7.8)	7.12
									156.54	116.63	130.00	123.41

9.2 Hz, H-2), 5.38 (dd, 1H, J = 7.8, 9.4 Hz, H-2′), 5.23 (m, 1H, H-3′), 5.22 (d, 1H, 7.7 Hz, H-1), 4.75 (d, 1H, J = 7.8 Hz, H-1′), 4.62 (dd, 1H, J = 1.8, 12.1 Hz, H-6a), 4.49 (dd, 1H, J = 5.9, 12.1 Hz, H-6b), 4.25 (t, 1H, J = 9.4 Hz, H-4), 2.25 (m, 1H, H-4a′), 1.61 (m, 1H, H-4b′). A solution of **15** (0.78 g, 759 μmol) in methanolic sodium methoxide (0.05 M, 65 mL) was stirred at rt overnight, then neutralized with Dowex-50 (H+) and concentrated. The residue was partitioned between EtOAc and water. The aqueous layer was washed with EtOAc, partially concentrated on a rotary evaporator and finally lyophilized. The residue was dissolved in water (10 mL) and the solution was slowly passed through a C-18 Isolute cartridge (5 g, pre-conditioned with first 40 mL of CH_3OH, then 100 mL of water). The cartridge was washed with water (20 mL) and then the desired material was eluted with 10–50% CH_3OH to give **16** (290 mg, 721 μmol, 95% from **15**), $[\alpha]_D$ −51 (*c* 0.5, CH_3OH); HRMS: Calcd for $C_{18}H_{27}O_{10}$: 403.1604. Found: 403.1617 [$(M+H^+)$] and calcd for $C_{18}H_{26}O_{10}Na$: 425.1424. Found: 425.1403 ($M+Na^+$). The NMR data for **16** are provided in Table 4.

3.10. Phenyl 4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (20)

Phenyl 4,6-*O*-benzylidene-β-D-glucopyranoside[9] (829 mg, 2.41 mmol) was dissolved in dry *N*,*N*-dimethylformamide (12 mL) and benzyl bromide (1.45 mL, 12.0 mmol) was added. The solution was cooled on an ice bath while sodium hydride (80% dispersion in oil, 361 mg, 11.8 mmol) was added and was then stirred at rt overnight, where after CH_3OH (5.4 mL) was added. The mixture was partitioned between $CHCl_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (toluene/EtOAc, 10:1) of the residue gave **18** (1.28 g, 2.41 mmol) as a white solid. This material was dissolved in dry tetrahydrofuran (15 mL) and sodium cyanoborohydride (1.31 g, 20.9 mmol) was added. A saturated solution of HCl in dry diethyl ether was added in small portions until gas development ceased and the mixture remained acidic. The mixture was concentrated to approximately 2 mL, CH_2Cl_2 was added and the mixture was neutralized by addition of aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, the combined organic phases were washed with water, dried ($MgSO_4$) and evaporated. Column chromatography (toluene/EtOAc, 10:1) of the residue gave **19** (465 mg, 880 μmol, 37%). The physical constants of this material matched those previously reported.[10] A mixture of **19** (375 mg, 0.712 mmol), ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[11] **17** (581 mg, 1.082 mmol) and powdered 4 Å molecular sieves (2.4 g) in dry CH_2Cl_2/diethyl ether/toluene (5.3:3.9:3.9 mL) was stirred at rt for 5 min and was then cooled in a ice bath while DMTST (577 mg, 2.23 mmol) was added in portions over 2 h. When TLC indicated no more change, pyridine (0.52 mL) was added to quench the reaction. The mixture was diluted with diethyl ether and the solids were filtered. The filtrate was washed with aq 2 M H_2SO_4, aq 1 M $NaHCO_3$, dried ($MgSO_4$) and concentrated. Column chromatography (petroleum ether/EtOAc, 3:1) of the residue gave **20** (497 mg, 0.496 mmol, 70%) as a colourless syrup, $[\alpha]_D$ +17 (*c* 0.8, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.33 (dd, 1H, J = 10.0, 8.1 Hz, H-2′), 5.03–4.89 (m, 4H, CH_2Ph, H-1), 4.80 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.75 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.65 (d, 2H, J = 12.1 Hz, CH_2Ph), 4.54–4.42 (m, 4H, CH_2Ph, H-1′), 4.29, 4.21 (two doublets, 2H, J = 11.7 Hz, CH_2Ph), 3.94–3.88 (m, 2H, H-3, H-4′), 3.65 (m, H-2), 3.51 (m, 1H, H-4), 3.36 (m, 1H, H-3′). HRMS: Calcd for $C_{62}H_{68}O_{12}N$: 1018.4742. Found: 1018.4749 ($M+NH_4^+$).

3.11. Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (21)

Methanolic sodium methoxide (0.5 M, 9.9 mL) was added to a solution of **20** (493 mg, 0.493 mmol) in CH_3OH (49 mL) was added. The mixture was stirred at rt overnight, then neutralized with Dowex-50 (H+)

and filtered. A drop of concd aq NH_3 was added (to ensure nonacidity) before the solution was concentrated to give **21** (464 mg, 0.484 mmol, 98%), $[\alpha]_D$ −12 (*c* 0.1, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 4.97 (br d, 3H, J = 10.7 Hz, CH_2Ph, H-1), 4.86 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.84 (d, 1H, J = 11.2 Hz, CH_2Ph), 4.77 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.71–4.51 (m, 6H, CH_2Ph, H-1′), 4.27, 4.21 (two doublets, 2H, J = 11.5 Hz, CH_2Ph), 4.05 (m, 1H, H-3), 3.97–3.81 (m, 4H, H-2′, H-5, H-6a, H-6b), 3.69 (m, 1H, H-2), 3.60 (m, 1H, H-4), 3.36 (m, 1H, H-3′), 3.24 (dd, 1H, J = 8.6, 4.8 Hz), 3.01 (br d, 1H, OH). HRMS: Calcd for $C_{60}H_{66}O_{11}N$: 976.4636. Found: 976.4651 ($M+NH_4^+$).

3.12. Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-β-D-*lyxo*-hexopyranosyl)-β-D-glucopyranoside (23)

A mixture of **21** (355 mg, 0.369 mmol), sodium hydride (80% dispersion in oil, 71 mg, 1.47 mmol,) and imidazole (3.6 mg, 0.037 mmol) in dry tetrahydrofuran (3.5 mL) was stirred for 2 h under nitrogen, then carbon disulfide (0.213 mL, 3.69 mmol) was added and the mixture was stirred overnight. Methyl iodide (0.227 mL, 3.69 mmol) was added and the solution was stirred for another 2 h. The mixture was concentrated, dissolved in toluene and the organic layer was washed with 1 M aq HCl, satd aq $NaHCO_3$, and brine, dried ($MgSO_4$) and concentrated. Column chromatography (petroleum ether/EtOAc 3:1) of the residue gave **22** (351 mg, 0.335 mmol, 90%). This material was dissolved in dry toluene (28 mL) and heated to 75 °C under nitrogen for 30 min, then 2,2′-azo-bis-isobutyronitrile (84 mg, 0.49 mmol) and tributyltin hydride (1.47 mL, 5.39 mmol) were added. After 8 h at 75 °C, the solution was concentrated and the residue purified on column of silica gel (toluene/EtOAc, 15:1) to give **23** (194 mg, 62%) as a syrup, $[\alpha]_D$ −15 (*c* 0.6, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.06–488 (m, 4H, CH_2Ph, H-1), 4.81 (d, 1H, J = 11.2 Hz, CH_2Ph), 4.78 (d, 1H, J = 11.2 Hz, CH_2Ph), 4.65–4.42 (m, 6H, CH_2Ph, H-1′), 4.35, 4.27 (two doublets, 2H, J = 11.8 Hz, CH_2Ph), 3.95 (1H, H-3), 3.86 (m, H-4′), 3.73 (m, 1H, H-2), 3.45 (m, 1H, H-3′), 2.09–1.91 (m, 2H, H-2′a, H-2′b). HRMS: Calcd for $C_{60}H_{66}O_{10}N$: 960.4687. Found: 960.4675 ($M+NH_4^+$).

3.13. Phenyl 4-*O*-(2-deoxy-β-D-*lyxo*-hexopyranosyl)-β-D-glucopyranoside (24)

A suspension of palladium on carbon (10%, 130 mg) in ethanol (5 mL) was added to a solution of **23** (184 mg, 0.195 mmol) in ethanol (5 mL). The mixture was flushed, first with nitrogen and then with hydrogen and stirred overnight at rt under a hydrogen atmosphere. After flushing the flask with nitrogen, the solution was filtered and concentrated. The residue was dissolved in water (5 mL) and the solution was slowly passed through a C-18 Isolute cartridge (1 g, pre-conditioned with first 10 mL of CH_3OH, then 50 mL of water). The cartridge was washed with water (10 mL) and then the desired material was eluted with 10–50% CH_3OH to give **24** (47 mg, 0.144 mmol, 74%), $[\alpha]_D$ −42 (*c* 0.5, CH_3OH). HRMS: Calcd for $C_{18}H_{26}O_{10}Na$: 425.1424. Found: 425.1422 ($M+Na^+$). The NMR data for **24** are provided in Table 5.

3.14. Phenyl 4-*O*-(3-*O*-allyl-β-D-galactopyranosyl)-β-D-glucopyranoside (25)

A mixture of **1** (2.00 g, 4.78 mmol), dibutyltin oxide (1.28 g, 5.12 mmol), 4 Å molecular sieves (4 g) and acetonitrile (85 mL) was heated at reflux overnight under nitrogen. Tetraethylammonium bromide (0.443 g, 2.11 mmol) and allyl bromide (12 mL) were added and the reaction was continued under the same conditions for 24 h. The molecular sieves were filtered off and the mixture was concentrated. Column chromatography ($CHCl_3/CH_3OH$, 5:1) of the residue gave **25** (1.03 g, 2.25 mmol, 47%) as a white solid, $[\alpha]_D$ −25 (*c* 0.6, CH_3OH); ^1H NMR data (CD_3OD): δ 5.98 (ddt, 1H, J = 5.8, 10.4, 17.2 Hz, CH_2=$CHCH_2$), 5.32 (ddd, 1H, J = 1.8, 3.2, 17.2 Hz, CH_2=$CHCH_2$), 5.15 (ddd, 1H, J = 1.8, 3.2, 10.4 Hz, CH_2=$CHCH_2$), 4.93 (d, 1H, J = 7.9 Hz, H-1), 4.40 (d, 1H, J = 7.9 Hz, H-1′), 4.22 (ddt, 1H, J = 1.5, 5.6, 12.6 Hz, CH_2=$CHCH_2$), 4.12 (ddt, 1H, J = 1.5, 5.6, 12.6 Hz, CH_2=$CHCH_2$), 4.00

Table 5. NMR data for **24** (D_2O, 323 K)

	H-1 (*J*) C-1	H-2_{ax} (*J*) C-2	H-2_{eq} (*J*) C-2	H-3 (*J*) C-3	H-4 (*J*) C-4	H-5 (*J*) C-5	H-6 (*J*) C-6	H-6′ (*J*) C-6	C	CH_o (*J*)	CH_m (*J*)	CH_p (*J*)
Glc	5.12 (7.9) 100.84	3.61 (2.4, 7.9) 73.48		3.72 74.97	3.76 78.95	3.74 75.60	3.89 60.89	3.72 60.89				
Gal	4.71 (2.2, 9.9) 101.16	1.72 (9.9, 12.3) 34.25	2.08 (2.2, 4.8, 12.3) 34.25	3.88 68.44	3.78 67.46	3.61 76.31	3.82 62.10	3.75 62.10				
OPh									157.24	7.15 117.44	7.41 130.65	7.16 124.09

(d, 1H, J = 2.9 Hz, H-4′), 3.64 (m, H-2′), 3.51 (m, H-2), 3.32 (dd, 1H, J = 2.9, 9.1 Hz, H-3′). HRMS: Calcd for $C_{21}H_{34}O_{11}N$: 476.2132. Found: 476.2123 ($M+NH_4^+$).

3.15. Phenyl 4-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (26)

A solution of **25** (2.30 g, 5.02 mmol) in *N*,*N*-dimethylformamide (80 mL) was stirred with NaH (3.3 g, 80% dispersion) and benzyl bromide (13 mL) overnight. MeOH (40 mL) and then water (53 mL) were added, the mixture was extracted with $CHCl_3$ and the extract was dried (Na_2SO_4) and concentrated. Column chromatography (hexane/EtOAc, 5:1) of the residue gave **26** (3.15 g, 3.15 mmol, 63%) as a white solid, $[\alpha]_D$ −15 (*c* 0.7, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.93 (ddt, 1H, J = 5.3, 10.5, 17.1 Hz, CH_2=$CHCH_2$), 5.32 (ddd, 1H, J = 1.8, 3.3, 17.1 Hz, CH_2=$CHCH_2$), 5.15 (ddd, 1H, J = 1.8, 3.2, 10.3 Hz, CH_2=$CHCH_2$), 5.05 (d, 1H, J = 10.7 Hz, CH_2Ph), 4.99 (m, 3H, CH_2Ph, H-1), 4.82 (m, 2H, CH_2Ph), 4.74 (m, 2H, CH_2Ph), 4.55 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.48 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.44 (d, 1H, J = 7.7 Hz, H-1′), 4.38 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.35 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.25 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.17 (bdd, 2H, J = 1.8, 5.3 Hz, CH_2=$CHCH_2$), 3.98 (dd, 1H, J = 8.8, 9.7 Hz, H-4), 3.88 (br d, 1H, J = 3.1 Hz, H-4′), 3.34 (dd, 1H, J = 3.1, 9.9 Hz, H-3′). HRMS: Calcd for $C_{63}H_{70}O_{11}N$: 1016.4949. Found: 1016.4932 ($M+NH_4^+$).

3.16. Phenyl 4-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (27)

A solution of **26** (3.00 g, 3.00 mmol), tris(triphenylphosphine)rhodium(I) chloride (172 mg, 0.186 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (51 μL, 0.342 mmol) in ethanol/toluene/water (13 mL, 30:10:5 by vol) was heated at reflux for 24 h, then concentrated and taken up in acetic acid/water (30 mL, 9:1 by vol). The solution was heated at 80 °C for 1 h, then cooled and concentrated. The concentrate was partitioned between diethyl ether (70 mL) and water (70 mL). The organic layer was washed with water, dried ($MgSO_4$) and concentrated. Column chromatography (toluene/EtOAc, 8:1) of the residue gave **27** (2.32 g, 2.42 mmol, 81%) as a white solid, $[\alpha]_D$ −14 (*c* 0.5, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.07 (d, 1H, J = 11.0 Hz, CH_2Ph), 5.01 (d, 1H, J = 11.0 Hz, CH_2Ph), 5.00 (d, 1H, J = 7.2 Hz, H-1), 4.85 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.84 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.78 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.77 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.71 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.63 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.55 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.44 (d, 1H, J = 7.0 Hz, H-1′), 4.43 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.41 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.30 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.03 (dd, 1H, J = 9.7 Hz, H-4), 3.86 (br d, 1H, J = 2.4 Hz, H-4′), 3.70 (m, 2H, H-2, H-3), 2.19 (d, 1H, J = 5.5 Hz, OH-3′). HRMS: Calcd for $C_{60}H_{66}O_{11}N$: 976.4636. Found: 976.4617 ($M+NH_4^+$).

3.17. Phenyl 4-*O*-(2,4,6-tri-*O*-benzyl-3-keto-β-D-*xylo*-hexopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (28)

A solution of DMSO (426 μL, 6.00 mmol) in dry CH_2Cl_2 (15 mL) was cooled to −78 °C, then oxalyl chloride (230 μL, 2.64 mmol) was added, followed by, after 15 min, a solution of **27** (2.30 g, 2.40 mmol) in dry CH_2Cl_2 (35 mL). The mixture was stirred for 15 min and then Et_3N (1.66 mL, 11.99 mmol) was added. After 20 min, the cooling bath was removed, and when the mixture had reached rt water was added (100 mL). The mixture was then extracted with CH_2Cl_2 (100 mL), dried ($MgSO_4$) and concentrated. Column chromatography (toluene/EtOAc, 10:1) of the residue gave **28** (2.13 g, 2.23 mmol, 93%) as a white solid, $[\alpha]_D$ −38 (*c* 0.7, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.01 (m, 3H, H-1, CH_2Ph), 4.83 (dd, 2H, J = 11.0, 10.7 Hz, CH_2Ph), 4.69 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.67 (d, 1H, J = 7.5 Hz, H-1′), 4.52 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.47 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.41 (m, 3H, CH_2Ph), 4.36 (m, 2H, H-2′, CH_2Ph), 4.24 (d, 1H, J = 12.1 Hz, CH_2Ph), 3.99 (dd, 1H, J = 9.2 Hz, H-4), 3.86 (m, 2H, H-4′, H-6a), 3.77 (dd, 1H, J = 5.0, 11.0 Hz, H-6b), 3.72–3.63 (m, 3H, H-2, H-3, H-6a′), 3.59 (ddd, 1H, J = 1.5, 5.0, 9.2 Hz, H-5), 3.45 (ddd, 1H, J = 1.1, 5.3 Hz, H-5′), 3.40 (dd, 1H, J = 5.3, 8.8 Hz, H-6b′). HRMS: Calcd for $C_{60}H_{64}O_{11}N$: 974.4480. Found: 974.4496 ($M+NH_4^+$).

3.18. Phenyl 4-*O*-(2,4,6-tri-*O*-benzyl-3-oximino-β-D-*xylo*-hexopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (29)

A mixture of **28** (2.00 g, 2.09 mmol) and hydroxylamine hydrochloride (800 mg, 11.5 mmol) in 1:1 pyridine/ethanol (40 mL) was stirred at rt overnight. The solvent was evaporated and the residue was mixed with water and extracted several times with diethyl ether. The ether solution was dried (Na_2SO_4) and concentrated to give **29** (1.78 g, 1.83 mmol, 88%) as a white solid, $[\alpha]_D$ −19 (*c* 0.5, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.08 (br d, 1H, J < 1 Hz, H-4′), 4.98 (m, 3H, H-1, CH_2Ph), 4.81 (d, 2H, J = 10.5 Hz, CH_2Ph), 4.78 (d, 1H, J = 10.5 Hz, CH_2Ph), 4.75 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.63 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.61 (d, 1H, J = 8.1 Hz, H-1′), 4.49–4.36 (m, 5H, CH_2Ph), 4.23 (d, 1H, J = 12.7 Hz, CH_2Ph), 4.21 (d, 1H, J = 8.1 Hz, H-2′), 3.99 (dd, 1H, J = 9.2 Hz, H-4), 3.84 (dd, 1H, J < 1, 11.0 Hz, H-6a), 3.76 (dd, 1H, J = 5.0, 11.0 Hz, H-6b),

3.72– 3.60 (m, 3H, H-2, H-3, H-6a′), 3.55 (m, 1H, H-5), 3.45 (m, 1H, H-5′), 3.40 (dd, 1H, J = 5.5, 9.0 Hz, H-6b′). HRMS: Calcd for $C_{60}H_{65}O_{11}N_2$: 989.4589. Found: 989.4576 ($M+NH_4^+$).

3.19. Phenyl 4-*O*-(3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (31) and phenyl 4-*O*-(3-benzamido-2,4,6-tri-*O*-benzyl-3-deoxy-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (32)

A solution of **29** (400 mg, 0.411 mmol) in dry tetrahydrofuran (8 mL) was stirred with $LiAlH_4$ (120 mg, 3.16 mmol) at rt overnight. The excess $LiAlH_4$ was destroyed by careful addition of water (1 mL) followed by 30% NaOH (85 mL). The mixture was extracted with ether (85 mL). The organic layer was washed with water, dried ($MgSO_4$) and concentrated to give crude **30**. Half of this material was dissolved in 2:1 pyridine/Ac_2O (3 mL) and stirred at rt overnight and then concentrated. Column chromatography (toluene/EtOAc, 4:1) of the residue gave **31** (77 mg, 0.077 mmol, 38% from **29**) as the only detectable reduction epimer, $[\alpha]_D$ +12 (c 0.5, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.06 (d, 1H, J = 10.5 Hz, CH_2Ph), 5.00 (d, 1H, J = 10.5 Hz, CH_2Ph), 4.99 (d, 1H, J = 7.7 Hz, H-1), 4.92 (d, 1H, J = 8.6 Hz, NH), 4.82 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.80 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.72 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.64 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.55–4.49 (m, 3H, H-1′, CH_2Ph), 4.46 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.42 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.31 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.30 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.04 (dd, 1H, J = 8.6 Hz, H-4), 3.95 (ddd, 1H, J = 3.3, 8.6, 10.7 Hz, H-3′), 3.86 (dd, 1H, J = 1.8, 11.0 Hz, H-6a), 3.81– 3.75 (m, 2H, H-6b, H-4′), 3.70 (m, 2H, H-2, H-3), 3.57 (ddd, 1H, J = 1.5, 4.8, 9.2 Hz, H-5), 3.31 (dd, 1H, J = 7.7, 11.0 Hz, H-2), 2.35 (s, 3H, NHAc). HRMS: Calcd for $C_{62}H_{69}O_{11}N_2$: 1017.4902. Found: 1017.4916 ($M+NH_4^+$).

The other half of crude **30** was dissolved in pyridine (4 mL) and benzoyl chloride (120 mL, 1.03 mmol) was added. The solution was stirred overnight and then concentrated. Column chromatography (toluene/EtOAc, 6:1) of the residue gave **32** (133 mg, 0.125 mmol, 61% from **29**) as the only detectable reduction epimer, $[\alpha]_D$ +14 (c 0.4, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.82 (d, 1H, J = 8.8 Hz, NH), 5.08 (d, 1H, J = 10.7 Hz, CH_2Ph), 5.01 (d, 1H, J = 10.7 Hz, CH_2Ph), 5.00 (d, 1H, J = 7.7 Hz, H-1), 4.84 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.81 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.72 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.65 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.59 (d, 1H, J = 7.2 Hz, H-1′), 4.58 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.55 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.48 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.44 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.38 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.30 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.24 (ddd, 1H, J = 3.3, 8.8, 10.5 Hz, H-3′), 4.09 (dd, 1H, J = 9.0 Hz, H-4), 3.94 (br d, 1H, J = 3.3 Hz, H-4′), 3.88 (dd, 1H, J = 1.8, 11.0 Hz, H-6a), 3.82 (dd, 1H, J = 4.8, 11.0 Hz, H-6b), 3.72 (m, 2H, H-2, H-3), 3.62–3.41 (m, 5H, H-5, H-2′, H-5′, H-6a′, H-6b′). HRMS: Calcd for $C_{67}H_{68}O_{11}N$: 1062.4793. Found: 1062.4772 ($M+H^+$).

3.20. Phenyl 4-*O*-(3-acetamido-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside (33)

Pd/C (10%, 100 mg) in ethanol (5 mL) was added to a solution of **31** (133 mg, 0.133 mmol) in ethanol (5 mL). The mixture was flushed with nitrogen and then with hydrogen and stirred overnight at rt under a hydrogen atmosphere. The solution was filtered and then concentrated. The residue was diluted with water to 5 mL and then slowly passed through a C-18 Isolute cartridge (1 g, previously washed with first 10 mL of CH_3OH, then 50 mL of water). The cartridge was washed with 10 mL water and then the desired material was eluted with 10–50% CH_3OH to give **33** (41 mg, 0.089 mmol, 67%) as a white solid, $[\alpha]_D$ −19 (c 0.1, CH_3OH). HRMS: Calcd for $C_{20}H_{30}NO_{11}$: 460.1819. Found: 460.1811 ($M+H^+$)] and calcd for $C_{20}H_{29}NO_{11}Na$: 482.1638. Found: 482.1615 ($M+Na^+$). The NMR data for **33** are provided in Table 6.

Table 6. NMR data for **33** (D_2O, 303 K)

	H-1 (J) C-1	H-2 (J) C-2	H-3 (J) C-3	H-4 (J) C-4	H-5 (J) C-5	H-6 (J) C-6	H-6′ (J) C-6	C	CH_o (J)	CH_m (J)	CH_p (J)	NHAc (C=O)	NHAc (CH_3)
Glc	5.10 (8.1)	3.59	3.74	3.73	3.74	3.94	3.80						
	100.07	72.74	74.25	78.08	74.99	59.99	59.99						
Gal	4.53 (7.7)	3.57	3.91 (3.3, 10.5)	3.86 (3.3)	3.77	3.75	3.71						2.01
	103.40	68.72	54.67	67.13	76.34	61.05	61.05					174.36	21.96
OPh									7.11	7.37 (8.1)	7.12		
								156.56	116.64	130.01	123.41		

3.21. Phenyl 4-*O*-(3-benzamido-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside (34)

Pd/C (10%, 100 mg) in ethanol (5 mL) was added to a solution of **32** (127 mg, 0.120 mmol) in ethanol (5 mL). The mixture was flushed with nitrogen and then with hydrogen and then stirred overnight at rt under a hydrogen atmosphere. The solution was filtered and then concentrated. The residue was diluted with water to 5 mL and then slowly passed through a C-18 Isolute cartridge (1 g, previously washed with first 10 mL of CH_3OH, then 50 mL of water). The cartridge was washed with 10 mL water and then the desired material was eluted with 10–50% CH_3OH to give **34** (49 mg, 0.094 mmol, 78%) as a white solid, $[\alpha]_D$ +2 (*c* 0.1, CH_3OH). HRMS: Calcd for $C_{25}H_{32}NO_{11}$: 522.1975. Found: 522.1998 $(M+H^+)$] and calcd for $C_{25}H_{31}NO_{11}Na$: 544.1795. Found: 544.1766 $(M+Na^+)$. The NMR data for **34** are provided in Table 7.

3.22. Phenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-deoxy-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (35)

A solution of **6** (9 mg, 22 μmol), UDP-GlcNAc (31 mg, 48 μmol) and BSA (19 mg) in buffer (0.5 M sodium cacodylate, 0.015 M $MnCl_2$, pH 7.3, 2.6 mL) was mixed with GlcNAc-T (0.4 U, 0.43 mL of a 1 U/mL stock solution), and the mixture was stirred overnight, after which TLC indicated complete conversion into product (lower rf). The mixture was purified on a pre-washed (water) C-18 Isolute cartridge (1 g), elution first with water then with 10–50% aqueous CH_3OH. Only pure fractions were collected to give **35** (9 mg, 14.9 μmol, 67%), $[\alpha]_D$ −37 (*c* 0.1, CH_3OH). HRMS: Calcd for $C_{26}H_{40}NO_{15}$: 606.2398. Found: 606.2386 $(M+H^+)$. The NMR data for **35** are provided in Table 8.

3.23. Enzymatic assay

The assay was performed in 200 mM cacodylate buffer, pH 7.5, containing 0.4 mg/mL BSA, 10 mM ATP, 10 mM $MnCl_2$ and 50 nmol of ^{3}H-UDP-GlcNAc with a specific activity of 10,000 cpm. In a final volume of 50 μL incubation assay, 2 mM of each acceptor substrate was incubated with 10 mU of the recombinant β-3-GlcNAc-transferase (5 U/mL) for 60 min at 37 °C. Reactions were stopped by immersing the incubation tubes in a dry ice bath. After thawing, the reaction mixtures were immediately diluted with buffer, and unreacted UDP-sugar was separated from the ^{3}H-GlcNAc-containing product by passing the solution through a C-18 Sep-Pac column, and eluting with first water, then CH_3OH. The radioactivity of the CH_3OH eluate was

Table 7. NMR data for **34** (D_2O, 303 K)

	H-1 (*J*) C-1	H-2 (*J*) C-2	H-3 (*J*) C-3	H-4 (*J*) C-4	H-5 (*J*) C-5	H-6 (*J*) C-6	H-6′ (*J*) C-6	C	CH_o (*J*)	CH_m (*J*)	CH_p (*J*)	NHBz (C=O) (*J*)	NHBz (C) (*J*)	NHBz (CH_o) (*J*)	NHBz (CH_m) (*J*)	NHBz (CH_p) (*J*)
Glc	4.96 (7.9) 100.74	3.56 (7.9, 9.0) 73.27	3.67 74.98	3.75 78.93	3.61 75.24	3.94 60.38	3.90 60.38									
Gal	4.57 (7.7) 104.30	3.81 68.67	4.16 (3.3, 10.7) 55.92	4.00 (3.3) 67.36	3.77 76.72	3.81 61.14	3.73 61.14							7.47 (7.5) 128.08	7.54 (7.5, 1.3) 131.29	7.89 (7.7) 127.15
OPh								157.68	7.10 (7.9) 116.43	7.28 (7.9) 129.02	7.01 (7.5) 122.07	169.28	134.39			

Table 8. NMR data for **35** (D_2O, 323 K)

	H-1 (*J*) C-1	H-2 (*J*) C-2	H-3 (*J*) C-3	H-4 (*J*) C-4	H-5 (*J*) C-5	H-6 (*J*) C-6	H-6′ (*J*) C-6	C	CH_o	CH_m	CH_p	NHAc (C=O)	NHAc (CH_3)
Glc	5.13 (7.6) 100.38	3.61 73.03	3.74 75.19	3.68 79.02	ND ND	3.96 60.39	3.79 60.39						
Gal	4.42 (7.6) 103.25	3.57 70.00	3.71 82.51	3.96 71.23	3.83 70.89	1.27 (5.9) 15.64	1.27 (5.9) 15.64						
GlcNAc	4.69 (8.1) 102.97	3.74 74.57	3.56 74.00	3.47 70.19	ND ND	3.89 60.99	3.73 60.99					175.21	2.03 22.55
OPh								156.85	7.15 117.04	7.40 130.06	7.16 123.70		

determined by a scintillation counting. The results are summarized in Table 1.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2004.11.017.

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