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Synthesis of Gal-β-(1→4)-GlcNAc-β-(1→6)-[Gal-β-(1→3)]-GalNAc-α-OBn oligosaccharides bearing O-methyl or O-sulfo groups at C-3 of the Gal residue: specific acceptors for Gal: 3-O-sulfotransferases*

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Dedicated to Roger W. Jeanloz on the occasion of his 80th birthday.

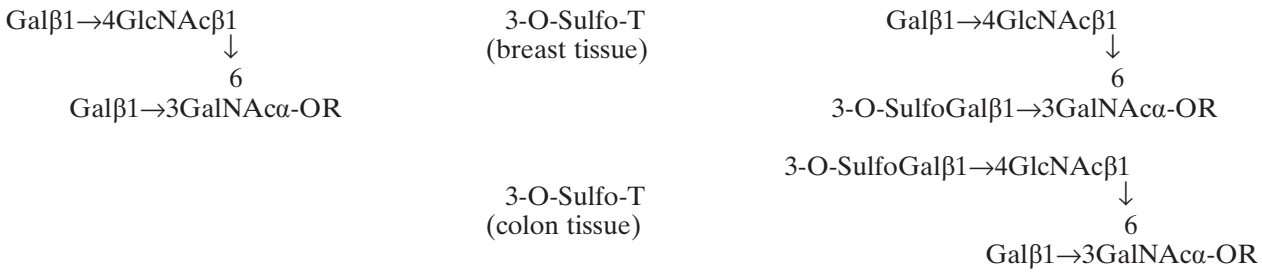
Our recent studies have revealed the existence of two distinct Gal: 3-O-sulfotransferases capable of acting on the C-3 position of galactose in a Core 2 branched structure, e.g., Galβ1→4GlcNAcβ1→6(Galβ1→3)GalNacα1→OBenzyl as acceptor to give 3-O-sulfoGalβ1→4GlcNAcβ1→3(Galβ1→3)GalNAcα1→OB 20 and Galβ1→4GlcNAcβ1→6(3-O-sulfoGalβ1→3)GalNAcα1→OB 23. We herein report the synthesis of these two compounds and also that of other modified analogs that are highly specific acceptors for the two sulfotransferases. Appropriately protected 1-thio-glycosides 7, 8, and 10 were employed as glycosyl donors for the synthesis of our target compounds.

Keywords: **acceptors, oligosaccharides, sulfotransferases, synthetic**

Introduction

The investigation of sulfotransferases involved in the construction of glycoproteins has now become an important aspect of research on selectin ligands. Sulfated carbohydrates form an integral part of the structure of glycoproteins associated with selectins and have been demonstrated to be essential for recognition and binding [2–6]. Our research has revealed that sulfate positioning on carbohydrate moieties directly influences both the level and manner of action by glycosyltransferases implicated in human glycoprotein biosynthesis [7–10].

We have very recently characterized Gal-3-O-sulfotransferase activity present in human colon, ovarian, and breast tumor tissues [11], and, by employing the synthetic branched acceptor, Galβ1→4GlcNAcβ1→6(Galβ1→3)GalNAcα1→OBn, (a representative of O-linked glycoprotein structures), we were able to demonstrate that this enzyme (as illustrated below) is tissue specific:



These remarkable results prompted us to synthesize some modified analogs of this tetrasaccharide structure in an effort to characterize sulfotransferase activities from different tissues. In this report, we describe the synthesis of some of those modified branched tetrasaccharides. The re-

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sults of our biochemical investigations on enzymic sulfation utilizing these compounds will be reported elsewhere.

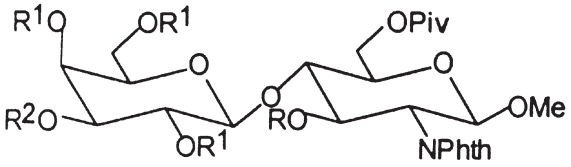
Results and Discussion

For the synthesis of compounds 12 and 16, we employed known benzyl O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)- (1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (6) [12] as a glycosyl acceptor. Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glycopyranoside (8) [13] was utilized as a glycosyl donor (Scheme 1) for the synthesis of 12. A regioselective glycosidation with 6 through utilization of 8 in the presence of NIS-triflic acid [14] afforded the protected trisaccharide 11 in 65% yield. The ^{1}H NMR spectrum of 11 displayed characteristic signals for H-1″, H-1 and H-1′ at δ 5.39 (d, J = 10.4 Hz), 5.31 (d, J = 1.0 Hz) and 4.38 (d, J = 8.0 Hz), respectively, confirming a β-configuration for the newly introduced glycosidic bond. The conversion of 11 into the trisaccharide 12 was then carried out in 3 steps: (1) Hydrazine hydrate-MeOH (phthalimido removal), (2) pyridine-acetic anhydride (N- and O-acetylation), and (3) MeOH-MeONa (de-O-acetylation). The structure of 12 was confirmed by ^{1}H and ^{13}C NMR spectroscopy (see Experimental section). A reaction sequence similar to that described for the preparation of 12 from 6 was adopted for the preparation of 16 from 6. Compound 15 was obtained in 74% yield by condensation of known glycosyl donor 10 [15], with 6 followed by purification on a column of silica gel. Compound 15 was converted into 16 as described for the preparation of 12 (from 11). The ^{1}H NMR spectrum of 16 showed four anomeric protons at δ 4.99 (d, J = 3.7 Hz, H-1), 4.58 (d, J = 8.2 Hz, 1 H, H-1″), 4.48 (d, J = 7.5 Hz, H-1‴) and 4.47 (d, J = 7.0 Hz, H-1′). The ^{13}C NMR spectrum of 16 was consistent with the structure assigned (see Experimental section).

For the synthesis of 14 and 25 (Schemes II and V, respectively), phenyl O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-1-thio-α/β-D-glucopyranoside (4) was used as the glycosyl donor. Regioselective methylation of known 1 [16] by the stannylene procedure [17] in the presence of tetrabutylammonium iodide produced the 3′-O-methyl derivative 2, which was subjected to acetolysis to afford compound 3 in 90% yield. The ^{1}H NMR spectrum of 3 contained a low-field signal at δ 6.42 (d, J = 9.6 Hz, 0.8 H, H-1), suggesting that it existed predominantly as the β-D-anomer. This predominance of the β-D-anomer could also be gleaned from the relative intensities of the CMe_3 signals (α/β ratio of 1:4). Treatment of 3 with thiophenol and BF_3 etherate [18] afforded 4 in 68% yield after silica gel column chromatography. Similarly, *N*-iodosuccinimide-triflic acid-catalyzed glycosylation of 5 with donor 4 afforded, in 68% yield, the protected tetrasaccharide 13. The synthesis of 14 from 13 was performed as described for the preparation of 12 from 11. The ^{1}H NMR spectrum of 14 showed four doublets at δ 4.99 (J = 3.7 Hz, H-1), 4.58 (J = 8.2 Hz, H-1″), 4.49 (J = 8.0 Hz, H-1‴), 4.45 (J = 7.7 Hz, H-1′). NIS-triflic acid catalyzed glycosylation of 9 with donor 4 (Scheme V), followed by the removal of the chloroacetyl group, provided an important intermediate 24 in 26% yield (on the basis of 9). The ^{1}H NMR spectrum of 24 was in conformity with the overall structure expected (see Experimental section). Reaction of 24 with SO_3-pyridine complex in *N,N*-dimethylformamide at 0°C, followed by removal of the protecting groups, as described in the preparation of 12, afforded 25 in 58% yield. The ^{1}H-NMR spectrum of 25 exhibited four anomeric proton signals at δ 5.02 (d, J = 3.6 Hz, H-1), 4.75 (d, J = 8.3 Hz, H-1′), 4.48 (d, J = 7.7 Hz, H-1‴) and 4.31 (d, J = 7.7 Hz, 1 H, H-1″). The structure of 25 was also confirmed by ^{13}C NMR spectroscopy (see Experimental section). Regioselective glycosylation of 5 with 7 [19] in the presence of NIS-triflic acid afforded the β-(1→6) linked tetrasaccharide 17 in 53% yield (Scheme III). The advantage of employing glycosyl donor 7 is apparent from the subsequent conversion of 17 into the key intermediate 19 in four steps: (1) NH_2-$NH_2.H_2O$/MeOH (phthalimido and O-acetyl group removal), (2) pyridine-acetic anhydride (N- and O-acetylation), (3) $CHCl_3$-TFA-H_2O (hydrolysis of isopropylidene group to afford diol 18), and (4) triethyl orthoacetate/80% aq. acetic acid (to convert diol 18 into the 3-hydroxy compound 19). The ^{1}H NMR spectrum of 18 displayed two low-field signals at δ 5.28 (d, J = 3.4 Hz, H-4) and 4.90 (d, J = 3.8 Hz, H-4′), confirming that compound 5 had been glycosylated at O-6. Similarly, the ^{1}H NMR spectrum of 19 exhibited three low-field chemical shifts at δ 5.11 (d, J = 3.1 Hz, H-4), 5.03 (d, J = 2.9 Hz, H-4‴), 4.92 (d, J = 3.3 Hz, H-4′), confirming that compound 19 had been acetylated at O-4‴. Sulfation of 19 with SO_3-pyridine complex in DMF, followed by de-O-acetylation with methanolic sodium methoxide and passage through IR-120 (Na+) resin, then gave the compound 20 as an amorphous sodium salt. The structure of 20 was confirmed by ^{13}C NMR and FAB mass spectroscopy (see Experimental section). A reaction sequence similar to that described for the preparation of 25 from 9 was enlisted for the synthesis of 23 from 9 (Scheme IV). The ^{13}C NMR and ^{1}H NMR spectra were in accordance with the structure assigned (see Experimental section).

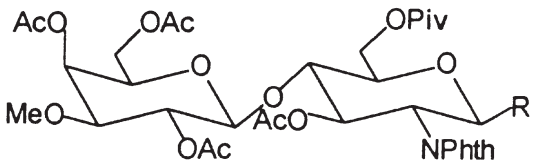
^{13}C NMR assignments

In the ^{13}C NMR spectra of 12, 14, 16, 20, 23, and 25, the resonance for C-6 of the GalNAc residue displayed a downfield shift (δ 67.54-68.39), confirming the site of glycosylation in these compounds. Similarly, the resonance of C-3 of β-D-Gal-(1→3) residue in 12, 16, 20, 23, and 25 was observed as δ 80.57-76.37, confirming that this was the site of methylation or sulfation. Similarly, the resonance of C-3 of β-D-Gal-(1→4) residue in compounds 14 and 25 displayed a downfield shift (δ 80.61 and 80.64), confirming that this position was the site of methylation.



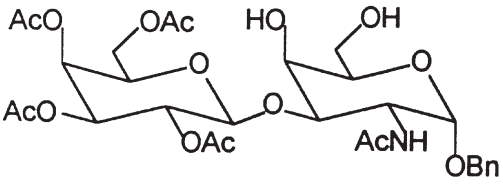
1. R = R^1 = R^2 = H

2. R = R^1 = H ; R^2 = Me



3. R = OAc

4. R = SPh



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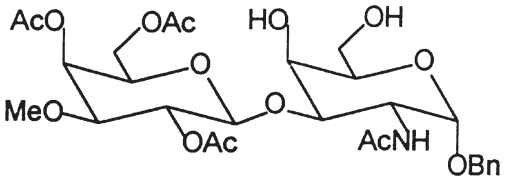

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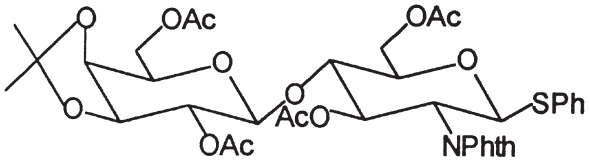

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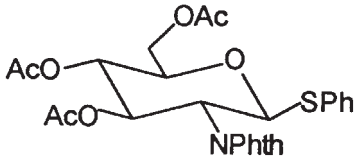

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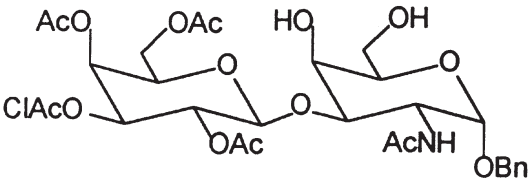

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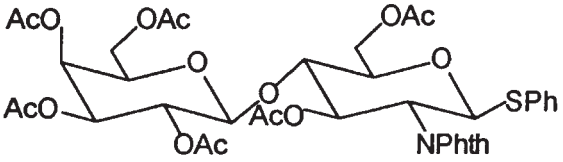

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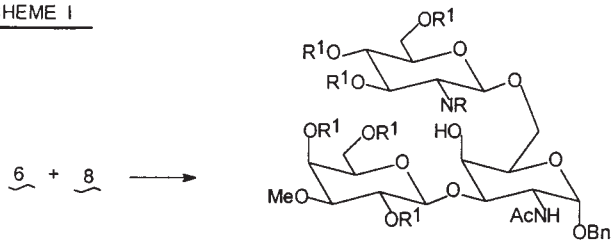
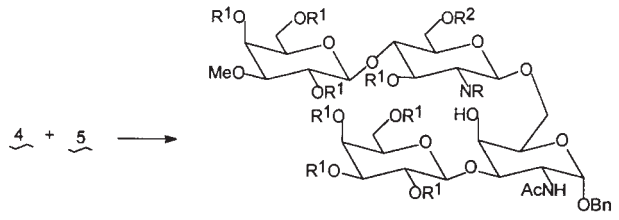
SCHEME I



11. R = Phth ; R^1 = Ac

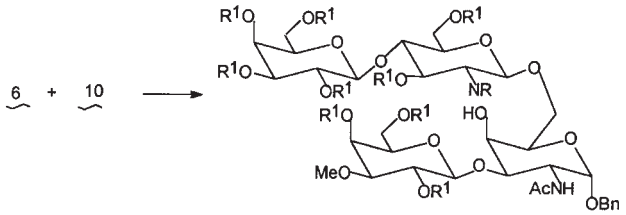
12. R = H, Ac ; R^1 = H

SCHEME II



13. R = Phth ; R^1 = Ac ; R^2 = Piv

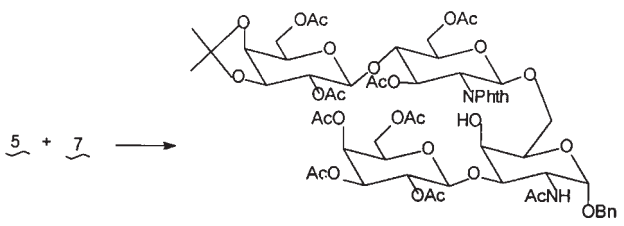
14. R = H, Ac ; $R^1 = R^2$ = H



15. R = Phth ; R^1 = Ac

16. R = H, Ac ; R^1 = H

SCHEME III



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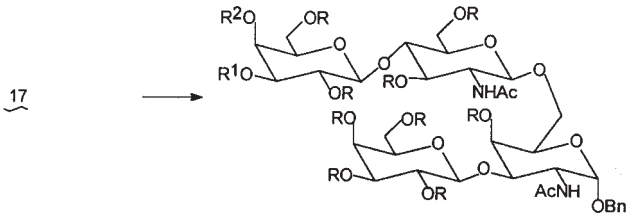
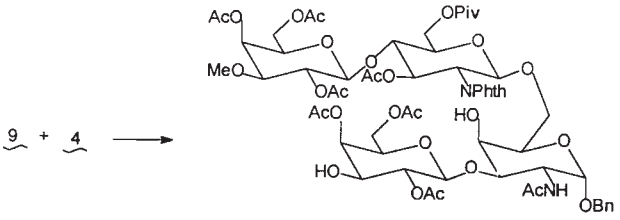


18. R = Ac ; $R^1 = R^2$ = H

19. R = R^2 = Ac ; R^1 = H

20. R = R^2 = H ; R^1 = SO_3Na

SCHEME V



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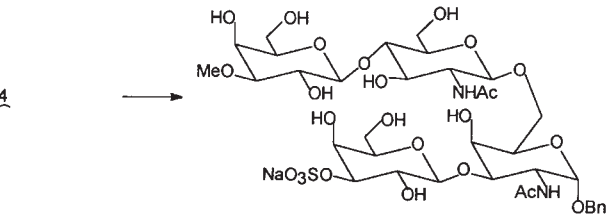

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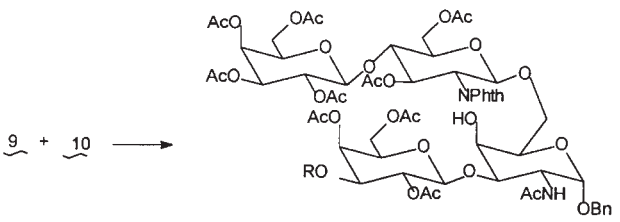
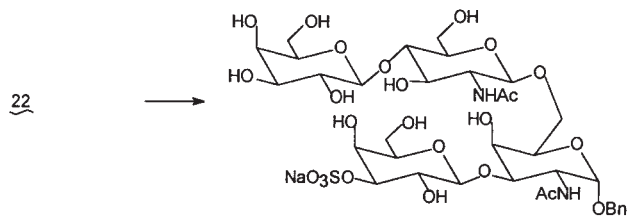
SCHEME IV



21. R = ClAc

22. R = H



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Experimental

General methods

Optical rotations were measured at ~25°C with a Perkin-Elmer 241 Polarimeter. TLC was conducted on glass plates, precoated with 0.25 mm layers of silica gel 60F-254 (Analtech GHLF uniplates). The compounds were located by exposure to u.v. light or by spraying with 5% H_2SO_4 in EtOH and charring, or by both techniques. The silica gel used for column chromatography was Baker Analyzed (60-200 mesh). NMR spectra were recorded at ~25°C, ^{1}H-spectra with a Varian EM-390 at 90 MHz and with a Bruker AM-400 at 400 MHz and ^{13}C-spectra with a Bruker AM-400 at 100.6 MHz. All chemical shifts are referenced to tetramethylsilane. Solutions in organic solvents were generally dried with anhydrous Na_2SO_4. Dichloromethane, *N,N*-dimethylformamide, 1,2-dichloroethane, benzene, and 2,2-dimethoxypropane were kept dried over 4A° molecular sieves. Elemental analyses were performed by the Robertson Laboratory, Madison, New Jersey, USA.

General procedure for glycosidation

A solution of 4 or 7 or 8 or 10 (1.2 mmol), acceptor sugars 5 or 6 or 9 (1.0 mmol) and *N*-iodosuccinimide (2.5-3.0 mmol) in CH_2Cl_2 (20 ml) was stirred for 0.5 h with 4A molecular sieves (3.0 g) under an argon atmosphere at −30°C. A dilute solution of trifluoromethanesulfonic acid (0.2 ml in 20 ml CH_2Cl_2) was then added dropwise, and stirring was continued for an additional 1 h at the same temperature. The acid was then neutralized with aq. $NaHCO_3$ solution and the mixture filtered through Celite, and the solids thoroughly washed with CH_2Cl_2 and the filtrate and washings were combined, successively washed with water, saturated $NaHCO_3$ solution, 10% $Na_2S_2O_3$ solution, dried and concentrated in vacuo.

Methyl O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranoside (2).

A mixture of 1 (4.5 g, 7.5 mmol) and dibutyltin oxide (2.4 g, 9.6 mmol) in benzene (250 ml) was heated for 16 h at reflux temperature with azeotropic distillation of water. The mixture was concentrated to about one half its volume, and, after addition of tetrabutylammonium iodide (3.3 g, 8.9 mmol) and methyl iodide (6.0 ml, 43 mmol), the stirring was continued at 70°C for 6 h. Evaporation of the solvent to dryness gave a residue that was purified on a column of silica gel with a solvent gradient consisting of 5% to 10% MeOH in $CHCl_3$ to afford 2 (1.8 g, 69% based on 1 consumed), amorphous, $[\alpha]_D$ + 10° (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.83-7.72 (m, 4 H, arom.), 5.09 (d, J = 9.0 Hz, 1 H, H-1), 4.39 (d, J = 8.0 Hz, 1 H, H-1′), 3.47 and 3.39 (each s, 6 H, 2 × OMe), 1.20 (s, 9 H, CMe_3).

Anal. Calcd. for $C_{27}H_{37}NO_{13}$: C, 55.57; H, 6.39; N, 2.40.
Found: C, 55.61; H, 6.19; N, 2.35.

O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→4)-1,3-di-O-acetyl-2- deoxy-2-phthalimido-6-O-trimethylacetyl-D-glucopyranose (3).

A solution of 2 (2.0 g) in acetic anhydride (40 ml) containing conc. H_2SO_4 (2.0 ml) was stirred for 16 h at 5°C. The mixture was diluted with CH_2Cl_2 (100 ml) and successively washed with water, saturated aq. $NaHCO_3$ solution, and water, dried, evaporated to dryness, and then dissolved in CH_2Cl_2. Addition of ether and hexane caused the precipitation of 3 (2.4 g, 90%); amorphous; $[\alpha]_D$ + 40° (*c* 1.5, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.86-7.75 (m, 4 H, arom.), 6.42 (d, J = 9.6 Hz, 0.8 H, H-1), 5.74 (d, J = 8.5 Hz, 1 H, H-3), 5.43 (d, J = 3.0 Hz, 1 H, H-4′), 3.38 (s, OMe-α), 3.32 (s, OMe-β, 2.01-1.89 (cluster of s, 15 H, 4 × OAc), 12.5 (s, CMe_3-β, 1.24 (τ, ΓΝε$_3$-α).

Anal. Calcd. for $C_{36}H_{45}NO_{18}$: C, 55.45; H, 5.82 N, 1.80
Found: C, 55.47; H, 5.76; N, 1.81.

Phenyl O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→4)-3-O- acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-1-thio-α/β-D-glucopyranoside (4).

To a stirred solution of 3 (2.2 g, 2.8 mmol) in CH_2CI_2 (25 ml) was added thiophenol (0.7 ml, 6.4 mmol) and BF_3 ethereate (1.26 ml, 9.0 mmol). Stirring was continued for 3 h at room temperature. The reaction mixture was washed with aq. $NaHCO_3$ solution, water, dried, and concentrated. The residue was dissolved in CH_2Cl_2 and addition of hexane gave 4 (1.6 g, 68%), amorphous, $[\alpha]_D$ + 38° (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.84- 7.74 (m, 4 H, arom.), 7.39-7.24 (m, 5 H, arom.), 5.71-5.65 (m, 2 H, H-1 andH-3), 5.42 (d, J = 3.0 Hz, 1 H, H-4′), 4.89 (dd, J = 10.0 Hz, 1 H, H-2′), 4.40 (d, J = 8.0 Hz, 1 H, H- 1′), 3.32 (s, OMe-α), 3.31 (s, OMe-β, 2.01-1.56 (cluster of s, 12 H, 4× OAc), 1.24 (s, CMe_3-β, 1.23 (s, CMe_3-α).

Anal. Calcd. for $C_{40}H_{47}NO_{16}S$: C, 57.89; H, 5.71; N, 1.69
Found: C, 58.01; H, 5.72; N, 1.56.

Benzyl O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→3)-O-[(3,4,6- tri-O-acetyl-2-deoxy-2-phthalimido-β-D- glucopyranosyl)-(1→6)]-2-acetamido-2-deoxy- α-D- galactopyranoside (11)

Glycosidation of 6 (1.1 g, 1.8 mmol) with 8 (1.1 g, 2.1 mmol), according to the general procedure, afforded 11 (1.2 g, 65%) after silica gel column chromatography (hexane-ethyl acetate, 3:2 (v/v); $[\alpha]_D$ + 57° (*c* 1.5, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.66-7.14 (m, 9 H, arom.), 5.78 (dd, J = 9.1 Hz, H-3′′), 5.42 (d, J = 4.0 Hz, 1 H, H-4′), 5.39 (d, J = 10.4 Hz, 1 H, H-1′′), 5.31 (d, J = 1.0 Hz, 1 H, H-1), 5.13 (dd, J = 9.9

Hz and 9.3 Hz, 1 H, H-2′), 4.92 (dd, J = 10.1 Hz, 1 H, H-2′′), 4.38 (d, J = 8.0 Hz, 1 H, H-1′), 3.30 (s, 3 H, OMe), 2.10-1.69 (7 s, 21 H, 6 × OAc and NAc).

Anal. Calcd. for $C_{48}H_{58}N_2O_{23}$: C, 56.42; H, 5.60; N, 2.68 . Found: C, 56.31; H, 5.49; N, 2.57.

Benzyl O-(3-O-methyl-β-D-galactopyranosyl)-(1→3)-O-[(2-acetamido-2-deoxy-β D-glucopyranosyl)-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranoside (12)

Compound 11 (0.7 g) was heated under reflux for 16 h in a mixture of MeOH (45 ml) and hydrazine hydrate (5 ml). The solvent was evaporated to give a residue which was dissolved in pyridine (40 ml) and Ac_2O (20 ml) and stirred overnight at room temperature. Pyridine and acetic anhydride were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with aq. $NaHCO_3$ solution, water, dried, and concentrated in vacuo. This residue in 0.025 M methanolic NaOMe (20 ml) was stirred at room temperature for 16 h. The base was neutralized with Amberlite IR- 120 (H^+) cation-exchange resin, which was then filtered off and thoroughly washed with MeOH. The filtrate was concentrated and purified in a column of silica gel with a solvent gradient consisting of $CHCl_3$-MeOH-H_2O (13:6:1→5:4:1) to afford 12 (0.3 g, 69%); $[\alpha]_D$ + 78° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 7.41-7.44 (m, 5 H, arom.), 4.99 (d, J = 3.7 Hz, 1 H, H-1), 4.74 (d, J = 10.7 Hz, 1 H, NH), 4.56 (d, J = 8.3 Hz, 1 H, H-1′′), 4.47 (d, J = 7.8 Hz, 1 H, H-1′), 3.44 (s, 3 H, OMe), 1.90 (s, 3 H, NAc); ^{13}C NMR: δ 3-O-Me- β-D-Gal-(1→3) Residue: 103.49 (C-1), 68.57 (C-2), 80.57 (C-3), 68.57 (C-4), 73.87 (C-5), 60.01 (C-6), 55.05 (OMe); β-D-GlcNAc-(1→6) Residue: 100.54 (C-1), 54.33 (C-2), 68.70 (C-3), 68.97 (C-4), 74.85 (C-5), 59.73 (C-6), 21.22 (NAc); α-D-GalNAc-OBn: 95.24 (C-1), 47.57 (C-2), 75.82 (C-3), 63.04 (C-4), 72.90 (C-5), 67.85 (C-6), 20.92 (NAc).

Anal. Calcd. for $C_{30}H_{46}N_2O_{16}H_2O$: C, 50.84; H, 6.84; N, 3.95. Found: C, 51.06; H, 6.91; N, 3.86.

Benzyl O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→4)-O-(3-O- acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranosyl)-(1→6)-O- [(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (13)

Compound 5 (0.77 g, 1.2 mmol) was treated with 4 (1.1 g, 1.33 mmol) as described for the preparation of 11 (from 6) to give 13 (1.1 g, 68%) after silica gel column chromatography (solvent gradient consisting of 30% to 40% acetone in $CHCl_3$); $[\alpha]_D$ + 43° (*c* 1.5, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.61-7.14 (m, 9 H, arom.), 5.43 (d, J = 2.8 Hz, 1 H, H-4′′′), 5.38 (dd, J = 9.1 Hz, 1 H, H-3′′), 5.29 (d, J = 1.5 Hz, 1 H, H-1), 5.28 (d, J = 1.8 Hz, 1 H, H-4′), 5.09 (dd, J = 10.4 Hz, H-2′′), 3.32 (s, 3 H, OMe), 2.11-1.62 (cluster of s, 27 H, 8 × OAc and NAc) 1.26 (s, 9 H, CMe_3).

Anal. Calcd. for $C_{63}H_{80}N_2O_{31}$: C, 55.58; H, 5.92; N, 2.05. Found: C, 55.39; H, 5.91; N, 2.12.

Benzyl O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-2-deoxy-β D-glucopyranosyl)-(1→6)-O-[(β-D-galactopyranosyl)-(1→3)] -2-acetamido-2-deoxy-α-D- galactopyranoside (14)

Compound 13 (0.8 g) was converted to 14 (0.38 g, 76%) by the same reaction sequence as described for the preparation of 12 (from 11). $[\alpha]_D$ + 61° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 7.51-7.44 (m, 5 H, arom.), 4.99 (d, J = 3.7 Hz, 1 H, H-1), 4.58 (d, J = 8.2 Hz, 1 H, H-1′′), 4.49 (d, J = 8.0 Hz, 1 H, H-1′′′), 4.45 (d, J = 7.7 Hz, 1 H, H-1′), 3.46 (s, 3 H, OMe), 1.98 and 1.97 (each s, 6 H, 2 × NAc); ^{13}C NMR: δ 3- O-Me-β-D-Gal-(1→3) Residue: 103.48 (C-1), 68.52 (C-2), 80.57 (C-3), 67.84 (C-4), 73.87 (C-5), 60.02 (C-6), 55.06 (OMe); β-D-Gal-(1→4) Residue: 101.88 (C-1), 68.56 (C-2), 68.68 (C-3), 68.40 (C-4), 73.74 (C-5), 59.99 (C-6); β-D-GlcNAc-(1→6) Residue: 100.45 (C-1), 54.07 (C-2), 69.95 (C-3), 77.57 (C-4), 74.34 (C-5), 59.08 (C-6), 21.25 (NAc); α-D-GalNAc-OBn Residue: 95.26 (C-1), 47.57 (C-2), 75.83 (C-3), 63.06 (C-4), 71.48 (C-5), 67.54 (C-6), 20.93 (NAc).

Anal. Calcd. for $C_{36}H_{56}N_2O_{21}.H_2O$: C, 49.65; H, 6.71; N, 3.22. Found: C, 49.81; H, 6.80; N, 3.16.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyl)-(1→4)-O-(3,6-di-O- acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-O [2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (15)

Glycosidation of 6 (0.49 g, 0.8 mmol) with 10 (0.85 g, 1.04 mmol) gave compound 15 (0.8 g, 74%) after silica gel column chromatography (solvent gradient consisting of 5–7% MeOH in $CHCl_3$); $[\alpha]_D$ +40° (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.32-7.14 (m, 9 H, arom.), 5.70 (dd, J = 9.0 Hz, 1 H, H-3′′), 5.40 (d, J = 8.3 Hz, 1 H, H-1′′), 5.39 (d, J = 1.0 Hz, 1 H, H-4′′′), 5.35 (d, J = 1.0 Hz, 1 H, H-4′), 5.34 (d, J = 2.7 Hz, 1 H, H-1), 5.07 (dd, J = 10.8 Hz, 1 H, H-2′′′), 3.30 (s, 3 H, OMe), 2.13-1.57 (cluster of s, 30 H, 9 × OAc and NAc).

Anal. Calcd. for $C_{60}H_{72}N_2O_{30}$: C, 54.62; H, 5.65; N, 2.12. Found: C, 54.42; H, 5.61; N, 2.05.

Benzyl O-(β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-2-deoxy-β-D- glucopyranosyl)-(1→6)-O-[(3-O-methyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-2- deoxy-α-D-galactopyranoside (16)

This compound was obtained from 15 by the same reaction sequence as described for the preparation of 12 (from 11),

amorphous (0.35 g, 80%); $[\alpha]_D$ +57° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 7.49-7.44 (m, 5 H, arom.), 4.99 (d, J = 3.7 Hz, 1 H, H-1), 4.58 (d, J = 8.2 Hz, 1 H, H-1′′), 4.48 (d, J = 7.5 Hz, 1 H, H-1′′′), 4.47 (d, J = 7.0 Hz, 1 H, H-1′), 3.44 (s, 3 H, OMe), 1.98 (bs, 6 H, 2 × NAc); ^{13}C NMR: δ β-D-Gal-(1→3) Residue: 100.57 (C-1), 68.68 (C-2), 68.90 (C-3), 67.82 (C-4), 73.93 (C-5), 60.04 (C-6); 3-O-Me-β-D-Gal-(1→4) Residue: 101.73 (C-1), 68.55 (C-2), 80.61 (C-3), 67.55 (C-4), 73.72 (C-5), 59.95 (C-6), 55.21 (OMe); β-D-GlcNAc-(1→6): 100.44 (C-1), 54.06 (C-2), 69.58 (C-3), 77.35 (C-4), 74.21 (C-5), 59.01 (C-6), 21.22 (NAc); α-D- GalNAc-OBn Residue: 95.23 (C-1), 47.53 (C-2), 75.92 (C-3), 63.06 (C-4), 71.46 (C-5), 68.37 (C-6), 20.92 (OAc).

Anal. Calcd. for $C_{36}H_{56}N_2O_{21}.H_2O$: C, 49.65; H, 6.71; N, 3.22. Found: C, 49.71; H, 6.65; N, 3.16.

Benzyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-O-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (17)

Glycosidation of 5 (0.6 g, 0.94 mmol) with 7 (0.85 g, 1.1 mmol) afforded 17 (0.65 g, 53%) after silica gel column chromatography (20% to 30% acetone in $CHCl_3$); $[\alpha]_D$ +53° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.63-7.16 (m, 9 H, arom.), 5.68 (dd, J = 8.7 Hz, 1 H, H-3′′), 5.38 (d, J = 8.6 Hz, 1 H, H-1′′), 5.29 (d, J = 3.3 Hz, 1 H, H-4′), 5.28 (d, J = 2.5 Hz, 1 H, H-1), 5.09 (dd, J = 8.0 Hz, 1 H, H-2′′), 2.12-1.29 (cluster of s, 33 H, 8 × OAc, NAc and CMe_2).

Anal. Calcd. for $C_{60}H_{74}N_2O_{30}$: C, 55.29; H, 5.72; N, 2.15. Found: C, 55.42; H, 5.69; N, 2.10.

Benzyl O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di- O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-4-O-acetyl-2-deoxy-α-D-galactopyranoside (18)

The removal of phthalimido from 17 (0.65 g) and N,O-acetylation with pyridine-Ac_2O, as described for the preparation of 12, afforded a crude intermediate. To a solution of this product in $CHCl_3$ (100 ml) were added trifluoroacetic acid (5.0 ml) and water (0.5 ml). After stirring for 2 h at room temperature, the solution was concentrated, and residual acid was removed by several co-evaporations with toluene. The residue was purified on a column of silica gel with 7% to 10% MeOH in $CHCl_3$ as the eluent to afford 18 (0.4 g, 71%); $[\alpha]_D$ +39° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.38-7.32 (m, 5 H, arom.), 5.67 (d, J = 9.3 Hz, 1 H, NH), 5.58 (d, J = 10.5 Hz, 1 H, NH), 5.28 (d, J = 3.4 Hz, 1 H, H-4), 5.05 (dd, J = 8.8 Hz, 1 H, H-3′′), 5.01 (d, J = 7.8 Hz, 1 H, H-1′′), 4.94 (d, J = 3.4 Hz, 1 H, H-1), 4.90 (d, J = 3.8 Hz, 1 H, H-4′), 4.83 (dd, J = 8.0 Hz, 1 H, H-2′′′), 4.56 (d, J = 7.7 Hz, 1 H, H-1′′′), 4.49 (d, J = 8.0 Hz, 1 H, H-1′), 2.13-1.59 (cluster of s, 33 H, 9 × OAc and 2 × NAc).

Anal. Calcd. for $C_{53}H_{72}N_2O_{30}$: C, 52.30; H, 5.96; N, 2.30. Found: C, 52.35; H, 6.01; N, 2.29.

Benzyl O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6- di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-[(2,3,4,6-tetra-O-acetyl-β-D- galactopyranosyl)-(1→3)]-2-acetamido-4-O-acetyl-2-deoxy-α-D-galactopyranoside (19)

To a solution of 18 (0.3 g) in dry benzene (40 ml) was added trietyl orthoacetate (10 ml) and 4-toluenesulfonic acid monohydrate (0.05 g), and the mixture was stirred for 1 h at room temperature. Triethylamine was added, and the solution was washed with cold water, dried, and concentrated under diminished pressure to give 3′′′,4′′′-orthoester in quantitative yield. It was dissolved in 80% aq. AcOH (50 ml), and the solution was stirred for 1 h at room temperature. Acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several added portions of toluene. The crude product was applied to a column of silica gel. Elution with a solvent gradient consisting of 5% to 7% MeOH in $CHCl_3$ and evaporation of the fractions corresponding to 19 (0.26 g, 84%) gave an amorphous solid; $[\alpha]_D$ +33° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.37-7.32 (m, 5 H, arom.), 5.11 (d, J = 3.1 Hz, 1 H, H-4), 5.08 (dd, J = 8.8 Hz, 1 H, H-3′′), 5.03 (d, J = 2.9 Hz, 1 H, H-4′′′), 5.01 (d, J = 7.7 Hz, 1 H, H-1′′), 4.95 (d, J = 3.5 Hz, H-1), 4.92 (d, J = 3.3 Hz, 1 H, H-4′), 4.83 (dd, J = 9.1 Hz, 1 H, H-2′′′), 4.69 (d, J = 8.0 Hz, 1 H, H-1′′′), 4.58 (d, J = 7.7 Hz, 1 H, H-1′), 2.11-1.82 (cluster of s, 36 H, 10 × OAc and 2 × NAc).

Anal. Calcd. for $C_{55}H_{74}N_2O_{31}$: C, 52.46; H, 5.92; N, 2.22. Found: C, 52.35; H, 5.86; N, 2.25.

Benzyl O-(3-O-sulfo-β-D-galactopyranosyl sodium salt)-(1→4)-O-(2-acetamido- 2-deoxy-β-D-glucopyranosyl)-(1→6)-O-[(β-D-galactopyranosyl)-(1→3)]-2-acetamido-2- deoxy-α-D-galactopyranoside (20)

To a solution of 19 (0.25 g, 0.2 mmol) in dry DMF (20 ml) was added SO_3-pyridine complex (0.3 g, 1.9 mmol). The mixture was stirred at room temperature for 2 h when TLC (4:1, $CHCl_3$-MeOH) revealed the presence of a slower migrating product. The reaction mixture was cooled to 0°C and treated with a few drops of methanol, followed by a few drops of pyridine, and then evaporated, and product purified in a small silica gel column using 4:1 $CHCl_3$-MeOH as an eluent.

The pure product so obtained was then dissolved in dry methanol (20 ml), 0.1 N sodium methoxide in methanol (1

ml) was added, and the reaction mixture stirred at room temperature for 48 h. It was neutralized with Amberlite IR120 (H^+) cation- exchange resin, filtered, and concentrated under reduced pressure. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of $CHCl_3$-MeOH-H_2O (13:6:1→4:5:1). Pure fractions were combined and evaporated, and the V residue dissolved in distilled water and passed through an Amberlite IR120 (Na^+) resin column, and then Iyophillized to afford 20 (0.17 g, 91%); $[\alpha]_D$ +53° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 7.49-7.44 (m, 5 H, arom.), 5.00 (d, J = 3.7 Hz, 1 H, H-1), 4.72 (d, J = 7.79 Hz, 1 H, H-1′′), 4.58 (d, J = 7.7 Hz, 1 H, H-1′′′), 4.46 (d, J = 7.8 Hz, 1 H, H-1′), 1.99 (bs, 6 H, 2 × NAc); ^{13}C NMR data; m/z: 917.4 $(M-Na)^-$: δ Gal-β-D-(1→3) Residue: 103.57 (C-1), 68.57 (C-2), 68.71 (C-3), 67.81 (C-4), 73.71 (C-5), 59.94 (C-6); 3-O-SO_3NaGal-β- (**1**→4): 101.46 (C-1), 67.48 (C-2), 76.37 (C-3), 65.81 (C-4), 71.52 (C-5), 59.98 (C-6); β-D-GlcNAc-(1→6): 100.48 (C-1), 54.07 (C-2), 69.59 (C-3), 77.47 (C-4), 73.90 (C-5), 59.03 (C-6), 21.22 (NAc); α-D-GalNAc-OBn: 95.23 (C-1), 47.54 (C-2), 75.93 (C-3), 65.81 (C-4), 71.46 (C-5), 68.08 (C-6), 20.91 (NAc).

Anal. Calcd. for $C_{35}H_{53}N_2O_{24}SNa.1.5\ H_2O$:
C, 43.43; H, 5.83; N, 2.89.
Found: C, 43.41; H, 5.91; N, 2.84.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-O- acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-O-[(2,4,6-tri-O-acetyl-3-O- chloroacetyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (21)

Glycosidation of 9 (0.7 g, 1.04 mmol) with 10 (0.96 g, 1.18 mmol) afforded compound 21 (1.2 g, 64%) after silica gel column chromatography (solvent gradient consisting of 30% to 40% acetone in $CHCl_3$); $[\alpha]_D$ +36° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.65-7.15 (m, 9 H, arom.), 5.70 (dd, J = 8.9 Hz, 1 H, H-3′′), 5.39 (d, J = 8.6 Hz, 1 H, NH), 5.38 (d, J = 9.7 Hz, 1 H, H-1′′), 5.34 (d, J = 3.1 Hz, 1 H, H-4′′′), 5.12 (d, J = 2.6 Hz, H-1), 5.11 (d, J = 3.4 Hz, 1 H, H-4′), 4.44 (d, J = 7.9 Hz, 1 H, H-1′′′), 4.20 (d, J = 7.2 Hz, 1 H, H-1′), 3.96 (s, 2 H, CH_2Cl), 2.13-1.59 (cluster of s, 30 H, 9 × OAc and NAc).

Anal. Calcd. for $C_{61}H_{73}N_2O_{32}Cl$: C, 53.02; H, 5.32; N, 2.03.
Found: C, 53.12; H, 5.42; N, 2.11.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-O- acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-O-[(2,4,6-tri-O-acetyl-β-D- galactopyranosyl)-(1→3)]- 2-acetamido-2-deoxy-α-D-galactopyranoside (22)

A solution of compound 21 (1.0 g, 0.55 mmol) in EtOH-CH_2Cl_2 (1:1, 30 ml) containing thiourea (0.46 g, 6.0 mmol) and lutidine (0.32 ml, 3.0 mmol) was stirred for 10 h at 70°C. The solvents were evaporated under reduced pressure and residue obtained was dissolved in CH_2Cl_2. The organic layer was washed with water, dried, and concentrated under diminished pressure. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 5% to 7% MeOH in $CHCl_3$ to give 22 (0.8 g, 84%); $[\alpha]_D$ +41° (*c* 1.5, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.65-7.14 (m, 9 H, arom.), 5.70 (dd, J = 8.5 Hz, 1 H, H-3′′), 5.42 (d, J = 9.5 Hz, 1 H, NH), 5.39 (d, J = 8.3 Hz, 1 H, H-1′′), 5.34 (d, J = 3.3 Hz, 1 H, H-4′′′), 5.23 (d, J = 3.4 Hz, 1 H, H-4′), 5.07 (dd, J = 7.9 Hz, 1 H, H-2′′′), 4.99 (d, J = 3.4 Hz, 1 H, H-1), 4.39 (d, J = 8.0 Hz, 1 H, H-1′′′), 4.19 (d, J = 7.4 Hz, 1 H, H-1′′), 2.13-1.64 (cluster of s, 30 H, 9 × OAc and NAc).

Anal. Calcd. for $C_{59}H_{72}N_2O_{31}$: C, 54.29; H, 5.56; N, 2.15.
Found: C, 54.31; H, 5.75; N, 2.07.

Benzyl O-(β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-[(3-O-sulfo-β-D-galactopyranosyl sodium salt)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (23)

A solution of 22 (0.68 g, 0.4 mmol) in dry DMF (20 ml) was sulfated using SO_3-pyridine complex (0.32 g, 2.0 mmol) at 0°C for 1 h, then treated in the manner prescribed for the preparation of 12 (from 11) to afford 23 (0.27 g, 73%); $[\alpha]_D$ +49° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 7.47-7.45 (m, 5 H, arom.), 4.98 (d, J = 3.8 Hz, 1 H, H-1), 4.57 (d, J = 8.7 Hz, 1 H, H-1′′), 4.55 (d, J = 8.4 Hz, 1 H, H-1′′′), 4.46 (d, J = 7.8 Hz, 1 H, H-1′), 1.99 (bs, 6 H, 2 × NAc); m/z: 917.3 $(M-Na)^-$: ^{13}C NMR; δ 3-O-SO_3Na-β-D-Gal-(1→3) Residue: 103.28 (C-1), 68.40 (C-2), 79.17 (C-3), 67.56 (C-4), 73.76 (C-5), 59.99 (C-6); β-D-Gal(1→4) Residue: 101.89 (C-1), 68.59 (C-2), 68.78 (C-3), 67.71 (C-4), 73.53 (C-5), 59.86 (C-6); β-D-GlcNAc-(1→6) Residue: 100.46 (C-1), 54.10 (C-2), 69.96 (C-3), 77.58 (C-4), 74.35 (C-5), 59.09 (C-6), 21.26 (NAc); α-D-GalNAc-OBn Residue: 95.26 (C-1), 48.38 (C-2), 78.98 (C-3), 67.55 (C-4), 71.46 (C-5), 67.75 (C-6), 20.93 (NAc).

Anal. Calcd. for $C_{35}H_{53}N_2O_{24}SNa.2H_2O$: C, 43.03;
H, 5.88; N, 2.87.
Found: C, 43.19; H, 6.03; N, 2.91.

Benzyl O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-galactopyranosyl)-(1→4)-O-(3-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranosyl)-(1→6)-O-[(2,4,6- tri-O-acetyl-β-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (24)

Glycosidation of 9 (0.43 g, 0.6 mmol) with 4 (0.49 g, 0.6 mmol), followed by removal of chloroacetyl, as described for the preparation of 22 (from 21), gave 24 (0.2 g, 26% on

the basis of 9); $[\alpha]_D$ +44° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.84 (d, J = 7.8 Hz, 1 H, NH), 7.60-7.13 (m, 9 H, arom.), 5.43 (d, J = 3.2 Hz, 1 H, H-4′′′), 5.39 (d, J = 3.4 Hz, 1 H, H-4′), 5.40-5.38 (m, 2 H, H-1′′ and H-3′′), 5.22 (d, J = 2.7 Hz, 1 H, H-1), 4.55 (d, J = 7.7 Hz, 1 H, H-1′′′), 4.18 (d, J = 7.3 Hz, 1 H, H-1′), 3.32 (s, 3 H, OMe), 2.19-1.57 (cluster of s, 24 H, 7 × OAc and NAc).

Anal. Calcd. for $C_{61}H_{78}N_2O_{30}$: C, 55.53; H, 5.95; N, 2.12.
Found: C, 55.69; H, 6.12; N, 2.08.

Benzyl O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-2-deoxy-β- D-glucopyranosyl)-(1→6)-O-[(3-O-sulfo-β-D-galactopyranosyl sodium salt)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (25)

This compound was obtained from 24 by the same reaction sequence as that described for the preparation of 23 (from 22), amorphous (0.08 g, 58%); %); $[\alpha]_D$ +50° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 7.53-7.46 (m, 5 H, arom.), 5.02 (d, J = 3.6 Hz, 1 H, H-1), 4.75 (d, J = 8.3 Hz, 1 H, H-1′′), 4.48 (d, J = 7.7 Hz, 1 H, H-1′′′), 4.31 (d, J = 7.7 Hz, 1 H, H-1′), 3.49 (s, 3 H, OMe), 2.00 (bs, 6 H, 2 × NAc); ^{13}C NMR: δ 3-O-SO_3Na-β-D-Gal-(1→3) Residue: 103.27 (C-1), 68.75 (C-2), 79.15 (C-3), 67.74 (C-4), 73.75 (C-5), 60.06 (C-6); 3-O-Me-β-D-Gal-(1→4) Residue: 101.74 (C-1), 68.75 (C-2), 80.64 (C-3), 67.52 (C-4), 73.52 (C-5), 59.85 (C-6), 55.24 (OMe); β-D-GlcNAc-(1→6) Residue: 101.46 (C-1), 54.09 (C-2), 68.92 (C-3), 77.37 (C-4), 74.24 (C-5), 59.06 (C-6), 21.26 (NAc); α-D-GalNAc-OBn Residue: 95.25 (C-1), 47.47 (C-2), 76.37 (C-3), 63.10 (C-4), 71.48 (C-5), 68.39 (C-6), 20.93 (NAc).

Anal. Calcd. for $C_{36}H_{55}N_2O_{24}SNa.H_2O$:
C, 44.40; H, 5.90; N, 2.88.
Found: C, 44.51; H, 5.85; N, 2.76.

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