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Synthesis of mono-, di- and trisulfated Lewis x trisaccharides

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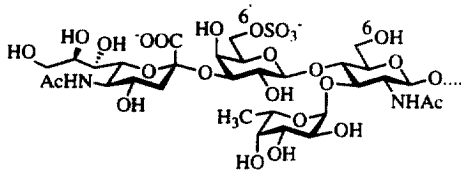
Abstract

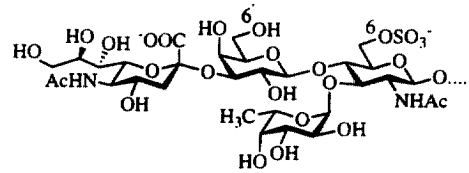
3′-Sulfated and 3′,6′-disulfated Lewis x trisaccharides have been prepared through selective sulfation of methyl 2-acetamido-6-*O*-benzyl-2-deoxy-4-*O*-β-D-galactopyranosyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside, followed by catalytic hydrogenolysis. In a similar manner, 3′,6-disulfated and 3′,6,6′-trisulfated Lewis x trisaccharides have been selectively obtained from methyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside.

1. Introduction

Sulfated Lewis x are structural trisaccharidic entities which have been found to be present at the extremity of sugar chains (capping oligosaccharides). They are frequently sialylated. A typical example is 6′-sulfated sialyl Lewis x, hereafter designated structure **A**, which is a main capping group[1] of the high endothelial venules (HEV) associated glycoprotein GLYCAM-1. The structure **B**, wherein the primary hydroxyl group of the GlcNAc residue is sulfated (6-sulfated sialyl Lewis x), and the 6,6′-disulfated sialyl Lewis x, may also[1] be determinants on GLYCAM-1.



Structure **A**



Structure **B**

These variously sulfated oligosaccharides are of current interest as potential physiological L-selectin ligands,[1] thus playing a role in the recruitment of leukocytes to sites of acute or chronic inflammation[2] and also in the process of lymphocyte recirculation.[3] On the other hand, non sialylated 3′-sulfated

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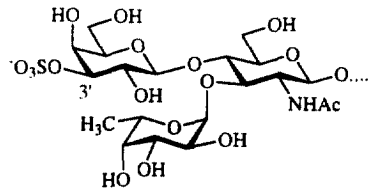
Lewis x (structure **C**) has also been identified in human foetal meconium[4] glycoproteins and in an ovarian cystodenoma glycoprotein.[5] Structure **C** has been shown to be a strong ligand for the inducible endothelial adhesion molecule E-selectin, which is involved in the leucocyte recruitment to inflammation sites.[6] Interestingly the structure of bovine articular cartilage keratan sulfate is partially made out of repeating units of sulfated Lewis x.[7]



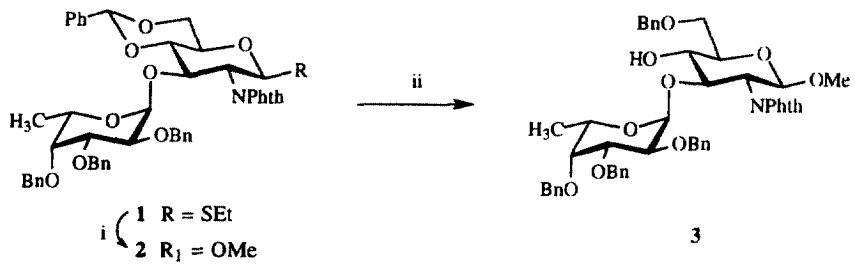
Structure **C**

It thus appears that the homogeneous linear poly (N-acetyl lactosamine) backbone, which is present in many glycoconjugates, is amenable to considerable structural heterogeneity, not only after α-L-fucosylation on position 3 of N-acetyl glucosamine or sialylation of the non-reducing end, but also through sulfation on various hydroxyl groups, especially the primary ones. Whether this heterogeneity, as in the well known case of heparin/heparan sulfate, is a key feature for the setting-up of a 'poly N-acetyl lactosamine' derived biologically relevant secret code, is so far an open question. For this reason, the synthesis of variously sulfated Lewis x containing oligosaccharides has recently been worked out.[8] On the other hand, close mimics have also been prepared,[9] together with more remote non fucosylated analogs.[10] We would like to report our own achievements in this area.

2. Results and discussion

2.1. *Synthesis of the mono- and disulfated Lewis x derivatives* **9** *and* **10**

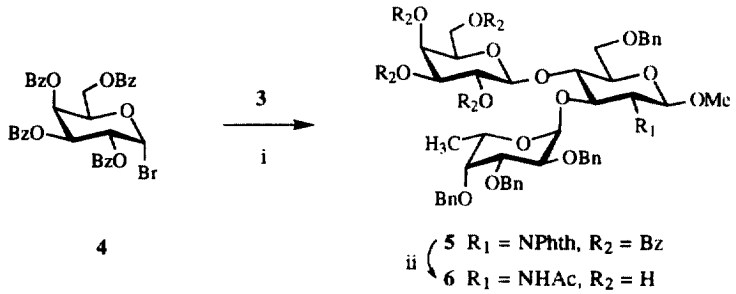
The selected starting material for the synthesis of the Lewis x skeleton was the disaccharide **1**. It has previously been efficiently prepared using a novel glycosylation reaction developed in our group.[11] Reaction of **1** with methanol in acetonitrile, promoted by tris (4-bromophenyl) ammoniumyl hexachloroantimonate,[12] provided **2** in high yield (Scheme 1). The β configuration of the newly introduced anomeric carbon was confirmed by the ^{1}H NMR spectrum that showed the H-1 as a doublet at δ 5.31 ($J_{1,2}$=8.5 Hz). Regioselective reductive opening of the benzylidene moiety with $NaCNBH_3$–HCl (gas in ether) in THF[13] gave the alcohol **3** in 86% yield.



Scheme 1. Reaction conditions: (i) 7 equiv. of MeOH, 1.5 equiv. of tris (4-bromophenyl) ammoniumyl hexachloroantimonate, 4 Å MS, CH_3CN, rt, 20 min, 90%; (ii) 10 equiv. of $NaCNBH_3$, HCl·Et_2O, 4 Å MS, THF, 0°C, 1 h, 86%

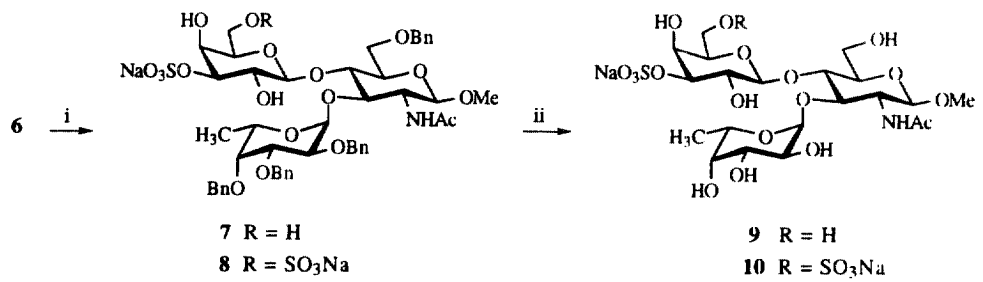
Condensation of **3** with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **4**,[14] in the presence of silver triflate, furnished the desired trisaccharide **5** in crystalline form in 87% yield (Scheme 2). The

β configuration of the newly introduced anomeric carbon was confirmed by the ^{1}H NMR spectrum that showed the H-1′ (galactose unit) as a doublet at δ 5.03 ($J_{1',2'}$=8.2 Hz). Treatment of **5** first with hydrazine hydrate in boiling ethanol, then with acetic anhydride in pyridine at room temperature, and finally with sodium methoxide in methanol at room temperature, afforded the trisaccharide **6** in crystalline form in 80% overall yield from **5**.



Scheme 2. Reaction conditions: (i) 1 equiv. of **3**, 2 equiv. of **4**, 3 equiv. of AgOTf, 1.9 equiv. of s-collidine, 4 Å MS, CH_2Cl_2, toluene, −20°C, 0.5 h, 87%; (ii) hydrazine hydrate, 90% aqueous ethanol, reflux 18 h; Ac_2O, pyridine, rt, 16 h; cat. NaOMe, MeOH, 80%

Sulfation was now accomplished using the stannylene procedure.[15] Thus, **6** was heated at reflux with one equivalent of dibutyltin oxide in methanol for 2 h then the residue was reacted, after removal of methanol, with two equivalents of trimethylamine–sulfur trioxide complex in dry THF, to give selectively the monosulfated derivative **7** (79%), which was easily separated from the disulfated derivative **8** (13%). Catalytic hydrogenolysis of **7** and **8**, followed by purification of the products on Sephadex G-10-120, and chromatography with a cation exchange resin (Na^+) provided quantitatively, after freeze-drying, the monosodium salt **9** and disodium salt **10**, respectively, as amorphous white powders (Scheme 3). Their structures were fully confirmed by NMR spectroscopy using COSY experiments. Sulfation of 3′-OH (galactose unit) caused a downfield shift of the signal of H-3′ of 0.64 and 0.68 ppm for **9** and **10** respectively, and sulfation of 6′-OH (galactose unit) caused a downfield shift of the signal of H-6′ of 0.42 ppm for **10**, compared to the non-sulfated parent compound Lewis x trisaccharide-β-OMe.[16]



Scheme 3. Reaction conditions: (i) 1 equiv. of Bu_2SnO, MeOH, reflux, 2 h; then 2 equiv. of $Me_3N \cdot SO_3$ complex, THF, rt, 40 h, Dowex resin (50x8-200, Na^+): **7** (79%), **8** (13%); (ii) H_2, 10% Pd–C, MeOH, 15°C, 15 h, quantitative

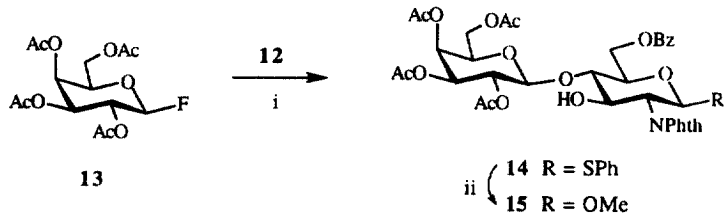
2.2. *Synthesis of the di- and trisulfated Lewis x derivatives 23 and 24*

Glycosylation of **12**, prepared as shown in Scheme 4, with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl fluoride **13**[17] in the presence of AgOTf–$SnCl_2$[11] selectively provided the β 1→4 linked disaccharide **14** in 70% yield. (Scheme 5). The β configuration of the newly introduced anomeric

centre was confirmed by the ^{1}H NMR spectrum that showed the H-1′ as a doublet at δ 4.59 ppm ($J_{1',2'}$=8.0 Hz). The preparation of **15** from **14** was achieved as described for **2** in 95% yield.

11 **12**

Scheme 4. Reaction conditions: (i) cat. NaOMe, MeOH; (ii) 0.75 equiv. of $(Bu_3Sn)_2O$, toluene, reflux with continuous removal of water, 14 h; (iii) 1.5 equiv. of BzCl, rt, 5 h, 92%



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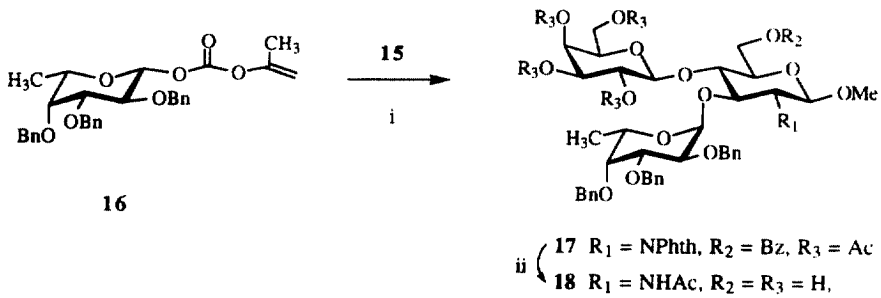
Scheme 5. Reaction conditions: (i) 1 equiv. of **12**, 2 equiv. of **13**, 1 equiv. of AgOTf, 1 equiv. of $SnCl_2$, 4 Å MS, CH_2Cl_2:toluene (5:1), −15°C to rt, 2 h, 70%; (ii) 10 equiv. of MeOH, 2 equiv. of tris(4-bromophenyl) ammoniumyl hexachloroantimonate, 4 Å MS, CH_3CN, rt, 1 h, 95%

Glycosylation of **15** with the fucosyl donor **16**,[11] in the presence of TMSOTf, gave the crystalline trisaccharide **17** in 81% yield (Scheme 6). Treatment of **17** with hydrazine hydrate in aqueous ethanol at 85°C followed by N-acetylation with acetic anhydride in dichloromethane–methanol afforded **18** in 70% yield.



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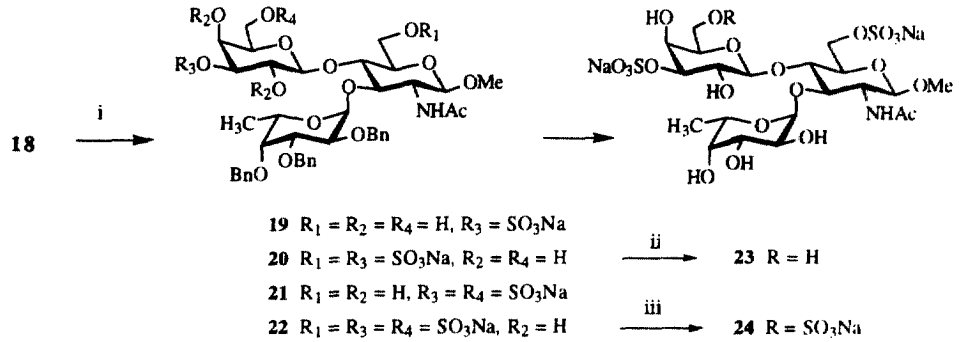
Scheme 6. Reaction conditions: (i) 1 equiv. of **15**, 1.1 equiv. of **16**, 1.1 equiv. of TMSOTf, Et_2O, 0°C, 40 min, 81%; (ii) hydrazine hydrate, 90% aqueous ethanol, reflux 18 h; Ac_2O, CH_2Cl_2:MeOH (1:1), rt, 3 h, 70%

The sulfation of **18** was achieved as described earlier for **7**, after stirring for 40 h with 2 equivalents of trimethylamine–sulfur trioxide complex in dry THF at room temperature. The THF was evaporated, and the residue was further reacted with 2 equivalents of trimethylamine–sulfur trioxide complex in dry DMF at room temperature for 60 h. Four compounds were separated and identified as **19** (26%), **20** (11%), **21** (52%), and **22** (7%). Reaction of the stannylene acetal intermediate with 6 equivalents of pyridine–sulfur trioxide complex in DMF gave the trisulfated compound **22** in 66% yield. Catalytic hydrogenolysis of **19** and **21** followed by purification of the products on Sephadex G-10-120, followed by chromatography with cation exchange resin (Na^+) provided quantitatively, after freeze-drying, the monosodium salt **9** and disodium salts **10**, identical with the samples prepared from compounds **7** and **8**. Debenzylation of **20** gave a trisaccharide disodium salt, characterized as **23**; in a similar manner, **22** was converted into the trisodium salt **24** (Scheme 7). The structures of **23** and **24** were confirmed by NMR spectroscopy using COSY experiments. In particular, sulfation of 3′-OH in the galactose unit caused a downfield shift of

0.64 and 0.69 ppm for **23** and **24** respectively, sulfation of 6′-OH in the galactose unit caused a downfield shift of the signal of H-6′ of 0.41 ppm for **24**, and sulfation of 6-OH in the glucosamine unit caused a downfield shift of the signal of H-6 of 0.49 and 0.52 ppm for **23** and **24** respectively, compared to the non-sulfated parent compound Lewis x trisaccharide-β-OMe.[16]



Scheme 7. Reaction conditions: (i) 1 equiv. of Bu_2SnO, MeOH, reflux 2 h; after evaporation of MeOH *in vacuo*: (a) 2 equiv. of $Me_3N{\cdot}SO_3$ complex, THF, rt, 40 h, then evaporation of THF, 2 equiv. of $Me_3N{\cdot}SO_3$ complex, DMF, rt, 60 h, Dowex resin (50x8-200, Na^+): **19** (26%), **20** (11%), **21** (52%), **22** (7%); (b) 6 equiv. of $Py{\cdot}SO_3$ complex, DMF, rt, 5 days, Dowex resin (50x8-200, Na^+): **22** (66%); (ii) H_2 (170 kPa), 10% Pd–C, MeOH, rt, 16 h, 97%; (iii) H_2 (180 kPa), 10% Pd–C, MeOH, rt, 20 h, 90%

3. Experimental

3.1. General

Melting points (m.p.) were determined with a Büchi model 510 m.p. apparatus and are uncorrected. Optical rotations were measured at 20±2°C with a Perkin–Elmer Model 241 digital polarimeter, using a 10 cm, 1 ml cell. CI (ammonia) mass spectra were obtained with a Nermag R10-10 spectrometer. Electrospray Ionisation (EI) mass spectra were recorded on a VG-Platform mass spectrometer (Fisons Instruments) in negative mode. Elemental analyses were performed by Service Central d'Analyse du C.N.R.S., BP 22, 69390 Vernaison, France, or by Service de Microanalyse de l'Université Pierre et Marie Curie, 4, Place Jussieu, 75005 Paris, France. ^1H NMR spectra were recorded with a Bruker AC 250 and a Bruker AM 400 spectrometer for solutions in $CDCl_3$, CD_3OD or D_2O at ambient temperature. Assignments were aided by COSY experiments. ^{13}C NMR spectra were recorded at 62.89 MHz with a Bruker AC 250 and at 100.57 MHz with a Bruker AM 400 for solutions in $CDCl_3$ adopting 77.00 ppm for the central line of $CDCl_3$. Assignments were aided by the J-mod technique and proton–carbon correlation. For trisaccharide, a single prime refers to the hydrogens or carbons of the galactose unit; and a double prime refers to the hydrogens or carbons of the fucose unit. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness, 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck).

3.2. *Methyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 2*

A suspension of **1** (1.2 g, 1.4 mmol), dry methanol (0.4 ml, 10 mmol) and molecular sieves (4 Å, 2 g) in dry acetonitrile (20 ml) was stirred for 0.5 h under argon at room temperature. Tris (4-bromophenyl) ammoniumyl hexachloroantimonate (1.63 g, 2 mmol) was added. The mixture was kept at room temperature for 20 min, when TLC showed the disappearance of **1**. The mixture was neutralized with triethylamine, diluted with dichloromethane, filtered through Celite, and concentrated. The residue was eluted from a column of silica gel with cyclohexane:ethyl acetate (4:1) to give **2** as an amorphous solid (1.05 g, 90%). R_f=0.36 (cyclohexane:ethyl acetate=3:1). $[\alpha]_D$ −19 (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.83–7.76, 7.65–7.62, 7.57–7.54, 7.39–7.21, 7.08–7.06 (5m, 24H, arom. H), 5.60 (s, 1H, PhCH), 5.31 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 4.87 (d, 1H, $J_{1',2'}$=3.1 Hz, H-1′), 4.84, 4.54 (2d, 2H, J=11.6 Hz, $PhCH_2$), 4.67 (dd, 1H, $J_{3,4}$=8.3, $J_{2,3}$=10.3 Hz, H-3), 4.49 (dd, 1H, $J_{6a,5}$=4.5, $J_{6a,6b}$=10.5 Hz, H-6a), 4.47, 4.42 (2d, 2H, J=11.5 Hz, $PhCH_2$), 4.37 (dd, 1H, H-2), 4.30, 3.88 (2d, 2H, J=12.7 Hz, $PhCH_2$), 4.11 (dq, 1H, $J_{5',6'}$=6.5, $J_{5',4'}$<1 Hz, H-5′), 3.73 (dd, 1H, $J_{2',3'}$=10.1 Hz, H-2′), 3.50 (s, 3H, OMe), 0.92 (d, 3H, $J_{6',5'}$=6.5 Hz, H-6′). ^{13}C NMR (62.89 MHz, $CDCl_3$): 168.21 (2O=C, Phth), 138.91, 138.59, 138.37, 137.21 (4C, Ph), 133.78, 132.11, 129.10, 128.79, 128.55–127.38, 126.10, 123.18 (arom. C), 101.15 (PhCH), 99.86 (C-1), 99.57 (C-1′), 82.23, 79.85, 78.09, 75.85, 75.63, 66.28 (ring C), 74.78, 73.15, 72.70 ($3PhCH_2$), 68.71 (C-6), 67.33 (C-5′), 57.08 (OMe), 55.70 (C-2), 16.49 (C-6′). MS (CI) m/z: 845 (40%) $M+NH_4^+$. Anal. calcd for $C_{49}H_{49}NO_{11}$: C, 71.08; H, 5.97; N, 1.69. Found: C, 70.97; H, 6.16; N, 1.61.

3.3. *Methyl 6-O-benzyl-2-deoxy-2-phthalimido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 3*

To a cold (0°C), stirred mixture of **2** (530 mg, 0.64 mmol), sodium cyanoborohydride (402 mg), molecular sieves (4 Å, 1 g), and methyl orange (0.1 g) in dry THF (10 ml) was added dropwise, a saturated solution of HCl in ether until the red color ceased to be discharged (about 2 ml), and stirring was continued for 30 min. The mixture was diluted with dichloromethane, and the solid was filtered through a Celite bed. The filtrate was washed with cold water, cold saturated $NaHCO_3$ solution, water, dried, and concentrated. The residue was applied to a column of silica gel and eluted with cyclohexane:ethyl acetate (3:1) to give **3** as an amorphous solid (457 mg, 86%). R_f=0.32 (cyclohexane:ethyl acetate=3:1). $[\alpha]_D$ +34 (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.89–7.87, 7.73–7.67, 7.61–7.57 (3m, 4H, Phth), 7.45–7.23, 7.02–6.99 (2m, 20H, 4Ph), 5.30 (d, 1H, $J_{1,2}$=8.4 Hz, H-1), 4.90, 4.56 (2d, 2H, J=11.5 Hz, $PhCH_2$), 4.72, 4.67 (2d, 2H, J=12.0 Hz, $PhCH_2$), 4.66 (d, 1H, $J_{1',2'}$=3.3 Hz, H-1′), 4.64, 4.57 (2d, 2H, J=11.8 Hz, $PhCH_2$), 4.30 (dd, 1H, $J_{2,3}$=10.8 Hz, H-2), 4.29 (d, 1H, $J_{OH,4}$=1 Hz, OH), 4.20 (dd, 1H, $J_{3,4}$=8.1 Hz, H-3), 4.15, 3.46 (2d, 2H, J=13 Hz, $PhCH_2$), 4.12 (dq, 1H, $J_{5',4'}$<1, $J_{5',6'}$=6.4 Hz, H-5′), 3.95 (dd, 1H, $J_{6a,5}$=1.8, $J_{6a,6b}$=10.7 Hz, H-6a), 3.82 (dd, 1H, $J_{3',4'}$=2.5, $J_{3',2'}$=10.2 Hz, H-3′), 3.80 (dd, 1H, $J_{6b,5}$=5.5 Hz, H-6b), 3.75 (dd, 1H, H-2′), 3.71 (ddd, 1H, $J_{5,4}$=9.6 Hz, H-5), 3.60 (ddd, 1H, H-4), 3.54 (dd, 1H, H-4′), 3.53 (s, 3H, OMe), 1.11 (d, 3H, H-6′). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 168.91, 168.11 (2C=O, Phth), 138.75, 138.49, 138.32, 138.14 (4C, Ph), 133.61 (2CH, Phth), 132.59, 132.02 (2C, Phth), 128.44–127.51 (CH, Ph), 122.95 (2CH, Phth), 100.80 (C-1′), 98.23 (C-1), 83.19 (C-3), 78.89 (C-3′), 77.92 (C-4′), 75.27 (C-5), 74.76 ($PhCH_2$), 73.99 (C-2′), 73.48 ($PhCH_2$), 73.43 ($PhCH_2$), 72.39 ($PhCH_2$), 71.25 (C-4), 69.29 (C-6), 68.47 (C-5′), 56.78 (OMe), 54.55 (C-2), 16.47 (C-6′). MS (CI): m/z 847 (65%) $M+NH_4^+$. Anal. calcd for $C_{49}H_{51}NO_{11}$: C, 70.91; H, 6.19; N, 1.69. Found: C, 70.66; H, 6.25; N, 1.60.

3.4. Methyl 6-O-benzyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 5

A solution of silver triflate (642 mg, 2.5 mmol) and s-collidine (0.25 ml) in dichloromethane (4.5 ml) and toluene (3 ml) was added dropwise at −20°C to a stirred solution of **3** (700 mg, 0.84 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **4** (1.11 g, 1.68 mmol) in toluene (7.5 ml) containing ground molecular sieves (4 Å, 2 g) under argon. When TLC indicated a complete reaction (0.5 h), 10% aqueous sodium thiosulfate (10 ml) and toluene (20 ml) were added. The mixture was filtered through Celite. The organic layer was separated and washed with water, dried, and concentrated. The residue was flash chromatographed on a column of silica gel (cyclohexane:ethyl acetate=2.5:1) to yield **5** (1.03 g, 87%) as a white solid. R_f=0.34 (cyclohexane:ethyl acetate=2:1). M.p. 178–179°C (from ether). $[\alpha]_D$ +3.7 (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18–7.07 (m, 44H, arom. H), 5.84 (dd, 1H, $J_{4',5'}$<1, $J_{4',3'}$=3.7 Hz, H-4′), 5.75 (dd, 1H, $J_{2',1'}$=8.2, $J_{2',3'}$=10.3 Hz, H-2′), 5.38 (dd, 1H, H-3′), 5.10 (d, 1H, $J_{1'',2''}$=3.5 Hz, H-1″), 5.03 (d, 1H, H-1′), 4.98 (d, 1H, $J_{1,2}$=8.6 Hz, H-1), 4.93, 4.50 (2d, 2H, J=12.1 Hz, $PhCH_2$), 4.82 (dd, 1H, $J_{3,4}$=8.9, $J_{3,2}$=10.5 Hz, H-3), 4.76 (dq, 1H, $J_{5'',4''}$<1, $J_{5'',6''}$=6.5 Hz, H-5″), 4.72, 4.24 (2d, 2H, J=11.2 Hz, $PhCH_2$), 4.40 (dd, 1H, H-2), 4.30 (dd, 1H, $J_{4,5}$=9.6 Hz, H-4), 3.95 (dd, 1H, $J_{3'',4''}$=2.6, $J_{3'',2''}$=10.2 Hz, H-3″), 3.83 (dd, 1H, H-2″), 3.60 (dd, 1H, H-4″), 3.37 (s, 3H, OMe), 1.46 (d, 3H, H-6″). ^{13}C NMR (100.57 MHz, $CDCl_3$): δ 167.79 (2O=C, Phth), 165.84, 165.74, 165.27, 164.70 (4O=C, Bz), 138.94, 138.82, 138.21, 137.78, 133.98, 133.50, 133.32, 133.24, 131.75, 129.84, 129.63, 129.60, 129.42, 129.19, 128.98, 128.76, 128.66, 128.63, 128.60, 128.48, 128.31, 128.24, 128.14, 127.96, 127.95, 127.86, 127.77, 127.16, 127.06, 126.89, 126.63, 123.45 (arom. C), 99.79 (C-1′), 99.07 (C-1), 96.71 (C-1″), 79.25 (C-4″), 79.15 (C-3″), 75.66, 75.37, 74.68, 72.36, 71.70, 71.28, 69.78, 68.24 (ring C), 75.05, 73.69, 72.66, 71.96, (4$PhCH_2$), 67.54 (C-6), 66.62 (C-5″), 61.30 (C-6′), 56.62 (OMe), 56.06 (C-2), 16.81 (C-6″). MS (CI): m/z 1425 (100%) M+NH_4^+. Anal. calcd for $C_{83}H_{77}NO_{20}$: C, 70.78; H, 5.51; N, 0.99. Found: C, 70.70; H, 5.46; N, 0.99.

3.5. Methyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-β-D-galactopyranosyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 6

Hydrazine hydrate (9 ml) was added to a stirred solution of compound **5** (450 mg, 0.319 mmol) in 90% aqueous ethanol (90 ml) and refluxed for 20 h. The solution was concentrated and the residue was acetylated using acetic anhydride (15 ml) and pyridine (30 ml) at room temperature overnight. The solution was concentrated and coevaporated with toluene. Then the residue was stirred with sodium (10 mg) in methanol:dichloromethane (25 ml, 3:2) overnight at room temperature. The base was neutralized by an IR-120 cation exchange resin. The resin was filtered off and thoroughly washed with methanol. The combined filtrate and washings were concentrated. The residue was flash chromatographed on a column of silica gel (dichloromethane:ethyl acetate:methanol=5:10:2) to yield **6** as a white solid (231 mg, 80%). R_f=0.38 (dichloromethane:ethyl acetate:methanol=5:10:2). M.p. 95°C (dichloromethane–ether). $[\alpha]_D$ −76 (c 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.62–7.45 (m, 20H, arom. H), 5.53 (d, 1H, $J_{1'',2''}$=3.8 Hz, H-1″), 5.08–4.98 (m, 5H, H-5″, $PhCH_2$, 2H from 2$PhCH_2$), 4.87, 4.75 (2d, 2H, J=11.8 Hz, $PhCH_2$), 4.77–4.73 (m, 2H from 2$PhCH_2$), 4.61 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.50 (d, 1H, $J_{1,2}$=7.9 Hz, H-1), 4.36 (m, 1H, H-4), 4.30 (dd, 1H, $J_{3'',4''}$=2.8, $J_{3'',2''}$=10.2 Hz, H-3″), 4.23 (dd, 1H, $J_{6a,5}$=3.4, $J_{6a,6b}$=11.2 Hz, H-6a), 4.22 (dd, 1H, $J_{2,3}$=8.2 Hz, H-2), 4.22–4.20 (m, 1H, H-3), 4.16 (d, 1H, H-2″), 4.13 (dd, 1H, $J_{6'a,5'}$=7.6, $J_{6'a,6'b}$=11.8 Hz, H-6′a), 4.10 (m, 1H, H-4″), 4.02 (dd, 1H, $J_{6b,5}$=1.9 Hz, H-6b), 3.90 (dd, 1H, $J_{4',5'}$<1, $J_{4',3'}$=3.4 Hz, H-4′), 3.77 (dd, 1H, $J_{6'b,5'}$=3.7 Hz, H-6′b), 3.75 (m, 1H, H-5), 3.67 (dd, 1H, $J_{2',3'}$=9.1 Hz, H-2′), 3.64 (s, 3H, OMe), 3.52 (dd, 1H, H-3′), 3.50 (m, 1H, H-5′), 2.17 (s,

3H, NHAc), 1.37 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6''). ^{13}C NMR (100.57 MHz, CD_3OD=49.30 ppm): δ 173.53 (O=C, NHAc), 140.75, 140.52, 139.95, 139.80 (4C, Ph), 129.84, 129.73, 129.47, 129.44, 128.96, 128.93, 128.85, 128.71, 128.63 (CH, Ph), 104.13 (C-1'), 103.80 (C-1), 98.05 (C-1''), 80.24, 80.24, 77.57, 77.41, 76.86, 75.86, 75.21, 74.85, 73.07, 70.41, 68.05 (11CH, ring C), 76.68, 74.39, 73.93, 73.91 (4PhCH$_2$), 69.61 (C-6), 63.70 (C-6'), 57.52 (C-2), 57.30 (OMe), 23.73 (CH_3, NHAc), 17.20 (C-6''). MS (CI): m/z 921 (40%) M+NH_4^+, 904 (100%) M+H^+. Anal. calcd for $C_{49}H_{61}NO_{15}\cdot 2H_2O$: C, 62.60; H, 6.97; N, 1.49. Found: C, 62.63; H, 6.80; N, 1.45.

3.6. Methyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-(3-O-sodium sulfonato-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 7 and methyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-(3,6-di-O-sodium sulfonato-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 8

A mixture of compound **6** (135 mg, 0.15 mmol) and dibutyltin oxide (37 mg, 0.15 mmol) in dry methanol (1.5 ml) was heated to reflux for 2 h (the solution becames clear) and then the solvent was distilled at 80°C to give a yellowish syrup, which was evaporated to dryness *in vacuo* (2 h). Sulfur trioxide–trimethylamine complex (41 mg, 0.3 mmol) and dry tetrahydrofuran (1.5 ml) were introduced. The mixture was stirred under argon for 40 h at room temperature. Methanol (1.5 ml) was added, after filtration, the filtrate was concentrated. Flash chromatography of the residue on a column of silica gel (dichloromethane:methanol=5:1) followed by cation exchange chromatography (Dowex 50X8-200, Na^+ form) using methanol afforded first **7** as a white amorphous mass (120 mg, 79%). R_f=0.36 (dichloromethane:methanol=5:1). $[\alpha]_D$ −58 (c 1, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 7.41–7.30 (m, 20H, arom. H), 5.52 (d, 1H, $J_{1'',2''}$=3.8 Hz, H-1''), 4.87, 4.74 (2d, 2H, J=12 Hz, PhCH$_2$), 4.67 (d, 1H, $J_{1,2}$=7.7 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1'), 3.65 (s, 3H, OMe), 1.33 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6''). ^{13}C NMR (62.89 MHz, CD_3OD=49.40 ppm): δ 173.70 (O=C, NHAc), 140.85, 140.60, 139.93, 139.87 (4C, Ph), 129.96, 129.91, 129.83, 129.61, 129.60, 129.53, 129.23, 129.12, 129.03, 128.89, 128.81, 128.71 (CH, Ph), 104.38 (C-1'), 103.58 (C-1), 98.17 (C-1''), 82.54 (C-3'), 80.34, 80.18, 77.36, 77.25, 76.80, 75.87, 74.81, 74.54, 74.06, 73.87, 71.52, 69.59, 68.78, 68.15 (4PhCH$_2$, C-6, ring C), 63.64 (C-6'), 57.54 (C-2), 57.54 (OMe), 23.83 (CH_3, NHAc), 17.24 (C-6''). Anal. calcd for $C_{49}H_{60}NO_{18}SNa\cdot 2.5H_2O$: C, 55.99; H, 6.23; N, 1.33. Found: C, 55.95; H, 6.17; N, 1.52.

Compound **8** was eluted second (after ion exchange), as a syrup (21 mg, 13%). R_f=0.18 (dichloromethane:methanol=4:1). This compound was characterized after debenzylation.

3.7. Methyl 2-acetamido-2-deoxy-3-O-α-L-fucopyranosyl-4-O-(3-O-sodium sulfonato-β-D-galacto-pyranosyl)-β-D-glucopyranoside 9

A solution of **7** (85 mg, 84.5 μmol) in methanol (8 ml) was reacted over Pd/C (10%, 160 mg) at 15°C under H_2 (160 kPa) for 15 h, filtered, and evaporated. The residue was purified on a Sephadex column (G10-120), using water as eluant. After ion exchange with Dowex 50X8-200 (Na^+ form) and freeze-drying, compound **9** was obtained as a white amorphous solid (54 mg, 99%). R_f=0.53 (ethyl acetate:isopropanol:water=3:3:2). $[\alpha]_D$ −63 (c 0.75, MeOH). ^{1}H NMR (400 MHz, D_2O): δ 5.10 (d, 1H, $J_{1'',2''}$=4.0 Hz, H-1''), 4.81 (dq, 1H, $J_{5'',4''}$<1, $J_{5'',6''}$=6.5 Hz, H-5''), 4.56 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1'), 4.46 (d, 1H, $J_{1,2}$=8.1 Hz, H-1), 4.32 (dd, 1H, $J_{3',4'}$=3.3, $J_{3',2'}$=9.9 Hz, H-3'), 4.26 (dd, 1H, $J_{4',5'}$<1 Hz, H-4'), 4.01 (dd, 1H, $J_{6a,5}$=2.0, $J_{6a,6b}$=12.0 Hz, H-6a), 3.91 (dd, 1H, $J_{2,3}$=10.0 Hz, H-2), 3.87 (dd, 1H, $J_{6b,5}$=4.0 Hz, H-6b), 3.73 (m, 2H, H-6'a, H-6'b), 3.68 (dd, 1H, $J_{2'',3''}$=10.5 Hz, H-2''), 3.62 (dd, 1H, H-2'), 3.50 (s, 3H, OMe), 2.02 (s, 3H, NHAc), 1.17 (d, 3H, H-6''). ^{13}C NMR (100.57 MHz, D_2O): δ

175.70 (C=O, NHAc), 103.05 (C-1), 102.74 (C-1′), 99.89 (C-1″), 81.46 (C-3′), 76.51, 76.11, 75.89, 74.74, 73.16, 70.48, 70.37, 68.96, 67.97, 67.97 (10CH, ring C), 62.63 (C-6′), 60.95 (C-6), 58.44 (OMe), 56.92 (C-2), 23.50 (CH_3, NHAc), 16.54 (C-6″). MS (EI) calcd for $C_{21}H_{36}NO_{18}SNa$: 645.38. Found: m/z 622 $(M-Na)^-$. Anal. calcd for $C_{21}H_{36}NO_{18}SNa \cdot 3.5H_2O$: C, 35.59; H, 6.12; N, 1.98. Found: C, 35.58; H, 6.38; N, 2.07.

3.8. Methyl 2-acetamido-2-deoxy-3-O-α-L-fucopyranosyl-4-O-(3,6-di-O-sodium sulfonato-β-D-galactopyranosyl)-β-D-glucopyranoside **10**

A solution of **8** (33 mg, 30 μmol) in methanol (5 ml) was reacted over Pd/C (10%, 140 mg) at 15°C under H_2 (160 kPa) for 15 h, filtered, and evaporated. The residue was purified on a Sephadex column (G10-120), using water as eluant. After ion exchange with Dowex 50X8-200 (Na^+ form) and freeze-drying, compound **10** was obtained as a white amorphous solid (22 mg, 99%). R_f=0.34 (ethyl acetate:isopropanol:water=3:3:2). $[\alpha]_D$ −41 (c 0.54, MeOH). ^1H NMR (400 MHz, D_2O): δ 5.10 (d, 1H, $J_{1'',2''}$=3.9 Hz, H-1″), 4.82 (dq, 1H, $J_{5'',6''}$=6.5, $J_{5'',4''}$<1 Hz, H-5″), 4.60 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.48 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.36 (dd, 1H, $J_{3',4'}$=3.4, $J_{3',2'}$=9.7 Hz, H-3′), 4.33 (dd, 1H, $J_{4',5'}$<1 Hz, H-4′), 4.19 (dd, 1H, $J_{6'a,5'}$=6.0, $J_{6'a,6'b}$=11.0 Hz, H-6′a), 4.15 (dd, 1H, $J_{6'b,5'}$=7.0 Hz, H-6′b), 4.03 (dd, 1H, $J_{6a,5}$=2.1, $J_{6a,6b}$=12.0 Hz, H-6a), 3.91 (dd, 1H, $J_{2,3}$=10.3 Hz, H-2), 3.90 (ddd, 1H, H-5′), 3.89 (dd, 1H, $J_{6b,5}$=5.0 Hz, H-6b), 3.66 (dd, 1H, $J_{2'',3''}$=10.2 Hz, H-2″), 3.65 (dd, 1H, H-2′), 3.51 (s, 3H, OMe), 2.03 (s, 3H, NHAc), 1.18 (d, 3H, H-6″). ^{13}C NMR (100.57 MHz, D_2O): δ 175.69 (C=O, NHAc), 103.04 (C-1), 102.71 (C-1′), 99.87 (C-1″), 81.15 (C-3′), 76.57, 76.32, 75.14, 73.26, 73.20, 70.41, 70.28, 69.12 (8CH, ring C), 68.25 (C-6′), 67.96 (C-5″), 67.57 (C-4′), 61.05 (C-6), 58.41 (OMe), 56.93 (C-2), 23.50 (CH_3, NHAc), 16.59 (C-6″). MS (EI) calcd for $C_{21}H_{35}NO_{21}S_2Na_2$: 747.64. Found: m/z 724 $(M-Na)^-$, 350.3 $[(M-2Na)/2]^{2-}$, 622 $(M-NaSO_3-Na+H)^-$.

3.9. Phenyl 6-O-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **12**

Sodium (23 mg) was added to a solution of phenyl 2-deoxy-2-phthalimido-1-thio-3,4,6-tri-O-acetyl-β-D-glucopyranoside **11** (1.05 g, 2 mmol) in methanol (20 ml). After stirring for 2 h at room temperature, the mixture was neutralized by Amberlite resin (IR120, H^+ form), filtered and concentrated to dryness. The obtained amorphous solid was heated to reflux in toluene (40 ml) with bis (tributyltin) oxide (1 g, 1.68 mmol) for 15 h, then allowed to cool. Benzoyl chloride (472 mg, 3.36 mmol) was added and the mixture was stirred at room temperature for 5 h. Methanol (0.5 ml) was introduced. After 1 h of stirring at room temperature, the solvent was evaporated. The residue was flash chromatographed on a column of silica gel (dichloromethane:ethyl acetate=2:1) to yield **12** (930 mg, 92%) as a white solid. R_f=0.25 (dichloromethane:ethyl acetate=2:1). M.p. 113°C (from cyclohexane:ethyl acetate). $[\alpha]_D$ +11.5 (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.08–7.15 (m, 14H, arom. H), 5.66 (d, 1H, $J_{1,2}$=10.5 Hz, H-1), 4.73 (dd, 1H, $J_{6a,5}$=5.0, $J_{6a,6b}$=12.2 Hz, H-6a), 4.66 (dd, 1H, $J_{6b,5}$=2.4 Hz, H-6b), 4.44 (ddd, 1H, $J_{3,OH}$=4.7, $J_{3,4}$=8.8, $J_{3,2}$=10.2 Hz, H-3), 4.23 (dd, 1H, H-2), 3.82 (ddd, 1H, $J_{5,4}$=9.8 Hz, H-5), 3.73 (d, 1H, $J_{OH,4}$=4.4 Hz, OH-4), 3.54 (ddd, 1H, H-4), 3.28 (d, 1H, OH-3). ^{13}C NMR (100.57 MHz, $CDCl_3$): δ 168.26, 167.83 (2O=C, Phth), 166.77 (O=C, Bz), 134.09, 133.13, 132.22, 131.94, 131.28, 129.68, 129.39, 128.63, 128.25, 127.53, 123.65, 123.23 (arom. C), 83.19 (C-1), 77.66, 72.43, 71.35 (C-3, 4, 5), 64.06 (C-6), 55.33 (C-2). MS (CI): m/z 523 (100%) $M+NH_4^+$. Anal. calcd for $C_{27}H_{23}NO_7S$: C, 64.15; H, 4.59. Found: C, 64.10; H, 4.61.

3.10. *Phenyl 6-O-benzoyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside* **14**

A solution of **12** (252 mg, 0.5 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl fluoride **13** (350 mg, 1 mmol) in 5 ml of CH_2Cl_2 and 1 ml of toluene was stirred with 4 Å ground molecular sieves (0.5 g) for 30 min at room temperature under an argon atmosphere. A mixture of stannous chloride (95 mg, 0.5 mmol) and silver triflate (128 mg, 0.5 mmol) was added at −15°C, then the reaction mixture was allowed to gradually warm to room temperature, and the stirring was continued for an additional 2 h. The mixture was filtered through Celite and the solids were washed with CH_2Cl_2. The combined filtrate and washings were washed with a saturated $NaHCO_3$ solution, then with water, dried over $MgSO_4$, and concentrated. The residue was applied to a column of silica gel and eluted with cyclohexane:ethyl acetate (1:1) to afford an amorphous solid (292 mg, 70%). R_f=0.34 (cyclohexane:ethyl acetate=1:1). $[\alpha]_D$ +17 (c 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 8.08–7.13 (m, 14H, arom. H), 5.66 (d, 1H, $J_{1,2}$=10.5 Hz, H-1), 5.38 (dd, 1H, $J_{4',5'}$<1, $J_{4',3'}$=3.4 Hz, H-4′), 5.28 (dd, 1H, $J_{2',1'}$=8.0, $J_{2',3'}$=10.4 Hz, H-2′), 4.97 (dd, 1H, H-3′), 4.67 (dd, 1H, $J_{6a,5}$=2, $J_{6a,6b}$=11.8 Hz, H-6a), 4.59 (d, 1H, H-1′), 4.48 (ddd, 1H, $J_{3,OH}$<1, $J_{3,4}$=8.1, $J_{3,2}$=10.2 Hz, H-3), 4.43 (d, 1H, OH-3), 4.33 (dd, 1H, $J_{6b,5}$=5 Hz, H-6b), 4.28 (dd, 1H, H-2), 4.11–3.93 (m, 4H, H-5, 5′, 6′a, 6′b), 3.68 (dd, 1H, $J_{4,5}$=9.7 Hz, H-4), 2.15, 2.12, 1.98, 1.85 (4s, 12H, 4Ac). ^{13}C NMR (100.57 MHz, $CDCl_3$): δ 169.98, 169.72, 169.58, 169.26 (4O=C, Ac), 167.80, 167.17 (2O=C, Phth), 165.77 (O=C, Bz), 133.98, 133.07, 132.49, 131.39, 131.29, 131.26, 129.41, 129.31, 128.52, 128.26, 127.69, 123.29, 123.07 (arom. C), 101.61 (C-1′), 83.01, 82.82 (C-1, C-4), 75.59, 71.04, 70.79, 70.53, 68.39, 66.55 (C-3, 5, 2′, 3′, 4′, 5′), 63.02, 61.50 (C-6, 6′), 54.47 (C-2), 20.33, 20.23, 20.15, 19.80 ($4CH_3$, Ac). MS (CI): m/z 853 (100%) M+NH_4^+. Anal. calcd for $C_{41}H_{41}NO_{16}S$: C, 58.92; H, 4.94. Found: C, 58.86; H, 5.15.

3.11. *Methyl 6-O-benzoyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside* **15**

A suspension of **14** (400 mg, 0.48 mmol), dry methanol (0.2 ml, 4.9 mmol) and molecular sieves (4 Å, 0.6 g) in dry acetonitrile (15 ml) was stirred for 0.5 h under argon at room temperature. Tris (4-bromophenyl) ammoniumyl hexachloroantimonate (800 mg, 0.98 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, TLC showed the disappearance of **14**. The mixture was neutralized with triethylamine, diluted with dichloromethane, filtered through Celite, and concentrated. The residue was eluted from a column of silica gel with dichloromethane:ethyl acetate (4:1) to give **15** as an amorphous solid (345 mg, 95%). R_f=0.26 (dichloromethane:ethyl acetate=5:1). $[\alpha]_D$ +5.5 (c 1.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.11–7.52 (m, 9H, arom. H), 5.38 (dd, 1H, $J_{4',5'}$=1, $J_{4',3'}$=3.4 Hz, H-4′), 5.30 (dd, 1H, $J_{2',1'}$=8.1, $J_{2',3'}$=10.5 Hz, H-2′), 5.28 (d, 1H, $J_{1,2}$=8.6 Hz, H-1), 4.97 (dd, 1H, H-3′), 4.66 (dd, 1H, $J_{6a,5}$=1.7, $J_{6a,6b}$=11.8 Hz, H-6a), 4.63 (d, 1H, H-1′), 4.49 (ddd, 1H, $J_{3,OH}$=1.8, $J_{3,4}$=8.2, $J_{3,2}$=10.7 Hz, H-3), 4.39 (d, 1H, OH-3), 4.38 (dd, 1H, $J_{6b,5}$=4.6 Hz, H-6b), 4.24 (dd, 1H, H-2), 4.12 (dd, 1H, $J_{6'a,5'}$=4.5, $J_{6'a,6'b}$=11.1 Hz, H-6′a), 4.07 (dd, 1H, $J_{6'b,5'}$=8.0 Hz, H-6′b), 4.01 (ddd, 1H, H-5′), 3.93 (ddd, 1H, $J_{5,4}$=9.7 Hz, H-5), 3.76 (dd, 1H, H-4), 2.18, 2.13, 2.00, 1.89 (4s, 12H, 4Ac). ^{13}C NMR (100.57 MHz, $CDCl_3$): δ 170.15, 169.85, 169.73, 169.45 (4O=C, Ac), 167.95 (2O=C, Phth), 166.02 (O=C, Bz), 133.95, 133.19, 131.57, 129.47, 129.45, 128.41, 123.19 (arom. C), 101.79 (C-1′), 99.03 (C-1), 83.34 (C-4), 71.86 (C-5), 71.13 (C-5′), 70.67 (C-3′), 69.81 (C-3), 68.49 (C-2′), 66.61 (C-4′), 62.98 (C-6), 61.61 (C-6′), 56.69 (OMe), 55.54 (C-2), 20.46, 20.38, 20.28, 19.95 ($4CH_3$, Ac). MS (CI): m/z 775 (80%) M+NH_4^+. Anal. calcd for $C_{36}H_{39}NO_{17}\cdot H_2O$: C, 55.74; H, 5.31; N, 1.80. Found: C, 55.53; H, 5.01; N, 1.75.

3.12. Methyl 6-O-benzoyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 17

To a cooled (0°C), stirred mixture of **15** (225 mg, 0.3 mmol), isopropenyl 2,3,4-tri-*O*-benzyl-α,β-L-fucopyronosyl carbonate **16** (171 mg, 0.33 mmol), activated 4 Å ground molecular sieves (0.6 g), and dry ether (9 ml) was added dropwise TMSOTf (64 μl, 0.33 mmol). Stirring was continued for 40 min at 0°C, then the mixture was neutralized with Et_3N, diluted with CH_2Cl_2, filtered through Celite, and concentrated. The residue was eluted from a column of silica gel with cyclohexane:ethyl acetate (1.5:1) to give **17**. Crystallization in ether gave a white solid (285 mg, 81%). R_f=0.5 (cyclohexane:ethyl acetate=1:1). M.p. 190–191°C (from ether). $[\alpha]_D$ +15 (c 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 8.12–7.07 (m, 24H, arom. H), 5.32 (dd, 1H, $J_{4',5'}$<1, $J_{4',3'}$=3.4 Hz, H-4′), 5.17 (dd, 1H, $J_{2',1'}$=8.3, $J_{2',3'}$=10.3 Hz, H-2′), 5.12 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 3.40 (s, 3H, OMe), 2.14, 2.01, 1.98, 1.89 (4S, 12H, 4Ac), 1.27 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6″). ^{13}C NMR (100.57 MHz, $CDCl_3$): δ 169.91, 169.82, 169.70, 168.84 (4O=C, Ac), 165.78 (O=C, Bz), 138.70, 138.51, 137.97, 134.07, 133.45, 131.69, 129.59, 129.43, 128.62, 128.26, 128.14, 128.10, 128.03, 127.96, 127.94, 127.76, 127.37, 127.23, 127.11, 126.88, 123.50 (arom. C), 99.91 (C-1′), 99.08 (C-1), 97.61 (C-1″), 79.65, 77.06, 76.23, 74.49, 73.21, 72.62, 70.86, 70.66, 68.85, 66.55, 66.51 (11CH, ring C), 74.11, 72.94, 72.29 (3$PhCH_2$), 62.59, 60.15 (C-6, C-6′), 56.68 (OMe), 56.00 (C-2), 20.64, 20.47, 20.41, 20.39 (4CH_3, Ac), 16.67 (C-6″). MS (CI): m/z 1191 (100%) M+NH_4^+. Anal. calcd for $C_{63}H_{67}NO_{21}$: C, 64.44; H, 5.75; N, 1.19. Found: C, 64.32; H, 5.85; N, 1.10.

3.13. Methyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside 18

Hydrazine hydrate (4 ml) was added to a stirred solution of compound **17** (220 mg, 0.19 mmol) in 90% aqueous ethanol (42 ml) and refluxed for 18 h. The solution was concentrated and the residue was taken by dichloromethane (2×30 ml), filtered and evaporated to dryness. The residue was stirred with acetic anhydride (1 ml) in 10 ml of dichloromethane:methanol (1:1) at room temperature for 3 h. The solution was concentrated and coevaporated with ethanol. The residue was flash chromatographed on a column of silica gel (dichloromethane:acetone:methanol=3:6:1) to yield **18** as a white amorphous solid (108 mg, 70%). R_f=0.26 (dichloromethane:acetone:methanol=3:6:1). $[\alpha]_D$ −75 (c 2.2, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 7.59–7.42 (m, 15H, arom. H), 5.52 (d, 1H, $J_{1'',2''}$=3.8 Hz, H-1″), 5.06 (m, 1H, H-5″), 5.04, 4.75 (2d, 2H, *J*=11.3 Hz, $PhCH_2$), 5.01 (m, 2H, $PhCH_2$), 5.00, 4.75 (2d, 2H, *J*=12.3 Hz, $PhCH_2$), 4.68 (d, 1H, $J_{1',2'}$=7.1 Hz, H-1′), 4.50 (d, 1H, $J_{1,2}$=7.5 Hz, H-1), 4.31 (dd, 1H, $J_{3'',4''}$=2.8, $J_{3'',2''}$=10.3 Hz, H-3″), 4.22–4.11 (m, 8H, H-2, H-4, H-5, H-6a, H-6b, H-2″, H-4″, H-6′a), 3.96 (dd, 1H, $J_{4',5'}$<1, $J_{4',3'}$=2.8 Hz, H-4′), 3.81 (dd, 1H, $J_{6'b,5'}$=3.7, $J_{6'b,6'a}$=11.8 Hz, H-6′b), 3.71 (dd, 1H, $J_{2',3'}$=9.7 Hz, H-2′), 3.67 (dd, 1H, H-3′), 3.66 (m, 1H, H-5′), 3.65 (s, 3H, OMe), 3.61 (m, 1H, H-3), 2.17 (s, 3H, NHAc), 1.38 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6″). ^{13}C NMR (100.57 MHz, CD_3OD=49.30 ppm): δ 173.59 (O=C, NHAc), 140.75, 140.53, 139.80 (3C, Ph), 129.87, 129.54, 129.49, 129.45, 128.95, 128.81, 128.72, 128.63-1 (CH, Ph), 104.26 (C-1), 103.99 (C-1′), 98.18 (C-1″), 80.25, 80.23, 77.66, 77.58, 77.39, 75.93, 75.20, 74.88, 73.21, 70.45, 68.06 (11CH, ring C), 76.72, 73.96, 73.88 (3$PhCH_2$), 63.72 (C-6′), 61.64 (C-6), 57.57 (C-2), 57.38 (OMe), 23.75 (CH_3, NHAc), 17.19 (C-6″). Anal. calcd for $C_{42}H_{55}NO_{15}\cdot 2H_2O$: C, 59.35; H, 7.00; N, 1.65. Found: C, 59.58; H, 6.94; N, 1.59.

3.14. Methyl 2-acetamido-2-deoxy-4-O-(3-O-sodium sulfonato-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside ***19****; methyl 2-acetamido-2-deoxy-6-O-sodium sulfonato-4-O-(3-O-sodium sulfonato-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside* ***20****; methyl 2-acetamido-2-deoxy-4-O-(3,6-di-O-sodium sulfonato-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside* ***21*** *and methyl 2-acetamido-2-deoxy-6-O-sodium sulfonato-4-O-(3,6-di-O-sodium sulfonato-β-D-galactopyranosyl)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside* ***22***

A mixture of compound **18** (105 mg, 0.13 mmol) and dibutyltin oxide (32 mg, 0.13 mmol) in dry methanol (1 ml) was heated to reflux for 2.5 h (the solution became clear), then the methanol was distilled at 80°C to give a yellowish syrup, which was evaporated to dryness *in vacuo* (2 h). Sulfur trioxide–trimethylamine complex (36 mg, 0.26 mmol) and dry tetrahydrofuran (1 ml) were introduced, and the mixture was stirred under argon for 40 h at room temperature. THF was evaporated and more sulfur trioxide–trimethylamine complex (36 mg, 0.26 mmol) was added, the mixture was stirred in 2 ml DMF for 60 h. Methanol (0.2 ml) was added, after filtration, the filtrate was concentrated. Flash chromatography of the residue on a column of silica gel (dichloromethane:methanol=2:1, then dichloromethane:methanol:H_2O=4:2:0.1) followed by cation exchange chromatography (Dowex 50X8-200, Na^+ form) using methanol afforded 4 compounds.

19: 31 mg (26%), R_f=0.57 (dichloromethane:methanol=3:1). Compound **19** (14 mg) was transformed into a triethylammonium salt by passage down a column of silica gel, eluted with dichloromethane:methanol:triethylamine (3.5:1:0.05) to give a dichloromethane soluble derivative (16 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.26 (m, 15H, 3Ph), 6.32 (br, 1H, NH), 5.26 (d, 1H, $J_{1'',2''}$=2.5 Hz, H-1″), 4.37 (dd, 1H, $J_{3',4'}$=3.1, $J_{3',2'}$=9.7 Hz, H-3′), 4.31 (dd, 1H, $J_{4',5'}$<1 Hz, H-4′), 3.44 (s, 3H, OMe), 3.15 (q, J=7.4 Hz, CH_2, Et_3N), 1.79 (s, 3H, Ac), 1.37 (t, CH_3, Et_3N), 1.18 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6″). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 170.61 (O=C, NHAc), 138.77, 138.66, 138.19 (3C, Ph), 128.21, 128.09, 127.91, 127.89, 127.85, 127.51, 127.20, 127.13, 127.08 (CH, Ph), 101.89, 101.75 (C-1, C-1′), 97.14 (C-1″), 80.17 (C-3′), 79.19, 77.61, 75.83, 75.59, 75.19, 75.07, 74.54, 70.27, 67.56, 66.61 (10CH, ring C), 74.81, 73.04, 71.75 ($3PhCH_2$), 61.96, 60.52 (C-6, C-6′), 57.11 (OMe), 56.70 (C-2), 46.08 (CH_2, Et_3N), 23.27 (CH_3, NHAc), 16.47 (C-6″), 8.52 (CH_3, Et_3N). Debenzylation of **19** gave a monosulfated trisaccharide identical with compound **9** prepared from **7**.

20: 15 mg (11%), R_f=0.22 (dichloromethane:methanol=3:1). Compound **20** (11 mg) was transformed into a triethylammonium salt by passage down a column of silica gel, eluted with dichloromethane:methanol:triethylamine (3:1:0.05) to give a dichloromethane soluble derivative (15 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.25 (m, 15H, 3Ph), 6.64 (br, 1H, NH), 5.36 (d, 1H, $J_{1'',2''}$=2.5 Hz, H-1″), 3.41 (s, 3H, OMe), 3.14 (q, J=7.4 Hz, CH_2, from Et_3N), 1.79 (s, 3H, Ac), 1.38 (t, CH_3, from Et_3N), 1.16 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6″). Further characterization of this compound was performed after debenzylation into the disulfated trisaccharide **23**.

21: 68 mg (52%), R_f=0.19 (dichloromethane:methanol=3:1). ^{1}H NMR (250 MHz, CD_3OD): δ 7.36–7.08 (m, 15H, 3Ph), 5.08 (d, 1H, $J_{1'',2''}$=3.0 Hz, H-1″), 3.38 (s, 3H, OMe), 1.87 (s, 3H, Ac), 1.08 (d, 3H, $J_{6'',5''}$=6.4 Hz, H-6″). Debenzylation of **21**, followed by ion exchange (Na^+ form), gave a disulfated trisaccharide identical to compound **10** prepared from **8**.

22: 10 mg (7%), R_f=0.31 (dichloromethane:methanol:H_2O=4:2:0.1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.35–7.10 (m, 15H, 3Ph), 5.07 (d, 1H, $J_{1'',2''}$=3.6 Hz, H-1″), 4.25 (dd, 1H, $J_{3',4'}$=3.0, $J_{3',2'}$=9.7 Hz, H-3′), 3.37 (s, 3H, OMe), 1.87 (s, 3H, Ac), 1.07 (d, 3H, $J_{6'',5''}$=6.5 Hz, H-6″). ^{13}C NMR (100.57 MHz, CD_3OD=49.30 ppm): δ 174.00 (O=C, NHAc), 140.77, 140.52, 139.74 (3C, Ph), 130.07, 130.03, 129.82, 129.58, 129.45, 129.41, 129.33, 129.06, 128.91, 128.79, 128.74, 128.37 (CH, Ph), 104.02, 103.45 (C-1,

C-1′), 98.83 (C-1″), 81.25 (C-3′), 80.17, 79.63, 76.16, 76.08, 75.61, 75.31, 74.08, 71.27, 68.08, 68.07 (10CH, ring C), 76.66, 73.75, 73.29 (3PhCH$_2$), 67.58, 67.45 (C-6, C-6′), 57.78 (OMe), 57.48 (C-2), 23.79 (CH$_3$, NHAc), 17.14 (C-6″). The trisulfated trisaccharide **22** was also obtained as the main compound by the following procedure.

A mixture of compound **18** (90 mg, 0.11 mmol) and dibutyltin oxide (27 mg, 0.11 mmol) in dry methanol (1 ml) was heated to reflux for 2.5 h (the solution became clear), then the methanol was distilled at 80°C to give a yellowish syrup, which was evaporated to dryness *in vacuo* (3 h). Sulfur trioxide–pyridine complex (57 mg, 0.36 mmol) and dry DMF (1.5 ml) were introduced and the mixture was stirred under argon at room temperature for 3 days. TLC showed complete disappearance of starting material, a mixture of **19**, **20**, **21**, and **22** was formed. More sulfur trioxide–pyridine complex (57 mg, 0.36 mmol) was added, and stirring was continued for 40 h at room temperature. DMF was evaporated under reduced pressure. Flash chromatography of the residue on a column of silica gel (dichloromethane:methanol:H$_2$O=4:2:0.2) followed by cation exchange chromatography (Dowex 50X8-200, Na$^+$ form) using methanol afforded **22** as an amorphous solid (82 mg, 66%).

3.15. Methyl 2-acetamido-2-deoxy-3-O-α-L-fucopyranosyl-6-O-sodium sulfonato-4-O-(3-O-sodium sulfonato-β-D-galactopyranosyl)-β-D-glucopyranoside 23

A solution of **20** (10 mg, 9.8 μmol) in methanol (3 ml) was reacted over Pd/C (10%, 30 mg) at 15°C under H$_2$ (170 kPa) for 16 h, filtered, and evaporated. The residue was purified on a Sephadex column (G10-120), using water as eluant. After ion exchange with Dowex 50X8-200 (Na$^+$ form) and freeze-drying, compound **23** was obtained as a white amorphous solid (7.3 mg, 97%). R_f=0.37 (ethyl acetate:isopropanol:water=3:3:2). $[\alpha]_D$ −19 (c 0.38, H$_2$O). ^{1}H NMR (400 MHz, D$_2$O): δ 5.10 (d, 1H, $J_{1'',2''}$=3.9 Hz, H-1″), 4.80 (dq, 1H, $J_{5'',4''}$<1, $J_{5'',6''}$=6.5 Hz, H-5″), 4.64 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.50 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.39 (m, 2H, H-6a, H-6b), 4.32 (dd, 1H, $J_{3',4'}$=3.3, $J_{3',2'}$=9.8 Hz, H-3′), 4.27 (dd, 1H, $J_{4',5'}$<1 Hz, H-4′), 3.90 (dd, 1H, $J_{2,3}$=8.8 Hz, H-2), 3.81 (m, 1H, H-5), 3.67 (dd, 1H, $J_{2'',3''}$=10.4 Hz, H-2″), 3.62 (dd, 1H, H-2′), 3.50 (s, 3H, OMe), 2.02 (s, 3H, Ac), 1.17 (d, 3H, H-6″). ^{13}C NMR (100.57 MHz, D$_2$O): δ 175.72 (C=O, NHAc), 103.08 (C-1), 102.50 (C-1′), 99.87 (C-1″), 81.49 (C-3′), 76.02, 75.96, 74.22, 74.20, 73.20, 70.52, 70.45, 69.05, 68.04, 68.03 (10CH, ring C), 67.28 (C-6), 62.82 (C-6′), 58.58 (OMe), 56.79 (C-2), 23.55 (CH$_3$, NHAc), 16.59 (C-6″). MS (EI) calcd for $C_{21}H_{35}NO_{21}S_2Na_2$: 747.64. Found: m/z 724 $(M–Na)^-$, 350.2 $[(M–2Na)/2]^{2-}$, 622 $(M–NaSO_3–Na+H)^-$.

3.16. Methyl 2-acetamido-2-deoxy-3-O-α-L-fucopyranosyl-6-O-sodium sulfonato-4-O-(3,6-di-O-sodium sulfonato-β-D-galactopyranosyl)-β-D-glucopyranoside 24

A solution of **22** (14 mg, 12.5 μmol) in methanol (5 ml) was reacted over Pd/C (10%, 35 mg) at room temperature under H$_2$ (170 kPa) for 20 h, filtered, and evaporated. The residue was purified on a Sephadex column (G10-120), using water as eluant. Further purification was made by anion exchange chromatography on DEAE-Sephacel eluting with a linear gradient of 4 mM to 2 M pyridine:AcOH (1:1). After lyophilization from water, the pyridinium salt was converted to the sodium salt by ion exchange with Dowex 50X8-200 (Na$^+$ form) and freeze-drying, compound **24** was obtained as a white amorphous solid (9.6 mg, 90%). R_f=0.24 (ethyl acetate:isopropanol:water=3:3:2). $[\alpha]_D$ −34 (c 0.35, H$_2$O). ^{1}H NMR (500 MHz, D$_2$O): δ 5.16 (d, 1H, $J_{1'',2''}$=4.0 Hz, H-1″), 4.85 (dq, 1H, $J_{5'',4''}$<1, $J_{5'',6''}$=6.5 Hz, H-5″), 4.70 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.58 (d, 1H, $J_{1,2}$=7.7 Hz, H-1), 4.49 (dd, 1H, $J_{6a,5}$=2.3, $J_{6a,6b}$=11 Hz, H-6a), 4.45 (dd, 1H, $J_{6b,5}$=4.4 Hz, H-6b), 4.43 (dd, 1H, $J_{3',4'}$=3.4, $J_{3',2'}$=9.8 Hz, H-3′), 4.38 (dd, 1H, $J_{4',5'}$<1 Hz, H-4′), 4.24 (dd, 1H, $J_{6'a,5'}$=5.7, $J_{6'a,6'b}$=10 Hz, H-6′a), 4.21 (dd, 1H, $J_{6'b,5'}$=6.5 Hz, H-6′b),

4.08 (m, 1H, H-4), 3.97, 3.87 (2m, 5H, H-2, 3, 3″, 4″, 5′), 3.91 (m, 1H, H-5), 3.72 (dd, 1H, $J_{2'',3''}$=10.4 Hz, H-2″), 3.70 (dd, 1H, H-2′), 3.57 (s, 3H, OMe), 2.10 (s, 3H, Ac), 1.25 (d, 3H, H-6″). ^{13}C NMR (75 MHz, D_2O): δ 175.66 (C=O, NHAc), 103.04 (C-1), 102.67 (C-1′), 99.92 (C-1″), 81.14 (C-3′), 76.24, 74.94, 74.39, 73.31, 73.22, 70.44, 70.36, 69.20, 68.03, 67.63 (10CH, ring C), 68.12 (C-6′), 67.46 (C-6), 58.49 (OMe), 56.76 (C-2), 23.52 (CH_3, NHAc), 16.63 (C-6″). MS (EI) calcd for $C_{21}H_{34}NO_{24}S_3Na_3$: 849.69. Found: m/z 401 $[(M-2Na)/2]^{2-}$, 260 $[(M-3Na)/3]^{3-}$.

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