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Chemoenzymatic synthesis of the 3-sulfated Lewis[a] pentasaccharide

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Abstract—The sulfated pentasaccharide benzyl *O*-(3-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-[(α-L-fucopyranosyl)-(1→4)]-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-β-D-glucopyranoside sodium salt was synthesized using a chemo-enzymatic approach. Lacto-*N*-tetraose, obtained from two disaccharides [4-methoxybenzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phtalimido-β-D-glucopyranoside and benzyl 2,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside], was regioselectively sulfated at the 3 OH position of the terminal galactose using the stannylene procedure. The fucosylation of the sulfated tetrasaccharide was performed using soluble or immobilized fucosyltransferase FucT-III to give the title compound.

Keywords: Chemo-enzymatic synthesis; Fucosyltransferase; Sulfated Lewis[a] pentasaccharide

1. Introduction

Sulfated and sialylated Lewis[a] and Lewis[x] compounds are known to be good ligands for selectins, a family of adhesion molecules that mediates the interaction of circulating leukocytes with endothelial cells, a key step in their recruitment to sites of inflammation.[1] The synthesis of these compounds has been a great challenge for many groups.[2]

Due to their regio- and stereoselectivity, enzymes have proved to be powerful tools as catalysts in carbohydrate chemistry avoiding the protection and deprotection steps.[3] In this respect, the enzymatic syntheses of these Lewis derivatives, or of some mimics, have been an intensive field of research.

The enzymatic synthesis of the sialylated Lewis[x] compounds was made easier by the availability of the three enzymes necessary to their synthesis: (a) β-(1→4) galactosyl transferase, (b) α-(2→3) sialyltransferase and (c) α-(1→3/4) fucosyltransferase.[4]

Regarding the Lewis[a] derivatives, the situation is quite different. β-(1→3) galactosyltransferase, one of the key enzymes for the synthesis of these compounds, has been cloned but is not easily available to be of use in a preparative scale. Thus, its use in synthesis has only been reported once[5] and the chemical synthesis remains the best way to obtain Gal-β-(1→3)-GlcNAc linkage. This has been demonstrated in our group where Lubineau et al. have reported the chemical synthesis of the 3^{IV}-sulfated Lewis[a] pentasaccharide, the most powerful monovalent ligand for human E-selectin known until now.[6,7]

Our interest in the chemo-enzymatic synthesis of oligosaccharides[4b,8] led us to use the fucosyltransferase FucT-III in this synthesis since it was already reported that natural and cloned fucosyltransferases were able to accept sulfated oligosaccharides as substrates.[9,10]

We thus describe here a chemo-enzymatic approach to the title compound based on the enzymatic fucosylation of the sulfated tetrasaccharide lacto-*N*-tetraose **11** using the FucT-III.

2. Results and discussion

The synthetic strategy adopted in this paper was based on the enzymatic fucosylation of tetrasaccharide **11** obtained from the two disaccharides (**4** and **8**).

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Disaccharide **4** was synthesized with 71% yield by glycosylation of the acceptor **2**[11] with the 2,3,4,6-tetra-*O*-acetyl-galactopyranosyl α-trichloroacetimidate **3**[12] in the presence of trimethylsilyl trifluoromethane sulfonate (Scheme 1).[13]

A *p*-methoxybenzyl group at the anomeric position was chosen for its facile introduction and the possibility to remove it before the activation step for tetrasaccharide synthesis. However during its cleavage (→**4**), we obtained a small quantity of the 4,6-diol by-product derived from the hydrolysis of the benzylidene group. We thus chose to remove this protecting group and to acetylate the diol. In this way, the cleavage of the *p*-methoxybenzyl group using ammonium and cerium nitrate in MeCN–water gave disaccharide **6** with 87% yield. Then, **6** was transformed in the usual way into trichloroacetimidate **7** (93%).

Glycosylation of **7** with the known protected derivative lactose **8**,[14] catalyzed by trimethylsilyl trifluoromethane sulfonate according to the Schmidt procedure,[15] provided tetrasaccharide **9** in 50% yield.

Conversion of the phtalimido group into the acetamido group under standard conditions afforded tetrasaccharide **10** in 86% yield after purification by reverse phase chromatography (C-18 column).

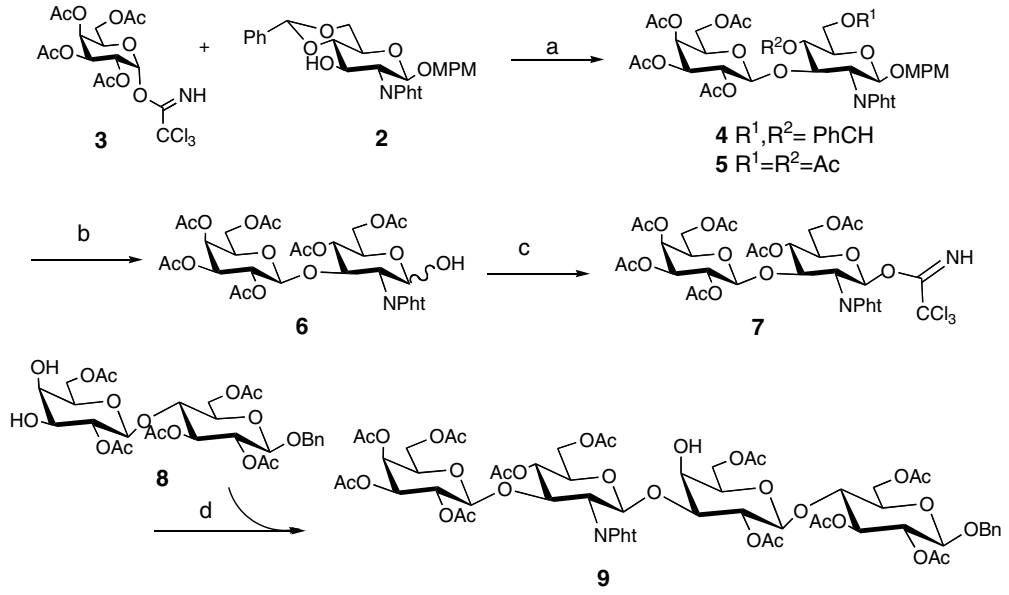
The sulfate group was then regioselectively introduced at position 3 of the galactose moiety by stannylene-directed sulfation.[6,16,17] Compound **10**, having 12 free hydroxyl groups, was thus heated at reflux with 1 equiv of dibutyltin oxide in 1:5 DMF–benzene for 16 h, then after removal of the benzene, 1.1 equiv of sulfur trioxide–trimethylamine complex was added. The 3^{IV}-sulfated tetrasaccharide **11** was thus obtained in 54% yield after purification on DEAE-Sephadex and elution with triethylammonium hydrogen carbonate buffer (Scheme 2).

The fucosylation of **11** was then achieved using a recombinant FucT-III, expressed in CHO cells.[18] Two sites of fucosylation are available on the sulfated tetrasaccharide **11** leading to an hexasaccharide. Indeed, disaccharide Gal-β-(1→3)-GlcNAc and the lactose residue are substrates for the enzyme, but we can expect to stop the reaction at the pentasaccharide stage, as the Gal-β(1→3)-GlcNAc residue is six times better substrate at 1 mM than disaccharide lactose according to de Vries.[19]

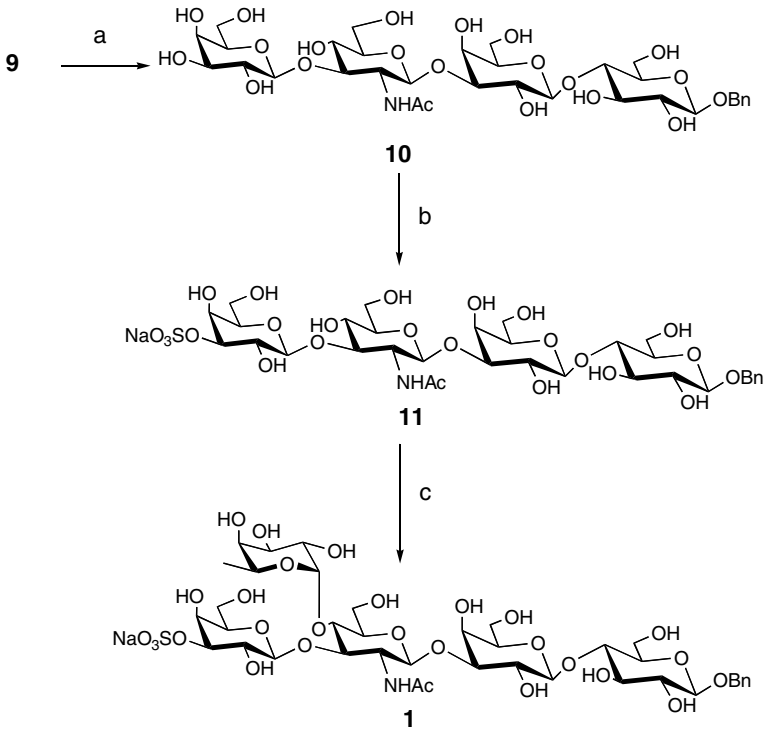
In order to minimize the formation of the hexasaccharide, fucosylation of acceptor **11** was therefore monitored by RP-HPLC at 1 mM using 1 equiv of GDP-fucose with 1 mU of enzyme for 1 μmol of substrate. The enzyme was used as a solution or immobilized on Ni^{2+}–NTA–Agarose through its 6His tag, as described previously in our group.[20]

After 18 h of incubation, we found 34% of starting material **11**, 64% of pentasaccharide **1** and 2% of hexasaccharide for both enzymatic preparations (soluble and immobilized). After 42 h, 80% of pentasaccharide **1**, 10% of tetrasaccharide **11** but also 10% of hexasaccharide could be detected.

For a preparative scale (20 μmol of tetrasaccharide), we chose to perform the incubations at 1 mM with soluble and immobilized enzyme and to stop the reaction after 18 h. After purification on reverse phase chromatography (C-18 column), **1** was isolated in 51% yield in the case of soluble enzyme and 62% yield with immobilized one. In each case, the unreacted tetrasaccharide **11**



Scheme 1. Synthesis of tetrasaccharide. Reagents and conditions: (a) **2** (1 equiv), **3** (1.5 equiv), Me_3SiOTf (0.1 equiv), CH_2Cl_2, rt, 3 h, 71%; (b) CAN, CH_3CN/H_2O, 0 °C, 15 min, 87%; (c) CCl_3CN, NaH, CH_2Cl_2, 0 °C, 30 min, 93%; (d) **7** (1.15 equiv), **8** (1 equiv), Me_3SiOTf, CH_2Cl_2, −10 °C, 5 h, 50%.



Scheme 2. Reagents and conditions: (a) (i) $CH_2CH_2NH_2$, EtOH, reflux, 20 h, (ii) Ac_2O, MeOH, 86%; (b) (i) Bu_2SnO (1.1 equiv), DMF–benzene reflux, 16 h, (ii) $SO_3{\cdot}Me_3N$ (1.1 equiv) 54%; (c) GDP-fucose (0.95 equiv), soluble FucT-III or immobilized FucT-III $MnCl_2$ (20 mM), 100 mM MES buffer pH 6.4, 37 °C, 7 h, 50–60%.

(45%) was recovered. Only traces of hexasaccharide were isolated.

In conclusion, the pentasaccharide 3-sulfated Lewis[a] has been synthesized using a chemo-enzymatic approach. We have shown that enzymatic fucosylation of the intermediate tetrasaccharide could be achieved with a minimum formation of the hexasaccharide by-product. We are currently extending the use of these conditions to the synthesis of Lewis[a] oligosaccharide on a soluble support.

3. Experimental

3.1. General methods

All moisture-sensitive reactions were performed under an argon atmosphere using oven-dried glassware. All solvents were dried over standard drying agents and freshly distilled prior to use. Flash column chromatography was performed on Silica Gel 60 A C.C (6–35 μm). Reactions were monitored by TLC on Silica Gel $60F_{254}$ plates with detection by UV at 254 nm and by charring with 10% H_2SO_4 in EtOH or 2% orcinol in 10% H_2SO_4 in EtOH. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were recorded on a Bruker IF S66. NMR spectra were recorded with Bruker AM-250 or AMX-360 spectrometers; ^{13}C spectra were performed at 60 or 100 MHz. The chemical shifts spectra are given relative to Me_4Si in $CDCl_3$ and to acetone (δ 2.22 and 30.5 ppm) for spectra performed in D_2O. Mass spectra were obtained with a Finnigan MATT 95 apparatus using ESI. Elemental analyses were performed at the CNRS (Gif sur Yvette, France).

FucT-III was obtained within a French network (G3) devoted to the production and studies of recombinant glycosyltransferases. GDP-fucose was synthesized according to published procedures.[21]

3.2. 4-Methoxybenzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-β-benzylidene-2-deoxy-2-phtalimido-β-D-glucopyranoside (4)

A soln of Me_4SiOTf (66 μL in 3.3 mL of CH_2Cl_2) was added dropwise to a cooled suspension (0 °C) of the alcohol **2** (3.34 g, 9.74 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl α-D-galactopyranosyl trichloroacetimidate **3** (4.8 g, 9.74 mmol) and 4 Å powdered molecular sieves in CH_2Cl_2 (20 mL). After 3 h at rt, the reaction mixture was neutralized with Et_3N. The solvent was evaporated and the residue was purified by flash chromatography (2:3–29:21 EtOAc–petroleum ether) to give **4** (3.9 g, 71.2%) as a powder. R_f 0.30 (2:1 toluene–EtOAc); $[\alpha]_D^{29}$ −32 (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): δ 7.75 (m, 4H, arom NPht), 7.60–7.40 (m, 5H, arom), 6.90 (d, 2H, *J* 9 Hz, H-9, H-9′), 6.45 (d, 2H, *J* 9 Hz, H-10, H-10′), 5.60 (s, 1H, H-7), 5.19 (d,

1H, $J_{3,4}$ 3.5 Hz, H-4^{II}), 5.12 (d, $J_{1,2}$ 9 Hz, H-1^{I}), 4.99 (dd, 1H, $J_{1,2}$ 8.5 Hz; $J_{2,3}$ 10.5 Hz, H-2^{II}), 4.74 (d, 1H, J 12.5 Hz, Ph–CH), 4.73 (dd, 1H, $J_{2,3}$ 11 Hz, $J_{3,4}$ 3.5 Hz, H-3^{II}), 4.73 (dd, 1H, $J_{2,3}$ 11 Hz, $J_{3,4}$ 10 Hz, H-3^{I}), 4.5 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1^{II}), 4.42 (d, 1H, $J_{1,2}$ 12.5 Hz, Ph–CH), 4.41 (dd, 1H, $J_{6,6'}$ 11 Hz, $J_{5,6}$ 4.5 Hz, H-6^{I}), 4.31 (dd, 1H, $J_{1,2}$ 9 Hz, $J_{2,3}$ 11 Hz, H-2^{I}), 4.06 (dd, 1H, $J_{6,6'}$ 11 Hz, $J_{5,6}$ 9 Hz, H-6^{II}), 3.67 (s, OCH_3), 3.63 (ddd, 1H, $J_{5,6}$ 4.5, $J_{5,6'}$ 10 Hz, $J_{4,5}$ 9 Hz, H-5^{I}), 3.48 (dd, 1 H, $J_{5,6}$ 6 Hz, $J_{5,6'}$ 8.5 Hz, H-5^{II}), 2.07, 1.91, 1.84, 1.52 (4s, 12H, CH_3CO). ^{13}C ($CDCl_3$, 60 MHz): δ 170.17, 169.95, 168.75 (C=O), 159.02 (C–Ph), 113.43 (2C–Ph), 101.47 (C-7), 100.40 (C-1^{II}), 97.26 (C-1^{I}), 80.74 (C-4^{I}), 60.67 (C-6^{II}), 55.24 (C-2^{I}), 54.90 (CH_3O), 20.57, 20.51, 20.40, 20.01 (CH_3CO); HRMS Calcd for $C_{44}H_{45}NO_{17}$ [M+Na$^+$]: 870.2580. Found 870.2589.

3.3. 4-Methoxybenzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (5)

A soln of **4** (3.5 g, 4.13 mmol) in 70% AcOH (60 mL) was stirred at 50 °C for 4.5 h. The solvent was evaporated and coevaporated with toluene. The residue was dissolved in pyridine (35 mL) and Ac_2O (15 mL) overnight at rt. Coevaporation with toluene followed by flash chromatography (1:1 petroleum ether–EtOAc) gave **5** (3.26 g, 94%) as a powder. R_f 0.26 (2:1 EtOAc–petroleum ether); $[\alpha]_D^{29}$ −27 (*c* 1.4, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.80 (m, 4H, arom NPht), 6.92 (d, 2H, J 9 Hz, H-9, H-9′), 6.52 (d, 2H, J 9 Hz, H-10, H-10′), 5.20 (d, 1H, $J_{3,4}$ 3.5 Hz, H-4^{II}), 5.08 (t, 1H, $J_{3,4} = J_{4,5}$ 10 Hz, H-4^{I}), 5.00 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{I}), 4.93 (dd, 1H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2^{II}), 4.72 (d, 1H, CH_2Ph), 4.67 (dd, 1H, $J_{2,3}$ 9 Hz, H-3^{I}), 4.61 (dd, 1H, H-3^{II}), 4.40 (d, 1H, Ph–CH), 4.13 (d, 1H, H-1^{II}), 3.80–3.72 (m, 2H, H-5, H-5^{II}), 3.70 (s, 3H, OCH_3), 2.14, 2.11, 2.05, 1.86, 1.84 (4s, 12H, CH_3CO). ^{13}C ($CDCl_3$, 60 MHz): δ 170.70, 170.13, 169.98, 169.10, 168.95 (C=O), 159.01 (C–Ph), 113.43 (2C–Ph), 100.23 (C-1^{II}), 96.40 (C-1^{I}), 60.48 (C-6^{II}), 55.33 (C-2^{I}), 54.85 (CH_3O), 20.59, 20.37, 20.23, 20.12 (CH_3CO). Anal. Calcd for $C_{43}H_{45}NO_{17}$: C, 56.92; H, 5.38; N, 1.66; O, 36.04. Found: C, 56.42; H, 5.41; N, 1.48; O, 36.33.

3.4. *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl trichloroacetimidate (7)

Cerium ammonium nitrate (21.8 g, 3.86 mmol, 10 equiv) was added to a vigorously stirred soln of **5** (3.26 g, 3.86 mmol) in 9:1 MeCN–water (25 mL) at 0 °C. After 15 min, the reaction mixture was diluted with CH_2Cl_2 then washed with $KHCO_3$ and water, dried and concentrated to give after flash chromatography **6** (2.43 g, 3.36 mmol, 87%) as a powder.

A mixture of **6** (2.43 g, 3.36 mmol), CCl_3CN (3.4 mL, 3.36 mmol) and NaH (cat) in CH_2Cl_2 (8 mL) was stirred for 30 min at 0 °C. Then, the mixture was filtered through a silica gel column (1:1 petroleum ether–EtOAc containing 0.1% Et_3N) to give **7** (2.7 g, 93%) as a powder. R_f 0.36 (1:1 EtOAc–petroleum ether); $[\alpha]_D^{29}$ +23 (*c* 1.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.6 (s, 1H, C=NH), 7.95–7.73 (m, 4H, Ph), 6.33 (d, 1H, $J_{1,2}$ 9 Hz, H-1^{I}), 5.25 (d, 1H, $J_{3,4}$ 3.5 Hz, H-4^{II}), 5.19 (dd, 1H, $J_{3,4} = J_{4,5}$ 10 Hz, H-4^{I}), 4.98 (dd, 1H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H-2^{II}), 4.85 (dd, 1H, $J_{2,3}$ 10.5 Hz, H-3^{I}), 4.67 (dd, 1H, H-3^{II}), 4.61 (dd, 1H, H-2^{I}), 4.25 (d, 1H, H-1^{II}), 2.13, 2.12, 2.08, 2.07, 1.87 (4s, 12H, CH_3CO). ^{13}C ($CDCl_3$, 60 MHz): δ 170.84, 170.25, 170.08, 169.25, 169.05 (C=O), 160.67 (OC=N), 100.49 (C-1^{II}), 93.60 (C-1^{I}), 90.07 (CCl_3), 61,79, 60.48 (C-6^{I}, C-6^{II}), 54.46 (C-2^{I}), 20.70–20.26 (CH_3CO).

3.5. Benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl-(1→3)-2,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (9)

A mixture of **7** (1.085 g, 1.25 mmol), **8** (0.69 g, 1.08 mmol) and powdered molecular sieves 4 Å in CH_2Cl_2 (5 mL) was stirred during 15 min at rt. Then, the temperature was decreased at −30 °C and a soln of Me_3SiOTf (11 μL, 0.05 mL) in CH_2Cl_2 (430 μL) was added dropwise and the mixture was stirred at −10 °C during 5 h. Et_3N was added and the mixture was concentrated. Flash chromatography (1:1 petroleum ether–EtOAc) gave **9** (715 mg, 50%) as a syrup. R_f 0.27 (1:2 petroleum ether–EtOAc); $[\alpha]_D^{29}$ −3 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8–7.80 (m, 4H, arom Ph), 7.40–7.20 (m, 5H, Ph), 5.30 (d, 1H, $J_{3,4}$ 3.5 Hz, H-4^{IV}), 5.15 (d, 1H, $J_{1,2}$, H-1^{III}), 4.43 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1^{IV}), 3.94 (d, 1H, $J_{3,4}$ 3 Hz, H-4^{IV}), 2.13, 2.10, 2.09, 2.07, 2.06, 1.99, 1.97, 1.87, 1.60 (9s, 18H, CH_3CO). ^{13}C ($CDCl_3$, 60 MHz): δ 171.35–169.14 (C=O), 100.21, 100.12, 98.96, 98.33 (C-1^{I}, C-1^{II}, C-1^{III}, C-1^{IV}), 62.80, 62.15, 61.78, 60.54 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}), 55.16 (C-2^{I}), 20.62–20.00 (CH_3CO). Anal. Calcd for $C_{61}H_{73}NO_{33}$: C, 54.33; H, 5.46; N, 1.04; O, 39.17. Found: C, 54.21; H, 5.46; N, 0.96; O, 39.19.

3.6. Benzyl *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranoside (10)

A mixture of ethylene diamine (2 mL) and **9** (0.67 g, 0.49 mmol) in EtOH (8 mL) was refluxed for 20 h. After cooling, the soln was concentrated and the residue was extracted with water, dried and treated with Ac_2O (1.5 mL) in MeOH (8 mL). After addition of Et_3N (50 μL), the mixture was stirred for 3 h at rt, then concentrated. Purification on a C-18 column (1:0–9:1

water–MeOH) gave **10** (338 mg, 0.42 mmol, 86.5%) as a powder. $[\alpha]_D^{29}$ −3 (*c* 1.3, water); ^{1}H NMR (D_2O, 200 MHz): δ 7.50–7.3 (m, 5H, arom Ph), 4.88 (d, 2H, CH_2Ph), 4.69 (d, 2H, CH_2Ph), 4.66 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{III}), 4.50 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1^{I}), 4.39 (d, 2H, $J_{1,2}$ 8 Hz, H-1^{II}, H-1^{IV}), 4.10 (d, 1H, $J_{3,4}$ 3 Hz, H-4^{II}), 3.29 (t, 1H, H-2^{I}), 1.96 (s, 3H, CH_3CO). ^{13}C (D_2O, 60 MHz): δ 172.80 (C═O), 126.55 (Ph), 101.30 (C-1^{IV}), 100.73, 100.36 (C-1^{II}, C-1^{III}), 98.82 (C-1^{I}), 79.80 (C-3^{II}), 76.17 (C-4^{I}), 58.80 (C-6^{II}, C-6^{IV}), 58.30 (C-6^{III}), 57.90 (C-6^{I}), 52.50 (C-2^{III}), 20.03 (CH_3CO); LRMS Calcd for $C_{33}H_{51}N_{21}$ [M+Na$^+$]: 820.27. Found 820.2.

3.7. Benzyl *O*-(3-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranoside sodium salt (11)

A mixture of **10** (152 mg, 0.19 mmol) and dibutyltinoxide (53 mg, 0.21 mmol) in DMF–benzene (1:5, 30 mL) was boiled for 16 h under reflux with continual removal of water using a Dean–Stark apparatus. Then, benzene was removed and the dibutylstannylene derivative was treated with the Me_3N–sulfur trioxide complex (29 mg, 0.21 mmol) at rt for 28 h. The reaction mixture was then diluted with MeOH and neutralized with $NaHCO_3$. After evaporation, the compound was extracted with water and purified on DEAE Sephadex A-25 column, eluted with a 0.1 M triethylammonium hydrogen carbonate buffer (pH 8). Fractions containing the starting compound were pooled to give **10** (21 mg, 14%). Fractions containing the sulfated tetrasaccharide **11** (99.7 mg, 54%) were pooled and twice lyophilized. $[\alpha]_D^{29}$ −8 (*c* 0.266, water); ^{1}H NMR (D_2O, 250 MHz): δ 7.50–7.30 (m, 5H, arom Ph), 4.95 (d, 2H, CH_2Ph), 4.77 (d, 2H, CH_2Ph), 4.74 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{III}), 4.57 (d, 2H, $J_{1,2}$ 8 Hz, H-1^{I}, H-1^{IV}), 4.44 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{II}), 4.33 (dd, 1H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3.5 Hz, H-3^{IV}), 4.30 (d, 1H, H-4^{IV}), 4.15 (d, 1H, $J_{3,4}$ 3.5 Hz, H-3^{II}), 3.35 (m, 1H, H-2^{I}), 2.04 (s, 3H, CH_3CO). ^{13}C (D_2O, 60 MHz): δ 126.22 (Ph), 100.94 (C-1^{IV}), 100.66, 100.21 (C-1^{II}, C-1^{III}), 98.76 (C-1^{I}), 80.17 (C-3^{II}), 77.86 (C-3^{IV}), 76.2 (C-4^{I}), 58.80 (C-6^{II}, C-6^{IV}), 58.30 (C-6^{III}), 57.80 (C-6^{I}), 52.31 (C-2^{III}), 19.95 (CH_3CO); LRMS (negative mode) Calcd for $C_{33}H_{50}NO_{24}SNa$ [M−Na$^+$] 876.24. Found 876.4.

3.8. Benzyl *O*-(3-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-[(α-L-fucopyranosyl)-(1→4)]-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranoside sodium salt (1)

3.8.1. Method A (using the soluble FucT-III). Tetrasaccharide **11** (20 mg, 20 μmol), GDP-fucose (15 mg, 19 μmol), soluble FucT-III (16 mU), $MnCl_2$ (80 mg) were incubated at 37 °C for 7 h in 100 mM MES buffer (20 mL, pH 6.4). After addition of EtOH, the precipitate was removed and the supernatant was concentrated. Purification on C-18 column (1:0–49:1 water–MeOH) gave **1**, which was converted to the sodium salt by passing through a column of Bio-Rad AG 50W-X8 resin (Na^+ form). The freeze-dried eluate afforded **1** (10.8 mg, 51.5%). Tetrasaccharide **11** (9 mg, 45%) was also recovered.

3.8.2. Method B (using immobilized FucT-III). Tetrasaccharide **11** (20 mg, 20 μmol), GDP-fucose (15 mg, 19 μmol), immobilized FucT-III (15 mU), and $MnCl_2$ (80 mg) were incubated at 37 °C for 7 h in 100 mM MES buffer (20 mL, pH 6.4). After centrifugation, the supernatant was concentrated. Purification on column C-18 (→1:0–98:2 water–MeOH) gave **1**, which was converted to the sodium salt by passing through a column of Bio-Rad AG 50W-X8 resin (Na^+ form). The freeze-dried eluate afforded **1** (13 mg, 62%) 6.5 mg of tetrasaccharide **11** (45%) was recovered.

Compound **1**: $[\alpha]_D^{29}$ −33 (*c* 0.6, water); ^{1}H NMR (D_2O, 400 MHz): δ 7.50–7.35 (m, 5H, arom Ph), 5.02 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1^{V}), 4.93 (d, 2H, CH_2Ph), 4.86 (q, 1H, H-5^{V}), 4.75 (d, 2H, CH_2Ph), 4.70 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{III}), 4.60 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{IV}), 4.55 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{I}), 4.42 (d, 1H, $J_{1,2}$ 8 Hz, H-1^{II}), 4.32–4.25 (m, 2H, H-4^{IV}, H-3^{IV}), 4.15 (d, 1H, $J_{3,4}$ 3.5 Hz, H-4^{II}), 3.30 (t, 1H, H-2^{I}), 2.04 (s, 3H, CH_3CO), 1.17 (d, 3H, CH_3). ^{13}C (D_2O, 100 MHz): δ 128.76 (Ph), 102.51, 102.12 (C-1^{II}, C-1^{III}, C-1^{IV}), 100.56 (C-1^{V}), 97.53 (C-1^{I}), 81.62 (C-3^{II}), 79.76 (C-3^{IV}), 77.90 (C-4^{I}), 75.51 (C-3^{III}), 61.08, 60.52, 59.62, 59.00 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}), 55.42 (C-2^{III}), 21.87 (CH_3CO), 14.89 (C-6^{V}). LRMS (negative mode) calcd for $C_{39}H_{60}NO_{28}SNa$ [M−Na] 1022.3. Found 1022.5.

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