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Synthesis of A Spacer-Armed Disulfated Tetrasaccharide of SB1a, A Carbohydrate Hapten Associated with Human Hepatocellular Carcinoma

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Synthesis of a spacer-armed disulfated tetrasaccharide of SB_{1a}, a carbohydrate hapten associated with human hepatocellular carcinoma

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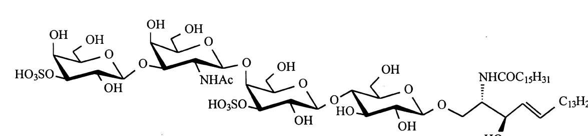
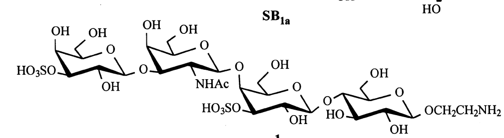
Introduction

Aberrant cell-surface glycosylation is often closely associated with tumor progression and malignancy.[1] In most cases, carbohydrate antigens may be rather specific to a certain type of tumor and are not overexpressed or recognized by the immune system in normal tissues.[2] Therefore, carbohydrate antigens have been greatly mesmerizing scientists in relevant fields because of their potential applications in tumor immunotherapy.[3] SB_{1a}, a glycosphinolipid with a disulfated tetrasaccharide moiety, was first isolated from rat kidney by Tadano and Ishizuka.[4] The normal human liver contains essentially no detectable amount of SB_{1a}. However, studies have shown that a remarkable accumulation of SB_{1a} exists, not only in the cultured human hepatocellular carcinoma (HCC) cell lines, but also in glycolipid fractions extracted from HCC tissues. Therefore, it is suggested that SB_{1a} is one of the most important cancer-associated carbohydrate antigens of HCC.[5,6]



SB_{1a}



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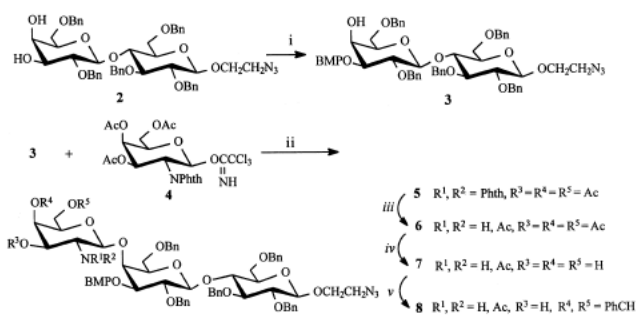
In order to elucidate the functions of SB_{1a} in detail, especially its mechanism involved in the onset, progression, and metastasis of HCC, and hence pursue optimal carbohydrate-based anticancer vaccines for HCC, we have synthesized the disulfated tetrasaccharide moiety of the SB_{1a} determinant, namely compound **1**, in which a 2-aminoethyl group is attached to the reducing terminal as a spacer arm, which could facilitate further formation of immunogenic glycoconjugates by the coupling of the spacer amino group and a carrier protein.

Results and discussion

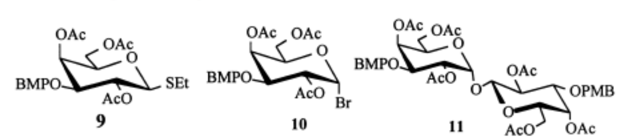
Of the various approaches available for the preparation of oligosaccharides, we adopted the stepwise synthetic strategy to build the target molecule. The reducing terminal D-lactosyl building block **3** of the target molecule was first synthesized in a good yield (89.6%) via the regioselective etherification of the 3′-OH of 2-azidoethyl 2,3,6-tri-*O*-benzyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**2**), which was prepared steadily through several steps from D-lactose.[7] In the synthesis of **3**, the *p*-methoxybenzyl group (PMB) was introduced to the 3-OH position of the galactosyl moiety via a dibutyltin oxide-mediated procedure,[8] followed by addition of *p*-methoxybenzyl chloride and tetrabutylammonium bromide in boiling toluene (Scheme 1).[9]



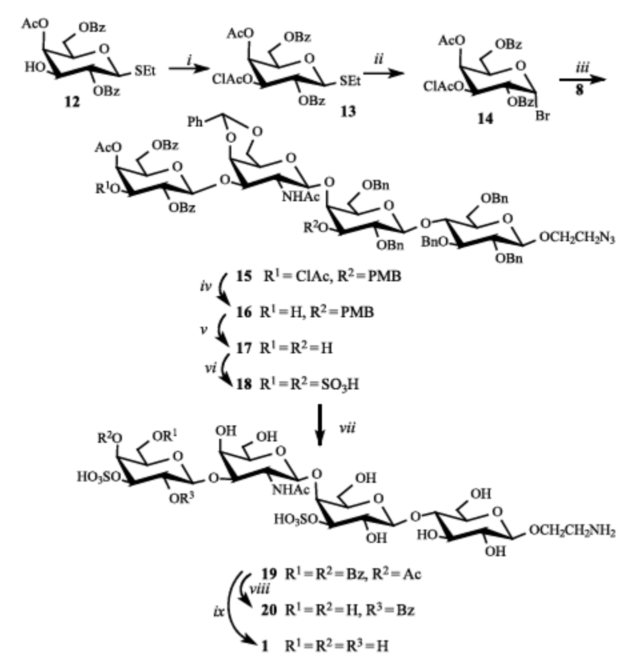
Scheme 1. Synthesis of the trisaccharide acceptor **8**. (i) (a) Bu_2SnO, MeOH, reflux; (b) toluene, $p\text{-}CH_3OC_6H_4CH_2Cl$, Bu_4NBr, 4 Å MS (89.6%); (ii) TMSOTf, toluene, −40 °C (83.2%); (iii) (a) 1,2-diaminoethane, *n*-butanol, 75 °C; (b) Ac_2O–Py (91.2%); (iv) NaOMe, MeOH; (v) $PhCH(OMe)_2$, camphorsulfonic acid, CH_3CN (90%).

Standard glycosylation of **3** and the glycosyl donor 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate (**4**)[10] in toluene at −40 °C gave the desired β-linked trisaccharide **5** (83.2%). Dephthaloylation of compound **5** with 1,2-diaminoethane[11] in *n*-butanol at 75 °C, followed by acetylation, resulted in the formation of **6**. Subsequent O-deacetylation and benzylidenation at the C-4″ and C-6″ hydroxy groups with benzaldehyde dimethyl acetal in acetonitrile under acidic conditions provided the trisaccharide acceptor **8** in excellent yield.

However, for the assembly of the tetrasaccharide backbone, some interesting results occurred. In our initial design, ethyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (**9**) or the corresponding glycosyl bromide **10** was chosen as the glycosyl donor to couple with the trisaccharide acceptor **8**. No reaction occurred when **9** and **8** were mixed and stirred at room temperature in nitromethane or DMF employing Bu_4NBr–$CuBr_2$ as the promoter. Neither did the coupling reaction of **9** and **8** using methyl triflate as the promoter in dichloromethane or diethyl ether. We next investigated the glycosylation of the donor **10** with the trisaccharide acceptor **8**, no desired tetrasaccharide was obtained when silver triflate was chosen to promote the coupling reaction. The main product is the asymmetric (1→1)-linked disaccharide **11**, in which two galactosyl groups were condensed to each other by α and β configurations at the anomeric center, respectively.



After a series of failures in the building of the tetrasaccharide backbone, we selected another type of glycosyl donor containing a benzoyl group at C-2 for coupling with acceptor **8**. Therefore, we chose the glycosyl bromide **14** as the glycosyl donor. Compound **14** was synthesized by the in situ transformation of ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (**13**), which was prepared from chloroacetylation of the known ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**12**).[12] To our surprise, the silver triflate-promoted glycosylation with **8** using donor **14** in dichloromethane at −20 °C gave the desired tetrasaccharide **15** in very high yield (89%) (Scheme 2).



Scheme 2. Synthesis of the target tetrasaccharide. (i) ClAcCl, CH_2Cl_2–pyridine (83.7%); (ii) Br_2, CH_2Cl_2, 0 °C→rt; (iii) AgOTf, 4 Å MS, CH_2Cl_2, −20 °C (89%); (vi) thiourea, 2,6-lutidine, CH_2Cl_2–EtOH, 58 °C (76.4%); (v) CAN, CH_3CN–H_2O, rt, (92.4%); (vi) SO_3·Pyr, Pyr (95%); (vii) H_2, 10% Pd/C, MeOH–H_2O, 1 M HCl; (viii) 0.012 M NaOMe–MeOH, rt; (ix) 0.5 M NaOMe–MeOH, 0 °C.

Deblocking of **15** to the target tetrasaccharide **1** includes several steps as in the following. At first, selective removal of the chloroacetyl group at the 3‴-OH position and the *p*-methoxybenzyl group at 3′-OH position with thiourea and cerium(IV) ammonium nitrate (CAN), respectively, gave **17**. Then, treatment of the diol **17** with sulfur trioxide·pyridine complex in pyridine furnished the disulfated compound **18** in 95% yield. But deprotection of **18** was rather complicated. Catalytic hydrogenolysis, using palladium-on-charcoal in different solvents (AcOH, 2:1 MeOH–AcOH) was sluggish and the yield was low. This problem may be ascribed to the catalyst passiveness due to the interaction with the aminoethyl fragment formed. A similar phenomenon has been observed by Spijker et al.[13] and Stahl et al.[14] To avoid this inhibitory effect, hydrochloric acid was added to the reaction mixture to convert the formed amine to its hydrochloride salt. This greatly increased the hydrogenolysis rate and yield. Then, deacylation of **19** with 0.012 M sodium methoxide in MeOH at room temperature provided product **20** with the 2‴-*O*-benzoyl group retained. Increasing base concentration and prolonging reaction time only led to decomposition of the product. When the O-deacylation was carried out with ammonia in MeOH, no O-deacylation but O-desulfonation was observed. Finally, the saponification of **19** was completed with 0.5 M sodium methoxide in MeOH at 0 °C for 6 h to give the title compound **1** in 90% yield.

Experimental

General methods.—All moisture-sensitive reactions were performed under argon atmosphere, and organic solvents were dried over standard drying agents and freshly distilled prior to use. Optical rotations were measured at 25 °C with an Optical Activity LTD AA-10R polarimeter in a 5-cm, 1-mL cell. Melting points were uncorrected. NMR spectra were recorded at room temperature with a JEOL 300, Bruker AM 400, and INOVA-600 spectrometers. Chemical shifts were expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$, CD_3OD and DMSO-d_6, or DSS in case of D_2O. MALDI-TOFMS analyses were performed with an LDI-1700 mass spectrometer. Column chromatography was performed on silica gel H 60, and fractions were monitored by TLC on silica gel 60 GF_{254} with detection by UV light and/or by charring with 10% H_2SO_4 in EtOH. Solutions were concentrated at or below 40 °C and dried with anhydrous Na_2SO_4.

*2-Azidoethyl 2,6-di-*O*-benzyl-3-*O*-p-methoxybenzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside* (**3**).—To a solution of 2-azidoethyl 2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**2**,[7] 3.53 g, 4.10 mmol) in dry MeOH (200 mL) was added Bu_2SnO (1.77 g, 7.11 mmol), and the mixture was stirred overnight at 60 °C under Ar. After cooling to room temperature, the mixture was concentrated, and the residue was dissolved in dry toluene (200 mL). Bu_4NBr (0.588 g, 1.8 mmol) and powdered 4 Å molecular sieves (5 g) were added, and the mixture was stirred for 1 h at room temperature under Ar. Then, *p*-methoxybenzyl chloride (1.77 mL) was added, and the mixture was stirred for 4 h at 120 °C, at the end of which time TLC (5:1 toluene–EtOAc) showed the disappearance of **2** and the formation of **3**. After cooling to room temperature, MeOH (10 mL) and Et_3N (2.5 mL) were added, and the stirring was continued for 15 min. After filtration through Celite, the filtrate was concentrated. Column chromatography (4:1 petroleum ether–acetone) of the residue afforded **3** as a white needles (3.60 g, 89.6%). mp 86.0–87.0 °C. $[\alpha]_D$ +15.8° (*c* 1.58, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.51–6.91 (m, 29 H, Ar-H), 5.12–3.43 (m, 32 H, sugar H, 5 × $PhCH_2$, $CH_3OC_6H_4CH_2$, $OCH_2CH_2N_3$), 2.64 (bs, 1 H, 4′-OH). ^{13}C NMR: δ 159.1, 113.6 ($CH_3OC_6H_4CH_2$), 138.9, 138.5, 138.4, 138.0, 129.8, 129.2, 128.2, 127.9, 127.8, 127.5, 127.4, 127.3, 127.1 (Ar-C), 103.4, 102.4 (C-1, C-1′), 82.6, 81.6, 79.2, 76.3, 75.1, 75.0, 74.9, 73.3, 72.9, 72.7, 71.7, 68.3, 68.0, 67.9, 65.9 (sugar C, 5 × $PhCH_2$, $CH_3OC_6H_4CH_2$, $OCH_2CH_2N_3$), 55.0 (OCH_3), 50.7 (CH_2N_3). MALDI-TOFMS: *m/z* 1002.8 $[M+Na]^+$. Anal. Calcd for $C_{17}H_{29}N_3O_{17}$: C, 69.72; H, 6.42; N, 3.79. Found: C, 69.40; H, 6.56; N, 3.79.

*2-Azidoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-p-methoxybenzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside* (**5**).—To a solution of **3** (1.00 g) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate (**4**,[10] 1.00 g) in dry toluene (50 mL) were added 4 Å molecular sieves (0.93 g), and the mixture was stirred for 1 h under argon. The mixture was cooled to −40 °C, and a solution of TMSOTf (60 μL) in dry CH_2Cl_2 (1 mL) was added. The mixture was stirred at −40 °C for 3 h and then overnight at room temperature. Et_3N (0.5 mL) was added, and the mixture was diluted with EtOAc (100 mL) and filtered (Celite). The filtrate was washed with water (100 mL), aq $NaHCO_3$ (100 mL) and water (100 mL), dried, concentrated. Column chromatography (4:1:0.1 C_6H_{12}–$CHCl_3$–acetone) afforded **5**, isolated as a colorless foam (1.12 g, 83.2%). $[\alpha]_D$ +8.5° (*c* 1.42, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.87–6.79 (m, 33 H, 5 × $PhCH_2$, Phth, $CH_3OC_6H_4CH_2O$), 6.61 (dd, 1 H, $J_{3'',4''}$ 3.90 Hz, $J_{2',3''}$ 11.70 Hz, H-3″), 5.55 (d, 1 H, H-4″), 5.35 (d, 1 H, $J_{1'',2''}$ 8.40 Hz, H-1″), 3.79 (s, 3 H, CH_3O), 2.20, 2.03, 1.85 (3 s, 3 H each, 3 × OAc). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.3, 169.8, 168.2, 167.4 (Phth, 3 × OAc), 159.4, 113.7 ($CH_3OC_6H_4CH_2O$), 139.1, 138.9, 138.7, 138.4, 138.3, 134.0, 133.7, 132.6, 131.7, 130.1, 130.0, 129.7, 28.3, 128.2, 128.0, 127.9, 127.5, 127.4, 127.3, 123.4, 123.3 (Ar-C), 103.7, 102.1 (C-1, C-1′), 99.8 (C-1″), 82.7, 81.7, 80.6, 80.1, 77.2, 76.4, 76.1, 75.7, 75.3, 75.1, 74.6, 73.2, 73.1, 72.4, 70.4, 68.9, 68.2, 68.1, 67.4, 66.6, 61.2 (sugar C, $CH_3OC_6H_4CH_2$, $5 \times PhCH_2$, $OCH_2CH_2N_3$), 55.3 (CH_3O), 51.5 (C-2″), 50.9 (CH_2N_3), 20.7, 20.5, 20.4 ($3 \times$ OAc). Anal. Calcd for $C_{77}H_{82}N_4O_{21}$: C, 66.04; H, 5.87; N, 4.01. Found: C, 65.90; H, 6.13; N, 3.74.

*2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-p-methoxybenzyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**6**).—A solution of **5** (2.30 g, 1.65 mmol) and 1,2-diaminoethane (32 mL) in *n*-butanol (117 mL) was stirred overnight at 75 °C under argon. After cooling to room temperature, the mixture was co-evaporated with toluene (3×50 mL). A solution of the residue in 1:1 Ac_2O–pyridine (130 mL) was stirred overnight at room temperature, then the mixture was co-evaporated with toluene (3×30 mL). Column chromatography of the residue (2.3:1 petroleum ether–acetone) afforded **6**, isolated as a colorless solid (1.96 g, 91.2%). $[\alpha]_D$ +12.6° (*c* 1.27, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.47–6.90 (m, 29 H, $5 \times PhCH_2$, $CH_3OC_6H_4CH_2$), 3.84 (s, 3 H, CH_3O), 2.18, 2.00, 1.93 (3 s, 3 H each, $3 \times$ OAc), 1.69 (s, 3 H, NHAc). ^{13}C NMR ($CDCl_3$): δ 170.3, 170.2, 169.7 ($4 \times$ Ac), 159.8, 114.3 ($CH_3OC_6H_4CH_2$), 103.5, 103.1, 102.5 (C-1, 1′, 1″), 55.3 (CH_3O), 50.8, 50.7 (C-2″, CH_2N_3), 23.2 (NHAc), 20.8, 20.6, 20.5 ($3 \times$ OAc). Anal. Calcd for $C_{71}H_{82}N_4O_{20}$: C, 65.04; H, 6.26; N, 4.27. Found: C, 65.39; H, 6.38; N, 4.21.

*2-Azidoethyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-p-methoxybenzyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**8**).—To a solution of **6** (1.30 g, 0.992 mmol) in dry MeOH (40 mL) was added NaOMe (108 mg). The mixture was stirred at room temperature for 14 h, then neutralized with cation-exchange resin (H^+ form), and filtered. The filtrate was concentrated affording **7** (1.25 g, quant). To a solution of **7** (1.25 g) and α,α-dimethoxytoluene (354 μL) in dry CH_3CN (15 mL) was added camphorsulfonic acid until pH 4 was attained. The mixture was stirred at room temperature for 24 h, then Et_3N (0.5 mL) was added, and the solvent was removed under reduced pressure. The residue was chromatographed (2:1 petroleum ether–acetone) to give **8** (1.21 g, 90%): ^1H NMR ($CDCl_3$): δ 7.59–6.79 (m, 34 H, Ar-H), 5.59 (s, 1 H, PhCH), 4.93–3.35 (m, 40 H, sugar H, $5 \times PhCH_2$, $CH_3OC_6H_4CH_2$, $OCH_2CH_2N_3$), 1.65 (s, 3 H, NHAc). ^{13}C NMR ($CDCl_3$): δ 173.6 (NHAc), 159.9, 114.1 ($CH_3OC_6H_4CH_2$), 138.5, 138.4, 138.1, 137.9, 137.8, 130.2, 129.6, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.7, 127.5, 127.2, 126.4 (Ar-C), 101.1 (PhCH), 103.5, 102.9, 102.5 (C-1, C-1′, C-1″), 82.4, 81.9, 81.5, 80.6, 76.5, 75.9, 75.5, 75.1, 74.7, 74.4, 73.1, 73.0, 72.7, 68.8, 68.0, 67.1, 61.1 (sugar C, $CH_3OC_6H_4CH_2$, $5 \times PhCH_2$, $OCH_2CH_2N_3$), 55.2, 55.1 (CH_3O, C-2″), 50.8 (CH_2N_3), 22.4 (NHAc). MALDI-TOFMS: 1294.0 $[M+Na]^+$, 1310 $[M+K]^+$. Anal. Calcd for $C_{72}H_{80}N_4O_{17}$: C, 67.92; H, 6.29; N, 4.40. Found: C, 67.76; H, 6.50; N, 4.39.

*Ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-chloroacetyl-1-thio-β-*D*-galactopyranoside* (**13**).—Monochloroacetyl chloride (0.53 mL, 6.67 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a cooled (0 °C) solution of ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**12**,[12] 2.22 g, 4.26 mmol) in 5:1 CH_2Cl_2–pyridine (60 mL). After 2 h the solution was washed with H_2O, dried, filtered and concentrated. After column chromatography (7:1 petroleum ether–EtOAc) **13** was obtained: $[\alpha]_D$ +7.1° (*c* 1.13, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.99–7.40 (m, 10 H, Ar-H), 5.60–5.52 (m, 2 H, H-2, 4), 5.36 (dd, 1 H, $J_{2,3}$ 9.90 Hz, $J_{3,4}$ 3.30 Hz, H-3), 4.72 (d, 1 H, $J_{1,2}$ 9.90 Hz, H-1), 4.54 (dd, 1 H, $J_{5,6a}$ 6.60 Hz, $J_{6a,6b}$11.40 Hz, H-6a), 4.32 (dd, 1 H, $J_{5,6b}$ 6.90 Hz, H-6b), 3.88 (m, 2 H, $ClCH_2CO$), 2.77–2.68 (m, 2 H, CH_3CH_2S), 2.19 (s, 3 H, Ac), 1.23 (t, 3 H, CH_3CH_2S). ^{13}C NMR ($CDCl_3$): δ 170.4, 166.6, 165.9, 165.2 (CO), 133.5 (2 C), 129.8, 129.6, 129.2, 129.0, 128.5 (Ar-C), 84.2 (C-1), 74.4, 73.6, 67.7, 67.3, 61.7 (C-2, 3, 4, 5, 6), 40.3 ($ClCH_2CO$), 24.6 (CH_3CH_2S), 20.7 (Ac), 14.8 (CH_3CH_2S).

*2-Azidoethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-chloroacetyl-β-*D*-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-p-methoxybenzyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**15**).—To a solution of **13** (402 mg, 0.731 mmol) in dry CH_2Cl_2 (16 mL) was added Br_2 (35 μL, 0.731 mmol) at 0 °C. The solution was stirred at 0 °C for 40 min, and the solvent was subsequently evaporated. After co-evaporation twice with benzene, the residue was dissolved in CH_2Cl_2 (16 mL) and added to a mixture of **8** (560 mg, 0.440 mmol), AgOTf (268 mg), 2,6-di-*tert*-butyl-4-methylpyridine (94.6 mg) and crushed 4 Å molecular sieves (950 mg) in CH_2Cl_2 (16 mL), which had been stirred under argon for 40 min, and then cooled to −20 °C. The mixture was allowed to warm to room temperature and to stir overnight. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and filtered through Celite. The filtrate was washed with aq $NaHCO_3$, 10% $Na_2S_2O_3$ and H_2O. The organic layer was dried and concentrated. Column chromatography (2.3:1 petroleum ether–acetone) of the residue afforded **15** (690 mg, 89.0%) as a white solid. $[\alpha]_D$ +41.2° (*c* 0.97, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.08–6.73 (m, 44 H, Ar-H), 5.56–3.23 (m, 50 H, PhCH, $5 \times PhCH_2$, $CH_3OC_6H_4CH_2$, $ClCH_2CO$, $OCH_2CH_2N_3$ and sugar H), 2.24 (s, 3 H, OAc), 1.30 (s, 3 H, NHAc). ^{13}C NMR ($CDCl_3$): δ 171.4, 170.9, 167.0, 166.3, 165.1

(2 × PhCO, $ClCH_2CO$, NHAc, OAc), 159.6, 114.1 ($CH_3OC_6H_4CH_2$), 138.9, 138.7, 138.6, 138.5, 133.9, 130.7, 130.2, 130.0, 129.8, 129.7, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.0, 127.9, 127.6126.7 (Ar-C), 104.0, 102.8, 102.6, 101.1, 99.9 (C-1, C-1′, C-1″, C-1‴, PhCH), 83.2, 82.0, 80.4, 76.6, 75.6, 75.4, 73.0, 72.5, 71.1, 69.6 (sugar C, 5 × $PhCH_2$, $CH_3OC_6H_4CH_2$, $OCH_2CH_2N_3$), 55.6 (CH_3O), 54.8 (C-2″), 51.3 ($OCH_2CH_2N_3$), 40.7 ($ClCH_2CO$), 23.7 (NHAc), 21.2 (OAc). MALDI-TOFMS: *m/z* 1783.5 $[M + Na]^+$. Anal. Calcd for $C_{96}H_{102}ClN_4O_{26}$: C, 65.45; H, 5.80; N, 3.18. Found: C, 65.41; H, 6.01; N, 3.15.

2-Azidoethyl 4-O-acetyl-2,6-di-O-benzoyl-3-O-chloroacetyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl-(1 → 4)-2,6-di-O-benzyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**16**).—To a solution of **15** (575 mg, 0.327 mmol) in 1:1 CH_2Cl_2–abs EtOH (16 mL) was added thiourea (115 mg) and 2,6-lutidine (69 μL). After stirring for 10 h at 58 °C, the mixture was cooled to room temperature and diluted with CH_2Cl_2 and washed with water. The organic layer was dried and concentrated. Purification of the residue by chromatography (2:1:0.1 petroleum ether–EtOAc–EtOH) gave **16** (420 mg, 76.4%). $[\alpha]_D$ +21.1° (*c* 1.14, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.06–6.80 (m, 44 H, Ar-H), 5.45–3.12 (m, 48 H, $PhCH_2$, PhCH, $CH_3OC_6H_4CH_2$, $OCH_2CH_2N_3$, sugar H), 2.14 (s, 3 H, OAc), 1.41 (s, 3 H, NHAc). ^{13}C NMR ($CDCl_3$): δ 171.0 (2 C), 166.4, 166.0 (2 × PhCO, OAc, NHAc), 159.3, 113.8 ($CH_3OC_6H_4CH_2$), 138.7, 138.6, 138.4, 138.3, 133.4, 130.4, 129.9, 129.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.5, 127.3, 126.4, 126.3 (Ar-C), 103.6, 102.5, 101.9, 100.6, 99.0 (C-1, C-1′, C-1″, C-1‴, PhCH), 82.8, 81.7, 81.2, 80.1, 76.2, 76.1, 75.6, 75.3, 75.2, 75.0, 73.4, 73.3, 73.2, 73.1, 72.2, 71.7, 71.2, 70.0, 68.9, 68.5, 68.0, 66.3, 62.7 (sugar C, 5 × $PhCH_2$, PhCH, CH_3O $C_6H_4CH_2$, $OCH_2CH_2N_3$), 55.3 (CH_3O), 54.6 (C-2″), 51.0 ($OCH_2CH_2N_3$), 23.6 (NHAc), 20.9 (OAc). MALDI-TOFMS: *m/z* 1708.9 $[M + Na]^+$, 1724.2 $[M + K]^+$. Anal. Calcd for $C_{94}H_{100}N_4O_{25}$: C, 66.98; H, 5.94; N, 3.32. Found: C, 66.69; H, 6.21; N, 3.11.

2-Azidoethyl 4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl-(1 → 4)-2,6-di-O-benzyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**17**).—To a solution of **16** (700 mg) in CH_3CN (27 mL) and water (3 mL) was added ammonium cerium(IV) nitrate (675 mg), and the mixture was stirred for 1.5 h at room temperature. TLC (1.3:1 petroleum ether–acetone) then showed the disappearance of **16** and the formation of **17**. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with aq $NaHCO_3$ (3 × 50 mL). The organic layer was dried, filtered, and concentrated. Column chromatography (1.3:1 petroleum ether–acetone) of the residue afforded **17**, isolated as a colorless solid (600 mg, 92.4%). $[\alpha]_D$ + 21.3 (*c* 2.07, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.04–6.81 (m, 40 H, Ar-H), 5.48–3.11 (m, 43 H, sugar H, 5 × $PhCH_2$, PhCH, $OCH_2CH_2N_3$), 2.09 (s, 3 H, OAc), 1.30 (s, 3 H, NHAc). ^{13}C NMR ($CDCl_3$): δ 171.4, 171.0, 165.9 (2 × PhCO, OAc, NHAc), 103.5, 102.2, 101.9, 100.4, 99.0 (C-1, C-1′, C-1″, C-1‴, PhCH), 54.7 (C-2″), 50.9 ($OCH_2CH_2N_3$), 23.1 (NHAc), 20.8 (OAc). Anal. Calcd for $C_{86}H_{92}N_4O_{24}$: C, 65.98; H, 5.88; N, 3.58. Found: C, 65.69; H, 6.17; N, 3.33.

2-Azidoethyl 4-O-acetyl-2,6-di-O-benzoyl-3-O-sulfo-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl-(→ 4)-2,6-di-O-benzyl-3-O-sulfo-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**18**).—To a solution of **17** (220 mg, 0.639 mmol) in dry pyridine was added sulfur trioxide·pyridine complex (668 mg, 4.20 mmol), and the mixture was stirred at room temperature for 36 h. MeOH (1 mL) was added, and stirring was continued for 10 min. The mixture was concentrated, and the residue was purified by flash chromatography (10:1 $CHCl_3$–MeOH) to give **18** (250 mg, 94.3%). $[\alpha]_D$ + 43.4° (*c* 1.29, MeOH). ^1H NMR (CD_3OD): δ 8.12–7.13 (m, 40 H, Ar-H), 5.85 (bs, 1 H, H-4‴), 5.46 (t, 1 H, $J_{2''',3'''}$ 8.62 Hz, H-2‴), 5.17 (s, 1 H, PhCH), 5.16 (d, 1 H, $J_{1''',2'''}$ 7.64 Hz, H-1‴), 4.98 (d, 1 H, H-3‴), 4.88–4.18 (m, 18 H), 3.96–3.80 (m, 1 H, $OCH_2CH_2N_3$), 3.80–3.34 (m, 19 H), 3.22 (t, 1 H, $J_{1,2}$ 8.10 Hz, H-2). ^{13}C NMR ($CDCl_3$): δ 174.9, 172.3, 167.8, 167.7 (2 × PhCO, OAc, NHAc), 140.1, 140.0, 139.5, 139.4, 134.7, 1‘34.4, 131.7, 131.3, 131.2, 131.1, 130.3, 129.9, 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 129.0, 128.7, 128.3, 127.9, 127.7 (Ar-C), 104.7 (C-1′, C-1″), 104.2 (C-1‴), 103.9 (C-1), 101.9 (PhCH), 83.9, 82.9, 81.9, 79.6, 78.0, 77.1, 76.7, 76.3, 76.1, 76.0, 75.3, 74.7, 74.2, 74.1, 72.5, 71.6, 71.0, 69.3, 69.2, 67.7, 67.5, 64.7 (sugar C, 5 × $PhCH_2$, $OCH_2CH_2N_3$), 52.2 (C-2″), 49.9 ($OCH_2CH_2N_3$), 23.1 (NHAc), 21.0 (OAc). MALDI-TOFMS: *m/z* 1742.8 $[M - 2H + Na]^-$, 1758.9 $[M - 2H + K]^-$ (negative-ion mode).

2-Aminoethyl 2-O-benzoyl-3-O-sulfo-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-3-O-sulfo-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (**20**).—A solution of **18** (100 mg) in 10:1 MeOH–H_2O (15 mL) and HCl (1 M, 160 μL) was hydrogenolysed at 0.42 MPa in the presence of palladium-on-charcoal (10%, 100 mg) for 60 h. The mixture was then filtered through Celite, and the solid was washed thoroughly with MeOH and water. The filtrate was then concentrated. Flash chromatography (5:4:0.6:1 $CHCl_3$–MeOH–H_2O–HOAc) of the residue afforded 2-aminoethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-sulfo-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-3-*O*-sulfo-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (**19**, 55 mg, 88%) as a white solid. MALDI-TOFMS: *m/z* 1184.2 $[M - 2H + Na]^-$ (negative-ion mode).

To a solution of **19** (50 mg) in dry MeOH was added NaOMe (10 mg). The mixture was stirred overnight at room temperature, then neutralized with HOAc until pH 7 was reached. The solution was then concentrated. Purification of the residue by passage through a Sephadex LH-20 column using water as eluent afforded, after lypophilization, **20** (44 mg, quant) as a white solid. $[\alpha]_D$ +7.4° (*c* 0.5, water). ^{1}H NMR (D_2O): δ 8.10–7.60 (m, 5 H, PhCO), 5.32 (t, 1 H, $J_{2''',3'''}$ 8.42 Hz, H-2‴), 4.96 (d, 1 H, $J_{1''',2'''}$ 7.61 Hz, H-1‴), 4.71 (dd, 1 H, $J_{3''',4'''}$ 3.30 Hz, $J_{2''',3'''}$ 6.47 Hz, H-3‴), 4.57 (d, 1 H, $J_{1'',2''}$ 8.06 Hz, H-1″), 4.53 (d, 1 H, $J_{1',2'}$ 8.06 Hz, H-1′), 4.49 (d, 1 H, $J_{1,2}$ 7.69 Hz, H-1), 4.34–4.31 (m, 3 H, H-3, H-4, H-4‴), 4.24 (d, 1 H, $J_{3'',4''}$ 2.20 Hz, H-4″), 4.13–4.10 (m, 1 H, $OCH_2CH_2NH_2$), 3.96–3.71 (m, 13 H), 3.67–3.57 (m, 9 H), 3.36 (t, 2 H, H-2, H-2′), 3.27 (t, 2 H, $OCH_2CH_2NH_2$), 1.20 (s, 3 H, NHAc). ^{13}C NMR (D_2O): δ 177.2 (NHAc), 170.3 (PhCO), 137.1, 132.9, 131.7, 131.5 (Ar-C), 105.6 (C-1′), 105.4 (C-1), 105.2 (C-1‴), 104.8 (C-1″), 83.2 (C-3″), 82.1 (C-3), 81.2 (C-3′), 80.7 (C-3‴), 77.0 (C-4), 75.5 (C-2), 73.3 (C-2‴), 72.1 (C-2′), 70.6 (C-4″), 68.7 ($OCH_2CH_2NH_2$), 63.7 (C-6, 6‴), 63.6 (C-6′), 63.4 (C-6″), 53.6 (C-2″), 42.3 ($OCH_2CH_2NH_2$), 24.3 (NHAc). MALDI-TOFMS: *m*/*z* 1035.9 $[M-2H+Na]^-$ (negative-ion mode).

*2-Aminoethyl 3-*O*-sulfo-β-*D*-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-*D*-galactopyranosyl-(1→4)-3-*O*-sulfo-β-*D*-galactopyranosyl-(1→4)-β-*D*-glucopyranoside* (**1**).—A solution of **19** (50 mg, 0.0422 mmol) in 0.5 M NaOMe–MeOH (10 mL) was stirred at 0 °C for 6 h. Work-up of the reaction mixture as described for compound **20** afforded **1** (35.2 mg, 90%) as a white solid. $[\alpha]_D$ +10.2° (*c* 0.8, water). ^{1}H NMR (D_2O): δ 4.69 (d, 1 H, $J_{1'',2''}$ 8.52 Hz, H-1″), 4.58 (d, 1 H, $J_{1''',2'''}$ 7.83 Hz, H-1‴), 4.563 (d, 1 H, $J_{1',2'}$ 8.00 Hz, H-1′), 4.560 (d, 1 H, $J_{1,2}$ 7.83 Hz, H-1), 4.43 (dd, 1 H, $J_{3',4'}$ 2.92 Hz, H-4′), 4.40 (dd, 1 H, $J_{2',3'}$ 9.89 Hz, H-3′), 4.33 (dd, 1 H, $J_{3''',4'''}$ 3.35 Hz, H-3‴), 4.30 (bs, 1 H, H-4‴), 4.18 (d, 1 H, $J_{3'',4''}$ 3.10 Hz, H-4″), 4.15–4.12 (m, 1 H, $OCH_2CH_2NH_2$), 4.10–4.08 (m, 1 H), 4.07 (dd, 1 H, $J_{2'',3''}$ 10.88 Hz, H-2″), 4.01–3.98 (m, 1 H, $OCH_2CH_2NH_2$), 3.93 (dd, 1 H, H-3″), 3.87–3.62 (m, 15 H), 4.16–4.12 (m, 1 H, $OCH_2CH_2NH_2$), 4.08 (dd, 1 H, H-2), 4.01–3.99 (m, 1 H, $OCH_2CH_2NH_2$), 3.87–3.62 (m, 15 H, H-2‴, H-3, H-4, H-5, H-5′, H-5″, H-5‴, H-6, H-6′, H-6″, H-6‴), 3.53 (dd, 1 H, H-2′), 3.38 (t, 1 H, $J_{2,3}$ 8.17 Hz, H-2), 3.28 (t, 2 H, $OCH_2CH_2NH_2$), 2.10 (s, 3 H, NHAc). ^{13}C NMR (D_2O): δ 177.8 (NHAc), 107.3 (C-1‴), 105.4 (2 C, C-1, C-1″), 104.8 (C-1′), 83.0 (C-3‴), 82.7 (C-3″), 82.3 (C-3′), 81.2 (C-3), 77.6 (C-2‴), 77.4, 77.3, 77.0, 76.9, 76.6 (C-4′, C-5, C-5′, C-5″, C-5‴), 75.5 (C-2), 75.3 (C-2′), 71.6 (C-4), 70.6 (C-4″), 69.6 (C-4‴), 68.7 ($OCH_2CH_2NH_2$), 63.8, 63.7, 63.5, 62.7 (C-6, C-6′, C-6″, C-6‴), 54.0 (C-2″), 42.3 ($OCH_2CH_2NH_2$), 25.3 (NHAc). MALDI-TOFMS: *m*/*z* 932.6 $[M-2H+Na]^-$ (negative-ion mode).

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