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

Qin Li, Hui Li, Qing Li, Qing-Hua Lou, Bin Su, Meng-Shen Cai, Zhong-Jun Li

**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.\(^5,6\)

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-methyl-β-D-galactopyranosyl(1 → 4)-β-D-glucopyranoside (2), which was prepared steadily through several steps from D-lactose. 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, followed by addition of p-methoxybenzyl chloride and tetrabutylammonium bromide in boiling toluene (Scheme 1).

Standard glycosylation of 3 and the glycosyl donor 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate (4) in toluene at −40 °C gave the desired β-linked trisaccharide 5 (83.2%). Dephaloylation of compound 5 with 1,2-diaminoethane in n-butanol at 75 °C, followed by acetylation, resulted in the formation of 6. Subsequent O-deacetylation and benzylideneation 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 Bu₄NBr–CuBr₂ 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 the 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-benzyl-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-benzyl-1-thio-β-D-galactopyranoside (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).
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 aminooethyl 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, decylation 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 AA10R 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 Me₄Si for solutions in CDCl₃, CD₃OD and DMSO-d₆, or DSS in case of D₂O. 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 plates (5:1 petroleum ether–acetone) of the residue afforded 3 as a white needles (3.60 g, 89.6%). mp 86.0–87.0 °C. [α]D +15.8° (c 1.58, CHCl₃).

1H NMR (CDCl₃): δ 7.51–6.91 (m, 29 H, Ar-H), 5.12–3.43 (m, 32 H, sugar H, 5 × PhCH₂, CH₂OC₆H₄CH₂, OCH₂CH₂N₃), 2.64 (bs, 1 H, 4′-OH). 13C NMR: δ 159.1, 113.6 (CH₂OC₆H₄CH₂), 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 × PhCH₂, CH₂OC₆H₄CH₂, OCH₂CH₂N₃), 55.0 (OCH₃), 50.7 (CH₂N₃). MALDI-TOFMS: m/z 1002.8 [M + Na]⁺. Anal. Calcéd for C₁₇H₂₉N₃O₁₇: 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-phthalimido-β-D-galactopyranosyl trichloroacetimidate (4) 100 g in dry toluene (50 mL) were added 4 Å molecular sieves (0.93 g), and the mixture was stirred for 1 h under Ar. The mixture was cooled to −40 °C, and a solution of TMSOTf (60 μL) in dry CH₂Cl₂ (1 mL) was added. The mixture was stirred at −40 °C for 3 h and then overnight at room temperature. Et₃N (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 NaHCO₃ (100 mL) and water (100 mL), dried, concentrated. Column chromatography (4:1:0.1 C₆H₁₂(CHCl₃-acetone) afforded 5, isolated as a colorless foam. [α]D +8.5° (c 1.42, CHCl₃).

1H NMR (CDCl₃): δ 7.87–6.79 (m, 33 H, 5 × PhCH₂), Phth., CH₂OC₆H₄CH₂OH), 6.61 (dd, 1 H, J₃,₂ = 3.90 Hz, J₂,₃ = 11.70 Hz, H-3″), 5.55 (d, 1 H, H-4″), 5.35 (d, 1 H, J₃,₂ = 8.40 Hz, H-1″), 3.79 (s, 3 H, CH₂O), 2.20, 2.03, 1.85 (3 s, 3 H each, 3 × OAc). 13C NMR (CDCl₃): δ 170.4, 170.3, 169.8, 168.2, 167.4 (Phth., 3 × OAc), 159.4, 113.7 (CH₂OC₆H₄CH₂O), 139.1, 138.9, 138.7, 138.4, 138.3, 134.0, 133.7, 132.6,
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 CH2Cl2 (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) (2.22 g, 4.26 mmol) in 5:1 CH2Cl2–pyridine (60 mL). After 2 h the solution was washed with H2O, dried, filtered, and concentrated. After column chromatography (7:1 petroleum ether–EtOAc) 13 was obtained: [x]D + 7.1° (c 1.13, CHCl3). 1H NMR (CDCl3): δ 7.99–7.40 (m, 10 H, Ar-H), 5.60–5.52 (m, 2 H, H-2, 4), 5.36 (d, 1 H, J1,2 9.90 Hz, H-1), 4.54 (dd, 1 H, J6a,6b 6.60 Hz, H-6a), 3.88 (m, 2 H, CH2CH2S), 2.77–2.68 (m, 2 H, CH2CH2S), 2.19 (s, 3 H, Ac), 1.23 (t, 3 H, CH3CH2S).

13C NMR (CDCl3): δ 170.4, 166.6, 165.9, 165.2 (CO), 133.5 (C-2), 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 (CICH2CO), 24.6 (CH3CH2S), 20.7 (Ac), 14.8 (CH3CH2S).

2-Azidoethyl 4-O-acetyl-2,6-di-O-benzoyl-3-O-chloroacetyl-β-D-galactopyranosyl-(1→3)-2-azidomethyl-4-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 CH2Cl2 (16 mL) was added Br2 (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 CH2Cl2 (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 CH2Cl2 (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 CH2Cl2 (100 mL) and filtered through Celite. The filtrate was washed with aq NaHCO3, 10% Na2S2O3 and H2O. 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. [x]D + 41.2° (c 0.97, CHCl3). 1H NMR (CDCl3): δ 8.08–6.73 (m, 44 H, Ar-H), 5.56–3.23 (m, 50 H, PhCH3, 5 × PhCH2, CH2OCH2CH2, CICH3CO, OCH2CH2N3, and sugar H), 2.24 (s, 3 H, OAc), 1.30 (s, 3 H, NHAc). 13C NMR (CDCl3): δ 171.4, 170.9, 167.0, 166.3, 165.1
(2 × PhCO, ClCH₂CO, NHAc, OAc), 159.6, 114.1 (CH₂OC₂H₅CH₂), 138.9, 138.7, 138.6, 138.5, 133.9, 130.7, 130.2, 129.8, 129.7, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.0, 127.9, 127.6, 126.7 (Ar-C), 104.0, 102.8, 102.6, 101.1, 99.9 (C-1, C-1', C-1''), 11°C (PhCH), 83.2, 82.0, 80.4, 76.6, 75.6, 75.4, 73.0, 72.5, 71.1, 69.6 (sugar C, 5 × PhCH₂, CH₂O₂C₂H₅CH₂, OCH₂CH₂N₃, sugar H), 2.14 (s, 3 H, OAc), 1.30 (OAc). MALDI-TOFMS: m/z 1783.5 [M + Na]+. Anal. Caled for C₉₀H₇₁NO₄₃C₈₂: C, 65.45; H, 5.80; N, 3.18. Found: C, 65.41; H, 6.01; N, 3.15.

2-Azidoethy 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-(1→4)-2,6-di-O-benzyl-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 CHCl₃–MeOH) to give 18 (250 mg, 94.3%). [α]D + 43.4° (c 1.29, MeOH). 1H NMR (CD₂OD): δ 8.12–7.13 (m, 40 H, Ar-H), 5.85 (bs, 1 H, H-4°), 5.46 (t, 1 H, J₂⁻,₂° 8.62 Hz, H-2°), 5.17 (s, 1 H, PhCH), 5.16 (d, 1 H, J₁⁻,₁° 7.64 Hz, H-1°), 4.98 (d, 1 H, H-3°), 4.88–4.18 (m, 18 H), 3.90–3.34 (m, 19 H), 3.22 (t, 1 H, J₂⁻,₂° 8.10 Hz, H-2°). 13C NMR (CD₂OD): δ 174.9, 172.3, 167.8, 167.7 (2 × PhCO, OAc, NHAc), 140.1, 140.0, 139.5, 139.4, 134.7, 134.4, 131.7, 131.3, 131.2, 131.1, 130.3, 129.9, 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 128.7, 128.3, 127.9, 127.7, 127.7 (Ar-C), 104.7 (C-1', C-1''), 104.2 (C-1°), 103.9 (C-1), 101.9 (PhCH₃), 83.9, 82.9, 81.9, 79.6, 78.0, 77.1, 76.7, 76.3, 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 × PhCH₂, OCH₂CH₂N₃), 52.2 (C-2°), 49.9 (OCH₂CH₂N₃), 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–H₂O (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 CHCl₃–MeOH–H₂O–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 lyophilization, 20 (44 mg, quant) as a white solid. 

\[ [\alpha]_D + 7.4^\circ \text{ (c 0.5, water)} \]. 1H NMR (D_2O): δ 8.10–7.60 (m, 5 H, PhCO), 5.32 (t, 1 H, J = 8.42 Hz, H-2"), 4.96 (d, 1 H, J = 7.61 Hz, H-1"), 4.71 (dd, 1 H, J = 3.30 Hz, H-3"), 6.47 Hz, H-3"), 4.57 (d, 1 H, J= 8.06 Hz, H-1"), 4.53 (d, 1 H, J = 8.06 Hz, H-1"), 4.49 (d, 1 H, J = 7.69 Hz, H-1), 4.34–4.31 (m, 3 H, H-3, H-4, H-4") on 1H 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", 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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References


