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Synthesis of a spacer-armed disulfated tetrasaccharide of SB\textsubscript{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.\textsuperscript{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.\textsuperscript{2} Therefore, carbohydrate antigens have been greatly mesmerizing scientists in relevant fields because of their potential applications in tumor immunotherapy.\textsuperscript{3} SB\textsubscript{1a}, a glycosphinolipid with a disulfated tetrasaccharide moiety, was first isolated from rat kidney by Tadano and Ishizuka.\textsuperscript{4} The normal human liver contains essentially no detectable amount of SB\textsubscript{1a}. However, studies have shown that a remarkable accumulation of SB\textsubscript{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\textsubscript{1a} is one of the most important cancer-associated carbohydrate antigens of HCC.\textsuperscript{5,6}

In order to elucidate the functions of SB\textsubscript{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\textsubscript{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 \(\text{D-lactosyl building block} 3\) of the target molecule was first synthesized in a good yield (89.6%) via the regioselective etherification of the 3'-OH of 2-azidoothyl 2,3,6-tri-O-benzyl-2,6-di-O-benzyl-\(\beta\)-\(\text{D-galactopyranosyl-(1 \rightarrow 4)}\)-\(\beta\)-\(\text{D-glucopyranoside}\) (2), which was prepared steadily through several steps from D-lactose. In the synthesis of 3, the \(\rho\)-methoxybenzyl group (PMB) was introduced to the 3-OH position of the galactosyl moiety via a dibutyltin oxide-mediated procedure, followed by addition of \(\rho\)-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-\(\beta\)-\(\text{D-galactopyranosyl trichloroacetimidate}\) (4)\(^{10}\) in toluene at \(-40\) °C gave the desired \(\beta\)-linked trisaccharide 5 (83.2%). Dephaloylation 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-\(\rho\)-methoxybenzyl-1-thio-\(\beta\)-\(\text{D-galactopyranoside}\) (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 \(\text{Bu}_4\text{NB}_{\text{r}} - \text{CuBr}_2\) 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 \(\rightarrow 1\))-linked disaccharide 11, in which two galactosyl groups were condensed to each other by \(\alpha\) and \(\beta\) 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-\(\beta\)-\(\text{D-galactopyranoside}\) (13), which was prepared from chloroacetylation of the known ethyl 4-O-acetyl-2,6-di-O-benzyl-1-thio-\(\beta\)-\(\text{D-galactopyranoside}\) (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 aminomethyl 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, deaclylation 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-deaclylation was carried out with ammonia in MeOH, no O-deaclylation 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. Column chromatography was performed on silica gel H 60, and fractions were monitored by TLC on silica gel plates. 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. [α]D 0 +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 × CH2OH, 7 × 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 × CH2OH, 2.20, 2.03, 1.85 (3 s, 3 H each, 3 × CH3). 13C NMR: δ 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, 10.00 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 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 C6H12O6-acetone) afforded 5, isolated as a colorless foam (1.12 g, 83.2%). [α]D 0 +8.5° (c 1.42, CHCl3). 1H NMR (CDCl3): δ 8.77–6.79 (m, 33 H, 5 × PhCH2, Phth, CH2OC6H4CH2O), 6.61 (dd, 1 H, J1, 3.90 Hz, aryl), 11.70 Hz, 3°), 5.55 (d, 1 H, H-4°), 5.35 (d, 1 H, J1, 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,
cation-exchange resin (H) and added NaOMe (108 mg). The mixture was stirred at room temperature, then the residue afforded 7 (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 Ac₂O/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–acetonitrile) afforded 6, isolated as a colorless solid (1.96 g, 91.2%). [x]D +12.6° (c 1.27, CHCl₃). ¹H NMR (CDCl₃): δ 7.47–6.90 (m, 29 H, 5 × PhCH₂, CH₂OC₆H₄CH₂, 5 × PhCH₂, OCH₂CH₂N₃), 1.65 (s, 3 H, CH₃O); 13C NMR (CDCl₃): 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, CH₂OC₆H₄CH₂, 5 × PhCH₂, OCH₂CH₂N₃), 55.3 (CH₂O), 51.5 (C-2'), 50.9 (CH₂N₃), 20.7, 20.5, 20.4 (3 × OAc). Anal. Caled for C₇₇H₈₂N₄O₂₁: 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 room temperature, then the residue afforded 7 (2.30 g, 1.65 mmol) s, 3 H, CH₂O), 2.18, 2.00, 1.93 (3 s, 3 H each, 3 × OAc), 1.69 (s, 3 H, NHAc). ¹³C NMR (CDCl₃): δ 171.4, 170.9, 167.0, 166.3, 165.1.

11C NMR (CDCl₃): δ 171.4, 170.9, 167.0, 166.3, 165.1.
(2 × PhCO, ClCH₂CO, NHAc, OAc), 159.6, 114.1
(CH₂OCH₂H₂CH₂CN), 138.9, 138.7, 138.6, 138.5, 133.9,
130.7, 130.2, 129.0, 129.8, 129.7, 129.0, 128.7, 128.7,
128.6, 128.4, 128.0, 127.9, 127.4, 127.2, 126.7, 126.5
(Ar-C), 104.0, 102.8, 102.6, 101.1, 99.9 (C-1, C-1', C-1″, C-1‴, PhCH₃), 83.2, 82.0, 80.4, 76.6, 75.6, 75.4, 73.0, 72.5,
71.1, 69.6 (sugar C, 5 × PhCH₃, CH₂OC₆H₄CH₂, OCH₂CH₂N₃), 55.6 (CH₂O), 54.8 (C-2") (3H, OCH₂CH₂N₃), 40.7 (CICH₂CO), 23.7 (NHAc), 21.2
Caled for C₉₀H₉₀O₄N₄Na₃: 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-sulfol-
β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benz-
ylidene-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 15 (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 by flash chromatography (10:1 CHCl₃–MeOH) to give 18 (250 mg, 94.3%). [δ]D +
43.4° (c 1.29, MeOH).

1H NMR (CD₃OD): δ 8.12–7.13
(m, 40 H, Ar-H), 5.85 (bs, 1 H, H-4′), 5.46
(t, 1 H, J₂-₂, 6.82 Hz, H-2″), 5.17 (s, 1 H, PhCH₃), 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), 3.96–3.80 (m, 1 H, OCH₂CH₂N₃),
3.80–3.34 (m, 19 H), 3.22 (t, 1 H, J₁, 8.10 Hz, H-2′).

13C NMR (CD₃OD): 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, 127.9 (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 × PhCH₃, OCH₂CH₂N₃), 55.3 (CHO), 54.6
(C-2′), 51.0 (OCH₂CH₂N₃), 23.6 (NHAc), 20.9 (OAc).

Anal. Caled for C₉₀H₈₉O₄N₄Na₃Cl: 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-3-O-sulfol-
β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benz-
ylidene-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 CDCl₃ (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 disappear-
ance of 16 and the formation of 17. The mixture was
diluted with CH₂Cl₂ (100 mL) and washed withaq
NaHCO₃ (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%). [δ]D +
21.3 (c 2.07, CHCl₃). 1H NMR (CDCl₃): δ 8.04–6.81
(m, 40 H, Ar-H), 5.48–3.11 (m, 43 H, sugar H, 5 ×
PhCH₂, PhCH₃, OCH₂CH₂N₃), 2.09 (s, 3 H, OAc), 1.30
(s, 3 H, NHAc). 13C NMR (CDCl₃): 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‴, PhCH₃), 54.7 (C-2″),
50.9 (OCH₂CH₂N₃), 23.1 (NHAc), 20.8 (OAc). Anal.
Caled for C₉₀H₈₉O₄N₄Cl: 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-sulfol-
β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benz-
ylidene-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 (19). —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
(5:4:0.61 CHCl₃–MeOH–H₂O–HOAc) of the residue
formed 2-aminoethyl 4-O-acetyl-2,6-di-O-benzoyl-3-O-
sulfol-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-
β-D-galactopyranosyl-(1→4)-3-O-sulfol-β-D-galactopy-
ranosyl-(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
(5:4:0.61 CHCl₃–MeOH–H₂O–HOAc) of the residue
formed 2-aminoethyl 4-O-acetyl-2,6-di-O-benzoyl-3-O-
sulfol-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-
β-D-galactopyranosyl-(1→4)-3-O-sulfol-β-D-galactopy-
ranosyl-(1→4)-β-D-glucopyranoside (20).
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. $[\alpha]_D^{25} + 7.4^\circ$ (c 0.5, water). $^1$H NMR (D$_2$O): $\delta$ 8.10–7.60 (m, 5 H, PhCO), 5.32 (t, 1 H, J$_{2,3}$ = 8.42 Hz, H-2$^\alpha$), 4.96 (d, 1 H, J$_{2,3}$ = 7.61 Hz, H-1$^\alpha$), 4.71 (dd, 1 H, J$_{3,4}$ = 3.30 Hz, J$_{2,3}$ = 6.47 Hz, H-3$^\alpha$), 4.57 (d, 1 H, J$_{2,3}$ = 8.06 Hz, H-1$^\alpha$), 4.53 (d, 1 H, J$_{3,4}$ = 8.06 Hz, H-1$^\alpha$), 4.49 (d, 1 H, J$_{3,4} = 7.69$ Hz, H-1), 4.34 – 4.31 (m, 3 H, H-3, H-4, H-4$^\alpha$), 4.24 (d, 1 H, J$_{3,4}$ = 2.20 Hz, H-4$^\alpha$), 4.13 – 4.10 (m, 1 H, OCH$_2$CH$_2$NH$_2$), 3.96 – 3.71 (m, 13 H), 3.67 – 3.57 (m, 9 H), 3.36 (t, 2 H, H-2, H-2$^\alpha$), 3.27 (t, 2 H, OCH$_2$CH$_2$NH$_2$), 1.20 (s, 3 H, NHAc). $^{13}$C NMR (D$_2$O): $\delta$ 177.2 (NHAc), 170.3 (PhCO), 137.1, 132.9, 131.7, 131.5 (Ar-C), 105.6 (C-1$^\alpha$), 105.4 (C-1), 105.2 (C-1$^\alpha$), 104.8 (C-1$^\alpha$), 83.2 (C-3$^\alpha$), 82.1 (C-3), 81.2 (C-3$^\alpha$), 80.7 (C-3$^\alpha$), 77.0 (C-4), 75.5 (C-2), 73.3 (C-2$^\alpha$), 72.1 (C-2$^\alpha$), 70.6 (C-4$^\alpha$), 68.7 (OCH$_2$CH$_2$NH$_2$), 63.7 (C-6, 6$^\alpha$), 63.6 (C-6$^\alpha$), 63.4 (C-6), 53.6 (C-2$^\alpha$), 42.3 (OCH$_2$CH$_2$NH$_2$), 24.3 (NHAc). MALDI-TOFMS: m/z 392.6 [M – 2H + Na$^+$] (negative-ion mode).

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**References**