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CARBOHYDRATE RESEARCH

Note

Synthesis of isomeric sulfated disaccharides. Methyl *O*-(2-acetamido-2-deoxy-3-*O*-, 4-*O*-, and 6-*O*-sulfo-β-D-glucopyranosyl sodium salt)-(1 → 3)-β-D-galactopyranoside ☆

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At present, there is an immense interest in the biosynthesis of sulfated glycoproteins. Moreover, since the reported successful employment of 3-*O*-sulfated Le^x and Le^a moieties as ligands for selectins, sulfated glycoproteins are attracting special attention [2–4]. In glycoproteins the sulfate group has been frequently found at the C-3 or C-6 positions of galactose and the C-6 position of GlcNAc, though, in some cases, sulfate at the C-4 position of galactose has also been reported [5–9]. Two high endothelial venules (HEV)-associated selectin ligands, Glycam I and CD_{34}, are *O*-linked glycoproteins containing sulfate, sialic acid, and fucose, and it has been determined that these elements are essential ingredients for binding with L-selectin. Information regarding the biosynthesis of complex carbohydrates containing fucose, sialic acid and sulfate functional groups will prove very important, as this knowledge can reveal the carbohydrate arrangements likely to serve as ligands for L-selectin. For example, our specificity studies on α1,3/4-L-fucosyltransferases with sulfated saccharides led us to synthesize the 3-*O*-sulfated Le^x and Le^a structures [10,11]. Reports on the synthesis of these noted compounds by other laboratories [12,13] have recently appeared in the literature. We have also recently investigated sialyltransferases utilizing a series of acceptors containing sulfate and sialic acid [14].

☆ Synthetic Studies in Carbohydrates, Part 95. For Part 94, see Ref. [1].

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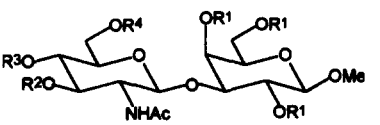
Spiro et al. [15] have studied a 3-*O*-sulfotransferase from thyroid microsomes which catalyzes the following reaction:

$$\beta\text{-Gal-}(1 \rightarrow 4)\text{-GlcNAc} \xrightarrow{3\text{-}O\text{-sulfotransferase}} \beta\text{-GalSO}_3\text{-3-}(1 \rightarrow 4)\text{-GlcNAc}$$

The structure of the reaction product was established with synthetic β-GalSO$_3$-3Gal-(1 → 4)-GlcNAc. According to Slomiany et al. [6], rat gastric 6-*O*-sulfotransferase can use β-GlcNAc-(1 → 3)-β-Gal-(1 → 3)-α-GalNAc-(1 → O) linked to mucin glycoprotein as an acceptor as illustrated in the equation:

$$\beta\text{-GlcNAc-}(1 \rightarrow 3)\text{-}\beta\text{-Gal-}(1 \rightarrow 3)\text{-}\alpha\text{-GalNAc-}(1 \rightarrow \text{O})\text{Ser/Thr protein} \xrightarrow{6\text{-}O\text{-sulfotransferase}} \beta\text{-GlcNAcSO}_3\text{-6-}(1 \rightarrow 3)\text{-}\beta\text{-Gal-}(1 \rightarrow 3)\text{-}\alpha\text{-GalNAc-OSer/Thr protein.}$$

In our continuing effort to provide pertinent structures as reference standards for biological investigations, we undertook the synthesis of the sulfated analogues of β-GlcNAc-(1 → 3)-β-Gal-(1 → OCH$_3$). To confirm the structure of the enzymatic product obtained by the action of sulfotransferase on the aforementioned disaccharide moiety, we synthesized the three isomeric sulfated disaccharides expected upon incorporation of a sulfate group into the GlcNAc residue. In addition, we also report the synthesis of 4-nitrophenyl *O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl sodium salt)-(1 → 6)-α-D-mannopyranoside which represents a part of the structure of gp120 glycoprotein [16]. This compound is required for use in immunological studies associated with our research program.



1 R^1 = Bn; R^2 = H; R^3, R^4 = PhCH

2 $R^1 = R^3 = R^4$ = H; $R^2 = SO_3Na$

3 $R^1 = R^2 = R^4$ = Bn; R^3 = H

4 $R^1 = R^2 = R^4$ = H; $R^3 = SO_3Na$

5 R^1 = Bn; $R^2 = R^3$ = H; R^4 = MeOPh(Ph)$_2$C

6 $R^1 = R^2 = R^3$ = Bn; R^4 = H

7 $R^1 = R^2 = R^3$ = H; $R^4 = SO_3Na$

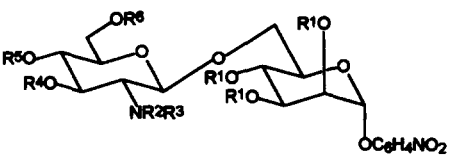
For the synthesis of sulfated disaccharides **2** and **4**, the previously reported compounds, methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside)-(1 → 3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside [17] (**1**) and methyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-β-D-galacto-

Table 1
Proposed ^{13}C NMR signal assignments [a]

Residue	Compound	C-1	C-2	C-3	C-4	C-5	C-6	NAc/OMe
3-*O*-SO_3Na-GlcNAc-ß-	**2**	101.0	53.4	80.3	68.6	74.2	59.9	21.3
(1 → 3)-Gal-ß-OMe		102.8	67.6	81.3	67.3	73.7	59.4	56.2
4-*O*-SO_3Na-GlcNAc-	**4**	101.5	54.5	71.1	75.8	73.7	59.9	21.2
ß-(1 → 3)-Gal-ß-OMe		102.8	68.7	81.3	67.3	73.1	59.3	56.2
6-*O*-SO_3Na-GlcNAc-	**7**	101.6	54.6	72.5	68.6	73.9	66.2	21.2
ß-(1 → 3)-Gal-ß-OMe		102.8	68.6	81.4	67.4	72.5	60.1	56.1
6-*O*-SO_3Na-GlcNAc	**10**	100.5	54.4	72.6	68.6	72.8	65.8	21.2
ß-(1 → 3)-Man-α-O-$C_6H_4NO_2$ (4)		96.9	68.7	69.3	68.0	72.6	66.1	

[a] For solutions in D_2O, Me_4Si as the external standard.

pyranoside [17] (**3**), were utilized as key intermediates. Reaction of **1** and **3** with five molar equivalents of SO_3–pyridine complex in *N*,*N*-dimethylformamide followed by hydrogenolysis of the benzyl groups in the presence of 10% palladium-on-carbon furnished compounds **2** and **4**, respectively, after passage through a cation-exchange resin column. The structures of **2** and **4** were confirmed by ^{13}C NMR and FAB mass spectroscopy (see Experimental section; Table 1). For the synthesis of compound **7**, methyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside [18] was reacted with 4′-methoxytrityl chloride in pyridine to afford compound **5** in 46% yield. Benzylation of compound **5** with sodium hydride–benzyl bromide was followed by hydrolysis of the methoxytrityl group with aq 80% acetic acid to give the key intermediate **6** in 64% yield. Sulfation of **6** as described for the preparation of **2** from **1** provided **7** as an amorphous solid in 48% yield. The ^{13}C NMR and FAB mass spectra of **7** were consistent with the structure expected (see Table 1; Experimental section).



8 $R^1 = Ac$; $R^2R^3 = Phth$; $R^4 = R^5 = R^6 = H$
9 $R^1 = R^4 = Ac$; R^2, $R^3 = H$, Ac; $R^5 = R^6 = H$
10 $R^1 = R^4 = R^5 = H$; R^2, $R^3 = H$, Ac; $R^6 = SO_3Na$

4-Nitrophenyl *O*-(2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside [19] (**8**) was converted into the key intermediate **9** in 4 steps: (*1*) reaction with acetone-2,2-dimethoxypropane-4-toluenesulfonic acid (4′,6′-*O*-isopropylidene formation), (*2*) NH_2-$NH_2 \cdot H_2O$/EtOH (phthalimido group removal), (*3*) pyridine–acetic anhydride (*N*- and *O*-acetylation), and (*4*) aq 70% acetic acid (hydrolysis of isopropylidene group). The selective sulfation of **9** at ice-cold temperature in a manner analogous to that described for the preparation of **2** from **1** followed by

O-deacetylation with methanolic sodium methoxide afforded compound **10**. The ^{13}C NMR spectrum of **10** was also in agreement with the proposed structure (see Table 1).

In the ^{13}C NMR spectra of compounds **2**, **4**, and **7**, the resonance for C-1 of the GlcNAc (δ 101.0–101.6) and Gal (δ 102.8) residues were all in the region characteristic of β-glycosidic linkages. The resonance for C-3 (δ 81.3–81.4) of the galactose residues suffered a downfield shift, confirming that O-3 was the site of glycosylation. Similarly, the resonances for C-3 (δ 80.3) in **2**, C-4 (δ 75.8) in **4**, and C-6 (δ 66.2) in **7** of the 2-acetamido-2-deoxy-β-D-glucopyranose residue suffered a downfield shift [20], evidencing that O-3, O-4, and O-6 were the sites of sulfation in these respective compounds. In the spectrum of **10** an analogous downfield shift was observed for the C-6 resonance (δ 66.1) of the α-D-Man-$C_6H_4NO_2$ (ρ) residue and the C-6 resonance (δ 65.8) of the β-D-GlcNAc residue, confirming these positions as the site of substitution.

1. Experimental

General methods.—These were exactly the same as described earlier [18].

Methyl O-*(2-acetamido-2-deoxy-3-*O-*sulfo-*β-D-*glucopyranosyl sodium salt)-(1 → 3)-*β-D-*galactopyranoside* (**2**).—To a stirred solution of **1** (1.0 g, 1.3 mmol) in dry *N,N*-dimethylformamide (20 mL) at ~ 0°C was added a solution of SO_3–pyridine complex (0.6 g) in dry *N,N*-dimethylformamide (10 mL). Stirring was continued for 2 h at the same temperature, then excess reagent was destroyed by the addition of MeOH (10 mL), followed by the addition of pyridine (10 mL). Solvents were evaporated under reduced pressure and the resultant residue fractionated on a small silica gel column using 10–15% MeOH in $CHCl_3$ as the eluent. The fractions corresponding to the product were combined, concentrated, and the residue used in the next step. The residue was taken up in 95% EtOH (50 mL) and treated with 10% Pd–C (1.5 g) under H_2 at ~ 345 kPa for 3 days. The suspension was filtered through a bed of Celite and the solids were thoroughly washed with aq 20% EtOH. The filtrate and washings were combined and evaporated. The residue was purified on a column of silica gel with 5:4:1 $CHCl_3$–MeOH–H_2O as the eluent. The fractions corresponding to **2** were combined and concentrated and the residue was dissolved in water and passed through Amberlite IR-120 (Na^+) cation-exchange resin. Lyophilization of the fraction corresponding to **2** gave an amorphous solid (0.35 g, 53% on the basis of **1**); $[\alpha]_D$ −10° (*c* 1.2, H_2O); ^1H NMR (D_2O): δ 4.90 (d, *J* 8.5 Hz, 1 H, H-1′), 4.38 (d, *J* 7.9 Hz, 1 H, H-1), 3.64 (s, 3 H, OMe), 2.08 (s, 3 H, NAc); *m/z*: 498.5 $[M - H]^-$; 476.4 $[M - Na]^-$; For ^{13}C NMR data see Table 1. Anal. Calcd. for $C_{15}H_{26}O_{14}NNaS$: C, 36.07; H, 5.25; N, 2.80. Found: C, 35.83; H, 5.31; N, 2.72.

Methyl O-*(2-acetamido-2-deoxy-4-*O-*sulfo-*β-D-*glucopyranosyl sodium salt)-(1 → 3)-*β-D-*galactopyranoside* (**4**).—Compound **3** (1.0 g, 1.18 mmol) was treated with SO_3–pyridine complex (0.4 g) in a manner analogous to that described for **1** (to give **2**). After the aforedescribed processing, the crude reaction product was applied to a column of silica gel and eluted with 5–10% MeOH in $CHCl_3$. The combined product fractions were concentrated under reduced pressure and the residue used in the next step. A solution of this intermediate in aq 95% EtOH (50 mL) was hydrogenolyzed in the

presence of 10% Pd–C (1 g) as described for the preparation of **2**. After purification over a silica gel column with 5:4:1 $CHCl_3$–MeOH–H_2O as the eluent, **4** (0.32 g; 54.3%, on the basis of **3**) was obtained as its sodium salt by passing through Amberlite IR 120 (Na^+) cation-exchange resin; $[\alpha]_D$ +6° (*c* 1.1, H_2O); ^1H NMR (D_2O): δ4.79 (d, *J* 7.9 Hz, 1 H, H-1′), 4.36 (d, *J* 8.0 Hz, 1 H, H-1), 3.62 (s, 3 H, OMe), 2.09 (s, 3 H, NAc); *m/z*: 498.5 $[M - H]^-$; 476.5 $[M - Na]^-$; For ^{13}C NMR data see Table 1. Anal. Calcd. for $C_{15}H_{26}O_{14}NNaS$: C, 36.07; H, 5.25; N, 2.80. Found: C, 36.13; H, 5.36; N, 2.94.

Methyl O-*(2-acetamido-2-deoxy-6-*O-*(4-methoxytrityl)-β-*D-*glucopyranosyl)-(1 → 3)-2,4,6-tri-*O-*benzyl-β-*D-*galactopyranoside* (**5**).—A mixture of methyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (1.0 g) and chloro-(4-methoxy)triphenylmethane (1.4 g) and *N,N*-dimethyl-4-aminopyridine (0.1 g) in pyridine (20 mL) was stirred for 2 days at room temperature. The mixture was poured into ice–water and extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed (5% MeOH in $CHCl_3$ containing 0.1% v/v Et_3N to give **5** in 46% yield; $[\alpha]_D$ −20° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.31–7.11 (m, 29 H, arom), 6.81–6.72 (m, 2 H, arom), 3.66 (s, 3 H, OMe), 3.49 (s, 3 H, OMe), 1.46 (s, 3 H, NAc). Anal. Calcd. for $C_{56}H_{61}NO_{12}$: C, 71.53; H, 6.54; N, 1.49. Found: C, 71.81; H, 6.29; N, 1.32.

Methyl O-*(2-acetamido-3,4-di-*O-*benzyl-2-deoxy-β-*D-*glucopyranosyl)-(1 → 3)-2,4,6-tri-*O-*benzyl-β-*D-*galactopyranoside* (**6**).—To a stirred solution of **5** (1.2 g) in *N,N*-dimethylformamide (30 mL) was added NaH (0.18 g), and stirring was continued for 0.5 h at room temperature. The mixture was then cooled (~ 0°C; bath), and benzyl bromide (0.34 ml) was added, and the stirring was continued for 2 h at room temperature. After careful addition of MeOH to decompose excess NaH, the mixture was evaporated under reduced pressure and the residue applied to a column of silica gel. On elution with $CHCl_3$, evaporation of the fractions corresponding to the product gave a solid which was utilized for the next step without any further characterization. A suspension of this crude product in aq 80% AcOH (50 ml) was heated for 0.5 h at ~ 60°C. After conventional processing, the crude product was chromatographed (4:1 hexane–EtOAc) to give **6** (0.7 g, 64%); $[\alpha]_D$ −7° (*c* 1.1, $CHCl_3$); ^1H NMR: δ 7.43–7.13 (m, 25 H, arom), 3.49 (s, 3 H, OMe), 1.47 (s, 3 H, NAc). Anal. Calcd. for $C_{50}H_{57}NO_{11}$: C, 70.81; H, 6.78; N, 1.65. Found: C, 70.89; H, 6.63; N, 1.69.

Methyl O-*(2-acetamido-2-deoxy-6-*O-*sulfo-β-*D-*glucopyranosyl sodium salt)-(1 → 3)-β-*D-*galactopyranosyl* (**7**).—A solution of **6** (0.6 g) in *N,N*-dimethylformamide (20 mL) was treated with SO_3–pyridine complex (0.2 g) for 1 h at room temperature. Processing as described for the preparation of **2** from **1** provided **7** (0.17 g, 48%); $[\alpha]_D$ +7° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 4.79 (d, *J* 8.4 Hz, 1 H, H-1′), 4.38 (d, *J* 8.1 Hz, 1 H, H-1), 3.64 (s, 3 H, OMe), 2.11 (s, 3 H, NAc); *m/z*: 476.4 $[M - Na]^-$, 498.5 $[M - H]^-$, 522.1 $(M + Na]^+$; For ^{13}C NMR data, see Table 1. Anal. Calcd. for $C_{15}H_{26}O_{14}NNaS$: C, 36.07; H, 5.25; H, 2.80. Found: C, 35.91; H, 5.32; N, 2.92.

4-Nitrophenyl O-*(2-acetamido-3-*O-*acetyl-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)-2,3,4-tri-*O-*acetyl-α-*D-*mannopyranoside* (**9**).—To a stirred solution of 4-nitrophenyl *O*-(2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-

mannopyranoside (3.0 g) in dry acetone (25 mL) were added 4-toluenesulfonic acid monohydrate (0.3 g) and 2,2-dimethoxypropane (25 mL) and stirring was continued for 4 h at room temperature. The acid was neutralized with Et_3N and the solution concentrated to dryness. The residue was dissolved in $CHCl_3$ and washed with water, dried over Na_2SO_4, and concentrated to give an amorphous solid. The solid (3.0 g) so obtained was heated under reflux for 2 h in a mixture of EtOH (80 mL) and hydrazine hydrate (20 mL). The liquids were then evaporated and co-evaporated several times with toluene to give a residue which was dissolved in pyridine (60 mL) and Ac_2O (30 mL), and stirred overnight at room temperature. Solvent and reagents were removed under reduced pressure and the residue was dissolved in $CHCl_3$ and washed with water, aq $NaHCO_3$, water, and evaporated under reduced pressure. To hydrolyze the acetal ring, the material was suspended in aq 70% AcOH (70 mL) and heated at 75°C for 3 h. After evaporation of the solvent the residue was purified on a silica gel column using a solvent gradient consisting of 4–6% MeOH in $CHCl_3$ to afford compound **9** (1.34 g; 51% yield); $[\alpha]_D$ +164° (*c* 1.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.21 (d, *J* 9.1 Hz, 2 H, arom), 7.20 (d, *J* 9.0 Hz, 2 H, arom), 6.20 (d, *J* 9.1 Hz, 1 H, NH), 5.68 (s, 1 H, H-1), 2.23–2.00 (cluster of s, 15 H, 4 × OAc and NAc); ^{13}C NMR ($CDCl_3$): δ 101.99 (C-1′), 95.25 (C-1), 68.33 (C-6), 61.89 (C-6′), 53.44 (C-2′), 24.80, 22.84, 20.80, 20.70, and 20.55 (4 × OAc and NAc). Anal. Calcd. for $C_{28}H_{36}O_{17}N_2$: C, 50.00; H, 5.39; N, 4.16. Found: C, 50.11; H, 5.51; N, 4.10.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-6-*O*-sulfo-β-*D*-glucopyranosyl sodium salt)-(1 → 6)-α-*D*-mannopyranoside* (**10**).—To an ice-cold solution of **9** (0.64 g) in *N*,*N*-dimethylformamide (20 mL) was added SO_3–pyridine complex. After stirring at the same temperature for 3 h, excess reagent was decomposed by addition of MeOH. The solvent was evaporated under reduced pressure and the residue passed through a small silica gel column using a solvent gradient consisting of 10–15% MeOH in $CHCl_3$. The fractions corresponding to the product were concentrated and *O*-deacetylated with methanolic NaOMe afford compound **10** after passing through Amberlite IR-120P (Na^+) resin; 0.18 g (81%); $[\alpha]_D$ +30° (*c* 0.7, H_2O); ^{1}H NMR (D_2O): 8.28 (d, *J* 9.3 Hz, 2 H, arom), 7.30 (d, *J* 9.3 Hz, 2 H, arom); 5.75 (d, *J* 1.7 Hz, 1 H, H-1), 4.56 (d, *J* 8.3 Hz, 1 H, H-1′), 2.06 (s, 3 H, NAc); For ^{13}C NMR data see Table 1. Anal. Calcd. for $C_{20}H_{27}O_{16}N_2NaS$: C, 39.61; H, 4.49; N, 4.62. Found: C, 39.11; H, 4.82; N, 3.46.

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