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Note

Synthesis of methyl 2-*O*- and 3-*O*-sulfo-D-glucopyranosiduronic acids

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In recent years, D-glucuronic acid 2- and 3-sulfate residues have been found to be constituents of the saccharide chains of several glycoconjugates. Thus, D-glucuronic acid 2-sulfate residues have been found in heparin[1], heparan sulfate[2], and chondroitin sulfate D[3]. D-Glucuronic acid 3-sulfate residues have been found in chondroitin sulfate K[3] and two acidic glycolipids in the human nervous system[4,5]. It has also been reported that the cell surface glycoproteins of halobacteria contain D-glucuronic acid 2- or 3-sulfate residues[6]. The *O*-sulfated positions on the D-glucuronic acid residues in these glycoconjugates were determined primarily by chromatography[1,2], periodate oxidation[3,6], or methylation analysis[4,5]. We describe herein the synthesis and n.m.r. spectra of methyl 2-*O*- (**1**) and 3-*O*-sulfo-α-D-glucopyranosiduronic acid (**2**), and their respective β-anomers (**3** and **4**), to be used in the structural analysis of glycoconjugates containing D-glucuronic acid sulfate residues.

In general, the methyl glycoside monosulfates were prepared by sulfation of a suitably substituted methyl glycoside with a sulfur trioxide–pyridine complex and subsequent removal of the protecting groups, and then conversion into the methyl D-glucopyranosiduronic acid sulfates by catalytic oxidation in the presence of platinum-on-carbon. The products were purified by chromatography on DEAE-cellulose and isolated as diammonium salts.

Preparation of **1** was performed by catalytic oxidation[7] of methyl 2-*O*-sulfo-α-D-glucopyranoside (**7**), synthesized from methyl 4,6-*O*-benzylidene-3-*O*-nitro-α-D-glucopyranoside (**8**) essentially by the same route as that reported by Guisley and Ruoff[8].

Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside[9] (**9**) was the starting material for synthesis of **2**. Sulfation of **9** gave the 3-sulfate ester **10**, from which acid hydrolysis and deacetylation removed the protecting benzylidene and acetyl groups to give methyl 3-*O*-sulfo-α-D-glucopyranoside (**11**). Subsequently, the sulfate ester was converted into **2** by catalytic oxidation[7].

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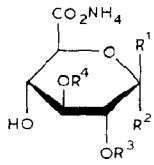
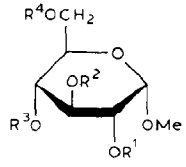
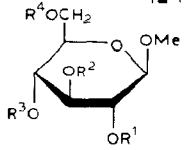




1 $R^1 = R^4 = H; R^2 = OMe; R^3 = SO_3NH_4$
2 $R^1 = R^3 = H; R^2 = OMe; R^4 = SO_3NH_4$
3 $R^1 = OMe; R^2 = R^4 = H; R^3 = SO_3NH_4$
4 $R^1 = OMe; R^2 = R^3 = H; R^4 = SO_3NH_4$
5 $R^1 = R^3 = R^4 = H; R^2 = OMe$
6 $R^1 = OMe, R^2 = R^3 = R^4 = H$

7 $R^1 = SO_3NH_4; R^2, R^3 = R^4 = H$
8 $R^1 = H; R^2 = NO_2; R^3, R^4 = CHC_6H_5$
9 $R^1 = Ac; R^2 = H; R^3, R^4 = CHC_6H_5$
10 $R^1 = Ac; R^2 = SO_3H; R^3, R^4 = CHC_6H_5$
11 $R^1, R^3, R^4, H; R^2 = SO_3NH_4$
12 $R^1 = SO_3NH_4; R^2 = NO_2; R^3, R^4 = H$



13 $R^1 = R^2 = NO_2; R^3, R^4 = CHC_6H_5$
14 $R^1 = NO_2; R^2 = H; R^3, R^4 = CHC_6H_5$
15 $R^1 = R^3 = R^4 = H; R^2 = SO_3NH_4$
16 $R^1 = H; R^2 = Ac; R^3, R^4 = CHC_6H_5$
17 $R^1 = NO_2; R^2 = Ac; R^3, R^4 = CHC_6H_5$
18 $R^1 = SO_3NH_4; R^2 = R^3 = R^4 = H$

Methyl 4,6-*O*-benzylidene-2,3-di-*O*-nitro-β-D-glucopyranoside (**13**), the starting material for the synthesis of the β anomers **3** and **4**, was prepared by the same method as that used for the preparation of the α anomer[9]. Unexpectedly, the 2,3-dinitrate **13** gave regioselectively methyl 4,6-*O*-benzylidene-2-*O*-nitro-β-D-glucopyranoside (**14**) by denitration with sodium nitrite in ethanol, although it had been shown that selective denitration of methyl 4,6-*O*-benzylidene- and 4,6-*O*-alkylidene-2,3-di-*O*-nitro-α-D-hexopyranosides with sodium nitrite in ethanol yields 3-nitrates[10,11]. The highly selective denitrations at O-3 or O-2 in these cases are difficult to rationalize. The 2-nitrate **14** was subjected in sequence to sulfation, acetal hydrolysis, and denitration by hydrogenolysis to give methyl 3-*O*-sulfo-β-D-glucopyranoside (**15**), which was then oxidized into **4**.

Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**16**) was the key compound for the synthesis of **3**. Compound **16** had already been prepaed by a different method[12,13], but this was not suitable for a large-scale preparation. Thus, we prepared **16** by hydrogenolysis of easily accessible methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-nitro-β-D-glucopyranoside (**17**), and compound **3** was obtained *via* methyl 2-*O*-sulfo-β-D-glucopyranoside (**18**) by the same sequence of reactions as that described for the synthesis of the 3-sulfate **2**.

The structures of the four methyl glucosiduronic acid sulfates **1**–**4** were confirmed by ^{13}C- and ^{1}H-n.m.r. spectroscopy. Sulfation of a hydroxyl group

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (δ) FOR AMMONIUM SALTS OF SULFATED AND NONSULFATED METHYL D-GLUCOPYRANOSIDURONIC ACIDS[a] (**1–6**)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*
5	101.87	73.67	75.46	74.22	74.49	178.72	57.74
1	100.01	79.93	73.36	74.18	74.53	178.71	57.96
2	101.77	72.46	84.54	73.16	74.71	178.47	57.83
6	105.70	75.47	78.31	74.33	78.21	178.04	59.65
3	104.10	82.76	76.95	74.29	78.31	178.05	59.83
4	105.38	74.34	86.48	73.05	78.50	177.68	59.83

[a]For solutions in D_2O, relative to the chemical shift of 2-methyl-2-propanol (δ 32.31).

TABLE II

^{13}C-N.M.R. SHIFT DIFFERENCES[a] IN WATER BETWEEN AMMONIUM SALTS OF SULFATED AND NONSULFATED METHYL D-GLUCOPYRANOSIDURONIC ACIDS (**1–6**)

Compounds	*Shift differences(p.p.m.)*						
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*
1–5	+1.86	−6.26	+2.10	+0.04	−0.04	+0.01	−0.22
3–6	+1.60	−7.29	+1.36	+0.04	−0.10	−0.01	−0.18
2–5	+0.10	+1.21	−9.08	+1.06	−0.22	+0.25	−0.09
4–6	+0.32	+1.13	−8.17	+1.28	−0.29	+0.36	+0.18

[a]Positive values are upfield with respect to the signal of tetramethylsilane.

TABLE III

^{1}H-N.M.R. CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (Hz) FOR AMMONIUM SALTS OF SULFATED AND NONSULFATED METHYL D-GLUCOPYRANOSIDURONIC ACIDS (**1–6**)[a]

Compound	*H-1* ($J_{1,2}$)	*H-2* ($J_{2,3}$)	*H-3* ($J_{3,4}$)	*H-4* ($J_{4,5}$)	*H-5*	*OCH_3*
5	4.81d (3.8)	3.59dd (9.4)	3.66t (9.4)	3.48dd (10.0)	3.88d	3.41s
1	5.11d (3.7)	4.19dd (9.5)	3.77t (9.5)	3.58dd (10.1)	3.91d	3.43s
2	4.85d (3.7)	3.77dd (9.5)	4.43t (9.5)	3.66dd (10.1)	3.95d	3.43s
6	4.37d (7.9)	3.29dd (9.1)	3.46–3.52m		3.71d	3.55s
3	4.52d (8.1)	4.04dd (9.4)	3.68t (9.4)	3.57dd (10.0)	3.73d	3.54s
4	4.46d (8.1)	3.48dd (9.1)	4.30t (9.1)	3.68dd (10.1)	3.78d	3.56s

[a]For solutions in D_2O, relative to the chemical shift of 2-methyl-2-propanol (δ 1.23).

causes[14–18] a downfield shift (6–10 p.p.m.) of the signal of the carbon atom to which it is attached, whereas the signals of adjacent carbon atoms are shifted upfield by 0.6–2.5 p.p.m. The results reported in Tables I and II are in agreement with this generalization. Thus, the sulfate group caused a downfield displacement of 6.26 and 7.29 p.p.m. of the signal of C-2 of the α-anomer 2-sulfate **1** and β-anomer 2-sulfate **3**, respectively. Similarly, sulfation of HO-3 caused a downfield shift of 9.08 and 8.17 p.p.m. of the signal of C-3 of the α-anomer 3-sulfate **2** and β-anomer 3-sulfate **4**, respectively. ^{1}H-N.m.r. spectroscopy has also been used to locate the sulfate groups in carbohydrate sulfates[17–19], and the relevant data for **1–4** (500 MHz) are summarized in Table III. Sulfation of HO-2 caused a downfield displacement of 0.60 and 0.75 p.p.m. of the H-2 signals of the 2-sulfates **1** and **3**, respectively. Similarly, sulfation of HO-3 caused a downfield shift of 0.76 and ~0.8 p.p.m. of the signals of H-3 of the 3-sulfates **2** and **4**, respectively. These data are in agreement with previous observations[17–19]. The aforementioned results indicate that ^{13}C- and ^{1}H-n.m.r. spectroscopy will be helpful in the structural study of glycoconjugates containing glucuronic acid sulfate residues.

EXPERIMENTAL

General methods. — Optical rotations were determined with a JASCO DIP-4 automatic polarimeter. T.l.c. was performed on Silica Gel 60 F_{254} (Merck) with 11:4:1:4 propanol–2-propanol–butanol–0.2M NaCl in 0.05M ammonia as solvent[20]. Compounds were detected by charring with H_2SO_4.

N.m.r. specctra. ^{1}H-N.m.r. spectra (at 500 MHz) and ^{13}C-n.m.r. spectra (at 125 MHz) were recorded with a JEOL GX-500 spectrometer at 30° for solutions in D_2O. Chemical shifts (δ) were measured relative to the methyl signal of internal 2-methyl-2-propanol (δ 1.23 for ^{1}H and 32.31 for ^{13}C). ^{1}H-N.m.r. spectra at 270 MHz were recorded with a JEOL GX-270 spectrometer at 25° for solutions in ($^2H_5$)pyridine with internal tetramethylsilane as reference.

Sulfation. To a 10% (w/v) solution of sugar derivative in dry pyridine was added sulfur trioxide–pyridine complex (1.5 molar equiv). The mixture was kept for 4 h at 55° and then for 15 h at 25°. An equal volume of water was added to the mixture, and the pH of the solution was adjusted to 7.5 with an aqueous $Ba(OH)_2$ solution. The resulting suspension was concentrated to dryness *in vacuo* at 40°, the residue was suspended in the original volume of water and the pH was adjusted to 7 with an aqueous $Ba(OH)_2$ solution. The neutralization and evaporation steps were repeated until the suspension of the residue had a pH of 7.0. Insoluble inorganic salts were removed by centrifugation.

Acetal hydrolysis. The aqueous solution of sugar acetal (0.07M) was percolated through a column of Dowex 50 (H^+) cation-exchange resin, which was then washed with water until the pH of the eluate had risen to 5. The combined eluate (pH ~2) was kept for 40 min at 60°, and then extracted with ether to remove benzaldehyde. The eluate was made slightly alkaline with aqueous ammonia and then taken to dryness.

Catalytic oxidation. To a 1.5% (w/v) aqueous solution of ammonium methyl glycoside monosulfate were added $NaHCO_3$ (1.6 molar equiv.) and 5% Pd–C (4.8 g per g of sugar). The mixture was vigorously shaken by bubbling O_2at 70° (ref. 7), and the progress of the reaction was monitored by t.l.c. When completed, the catalyst was filtered off, and the filtrate taken to dryness after percolation through Dowex 50 (H^+) cation-exchange resin and neutralization with ammonia.

Purification of crude sulfates. Crude methyl D glucoside or methyl D-glucosiduronic acid sulfates were purified by column chromatography on DEAE-cellulose by the method of Archbald *et al.*[16]. For the elution of the methyl-D-glycosiduronic acid sulfates, 0.1M $(NH_4)HCO_3$ was used in the place of 0.05M $(NH_4)HCO_3$. The pure ammonium salt of the sulfate ester was isolated by the method of Archbald *et al.*[16].

Ammonium methyl 2-O-sulfo-α-D-glucopyranoside (**7**). — Methyl 4,6-*O*-benzylidene-3-*O*-nitro-α-D-glucopyranoside[10] (**8**; 3.2 g) was subjected in sequence to sulfation, acetal hydrolysis, and chromatography on DEAE-cellulose to give methyl 3-*O*-nitro-2-*O*-sulfo-α-D-glucopyranoside[8] (**12**), isolated as a glassy ammonium salt (1.8 g, 56%), $[\alpha]_D$ +90° (*c* 1.8, water). A solution of **12** (1.8 g) in water (50 mL) was hydrogenolyzed in the presence of 10% Pd–C (1.8 g) for 3 h, then filtered, and concentrated to give **5**, isolated from water–ethanol–ether[16] as a very hygroscopic amorphous solid (1.5 g, 94%), $[\alpha]_D^{25}$ +89° (*c* 1.5, water); ^{1}H-n.m.r. (500 MHz): δ 5.13 (d, 1 H, H-1) , 4.18 (dd, 1 H, H-2), 3.90 (dd, 1 H, H-6a), 3.80 (t, 1 H, H-3), 3.78 (dd, 1 H, H-6b), 3.69 (m, 1 H, H-5), 3.52 (t, 1 H, H-4), and 3.46 (s, 3 H, OMe); $J_{1,2}$ 3.8, $J_{2,3} = J_{3,4}$ 10.0, $J_{4,5}$ 9.6, $J_{5,6a}$ 2.2, $J_{5,6b}$ 5.6, and $J_{6a,6b}$ 12.5 Hz.

Diammonium methyl 2-O-sulfo-α-D-glucopyranosiduronic acid (**1**). — Compound **7** (1.5 g) was subjected to catalytic oxidation for 8.5 h, and then purification by chromatography on DEAE-cellulose to give **1** as a very hygroscopic crystalline solid (1.2 g, 73%), m.p. 149–152° (dec.), $[\alpha]_D^{25}$ +78° (*c* 1.0, water); ^{13}C- and ^{1}H-n.m.r., see Tables I and III.

Anal. Calc. for $C_7H_{18}N_2O_{10}S$: C, 26.09; H, 5.62; N, 8.69; S, 9.95. Found: C, 26.37; H, 5.91; N, 8.51; S, 9.46.

Ammonium methyl 3-O-sulfo-α-D-glucopyranoside[9] (**11**). — Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**9**; 2.2 g) was sulfated, and the resulting crude barium glucoside sulfate (3.3 g) was dissolved in ice-cold 0.15M barium methoxide in methanol[16] (200 mL). The solution was kept at 4°, and the progress of the deacetylation reaction was monitored by t.l.c. When completed, excess solid CO_2 was added, and the resulting precipitate was removed by centrifugation. The supernatant solution was concentrated and the crude residue subjected to acetal hydrolysis, followed by chromatography on DEAE-cellulose. The ammonium salt **11** was isolated as an extremely hygroscopic, amorphous solid (1.5 g, 79%), $[\alpha]_D^{25}$ +107° (*c* 4.4, water); ^{1}H-n.m.r. (500 MHz): δ 4.88 (d, 1 H, H-1), 4.46 (t, 1 H, H-3), 3.90 (dd, 1 H, H-6a), 3.81 (dd, 1 H, H-6b), 3.76 (dd, 1 H, H-2), 3.73 (m, 1 H, H-5), 3.63 (t, 1 H, H-4), and 3.47 (s, 3 H, OMe); $J_{1,2}$ 3.8, $J_{2,3} = J_{3,4}$ 10.0, $J_{4,5}$ 9.0, $J_{5,6a}$ 2.0, $J_{5,6b}$ 5.0, and $J_{6a,6b}$ 12.5 Hz.

The ammonium salt was too hygroscopic for elemental analysis.

Diammonium methyl 3-O-sulfo-α-D-glucopyranosiduronic acid (**2**). — Compound **11** (1.8 g) was subjected to catalytic oxidation for 7.5 h and then purified on DEAE-cellulose to give **2** as a very hygroscopic, crystalline solid (1.5 g), m.p. 156–159° (dec.), $[\alpha]_D^{25}$ +99° (*c* 0.71, water); ^{13}C- and ^1H-n.m.r., see Tables I and III.

Anal. Calc. for $C_7H_{18}N_2O_{10}S$: C, 26.09; H, 5.62; N, 8.69; S, 9.95. Found: C, 26.13; H, 5.81; N, 8.59; S, 9.95.

Methyl 4,6-O-benzylidene-2,3-di-O-nitro-β-D-glucopyranoside (**13**). — Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (25 g) was nitrated by the method reported for the preparation of the α-D anomer[9] to give crystalline **13** (29 g, 88%), m.p. 175–176°, $[\alpha]_D^{25}$ −60° (*c* 1.2, chloroform); ^1H-n.m.r. data (270 MHz): δ 7.63–7.15 (m, 5 H, Ph), 6.19 (t, 1 H, H-3), 5.80 (s, 1 H, PhC*H*), 5.72 (dd, 1 H, H-2), 5.01 (d, 1 H, H-1), 4.52 (dd, 1 H, H-6*e*), 4.00–3.92 (superimposed, 2 H, H-5 and H-6*a*), and 3.50 (s, 3 H, OMe); $J_{1,2}$ 7.6, $J_{2,3}$ 9.9, $J_{3,4} = J_{4,5}$ 9.6, $J_{5,6e}$ 3.3, and $J_{6a,6e}$ 8.6 Hz.

Anal. Calc. for $C_{14}H_{16}N_2O_{10}$: C, 45.16; H, 4.33; N, 7.53. Found: C, 45.18; H, 4.50; N, 7.34.

Methyl 4,6-O-benzylidene-2-O-nitro-β-D-glucopyranoside (**12**). — The selective denitration of **13** (10 g) was achieved by the same method as that reported for the selective denitration of the α-D anomer[10], and **14** was isolated in crystalline form (7.7 g, 87%), m.p. 199–200° (dec.), $[\alpha]_D^{25}$ −55° (*c* 0.4, chloroform); ^1H-n.m.r. (270 MHz): δ 7.63–7.15 (m, 5 H, Ph), 5.80 (s, 1 H, PhC*H*), 5.63 (dd, 1 H, H-2), 4.79 (d, 1 H, H-1), 4.48 (dd, 1 H, H-6*e*), 4.40 (m, 1 H, H-3), 4.00 (t, 1 H, H-4), 3.94 (t, 1 H, H-6*a*), 3.72 (m, 1 H, H-5), and 3.50 (s, 3 H, OMe); $J_{1,2}$ 8.1, $J_{2,3} = J_{3,4} = J_{4,5} = J_{5,6a}$ 9.5, $J_{5,6e}$ 4.9, and $J_{6e,6a}$ 10.0 Hz.

Anal. Calc. for $C_{14}H_{17}NO_8$: C, 51.37; H, 5.23; N, 4.28. Found: C, 51.32; H, 5.26; N, 4.27.

Methyl 3-O-acetyl-4,6-O-benzylidene-2-O-nitro-β-D-glucopyranoside (**17**). — A mixture of **14** (4.5 g), pyridine (43 mL), and acetic anhydride (0.73 g) was kept for 20 h at 20°, and then poured into ice–water. The resulting solid crystallized from methanol to give **17**, thin plates (4.5 g, 88%), m.p. 134–135°, $[\alpha]_D^{25}$ −66° (*c* 1.0, chloroform); ^1H-n.m.r. (270 MHz): δ 7.65–7.33 (m, 5 H, Ph), 5.90 (t, 1 H, H-3), 5.79 (s, 1 H, PhC*H*), 5.59 (dd, 1 H, H-2), 4.88 (d, 1 H, H-1), 4.48 (dd, 1 H, H-6*e*), 4.11 (t, 1 H, H-4), 3.94 (t, 1 H, H-6*a*), 3.83 (m, 1 H, H-5), 3.49 (s, 3 H, OMe), and 2.00 (s, 3 H, OAc); $J_{1,2}$ 8.2, $J_{2,3} = J_{3,4} = J_{4,5} = J_{5,6a}$ 9.4, $J_{5,6e}$ 4.7, and $J_{6e,6a}$ 10.6 Hz.

Anal. Calc. for $C_{16}H_{19}NO_9$: C, 52.03; H, 5.19; N, 3.79. Found: C, 52.21; H, 5.24; N, 3.80.

Methyl 3-O-acetyl-4,6-O-benzylidene-β-D-glucopyranoside (**16**). — A solution of **17** (3.7 g) in ethanol (300 mL) was hydrogenolyzed in the presence of 10% Pd–C (8 g) for 5 h. The catalyst was filtered off and the solvent removed *in vacuo*. The residue crystallized from methanol–water to give **16** (2.3 g, 71%), m.p. 155–156° (lit.[13] m.p. 154–156°), $[\alpha]_D^{25}$ −55° (*c* 0.6, chloroform) (lit.[12] $[\alpha]_D$ −55.2°); ^1H-n.m.r. (270 MHz): δ 7.70–7.33 (m, 5 H, Ph), 5.85 (t, 1 H, H-3), 5.75 (s, 1 H, PhC*H*), 4.73

(d, 1 H, H-1), 4.48 (dd, 1 H, H-6*e*), 4.05 (dd, 1 H, H-2), 3.96 (t, 1 H, H-4), 3.93 (t, 1 H, H-6*a*), 3.75 (m, 1 H, H-5), 3.58 (s, 3 H, OMe), and 2.01 (s, 3 H, OAc); $J_{1,2}$ 7.6, $J_{2,3} = J_{3,4} = J_{4,5} = J_{5,6a}$ 9.6, $J_{5,6e}$ 4.6, and $J_{6e,6a}$ 10.2 Hz.

Ammonium methyl 2-O-sulfo-β-D-glucopyranoside (**18**). — Compound **16** (2.1 g) was converted into **18**, by the same reaction sequence as that described for the synthesis of **9**; **18** was isolated as a very hygroscopic, crystalline solid (1.7 g, 89%), $[\alpha]_D^{25}$ −32° (*c* 3.0, water); ^{1}H-n.m.r. (500 MHz): δ 4.51 (d, 1 H, H-1), 4.01 (dd, 1 H, H-2), 3.91 (dd, 1 H, H-6a), 3.72 (dd, 1 H, H-6b), 3.67 (t, 1 H, H-3), 3.54 (s, 3 H, OMe), and 3.44–3.49 (superimposed, 2 H, H-4, 5); $J_{1,2}$ 8.0, $J_{2,3} = J_{3,4}$ 9.1, $J_{5,6a}$ 2.0, $J_{5,6b}$ 6.3, and $J_{6a,6b}$ 12.5 Hz.

Anal. Calc. for $C_7H_{17}NO_9S$: C, 28.87; H, 5.88; N, 4.81; S, 11.01. Found: C, 28.51; H, 5.94; N, 5.14; S, 11.00.

Diammonium methyl 2-O-sulfo-β-D-glucopyranosiduronic acid (**3**). — Compound **17** (1.6 g) was oxidized to give **3**, isolated as a hygroscopic, crystalline solid (1.4 g, 78%), m.p. 164–167° (dec.), $[\alpha]_D^{25}$ −52° (*c* 1.1, water); ^{13}C- and ^{1}H-n.m.r., see Tables I and III.

Anal. Calc. for $C_7H_{18}N_2O_{10}S$: C, 26.09; H, 5.63; N, 8.69; S, 9.95. Found: C, 26.02; H, 5.82; N, 8.27; S, 9.95.

Ammonium methyl 3-O-sulfo-β-D-glucopyranoside (**15**). — Compound **14** was converted into **13** by the same method as that described for the preparation of **7**; **15** was isolated as a very hygroscopic, crystalline solid (1.8 g, 44%), $[\alpha]_D^{25}$ −14° (*c* 3.1, water); ^{1}H-n.m.r. data (500 MHz): δ 4.46 (d, 1 H, H-1), 4.30 (t, 1 H, H-3), 3.92 (dd, 1 H, H-6a), 3.74 (dd, 1 H, H-6b), 3.59 (t, 1 H, H-4), 3.56 (s, 3 H, OMe), 3.52 (m, 1 H, H-5), and 3.44 (dd, 1 H, H-2); $J_{1,2}$ 8.0; $J_{2,3} = J_{3,4}$ 9.2, $J_{4,5}$ 9.8, $J_{5,6a}$ 2.2, $J_{5,6b}$ 5.4, and $J_{6a,6b}$ 13 Hz.

Anal. Calc. for $C_7H_{17}NO_9S \cdot H_2O$: C, 27.18; H, 5.54; N, 4.53; S, 10.36. Found: C, 27.45; H, 5.35; N, 4.49; S, 10.0

Diammonium methyl 3-O-sulfo-β-D-glucopyranosiduronic acid (**4**). — Compound **15** (1.6 g) was subjected to catalytic oxidation to give **4** as a hygroscopic, crystalline solid (1.0 g, 59%), m.p. 177–179° (dec.), $[\alpha]_D^{25}$ −24° (*c* 0.69, water); ^{13}C- and ^{1}H-n.m.r., see Tables I and III.

Anal. Calc. for $C_7H_{18}N_2O_{10}S$: C, 26.09; H, 5.63; N, 8.69; S, 9.95. Found: C, 26.23; H, 5.55; N, 8.40; S, 10.3.

Ammonium methyl α-D-glucopyranosiduronic acid (**5**). — Methyl α-D-glucopyranoside (3 g) in water (80 mL) was oxidized as described under "catalytic oxidation". The product was purified by chromatography on DEAE-cellulose to give chromatographically pure **5** (3.2 g, 91%) as a colorless syrup, $[\alpha]_D$ +65° (*c* 1.5, water); ^{13}C- and ^{1}H-n.m.r., see Tables I and III.

Ammonium methyl β-D-glucopyranosidurnic acid (**6**). — Methyl β-D-glucopyranoside (3 g) was treated as just described to give chromatographically pure **6** (3.2 g, 91%), syrup, $[\alpha]_D^{25}$ −24° (*c* 1.8, water); ^{13}C- and ^{1}H-n.m.r., see Tables I and III.

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