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Note

^{1}H- and ^{13}C-n.m.r. spectroscopy of synthetic monosulphated methyl α-D-galactopyranosides*

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In our studies of the structure of sulphated *N*- and *O*-linked carbohydrate chains derived from *N,O*-glycoproteins and of sulphated polysaccharides, reference sulphated monosaccharides were required. Methyl glycosides are suitable reference compounds[1] in the ^{1}H- and ^{13}C-n.m.r. analyses of sulphated oligosaccharides, and we now present the complete assignments of the ^{1}H-(500 MHz) and ^{13}C-(50 MHz)n.m.r. spectra of the sodium salts of methyl α-D-galactopyranoside 2-, 3-, 4-, and 6-sulphate (**5, 9, 12**, and **13**, respectively).

Following the route for the synthesis of methyl 3,4-*O*-isopropylidene-6-*O*-trityl-β-D-galactopyranoside[2], methyl α-D-galactopyranoside (**1**) was isopropylidenated (→**2**) and then tritylated (→**3**). 2-Sulphation of **3** with the pyridine–sulphur trioxide complex gave **4**, which was converted, after removal of the protecting groups, into methyl α-D-galactopyranoside 2-(sodium sulphate) (**5**).

Benzylation of **1**, as described to obtain methyl 3-*O*-benzyl-β-D-galactopyranoside[3], gave **6**, which was acetylated (→**7**) and then debenzylated (→**8**). 3-Sulphation of **8** and subsequent deacetylation yielded methyl α-D-galactopyranoside 3-(sodium sulphate) (**9**).

The 4-(sodium sulphate) of methyl α-D-galactopyranoside (**12**) was synthesised essentially as reported[4,5]. Selective benzoylation of **1** gave the 2,3,6-tribenzoate **10**, 4-sulphation of which afforded **11**, and subsequent debenzoylation yielded **12**.

Comparison of the ^{1}H-n.m.r. data (Table I) of the monosulphated derivatives **5, 9, 12**, and **13** with those of the parent compound **1** demonstrates that the sulphate group deshields the geminal and vicinal protons. The secondary sulphate groups in

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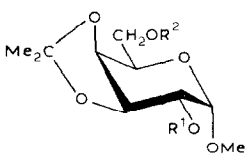

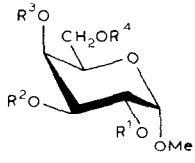

2 $R^1 = R^2 = H$
3 $R^1 = H, R^2 = Tr$
4 $R^1 = SO_3Ba, R^2 = Tr$

5 $R^1 = SO_3Na_2, R^2 = R^3 = R^4 = H$
6 $R^1 = R^3 = R^4 = H, R^2 = Bn$
7 $R^1 = R^3 = R^4 = Ac, R^2 = Bn$
8 $R^1 = R^3 = R^4 = Ac, R^2 = H$
9 $R^1 = R^3 = R^4 = H, R^2 = SO_3Na_2$
10 $R^1 = R^2 = R^4 = Bz, R^3 = H$
11 $R^1 = R^2 = R^4 = Bz, R^3 = SO_3Ba$
12 $R^1 = R^2 = R^4 = H, R^3 = SO_3Na_2$

5, 9, and **12** cause α-effects of 0.6–0.7 p.p.m., whereas the primary sulphate group in **13** shows 0.4–0.5 p.p.m. for this effect. The β-effects are 0.1–0.4 p.p.m. depending on the axial or equatorial orientation of the vicinal proton. The resonance for an equatorial proton next to an equatorial sulphate group is shifted more downfield as compared to other situations. The values of $^3J_{H,H}$ for **1, 5, 9, 12**, and **13** are similar, indicating that sulphation does not cause significant distortion of the $^4C_1$ chair conformation or change in the rotamer populations around the C-5–C-6 bond[6] (Table I).

The ^{13}C-n.m.r. data for **1, 5, 9, 12**, and **13** are summarised in Table II. The signals in the spectra **5, 9, 12**, and **13** were assigned by comparison with the data for the corresponding reducing monosaccharide derivatives[7]. Specific downfield shifts of 6–8 p.p.m. for the signals of the carbon atoms bearing the sulphate group were observed. A sulphate group at C-6 gives rise to the smallest downfield shift (6.33 p.p.m.), whereas sulphate groups at C-3 or C-4 cause the largest shifts (8.28–8.24 p.p.m.). An equatorial (C-3) or axial (C-4) orientation does not influence markedly the magnitude of the shift. The signals from the β-carbon atoms were shifted upfield by 1–2 p.p.m. An axial sulphate group (C-4) caused a smaller upfield shift (0.6–1.0 p.p.m.) of the resonance of the β-carbon atoms than an equatorial sulphate group (1.8–2.2 p.p.m.). The various shift effects observed accorded with those reported earlier for the reducing α-analogues[7]. The presence of the α-methoxyl group at C-1 does not really influence these effects.

The above results indicate that ^{1}H- and ^{13}C-n.m.r. data will be helpful in assigning the positions of sulphate groups in oligosaccharides. This has been demonstrated recently using ^{1}H-n.m.r. data for the acidic *N*-linked carbohydrate chain of the hemocyanin from the spiny lobster *Panulirus interruptus*, which has a 6-sulphated α-D-Man residue[1].

TABLE I

^{1}H-N.M.R. DATA FOR THE MONOSULPHATED METHYL α-D-GALACTOPYRANOSIDES **5, 9, 12**, AND **13**, AND METHYL α-D-GALACTOPYRANOSIDE (**1**)

Compound	*Chemical shift[a] (and shift relative to 1)*							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*OCH_3*
Methyl α-D-galactopyranoside[b] (**1**)	4.837	3.819	3.811	3.968	3.897	3.741	3.752	3.414
–, 2-sulphate (**5**)	5.131 (+0.294)	4.443 (+0.624)	3.940 (+0.129)	4.048 (+0.080)	3.923 (+0.026)	3.749 (+0.008)	3.763 (+0.011)	3.428 (+0.014)
–, 3-sulphate (**9**)	4.899 (+0.062)	3.982 (+0.163)	4.477 (+0.666)	4.324 (+0.356)	3.935 (+0.038)	3.751 (+0.010)	3.759 (+0.007)	3.430 (+0.016)
–, 4-sulphate (**12**)	4.867 (+0.030)	3.855 (+0.036)	3.941 (+0.130)	4.713 (+0.745)	4.030 (+0.133)	3.810 (+0.069)	3.755 (+0.003)	3.425 (+0.011)
–, 6-sulphate[c] (**13**)	4.842 (+0.005)	3.830 (+0.011)	3.828 (+0.017)	4.018 (+0.050)	4.148 (+0.251)	4.214 (+0.473)	4.158 (+0.406)	3.421 (+0.007)
	Coupling constant[d] (Hz)							
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	
Methyl α-D-galactopyranoside (**1**)	3.2	10.2	2.7	1.2	4.1	8.3	−11.8	
–, 2-sulphate (**5**)	3.8	10.2	3.7	1.0	4.2	8.1	−11.8	
–, 3-sulphate (**9**)	3.8	10.3	3.2	1.1	4.1	8.2	−11.8	
–, 4-sulphate (**12**)	3.9	10.4	3.3	n.d.	4.1	8.3	−12.0	
–, 6-sulphate (**13**)	3.0	10.3	3.3	0.8	4.1	8.3	−10.8	

[a]In p.p.m. relative to internal DSS in D_2O (using internal acetone at δ 2.225) at 27°. [b]For reference data, see also ref. 10. [c]Obtained by computer simulation of the experimental spectrum. [d]Refinement of the couplings between H-5, H-6, and H-6' was carried out by computer simulation.

TABLE II

^{13}C-N.M.R. DATA FOR THE MONOSULPHATED METHYL α-D-GALACTOPYRANOSIDES **5, 9, 12**, AND **13**, AND METHYL α-D-GALACTOPYRANOSIDE (**1**)

Compound	*Chemical shift[a] (and shift relative to* **1***)*						
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*
Methyl α-D-galactopyranoside[b] (**1**)	100.67	69.47	70.76	70.50	71.99	62.50	56.31
–, 2-sulphate (**5**)	98.63	76.64	68.61	70.60	71.81	62.32	56.32
	(−2.04)	(+7.17)	(−2.15)	(+0.10)	(−0.18)	(−0.18)	(+0.01)
–, 3-sulphate (**9**)	100.43	67.39	79.04	68.75	71.63	62.29	56.25
	(−0.24)	(−2.08)	(+8.28)	(−1.75)	(−0.36)	(−0.21)	(−0.06)
–, 4-sulphate (**12**)	100.50	69.53	69.78	78.74	71.35	62.44	56.42
	(−0.17)	(+0.06)	(−0.98)	(+8.24)	(−0.64)	(−0.06)	(+0.11)
–, 6-sulphate (**13**)	100.62	69.30	70.23	70.49	69.73	68.83	56.36
	(−0.05)	(−0.17)	(−0.53)	(−0.01)	(−2.26)	(+6.33)	(+0.05)

[a]In p.p.m. relative to internal acetone in D_2O (δ 31.55) at 27°. [b]For reference data, see also ref. 10.

EXPERIMENTAL

Methyl α-D-galactopyranoside 6-(sodium sulphate) (**13**) was donated by Mrs. C. E. M. Kolsteeg.

Melting points were determined with an Electrothermal melting-point apparatus and are uncorrected. Optical rotations were measured at 20° with a Perkin–Elmer 241 polarimeter, using a 10-cm microcell. Elemental analyses were carried out at the Institute for General Organic Chemistry, Madrid, Spain. Evaporations were conducted *in vacuo* at 50° (bath).

T.l.c. was performed on Kieselgel 60 F_{254} (Merck) with *A*, 7:1 tetrachloromethane–acetone; *B*, 3:2 dichloromethane–methanol; *C*, 4:1 chloroform–methanol; and *D*, 3:1 tetrachloromethane–acetone. Detection was effected by charring with sulphuric acid after examination under u.v. light. Column chromatography was performed on Kieselgel 60 (Merck, 70–230 mesh) and fractions were monitored by t.l.c.

N.m.r. data of monosaccharide intermediates were obtained at 27° at the SON hf-NMR facility (Bruker WM-200), Department of Biophysical Chemistry, Nijmegen University, The Netherlands, and expressed in p.p.m. relative to internal Me_4Si. 50-MHz ^{13}C-n.m.r. spectra (Bruker WM-200) of **1, 5, 9, 12**, and **13** were recorded for solutions in D_2O (internal acetone, δ 31.55). 500-MHz ^1H-n.m.r. spectra (Bruker AM-500) of **1, 5, 9, 12**, and **13** were recorded for solutions in D_2O after repeated exchange against D_2O (99.96 atom %). Chemical shifts (δ) are given in p.p.m. relative to sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) but measured indirectly to acetone in D_2O (δ 2.225) with an accuracy of 0.002 p.p.m.[8].

Methyl 3,4-O-isopropylidene-6-O-trityl-α-D-galactopyranoside (**3**). — A solution of **2**[2,9] (2.5 g, 10.7 mmol) and chlorotriphenylmethane (3.0 g, 11.0 mmol) in dry pyridine (30 mL) was kept overnight at room temperature and then heated for 1 h at 70°. The cooled solution was poured into ice–water, the white solid material was collected and extracted with acetone, and the extract was filtered and concentrated to dryness. Elution of the residue from Kieselgel 60 (150 g) with 1:5 acetone–tetrachloromethane gave triphenylmethanol, and then **3** (R_F 0.40, solvent *A*), isolated as a colourless foam (2.9 g, 56%), m.p. 60–61°, $[\alpha]_D$ +50° (*c* 1.1, methanol). N.m.r. data ($CDCl_3$): ^1H, δ 7.51–7.14 (m, 15 H, 3 Ph), 4.73 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 3.45 (s, 3 H, OMe), 1.44 and 1.33 (2 s, each 3 H, CMe_2); ^{13}C, δ 144.02, 128.74, 127.70, 126.93 [$(C_6H_5)_3C$], 109.41 [$(CH_3)_2C$], 98.39 (C-1), 86.72 [$(C_6H_5)_3C$], 76.21, 73.34, 69.55, 67.43 (C-2/5), 63.00 (C-6), 55.29 (OCH_3), 27.76 and 25.97 [$(CH_3)_2C$].

Anal. Calc. for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 73.14; H, 6.53.

Methyl 3,4-O-isopropylidene-6-O-trityl-α-D-galactopyranoside 2-(barium sulphate) (**4**). — To a solution of **3** (2.4 g, 5 mmol) in dry pyridine (50 mL) was added pyridine–sulphur trioxide (2.4 g, 15 mmol). The mixture was heated at 65–70° until t.l.c. showed that the reaction was complete (1–1.5 h) (R_F 0.64, solvent *C*). The mixture was cooled to room temperature, water (50 mL) was added, the solution

was kept for 1 h at room temperature, the pH was then adjusted to 9 by adding saturated aqueous barium hydroxide, the barium sulphate was removed, and the filtrate was concentrated to dryness. Traces of pyridine were eliminated by co-evaporation with water, the excess of Ba^{2+} was removed with carbon dioxide, and the filtrate was concentrated until **4** precipitated. Recrystallisation from ethanol afforded **4** (2.9 g, 83%), m.p. 170–171° (dec.), $[\alpha]_D$ +50° (*c* 1.2, methanol). N.m.r. data (methanol-d_4): ^{1}H, δ 7.50–7.14 (m, 15 H, 3 Ph), 5.13 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 3.40 (s, 3 H, OMe), 1.48 and 1.29 (2 s, each 3 H, CMe_2); ^{13}C, 145.06, 129.54, 128.61, 127.95 [$(C_6H_5)_3C$], 110.26 [$(CH_3)_2C$], 98.90 (C-1), 87.64 [$(C_6H_5)_3C$], 76.87, 75.17, 74.96, 67.69 (C-2/5), 64.10 (C-6), 55.71 (OCH_3), 28.37 and 26.68 [$(CH_3)_2C$].

Anal. Calc. for $C_{29}H_{31}BaO_9S$: C, 50.26; H, 4.51; S, 4.63. Found: C, 49.89; H, 4.71; S, 4.58.

Methyl α-D-*galactopyranoside 2-(sodium sulphate)* (**5**). — A solution of **4** (2.7 g, 3.9 mmol) in ethanol (10 mL) and aqueous 5% acetic acid (10 mL) was heated at 60–70°. After 10 h, t.l.c. (solvent *B*) showed that **4** was absent and a new compound of R_F 0.56 was present. The acetic acid was eliminated by co-evaporation with ethanol, the triphenylmethanol was removed, and the filtrate was concentrated to dryness. A solution of the residue in aqueous ethanol was brought to pH 4 by adding Dowex-50 (H^+) resin, then filtered, neutralised with saturated aqueous sodium hydrogencarbonate, and concentrated to dryness. The residue was eluted from Kieselgel 60 (50 g) with 2:1 dichloromethane–methanol, to give a small amount of triphenylmethanol and then **5**. Crystallisation from methanol and dichloromethane gave **5** (1.1 g, 87%), m.p. 203–204° (dec.), $[\alpha]_D$ +120° (*c* 1, water). For the ^{1}H- and ^{13}C-n.m.r. data, see Tables I and II, respectively.

Anal. Calc. for $C_7H_{13}Na_2O_9S$: C, 26.34; H, 4.11; S, 10.04. Found: C, 26.10; H, 4.50; S, 9.89.

*Methyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-*α*-*D*-galactopyranoside* (**7**). — To a solution of methyl α-D-galactopyranoside monohydrate (**1**; 2.0 g, 9.4 mmol) in dry benzene (60 mL) was added dibutyltin oxide (2.3 g, 9.4 mmol). The mixture was boiled under reflux for 14–15 h in a Soxhlet apparatus containing molecular sieves 4Å, and then cooled to room temperature, benzyl bromide (1.1 mL, 9.4 mmol) and tetrabutylammonium iodide (3.5 g, 9.4 mmol) were added, and the mixture was boiled under reflux with stirring for 3 h. T.l.c. (solvent *C*) then revealed **1** and several faster moving compounds, the main one having R_F 0.8. The solution was concentrated to dryness and the red syrup was dissolved in methanol. From the solution, a crystalline mass precipitated, which was shown by t.l.c. not to be a sugar derivative. The mother liquor was concentrated to a syrup, which was eluted from Kieselgel 60 (100 g) with 7:1 dichloromethane–methanol. Impurities were eluted first, followed by **6**. Crystallisation from ethyl acetate gave **6** (1.9 g, 71%), m.p. 146–147°. N.m.r. data ($CDCl_3$): ^{1}H, δ 7.30–7.38 (m, 5 H, Ph), 4.85 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.73 (s, 2 H, $PhCH_2O$), 3.42 (s, 3 H, OMe); ^{13}C, 128.63, 128.11, 127.85 ($C_6H_5CH_2$), 99.63 (C-1), 78.18 (C-3), 72.24 ($C_6H_5CH_2$), 69.39, 68.57, 68.29 (C-2,4,5), 63.05 (C-6), 55.42 (OCH_3).

Anal. Calc. for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 59.31; H, 6.76.

Conventional treatment of **6** (1.7 g, 6 mmol) with 2:1 pyridine–acetic anhydride (15 mL) for 2 h at room temperature gave the 2,4,6-triacetate **7** as colourless needles (2.3 g, 92%), m.p. 80–81° (from aqueous ethanol), $[\alpha]_D$ +160° (*c* 1.1, methanol). N.m.r. data ($CDCl_3$): ^1H, δ 7.27–7.35 (m, 5 H, Ph), 4.98 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.70 and 4.46 (2 d, 2 H, PhCH_2O), 3.38 (s, 3 H, OMe), 2.13, 2.09, 2.07 (3 s, each 3 H, 3 Ac); ^{13}C, δ 137.69, 128.32, 127.72, 127.68 ($C_6H_5CH_2$), 97.35 (C-1), 73.26 (C-3), 71.87 ($C_6H_5CH_2$), 70.09, 67.38, 66.52 (C-2,4,5), 62.40 (C-6), 55.35 (OCH_3), 20.92, 20.75 (2C) (3 CH_3CO).

Anal. Calc. for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39. Found: C, 58.50; H, 6.39.

Methyl α-D-galactopyranoside 3-(sodium sulphate) (**9**). — A solution of **7** (2.0 g, 4.9 mmol) in 2-methoxyethanol (40 mL) was stirred in a hydrogen atmosphere for 20 h at room temperature in the presence of 10% Pd/C (0.5 g), then filtered, and concentrated to dryness. The syrupy residue (**8**; 1.5 g, 94%) was sufficiently pure by t.l.c. (solvent *D*; R_F 0.5) for use in the next step. A solution of **8** (1.2 g, 3.6 mmol) in dry pyridine (25 mL) was sulphated with pyridine–sulphur trioxide (1.8 g, 11 mmol) as described for **4**. The crude product was deacetylated with methanolic 0.5M sodium methoxide (5 mL). The reaction was monitored by t.l.c. (solvent *B*), which showed an almost exclusive formation of a more polar compound (R_F 0.62). After 2 h at room temperature, the solution was adjusted to pH 4 by adding Dowex-50 (H^+) resin, filtered, neutralised by saturated aqueous sodium hydrogencarbonate, and concentrated to dryness. The residue was eluted from Kieselgel 60 (45 g) with 2:1 dichloromethane–methanol. The product was crystallised from methanol and dichloromethane to afford **9** (0.9 g, 81%), m.p. 166–167°, $[\alpha]_D$ +146° (*c* 1.1, water). For the ^1H- and ^{13}C-n.m.r. data, see Tables I and II, respectively.

Anal. Calc. for $C_7H_{13}Na_2O_9S$: C, 26.34; H, 4.11; S, 10.04. Found: C, 26.07; H, 4.23; S, 9.78.

Methyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside 4-(barium sulphate) (**11**). — A solution of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside[5] (**10**; 2.3 g, 4.5 mmol) in dry pyridine (60 mL) was mixed with pyridine–sulphur trioxide (2.2 g, 13.5 mmol) and heated for 2 h at 65–70°. T.l.c. (solvent *C*) then revealed a main product having R_F 0.7. Water (60 mL) was then added and, after 1 h at room temperature, the pH was adjusted to 9 by adding saturated aqueous barium hydroxide. Insoluble material was collected and extracted with hot ethanol, the extract was concentrated to dryness, and the residue crystallised from aqueous ethanol to yield **11** (1.5 g, 48%), m.p. 101–103°. N.m.r. data (methanol-d_4): ^1H, δ 7.30–8.08 (m, 15 H, 3 Ph), 5.20 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.41 (s, 3 H, OMe); ^{13}C, δ 167.95, 167.69, 167.17 (3 C_6H_5CO), 134.48–128.17 (3 C_6H_5CO), 98.55 (C-1), 75.33, 70.28 (2 C), 69.06 (C-2/5), 65.83 (C-6), 55.77 (OCH_3).

Anal. Calc. for $C_{28}H_{25}BaO_{12}S \cdot 2\,H_2O$: C, 48.69; H, 4.16. Found: C, 48.88; H, 4.24.

Methyl α-D-galactopyranoside 4-(sodium sulphate) (**12**). — A solution of **11** (1.3 g, 1.9 mmol) in methanol (30 mL) was debenzoylated with methanolic 0.5M

sodium methoxide (3 mL) at room temperature. After 2–3 h, the reaction was complete, as monitored by t.l.c. (solvent *B*, R_F 0.60). The pH was adjusted to 4 by adding Dowex-50 (H^+) resin, and the mixture was filtered, neutralised with saturated aqueous sodium hydrogencarbonate, and concentrated to dryness. The residue was eluted from Kieselgel 60 (30 g) with 2:1 dichloromethane–methanol and the product was crystallised from methanol and dichloromethane to afford **12** (0.5 g, 73%), m.p. 190–191° (dec.), $[\alpha]_D$ +116° (*c* 1.4, water). For the ^1H- and ^{13}C-n.m.r. data, see Tables I and II, respectively.

Anal. Calc. for $C_7H_{13}Na_2O_9S$: C, 26.34; H, 4.11; S, 10.04. Found: C, 26.10; H, 4.50; S, 9.89.

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