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^{1}H- and ^{13}C-NMR spectroscopy of synthetic monosulfated methyl α-D-mannopyranosides

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Abstract. The syntheses of methyl α-D-mannopyranoside 2-, 3-, 4-, and 6-(sodium monosulfate) are presented. The various monosulfated methyl α-D-mannopyranosides have been studied by ^{1}H- and ^{13}C-NMR spectroscopy, affording insight into the ^{1}H- and ^{13}C-shift effects caused by sulfation at particular positions. The NMR parameters of these sulfated monosaccharide derivatives are important, for example, in the structural analysis of sulfated carbohydrate chains of N-glycoproteins.

Introduction

In the framework of our studies on the structural analysis of sulfated carbohydrate chains derived from N,O-glycoproteins and of sulfated polysaccharides, adequate reference sulfated monosaccharides are required for comparison purposes in NMR and FAB-MS studies. As reference compounds in the analysis of sulfated oligosaccharides, methyl glycosides have been shown to be very suitable. In previous publications, we have reported NMR[1] and FAB-MS[2] data of a series of synthetic monosulfated methyl α-D--galactopyranosides. The ^{1}H-NMR data were useful in the structural elucidation of the carbohydrate chains of porcine thyroglobulin, which among other things, consist of N-linked carbohydrate chains with 3-sulfated galactose units[3]. In view of the occurrence of N-linked carbohydrate chains containing sulfated mannose residues[4,5], we now present the synthesis and complete assignments of the 360/500-MHz ^{1}H- and 50-MHz ^{13}C-NMR spectra of the sodium salts of methyl α-D-mannopyranoside 2-, 3-, 4-, and 6-sulfate (**5**, **9**, **13**, and **18**, respectively).

Results and discussion

To obtain methyl α-D-mannopyranoside 2-(sodium sulfate) (**5**), methyl α-D-mannopyranoside (**1**) was regiospecifically benzylated at C-3 via the cyclic dibutyltin intermediate[6-8] (→**2**), and then benzylidenated using (dimethoxymethyl)-benzene[9] at C-4,6 (→**3**). Subsequent sulfation at C-2 of **3** with the pyridine–sulfur-trioxide complex led to the formation of **4**, which was converted into **5** by hydrogenolysis. Acetylation of **2** (→**6**), followed by debenzylation (→**7**), sulfation at C-3 (→**8**), and deacetylation yielded methyl α-D--mannopyranoside 3-(sodium sulfate) (**9**).

For the synthesis of methyl α-D-mannopyranoside 4-(sodium sulfate) (**13**), **1** was isopropylidenated at C-2,3 (→**10**[10]), and then tritylated (→**11**). Subsequently, sulfation at C-4 of **11** yielded **12**, which was converted, after acid hydrolysis, into **13**.

Methyl α-D-mannopyranoside 6-(sodium sulfate) (**18**) was synthesized as follows: Compound **1** was tritylated at C-6 (→**14**[11]), then benzylated with benzyl bromide (→**15**[12]), and detritylated (→**16**[12]). Sulfation at C-6 of **16** gave **17**, which was debenzylated to yield **18**.

The ^{1}H-NMR data of **1**, **5**, **9**, **13**, and **18** are presented in Table I. Comparison of the ^{1}H chemical shifts of monosulfated methyl α-D-mannopyranosides with those of methyl α-D-mannopyranoside demonstrates that the sulfate group causes clear "deshielding" effects on the geminal and vicinal protons involved. α-Effects of 0.59–0.72 ppm were observed for the secondary sulfate groups in **5**, **9**, and **13**, whereas the primary sulfate group in **18** causes an α-effect of about 0.45 ppm. The β-effects are in the range of 0.14–0.35 ppm, depending on the relative orientation of the sulfate group. For an equatorial proton next to an equatorial sulfate group, the downfield shift is larger than for other orientations. Similar effects have been noticed in our earlier study on methyl α-D-galactopyranoside 2-, 3-, 4-, and 6-(sodium monosulfate)[1]. The ^{1}H coupling constants in **1**, **5**, **9**, **13**, and **18** are for all these compounds in the same order of magnitude. This means that sulfation does not lead to significant distortion in the $^4C_1$ chair conformation or to strong changes in the rotamer populations around the C-5/C-6 bond. As compared to **1**, the largest distortion is observed for the 4-sulfate **13**. The decrease of $J_{6,6'}$ in the 6-sulfate **18** is in line with the previous observations for methyl α-D-galactopyranoside 6-(sodium sulfate)[1].

The typical shifts of the highly characteristic proton peak patterns of the monosulfated mannosides have successfully been used in the ^{1}H-NMR analysis of an acidic N-linked carbohydrate chain of the hemocyanin from *Panulirus interruptus*, having a 6-sulfated α-mannose residue[4].

The ^{13}C-NMR data of **1**, **5**, **9**, **13**, and **18** are summarized in Table II. Comparison of the ^{13}C-NMR data of the monosulfated methyl α-D-mannopyranosides with those of methyl

Table I ^{1}*H-NMR data for the monosulfated methyl α-D-mannopyranosides* **5**, **9**, **13**, *and* **18**, *and methyl α-D-mannopyranoside* **1**.

Methyl α-D-mannopyranoside	Chemical shift[a] (and shift relative to **1**)							
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	OMe
non-sulfated (**1**)[b]	4.761	3.929	3.751	3.640	3.604	3.898	3.755	3.407
2-sulfated (**5**)	5.038 (+0.277)	4.521 (+0.592)	3.899[c] (+0.148)	3.63[c] (−0.01)	3.63[c] (+0.03)	3.897[c] (−0.001)	3.775[c] (+0.020)	3.431 (+0.024)
3-sulfated (**9**)	4.790 (+0.029)	4.278 (+0.349)	4.450 (+0.699)	3.802 (+0.162)	3.704 (+0.100)	3.913 (+0.015)	3.789 (+0.034)	3.420 (+0.013)
4-sulfated (**13**)	4.780 (+0.019)	3.988 (+0.059)	3.963 (+0.212)	4.363 (+0.723)	3.743 (+0.139)	3.939 (+0.041)	3.785 (+0.030)	3.414 (+0.007)
6-sulfated (**18**)	4.762 (+0.001)	3.940 (+0.011)	3.774[d] (+0.023)	3.696 (+0.056)	3.829 (+0.225)	4.346 (+0.448)	4.230 (+0.475)	3.415 (+0.008)
	Coupling constant in Hz							
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	
non-sulfated (**1**)	1.8	3.5	9.7	10.0	2.6	5.8	12.3	
2-sulfated (**5**)	1.8	3.6	9.4	n.d.	2.0	5.7	12.2	
3-sulfated (**9**)	1.9	3.3	9.5	9.9	2.3	5.8	12.3	
4-sulfated (**13**)	1.8	3.4	9.0	9.4	1.9	6.4	12.0	
6-sulfated (**18**)	1.8	3.4	9.5	9.8	2.2	5.6	11.2	

[a] In ppm relative to internal acetone in D_2O (δ 2.225) at 27°C. [b] For reference data, see also ref. 13. [c] Assignments based on ^{1}H−^{1}H and ^{1}H−^{13}C COSY measurements; virtual couplings are observed for H-6 and H-6′. [d] The earlier reported value[4] of 3.626 is not correct.

α-D-mannopyranoside shows specific downfield shifts of 6.5–8.5 ppm for the signals of the sulfated carbon atoms. The magnitude of the shift increment is influenced by the specific position of the sulfate group in the monosaccharide derivative. A sulfate group at C-6 gives rise to the smallest downfield shift for the involved carbon atom (Δδ + 6.5 ppm), and sulfate groups at C-3 or C-4 cause the largest ones (Δδ + 8.5 ppm). The shift increments found for C-2, C-3, C-4, and C-6 upon monosulfation of methyl α-D--galactopyranoside [2-, 3-, 4-, and 6-(sodium monosulfate), respectively] are similar to those found in the present mannoside series, showing that equatorial or axial orientations of the sulfate group do not influence the magnitude of the shift increment. The signals of the carbon atoms adjacent to sulfated carbons are shifted upfield 1–2 ppm in the specific derivatives.

Experimental

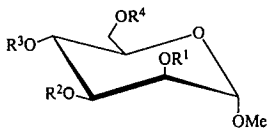
General procedures

Melting points were determined with an electrothermal melting-point apparatus and are uncorrected. Optical rotation was measured at 20°C 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. Evaporation was conducted *in vacuo* at 50°C (bath). All solvents were distilled from appropriate drying agents and stored over molecular sieves.

Table II ^{13}*C-NMR data for the monosulfated methyl α-D-mannopyranoside* **5**, **9**, **13**, *and* **18**, *and methyl α-D-mannopyranoside* **1**.

Methyl α-D-mannopyranoside	Chemical shift[a] (and shift relative to **1**)						
	C-1	C-2	C-3	C-4	C-5	C-6	OMe
non-sulfated (**1**)[b]	101.9	71.2	71.8	68.0	73.7	62.1	55.9
2-sulfated (**5**)	99.8 (−2.1)	78.1 (+6.9)	70.4 (−1.4)	68.0 (0)	73.8 (+0.1)	62.1 (0)	56.3 (+0.4)
3-sulfated (**9**)	101.9 (0)	69.6 (−1.6)	80.3 (+8.5)	66.0 (−2.0)	73.9 (+0.2)	62.2 (+0.1)	56.1 (+0.2)
4-sulfated (**13**)	101.8 (−0.1)	71.3 (+0.1)	70.7 (−1.1)	76.5 (+8.5)	72.1 (−1.6)	62.0 (−0.1)	56.1 (+0.2)
6-sulfated (**18**)	102.2 (+0.3)	71.1 (−0.1)	71.8[c] (0)	67.7 (−0.3)	71.7[c] (−2.0)	68.6 (+6.5)	56.1 (+0.2)

[a] In ppm relative to internal acetone in D_2O (δ 31.55) at 27°C. [b] For reference data, see also ref. 13. [c] Assignments may have to be interchanged.



1 $R^1 = R^2 = R^3 = R^4 = H$
2 $R^1 = R^3 = R^4 = H$; $R^2 = Bn$
3 $R^1 = H$; $R^2 = Bn$; $R^3, R^4 = CHPh$
4 $R^1 = SO_3Na$; $R^2 = Bn$; $R^3, R^4 = CHPh$
5 $R^1 = SO_3Na$; $R^2 = R^3 = R^4 = H$
6 $R^1 = R^3 = R^4 = Ac$; $R^2 = Bn$
7 $R^1 = R^3 = R^4 = Ac$; $R^2 = H$
8 $R^1 = R^3 = R^4 = Ac$; $R^2 = SO_3Na$
9 $R^1 = R^3 = R^4 = H$; $R^2 = SO_3Na$
10 $R^1, R^2 = CMe_2$; $R^3 = R^4 = H$
11 $R^1, R^2 = CMe_2$; $R^3 = H$; $R^4 = CPh_3$
12 $R^1, R^2 = CMe_2$; $R^3 = SO_3Na$; $R^4 = CPh_3$
13 $R^1 = R^2 = R^4 = H$; $R^3 = SO_3Na$
14 $R^1 = R^2 = R^3 = H$; $R^4 = CPh_3$
15 $R^1 = R^2 = R^3 = Bn$; $R^4 = CPh_3$
16 $R^1 = R^2 = R^3 = Bn$; $R^4 = H$
17 $R^1 = R^2 = R^3 = Bn$; $R^4 = SO_3Na$
18 $R^1 = R^2 = R^3 = H$; $R^4 = SO_3Na$

TLC was performed on Kieselgel 60 F_{254} (Merck) with dichloromethane/methanol 4/1, v/v (A); dichloromethane/acetone 1/1, v/v (B); dichloromethane/ethyl-acetate 9/1, v/v (C); dichloromethane/methanol 2/1, v/v (D); and dichloromethane/hexane 2/1, v/v (E). Detection was effected by charring with aqueous 50% sulfuric acid after examination under UV light. Column chromatography was performed on Kieselgel 60 (Merck, 70–230 mesh) and fractions were monitored by TLC. Electron-impact (EI) mass spectra were recorded with a Kratos MS-80 RFA, high-resolution mass spectrometer at an ionizing potential of 70 eV and an ion-source temperature (direct-inlet system) of 200°C. ^{1}H-NMR spectra were recorded at 360 MHz using a Bruker HX 360 or at 500 MHz using a Bruker AM 500 apparatus at 25°C; ^{13}C-NMR spectra at 50 MHz using a Bruker WP 200 spectrometer at 25°C. Chemical shifts (δ) are given in ppm relative to internal Me_4Si ($CDCl_3$) or internal sodium 3-(trimethylsilyl)propane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225) for ^{1}H, and to external Me_4Si (D_2O; indirectly to internal acetone, δ 31.55) for ^{13}C-NMR data.

Methyl 3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (**3**)

A suspension of methyl α-D-mannopyranoside **1** (3.0 g, 15.4 mmol) and dibutyltin oxide (3.9 g, 15.4 mmol) in dry methanol (40 ml) was boiled under reflux with stirring until the solution became clear (2 h)[8]. After concentration, the residue was dissolved in dry benzene (40 ml), benzyl bromide (1.8 ml, 15.4 mmol) and tetrabutylammonium bromide (5.0 g, 15.4 mmol) were added, and the mixture was boiled under reflux for 7 h. TLC (solvent B) revealed a small amount of **1** and several faster moving compounds, the main one having R_f 0.50. The solution was concentrated, the residue dissolved in methanol, from which a crystalline non-carbohydrate material precipitated. The mother liquor was concentrated and the residue purified by column chromatography on Kieselgel 60 (120 g) (dichloromethane/hexane 8/1, v/v) to yield **2**, isolated as a syrup (2.54 g, 58%), $[\alpha]_D$ +36° (*c* 1.2, ethanol). Anal. calcd. for $C_{14}H_{20}O_6$ (284.31): C 59.14, H 7.09; found: C 58.73, H 6.91%. EI-MS data: *m/z* 284 (4.0%, M), 252 (7.3%, M−MeOH), 193 (5.0%, M−$C_6H_5CH_2$), 107 (26.7%, $C_6H_5CH_2O$), 91 (100%, $C_6H_5CH_2$). ^{1}H-NMR data ($CDCl_3$): δ 7.26–7.38 (m, 5H, $C_6H_5CH_2O$), 4.758 (d, 1H, H-1), 4.698 and 4.590 (2d, 2H, $C_6H_5CH_2O$), 3.99 (m, 1H, H-2), 3.953 (t, 1H, H-4), 3.82 (2H, H-6 and H-6'), 3.678 (dd, 1H, H-3), 3.579 (m, 1H, H-5), 3.355 (s, 3H, OMe), $J_{1,2}$ 1.3, $J_{2,3}$ 3.2, $J_{3,4}$ 9.3, $J_{4,5}$ 9.8 Hz.

A mixture of **2** (1.5 g, 5.3 mmol), (dimethoxymethyl)benzene (0.80 ml) and 4-toluenesulfonic acid monohydrate (20 mg) in *N,N*-dimethylformamide (5 ml) was placed in a round-bottomed flask, attached to a Büchi evaporator, and stirred in a water bath for 1½ h at 65–70°C. TLC (solvent B) showed the absence of **2**, and the mixture was concentrated *in vacuo* (oil pump). The residue was purified by column chromatography on Kieselgel 60 (40 g) (dichloromethane/ethyl-acetate 9/1, v/v) to give **3**, isolated as a syrup (1.46 g, 74%), $[\alpha]_D$ +38° (*c* 0.9, ethanol) (lit.[14] $[\alpha]_D$ +38°, lit.[15] $[\alpha]_D$ +38.3°, lit.[16] $[\alpha]_D$ +33°; ethanol), R_f 0.58 (solvent C). Anal. calcd. for $C_{21}H_{24}O_6$ (372.42): C 67.73, H 6.50; found: C 67.25, H 6.51%. EI-MS data: *m/z* 372 (5.8%, M), 341 (0.9%, M−OMe), 281 (0.6%, M−$C_6H_5CH_2$), 265 (0.4%, M−$C_6H_5CH_2O$), 107 (22.4%, $C_6H_5CH_2O$), 91 (100%, $C_6H_5CH_2$), 77 (19.4%, C_6H_5). ^{1}H-NMR data ($CDCl_3$): δ 7.26–7.51 (m, 10H, C_6H_5CH and $C_6H_5CH_2O$), 5.618 (s, 1H, C_6H_5CH), 4.772 (d, 1H, H-1), 4.856 and 4.716 (2d, 2H, $C_6H_5CH_2O$), 3.379 (s, 3H, OMe), $J_{1,2}$ 1.2 Hz.

Methyl 3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside 2-(sodium sulfate) (**4**)

Pyridine–sulfur-trioxide (1.0 g, 6 mmol) was added to a solution of **3** (1.12 g, 3 mmol) in dry pyridine (10 ml). The mixture was heated at 65–70°C with stirring until TLC showed the reaction to be complete (1–1½ h) (R_f 0.72; solvent A). After cooling the mixture to room temperature, water (10 ml) was added, and the solution was kept for 1 h at room temperature. The pH was then adjusted to 9 by adding aqueous saturated barium hydroxide, precipitating barium sulfate was removed, and the filtrate was concentrated. Traces of pyridine were eliminated by co-concentration with water. The residue was dissolved in water, Dowex-50 (H^+) resin was added (pH 4), and the mixture was filtered, neutralized with aqueous saturated sodium hydrogen carbonate, and concentrated. The residue was purified by column chromatography on Kieselgel 60 (40 g) (dichloromethane/methanol 4/1, v/v) to yield **4** (1.21 g, 85%), m.p. 119–121°C (dec.), $[\alpha]_D$ +15° (*c* 1.4, ethanol). Anal. calcd. for $C_{21}H_{23}O_9SNa \cdot 2H_2O$ (510.49): C 49.41, H 5.33; found: C 49.76, H 5.48%. ^{1}H-NMR data ($CDCl_3$): δ 7.06–7.37 (m, 10H, C_6H_5CH and $C_6H_5CH_2O$), 5.455 (s, 1H, C_6H_5CH), 5.168 (bs, 1H, H-1), 4.824 (m, 1H, H-2), 4.769 and 4.644 (2d, 2H, $C_6H_5CH_2O$), 3.191 (s, 3H, OMe).

Methyl α-D-mannopyranoside 2-(sodium sulfate) (**5**)

A solution of **4** (0.95 g, 2 mmol) in methanol (20 ml) was hydrogenolysed in a Parr pressure reactor using 10% Pd/C (0.3 g) at 4 atm at room temperature. After 24 h, TLC showed the formation of a more polar compound (R_f 0.41; solvent D). The mixture was filtered and concentrated to give **5**, isolated as a colourless foam (0.5 g, 82%), m.p. 210–212°C (dec.), $[\alpha]_D$ +29° (*c* 1.1, methanol). Anal. calcd. for $C_7H_{13}O_9SNa$ (296.23): C 28.38, H 4.42, S 10.82; found: C 27.94, H 4.80, S 10.47%. For ^{1}H- and ^{13}C-NMR data, see Tables I and II, respectively.

Methyl 2,4,6-tri-O-acetyl-3-O-benzyl-α-D-mannopyranoside (**6**)

Compound **2** (1.56 g, 5.5 mmol) in pyridine (10 ml) was acetylated with acetic anhydride (5 ml) for 16 h at room temperature. The mixture was then poured into ice-water, and extracted with dichloromethane (3 × 20 ml). The combined organic phase was washed with aqueous saturated sodium hydrogen carbonate and water, dried (Na_2SO_4), and concentrated to yield **6**, isolated as a syrup (2.1 g, 93%), $[\alpha]_D$ +7° (*c* 1.0, ethanol) (lit.[17] $[\alpha]_D$ −1.0° in chloroform), R_f 0.60 (solvent C). Anal. calcd. for $C_{20}H_{26}O_9$ (410.42): C 58.53, H 6.39; found: C 58.34, H 6.19%. EI-MS data: *m/z* 410 (0.1%, M), 379 (4.3%, M−OMe), 367 (3.0%, M−Ac), 91 (100%, $C_6H_5CH_2$). ^{1}H-NMR data ($CDCl_3$): δ 7.25–7.33 (m, 5H, $C_6H_5CH_2O$), 5.346 (dd, 1H, H-2), 5.219 (t, 1H, H-4), 4.729 (d, 1H, H-1), 4.641 and 4.411 (2d, 2H, $C_6H_5CH_2O$), 4.241 (dd, 1H, H-6'), 4.105 (dd, 1H, H-6), 3.852 (dd, 1H, H-3), 3.848 (m, 1H, H-5), 3.373 (s, 3H, OMe), 2.147, 2.091, and 1.999 (3s, each 3H, 3 Ac), $J_{1,2}$ 1.8, $J_{2,3}$ 3.5, $J_{3,4}$ 9.6, $J_{4,5}$ 9.9, $J_{5,6}$ 2.4, $J_{5,6'}$ 5.7, $J_{6,6'}$ 12.2 Hz.

Methyl α-D-mannopyranoside 3-(sodium sulfate) (**9**)

A solution of **6** (1.76 g, 4.3 mmol) in methanol (40 ml) was hydrogenolysed using 10% Pd/C (1.5 g) at 4 atm at room temperature. After 20 h, the mixture was filtered and concentrated, yielding **7**, isolated as a syrup (1.22 g, 89%), sufficiently pure (TLC in solvent C; R_f 0.24) for use in the next step. Pyridine–sulfur-trioxide (1.1 g, 6.5 mmol) was added to a solution of **7** (1.0 g, 3.12 mmol) in dry pyridine (30 ml), and worked-up as described for **4**. The crude product **8** (R_f 0.56; solvent A) was suspended in methanolic 0.5M

sodium methoxide (4 ml). After 2 h at room temperature, deacetylation was complete on TLC (R_f 0.43; solvent D). The pH was adjusted to 4 by adding Dowex-50 (H^+) resin, and the mixture was filtered, neutralized with aqueous saturated sodium hydrogen carbonate, and concentrated. The residue was purified by column chromatography on Kieselgel 60 (30 g) (dichloromethane/methanol 2/1, v/v) to give **9** (0.70 g, 76%), m.p. 246–248°C (dec.), $[\alpha]_D$ +55° (*c* 0.9, methanol). Anal. calcd. for $C_7H_{13}O_9SNa$ (296.23): C 28.38, H 4.42, S 10.82; found: C 28.56, H 4.35, S 10.43%. For ^{1}H- and ^{13}C-NMR data, see Tables I and II, respectively.

Methyl α-D-mannopyranoside 4-(sodium sulfate) (**13**)

Chlorotriphenylmethane (3.0 g, 11 mmol) was added to a solution of **10**[10] (2.35 g, 10 mmol) in dry pyridine (30 ml), and the mixture was stirred for 24 h at room temperature. The solution was then poured into ice-water, the white solid material collected and extracted with acetone. The extract was filtered, concentrated, and the residue was purified by column chromatography on Kieselgel 60 (100 g) (dichloromethane). After elution of triphenylmethanol, **11** was obtained, isolated as a colourless foam (3.9 g, 81%), m.p. 59–61°C, $[\alpha]_D$ +9° (*c* 1.0, ethanol) (litt.[18] $[\alpha]_D$ +11° in ethanol), R_f 0.38 (dichloromethane). Anal. calcd. for $C_{29}H_{32}O_6$ (476.57): C 73.09, H 6.77; found: C 72.83, H 6.66%. ^{1}H-NMR data ($CDCl_3$): δ 7.15–7.56 (m, 15H, $(C_6H_5)_3CO$), 4.917 (s, 1H, H-1), 3.414 (s, 3H, OMe), 1.480 and 1.329 (2s, each 3H, CMe_2).

A solution of **11** (2.4 g, 5 mmol) in dry pyridine (40 ml) was mixed with pyridine–sulfur-trioxide (1.63 g, 10 mmol) and heated for 2 h at 65–70°C. After the addition of water (40 ml), the solution was kept for 1 h at room temperature, and the pH was then adjusted to 9 by adding aqueous saturated barium hydroxide. Precipitating barium sulfate was removed, the filtrate concentrated, and traces of pyridine eliminated by co-concentration with water. The crude compound **12** was dissolved in ethanol (25 ml) and aqueous 5% acetic acid (25 ml), and the solution was heated at 75–80°C. After 4 h, TLC showed the reaction to be complete (R_f 0.46; solvent D), and the mixture was co-concentrated with ethanol, triphenylmethanol was removed, and the filtrate concentrated. The residue in aqueous ethanol was brought to pH 4 by adding Dowex-50 (H^+) resin and, after filtration, the solution was neutralized with aqueous saturated sodium hydrogen carbonate, and concentrated. The residue was purified by column chromatography on Kieselgel 60 (60 g) (dichloromethane/methanol 2/1, v/v) to give a small amount of triphenylmethanol and then **13** (0.9 g, 64%), m.p. 228–230°C (dec.), $[\alpha]_D$ +54° (*c* 1.4, methanol). Anal. calcd. for $C_7H_{13}O_9SNa$ (296.23): C 28.38, H 4.42, S 10.82; found: C 27.87, H 4.25, S 11.01%. For ^{1}H- and ^{13}C-NMR data, see Tables I and II, respectively.

Methyl 2,3,4-tri-O-benzyl-α-D-mannopyranoside 6-(sodium sulfate) (**17**)

A solution of **14**[11] (1.76 g, 4 mmol) and benzyl bromide (7.2 ml, 60 mmol) in dry tetrahydrofuran (35 ml) was added dropwise to sodium hydride (0.8 g, 20 mmol) (washed with dry hexane), and the mixture was heated for 20 h at 55–60°C with stirring. The solution was then concentrated and the residue purified by column chromatography on Kieselgel 60 (80 g) (dichloromethane/hexane 2/1, v/v), yielding first the excess of benzyl bromide, followed by **15** (R_f 0.60; solvent E). Crystallization from aqueous ethanol gave **15** (2.1 g, 74%), m.p. 114–115°C (lit.[12] m.p. 116–118°C), $[\alpha]_D$ +19° (*c* 1.3, chloroform) (lit.[12] $[\alpha]_D$ +20° in chloroform). Anal. calcd. for $C_{47}H_{46}O_6$ (706.88): C 79.86, H 6.56; found: C 80.00, H 6.55%. ^{1}H-NMR data ($CDCl_3$): δ 6.88–7.53 (m, 30H, $(C_6H_5)_3CO$ and 3 $C_6H_5CH_2O$), 4.817 (bs, 1H, H-1), 4.834 (d), 4.726 (d), 4.714 (d), 4.638 (s), and 4.269 (d) (6H, 3 $C_6H_5CH_2O$), 4.010 (t, 1H, H-4), 3.870 (dd, 1H, H-3), 3.815 (dd, 1H, H-2), 3.769 (m, 1H, H-5), 3.511 (dd, 1H, H-6), 3.383 (s, 3H, OMe), 3.270 (dd, 1H, H-6′), $J_{1,2}$ 1.8, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3, $J_{4,5}$ 9.8, $J_{5,6}$ 1.7, $J_{5,6'}$ 5.3, $J_{6,6'}$ 9.8 Hz.

A solution of **15** (1.90 g, 1.42 mmol) in ethanol (30 ml) was kept in aqueous 5% acetic acid (30 ml) for 5 h at 70–80°C. TLC showed the detritylation to be complete and a new product was formed (R_f 0.55; solvent C). The solution was co-concentrated with ethanol, and the residue was purified by column chromatography on Kieselgel 60 (50 g) (dichloromethane) to yield **16**, isolated as a syrup (1.15 g, 92%), $[\alpha]_D$ +30° (*c* 1.2, chloroform) (lit.[12] $[\alpha]_D$ +30° in chloroform). Anal. calcd. for $C_{28}H_{32}O_6$ (464.56): C 72.39, H 6.94; found: C 72.02, H 6.81%. EI-MS data: *m/z* 433 (0.1%, M−OMe), 373 (22.0%, M−$C_6H_5CH_2$), 107 (12.0%, $C_6H_5CH_2O$), 91 (100%, $C_6H_5CH_2$), 77 (16.0%, C_6H_5). ^{1}H-NMR data ($CDCl_3$): δ 7.26–7.35 (m, 15H, 3 $C_6H_5CH_2O$), 4.706 (bs, 1H, H-1), 4.941 (d), 4.784 (d), 4.686 (d), 4.648 (d), and 4.635 (s) (6H, 3 $C_6H_5CH_2O$), 3.303 (s, 3H, OMe).

Conventional treatment of **16** (1.1 g, 2.4 mmol) with the pyridine–sulfur-trioxide complex (0.70 g, 4.3 mmol) in dry pyridine (20 ml) gave crude methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 6-(barium sulfate), which was dissolved in aqueous ethanol. The solution was adjusted to pH 4 by adding Dowex-50 (H^+) resin, then filtered, neutralized with aqueous saturated sodium hydrogen carbonate, and concentrated. The residue was purified by column chromatography on Kieselgel 60 (50 g) (dichloromethane/methanol 7/1, v/v) to give **17** (1.2 g, 89%), m.p. 120–122°C (dec.), $[\alpha]_D$ +7° (*c* 1.2, ethanol). Anal. calcd. for $C_{28}H_{31}O_9SNa \cdot H_2O$ (584.62): C 57.53, H 5.69; found: C 57.91, H 5.83%. ^{1}H-NMR data ($CDCl_3$): δ 7.12–7.31 (m, 15H, 3 $C_6H_5CH_2O$), 3.150 (s, 3H, OMe).

Methyl α-D-mannopyranoside 6-(sodium sulfate) (**18**)

A solution of **17** (1.0 g, 1.8 mmol) in methanol (50 ml) was hydrogenolysed using 10% Pd/C (0.3 g) at 4 atm at room temperature. After 20 h, TLC showed one main product having R_f 0.34 (solvent D). The mixture was then filtered, concentrated, and the residue was purified by column chromatography on Kieselgel 60 (30 g) (dichloromethane/methanol 2/1, v/v) to yield **18**, isolated as a colourless foam (0.4 g, 77%), m.p. 158–160°C (dec.), $[\alpha]_D$ +54° (*c* 1.2, methanol). Anal. calcd. for $C_7H_{13}O_9SNa$ (296.23): C 28.38, H 4.42, S 10.82; found: C 28.14, H 4.59, S 10.31. For ^{1}H- and ^{13}C-NMR data, see Tables I and II, respectively.

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