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Conformational flexibility in highly sulfated β-D-glucopyranoside derivatives

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Abstract

Triggered by findings on heparin-like disaccharides, the conformation of sulfated glucopyranosides was investigated. Sodium (methyl 2,3,4-tri-*O*-sulfonato-β-D-glucopyranosid)uronate tetrasodium salt is in a conformational equilibrium, to which a non-chair conformation contributes. The same is true for methyl (methyl 2,3,4-tri-*O*-sulfonato-β-D-glucopyranosid)uronate trisodium salt, methyl 2,3,4,6-tetra-*O*-sulfonato-β-D-glucopyranoside tetrasodium salt, and octa-*O*-sulfonato-β,β-trehalose octasodium salt, with less obvious non-chair contributions. The effect is charge related. The conformational effect, which does not occur in analogous α-D-glucopyranoside derivatives, is discussed in terms of the anomeric effect.

Keywords: β-D-Glucopyranosides; Glucuronic acids; Carbohydrate sulfates; Conformational analysis; Conformational flexibility

1. Introduction

The conformational flexibility of heparin and related glycosaminoglycans was suggested to be important for their binding properties and biological activities [1], the responsible monosaccharide unit being L-iduronic acid. While the glucosamine and glucuronic acid units in glycosaminoglycans have until now been described in the regular $^4C_1$ conformation, L-iduronic acid may occur in $^1C_4$, $^2S_0$, and $^4C_1$ conformations [2]. The conformational equilibrium is influenced by substitution of the L-iduronic acid pyranose ring, notably sulfation [3] in position 2, as well as by the substitution pattern in the neighbouring rings [4,5] or even at remote sites [6], and by the ionic strength of the solvent [3].

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We have already reported [7] that, according to the NMR spectra, the glucuronic acid residues of the highly sulfated heparin-related model disaccharides **1** and **2** do not occur solely in chair conformations but that other conformations, such as $^{3,0}B$, contribute to the conformational equilibrium. Similar highly sulfated monosaccharide units are found in natural glycosaminoglycans such as heparin, in chemically modified polysaccharides (so-called "oversulfated", "supersulfated", or "extrasulfated" saccharides), and in biologically active sulfated oligosaccharides [8,9]. While a number of low sulfated saccharides [10], and particularly glycosaminoglycan-related sulfated oligosaccharides [11], have been described, highly sulfated or persulfated saccharides have received little attention. We now report on conformational investigations of simple model compounds and, in particular, on the unexpected conformational differences between α-D- and β-D-glucopyranoside derivatives.

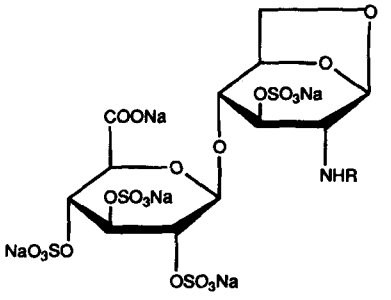
2. Results and discussion

To determine whether the unusual conformations found in the glucuronic acid moieties of the disaccharides **1** and **2** depend on the type of glycoside, a simple monosaccharide analogue was prepared. Sodium (methyl 2,3,4-tri-*O*-sulfonato-β-D-glucopyranosid)uronate tetrasodium salt (**4**) was obtained by sulfation of the known [12] unsulfated precursor methyl β-D-glucopyranosiduronic acid (**3**), using the sulfur trioxide–trimethylamine complex. A comparison of ^{1}H NMR data (Table 1) reveals that the conformations in water of **4** and the respective uronic acid moieties of **1** and **2** are

Table 1
Selected coupling constants [Hz]

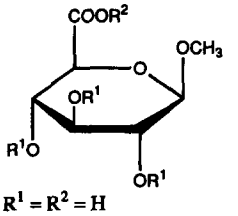
Compound	Solvent	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{2,4}$
1 [a]	D_2O	6.4	3.2	4.0	2.8	
2 [a]	D_2O	6.7	4.1	4.9	4.2	
4	D_2O	5.8	3.0	4.8	3.2	0.8
4	Me_2SO-d_6	5.0	≤1.5	2	2	
5	D_2O	6.1	3.0	5.0	4.0	0.5
7	D_2O	5.8	4.2	5.8	4.5	
7	Me_2SO-d_6	3.7	1	3.5	3.3	1
9	D_2O	5.7	5.2	6.8	6.0	>0
9	Me_2SO-d_6	3.5	≤1.5	2.6	3.3	
10	D_2O	7.9	9.2	8.1	9.8	
14	D_2O	5.8	4.8	6.1	6.2	
18 [b]	D_2O	4.1	3.9	5.0	7.0	
12	D_2O	3.6	9.8	8.8	—	
12	Me_2SO-d_6	3.4	9.8	8.3	9.7	
16	D_2O	3.7	10.0	8.7	10.2	
18 [c]	D_2O	3.5	8.8	8.2	8.5	

[a] Data for the glucuronate moiety.
[b] Data for the β-D-glucopyranoside moiety.
[c] Data for the α-D-glucopyranoside moiety.



1 R = SO_3Na

2 R = Ac



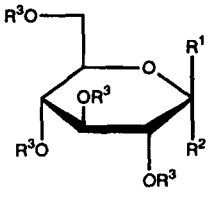
3 $R^1 = R^2 = H$

4 $R^1 = SO_3Na$, $R^2 = Na$

5 $R^1 = SO_3K$, $R^2 = H$

6 $R^1 = H$, $R^2 = CH_3$

7 $R^1 = SO_3Na$, $R^2 = CH_3$



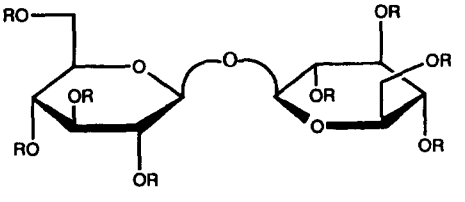
8 $R^1 = OCH_3$, $R^2 = R^3 = H$

9 $R^1 = OCH_3$, $R^2 = H$, $R^3 = SO_3Na$

10 $R^1 = OCH_3$, $R^2 = H$, $R^3 = SO_2NH_2$

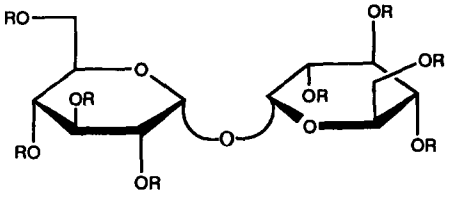
11 $R^1 = H$, $R^2 = OCH_3$, $R^3 = H$

12 $R^1 = H$, $R^2 = OCH_3$, $R^3 = SO_3Na$



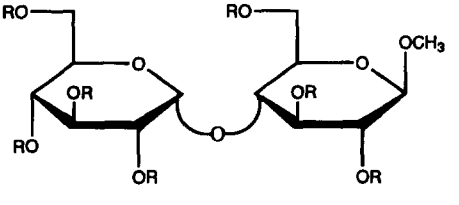
13 R = H

14 R = SO_3Na



15 R = H

16 R = SO_3Na



17 R = H

18 R = SO_3Na

comparable, indicating very similar conformational equilibria. The vicinal coupling constants in the ring are neither all large ($J \geq 8$ Hz) as expected for a $^4C_1$ conformation nor all small as expected for the inverted $^1C_4$ conformation, but the occurrence of

coupling constants of significantly different size ($J_{1,2}$ 5.8, $J_{4,5}$ 3.2 Hz) indicates the involvement of a non-chair conformation. The long-range coupling $^4J_{2,4}$ 0.8 Hz points to the W-arrangement of the H–C-2–C-3–C-4–H segment with axial orientation of the sulfate groups. Passing a solution of **4** over an ion-exchange column furnished the uronic acid **5**, as shown by mass spectrometry (m/z 513) and by the upfield shift (0.2 ppm) of H-5 in the ^{1}H NMR spectrum. The NMR spectroscopic and thus conformational characteristics of **5** are very similar to **4**, with only a slight difference in $J_{4,5}$ ($\Delta J = 0.8$ Hz).

Sulfation of known [13] methyl (methyl β-D-glucopyranosid)uronate (**6**), the synthetic precursor of glucuronic acid **3**, furnished **7**. Inspection of the NMR data of **7** in water (Table 1) reveals that all coupling constants are of medium size (J 4.2–5.8 Hz), so that the occurrence of a non-chair conformation is not as obvious. However, in the case of a chair–chair equilibrium the coupling constants $J_{2,3}$ and $J_{4,5}$ would not be expected to be smaller than $J_{1,2}$ and $J_{3,4}$ (cf. coupling constants for compound **10**). The potassium salt analogue of **7** gave, within experimental error, NMR data identical to those of the sodium salt (data not shown). Thus, the C-5 substituent is not necessarily a carboxylic acid to obtain a conformational effect, but may be a non-charged group such as a carboxylic ester.

Methyl 2,3,4,6-tetra-*O*-sulfonato-β-D-glucopyranoside (**9**) was obtained from methyl β-D-glucopyranoside (**8**) with the sulfur trioxide–base complex. While, according to earlier publications [14], only low sulfated saccharides, mainly monosulfates, could be prepared in pyridine as solvent, the use of *N*,*N*-dimethylformamide permitted persulfation of this and the other saccharides. The vicinal coupling constants of **9** are similar to, but slightly larger than, those of methyl glucosiduronate **7**, of medium size (J 5.2–6.8 Hz) so that the contribution of a $^4C_1$ conformation seems to be more important. In the ^{1}H NMR spectrum of **9** no long-range coupling is visible, but the COSY spectrum clearly reveals a strong H-2–H-4 cross-peak (clear assignments were made through double resonance experiments saturating H-5 and H-4), pointing to the existence of a W-arrangement of H-2–H-4 similar to that in **4**.

To investigate whether these conformational effects are related to steric or charge effects, the sulfamoyl derivative of **8** was prepared because the sulfamoyl group can be viewed as a non-charged sulfate mimetic. Thus, reaction of the alcoholate of **8** with sulfamoyl chloride [15] gave the tetra-*O*-aminosulfonyl derivative **10**, a non-charged analogue of **9**. In the ^{1}H NMR spectrum, the effected protons H-2, H-3, H-4, H-6a, and H-6b of **10** were strongly shifted to low field, even in comparison to the sulfated derivative **9** ($\Delta\delta = 0.13$–0.29 ppm), demonstrating the electronic similarity of sulfamoyl and sulfate groups. According to the ^{1}H NMR data, compound **10** exists in a $^4C_1$ conformation, and the conformational effects described above are without doubt related to the charge of sulfate groups. It thus seems that, due to charge interactions, the proximity of neighbouring equatorially oriented sulfate groups cause a conformational change to solution conformations which are usually of higher energy but which allow a bigger distance between sulfates.

Most surprising was the conformational investigation of methyl 2,3,4,6-tetra-*O*-sulfonato-α-D-glucopyranoside tetrasodium salt (**12**), which was obtained by standard sulfation of methyl α-D-glucopyranoside (**11**). The ^{1}H NMR data show that compound

12 occurs exclusively in the normal $^4C_1$ conformation. One explanation for this behaviour may be that the anomeric effect [16] of the *O*-methyl group of **12** can counterbalance the charge interaction of neighbouring equatorially oriented sulfates. This charge interaction must be smaller than the anomeric effect of the *O*-methyl group, which was estimated to be 5.8 kJ mol^{-1} for hexopyranoses [17]. In the β-D-glucoside series, the normal $^4C_1$ conformation obviously is destabilized by charge interactions and, in addition, the inverted $^1C_4$ (or a related twist–boat) conformation with an axial or quasi-axial *O*-methyl group would be favoured by the anomeric effect. This explanation on the role of the anomeric effect is in keeping with the observed solvent effect. Usually the anomeric effect increases with decreasing dielectric constant of the solvent [18]; in dimethyl sulfoxide it is stronger than in water. Accordingly, in the β-D-glycoside series, the conformational effect is more pronounced in dimethyl sulfoxide (Table 1) than in water.

The different behaviour of sulfated β-D-linked and α-D-linked saccharides was confirmed with trehalose derivatives. Sulfation of β,β-trehalose (**13**), prepared according to Cook et al. [19], yielded the octa-*O*-sulfonato derivative **14**. The ^{1}H NMR spectrum of this compound is monosaccharidic due to the C_2 symmetry of the molecule and shows, within the detection limits, practically the same coupling constants as the methyl β-D-glucoside analogue **9**, demonstrating a diversion from the normal $^4C_1$ conformation. In contrast, octa-*O*-sulfonato-α,α-trehalose octasodium salt (**16**), synthesized from α,α-trehalose (**15**) by standard sulfation, does not show a conformational effect but occurs in the $^4C_1$ conformation as the α-D-glucoside analogue **12**.

A very interesting example is methyl hepta-*O*-sulfonato-β-maltoside heptasodium salt (**18**) containing a β-D-glucopyranoside and an α-D-glucopyranoside in one molecule. The known [19] methyl β-maltoside (**17**) was sulfated, and the product was converted into the sodium as well as the potassium salt. Although the ^{1}H NMR spectra of both compounds were similar, signals of the heptapotassium salt **18** were better resolved and could be fully assigned with the help of 1D TOCSY [20]. The pyranose rings were readily discriminated by a ROESY experiment, showing a strong inter-ring ROE (H-4) upon conversion of H-1′. As expected, the α-D-glucopyranoside moiety of **18** is in a normal $^4C_1$ conformation which may be slightly distorted ($J_{4',5'}$ 8.5 Hz). For the β-D-glucopyranoside moiety, again a conformational equilibrium is found which is similar to the fully sulfated methyl β-D-glucopyranoside, although the 4-*O*-sulfonato group is replaced by a (charged) pyranosyl substituent.

If it is correct for glycosaminoglycans that the conformational flexibility of iduronic acid is linked to biological activity, then sulfated β-D-linked glucuronic acid or glucose moieties may substitute iduronic acid. In this context, it is interesting to note the biological data of sulfated oligosaccharides recently published by us [9]: highly sulfated β-maltosyl-(1 → 4)-α,α-trehalose has high heparin-like smooth muscle cell antiproliferative activity, whereas the analogous all-α-D-linked sulfated α-maltosyl-(1 → 4)-α,α-trehalose is virtually inactive.

3. Experimental

General.—Solvents and reagents were bought from Fluka. Solutions were evaporated below 50°C on a Büchi rotary evaporator. Qualitative TLC was performed with

precoated Silica Gel 60F-254 plates (Merck); compounds were detected by UV light (254 nm) and spraying with a 10% solution of sulfuric acid in MeOH followed by charring. Melting points were determined with a Büchi 510 capillary apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 spectrometer in a 1 dm cell. ^{1}H NMR spectra were recorded on Bruker AC 250 (250 MHz) and AM-400 (400 MHz) spectrometers with Aspect 3000 and process controller. Chemical shifts are given in ppm relative to tetramethylsilane or sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate as internal standard. Mass spectra were recorded on API III Sciex, Perkin–Elmer (ionspray) equipment, for sulfated compounds reconstructs were determined according to the Fenn method [21].

General procedure for sulfation.—A solution of carbohydrate compound in dry DMF (6 mL/g) was reacted with SO_3–Me_3N complex (2 equiv per OH group of the carbohydrate compound) for 17 h at 70°C. The cooled solution was then treated with an excess of 10% aq sodium acetate solution and evaporated. The residue was taken up in water and evaporated several times. The crude product was desalted by gel filtration over a Sephadex LH 20 column using doubly-distilled water as an eluent.

(Methyl β-D-glucopyranosid)uronic acid (**3**).—Prepared according to [12]. Mp 78–80°C [12]: mp 68–70°C; ^{1}H NMR (D_2O, 250 MHz [22]): δ 4.42 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.93 (d, 1 H, $J_{4,5}$ 9.7 Hz, H-5), 3.56 (s, 3 H, OCH_3), 3.54 (m, 2 H, H-3, H-4), 3.31 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2).

Sodium (methyl 2,3,4-tri-O-sulfonato-β-D-glucopyranosid)uronate trisodium salt (**4**). —Sulfation of **3** (300 mg, 1.44 mmol), according to the general procedure, furnished **4** (162 mg, 22%) as a colourless solid, $[\alpha]_D^{20}$ −13.0° (*c* 0.1, water); MS (ionspray): *m/z* 513 (8%, [M − Na]), 491 (30%, [M + H − 2Na]); ^{1}H NMR (D_2O, 250 MHz): δ 5.08 (ddd ~ dt, 1 H, H-4), 4.85 (dd, 1 H, $J_{3,4}$ 4.8 Hz, H-3), 4.80 (d, 1 H,$J_{1,2}$ 5.8 Hz, H-1), 4.52 (d, 1 H, $J_{4,5}$ 3.2 Hz, H-5), 4.43 (ddd, 1 H, $J_{2,3}$ 3.0, $J_{2,4}$ 0.8 Hz, H-2), 3.55 (s, 3 H, OCH_3); ^{1}H NMR (Me_2SO-d_6, 400 MHz; COSY): δ 4.94 (br s, 1 H, $J_{3,4} \approx 2$ Hz, H-4), 4.57 (br s, 1 H, H-3), 4.565 (d, 1 H, H-1, assignments of H-1 and H-3 might be interchanged, here based on $J_{1,2} > J_{2,3}$ in comparison to the D_2O spectrum), 4.42 (br d, 1 H, $J_{4,5}$ ~ 2 Hz, H-5), 4.32 (d, 1 H, $J_{1,2}$ 5.0, $J_{2,3} \leq 1.5$ Hz, H-1), 3.35 (s, 3 H, OCH_3). Anal. Calcd for $C_7H_8O_6S_3Na_4$: S, 17.95. Found: S, 17.45.

(Methyl 2,3,4-tri-O-sulfonato-β-D-glucopyranosid)uronic acid tripotassium salt (**5**). —A concd aq solution of **4** (30 mg, 0.056 mmol) was passed over a column of Sephadex C-25 (K^+/H^+) using doubly-distilled water as an eluent. The main product fractions were pooled and lyophilized to obtain **5** (27 mg, 87%) as colourless solid; MS (ionspray): *m/z* 561.99 (reconstructed M); ^{1}H NMR (D_2O, 250 MHz): δ 5.02 (ddd ~ br. t, 1 H, H-4), 4.87 (dd, 1 H, $J_{3,4}$ 5.0 Hz, H-3), 4.77 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1), 4.44 (ddd, 1 H, $J_{2,3}$ 3.0, $J_{2,4}$ ~ 0.5 Hz, H-2), 4.32 (d, 1 H, $J_{4,5}$ 4.0 Hz, H-5), 3.56 (s, 3 H, OCH_3). Anal. Calcd for $C_7H_9O_{16}S_3K_3$: S, 17.10. Found: S, 17.01.

Methyl (methyl 2,3,4-tri-O-sulfonato-β-D-glucopyranosid)uronate trisodium salt (**7**). —Sulfation of **6** (680 mg, 3.04 mmol), according to the general procedure, furnished **7** (1.29 mg, 86%) as a colourless solid, $[\alpha]_D^{20}$ −11.0° (*c* 0.2, water); $[\alpha]_{365}^{20}$ −33.5° (*c* 0.2, water); MS (ionspray); *m/z* 505 (15%, $[M - Na]^-$), 483 (30%, $[M + H - 2Na]^-$), 461 (100%, $[M + 2H–3Na]^-$); ^{1}H NMR (D_2O, 400 MHz): δ 5.01 (dd ~ t, 1 H, H-4, assignment proven by selective irradiation on H-2), 4.86 (d, 1 H, $J_{1,2}$ 5.8 Hz, H-1), 4.80

(dd, 1 H, $J_{3,4}$ 5.8 Hz, H-3), 4.71 (d, 1 H, $J_{4,5}$ 4.5 Hz, H-5), 4.38 (dd, 1 H, $J_{2,3}$ 4.2 Hz, H-2), 3.82 (s, 3 H, OCH_3 ester), 3.56 (s, 3 H, 1-OCH_3); ^{1}H NMR (Me_2SO-d_6, 400 MHz; COSY): δ 4.93 (dd ~ t, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 4.77 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.61 (d, 1 H, $J_{4,5}$ 3.3 Hz, H-5), 4.47 (ddd ~ br t, 1 H, $J_{1,3} \leq 1.5$ Hz, H-3), 4.30 (ddd ~ br dt, 1 H, $J_{2,3} \approx 1$, $J_{2,4} \approx 1$ Hz, H-2), 3.59 (s, 3 H, OCH_3 ester), 3.38 (s, 3 H, 1-OCH_3); ^{13}C NMR (D_2O, 100 MHz; hetero-COSY): δ 173.15 (*C*OO), 103.41 (C-1), 79.04 (C-2), 77.80 (C-3), 76.71 (C-5), 75.98 (C-4), 60.37 (OCH_3), 56.16 ($COOCH_3$). Anal. Calcd for $C_8H_{11}O_{16}S_3Na_3$: S, 18.21. Found: S, 18.19.

*Methyl 2,3,4,6-tetra-*O*-sulfonato-β-*D*-glucopyranoside tetrasodium salt* (**9**).—Sulfation of **8** (5.0 g, 24.6 mmol) according to the general procedure furnished, after a second gel filtration of the main fraction from the first separation, **9** (6.1 g, 41%) as a colourless solid, $[\alpha]_{365}^{20}$ −5.5° (*c* 0.2, water); MS (ionspray): *m*/*z* 602 (100%, reconstructed M), 580 (30%, $[M + H - 2Na]^-$); ^{1}H NMR (D_2O, 400 MHz): δ 4.78 (d, 1 H, H-1), 4.74 (dd ~ t, 1 H, $J_{2,3}$ 5.2 Hz, H-3), 4.51 (dd ~ t, 1 H, $J_{3,4}$ 6.8 Hz, H-4), 4.46 (dd, 1 H, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 10.8 Hz, H-6a), 4.41 (dd ~ t, 1 H, $J_{1,2}$ 5.7 Hz, H-2), 4.24 (dd, 1 H, $J_{5,6b}$ 8.0 Hz, H-6b), 4.15 (ddd ~ dt, 1 H, $J_{4,5}$ 6.0 Hz, H-5), 3.57 (s, 3 H, 1-OCH_3); ^{1}H NMR (Me_2SO-d_6, 400 MHz; COSY); δ 4.60 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.53 (dd ~ br s, 1 H, H-3), 4.32 (dd ~ t, 1 H, $J_{3,4}$ 2.6 Hz, H-4), 4.28 (dd ~ br d, 1 H, $J_{2,3} \leq 1.5$ Hz, H-2), 4.04 (ddd ~ dt, 1 H, $J_{4,5}$ 3.3 Hz, H-5), 3.90 (dd ~ t, 1 H, H-6a), 3.87 (dd, 1 H, $J_{5,6b}$ 3.5, $J_{6a,6b}$ 10.7 Hz, H-6b), 3.32 (s, 3 H, 1-OCH_3); ^{13}C NMR (D_2O, 100 MHz; hetero-COSY): δ 104.04 (C-1), 78.92 (C-2), 78.65 (C-3), 76.07 (C-5), 75.51 (C-4), 70.66 (C-6), 60.05 (OCH_3). Anal. Calcd, for $C_7H_{10}O_{18}S_4Na_4$: S, 21.29. Found: S, 21.02.

*Methyl 2,3,4,6-tetra-*O*-aminosulfonyl-β-*D*-glucopyranoside* (**10**).—A solution of **8** (400 mg, 2.1 mmol) in DMF (3 mL) was added to a suspension of NaH (302 mg, 12.6 mmol) in DMF (6 mL). While stirring, sulfamoyl chloride (1.10 g, 9.5 mmol) was added in portions. After 4 days the reaction mixture was poured into an aq $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with water, dried over sodium sulfate, and concentrated. The residue was chromatographed twice over silica gel using 10:3:1 EtOAc–MeOH–water and 4:1 EtOAc–toluene as eluents to give **10** (90 mg, 8%) as a colourless solid; $[\alpha]_D^{20}$ −4.0° (*c* 0.1, water); $[\alpha]_{365}^{20}$ −24° (*c* 0.1, water); MS (ionspray): *m*/*z* 528 (100%, $[M + NH_4]^+$); ^{1}H NMR (D_2O, 250 MHz): δ 5.01 (dd ~ t, 1 H, $J_{3,4}$ 8.1 Hz, H-3), 4.80 (m, 2 H, H-1, H-4), 4.61 (dd, 1 H, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.54 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.2 Hz, H-2), 4.13 (ddd, 1 H, $J_{4,5}$ 9.8 Hz, H-5), 3.62 (s, 3 H, OCH_3). Anal. Calcd for $C_7H_{18}O_{14}N_4S_4$: S, 25.12. Found: S, 25.08.

*Methyl 2,3,4,6-tetra-*O*-sulfonato-α-*D*-glucopyranoside tetrasodium salt* (**12**).—Sulfation of **11** (2.0 g, 20 mmol) according to the general procedure furnished, after chromatography on a Sephadex SP C25 (Na^+) ion-exchange column of the main fraction from the first separation, **12** (2.1 g, 35%) as a colourless solid, $[\alpha]_{365}^{20}$ +69.6° (*c* 0.2, water); MS (ionspray): *m*/*z* 602 (100%, reconstructed M), 580 (30%, $[M + H - 2Na]^-$); ^{1}H NMR (D_2O, 400 MHz): δ 5.18 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.67 (dd ~ t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.60 (m_c, 1 H, H-6a), 4.12 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 4.31 (m_c, 1 H, H-4), 4.18–4.11 (m, 2 H, H-5, H-6b), 3.48 (s, 3 H, OCH_3); ^{1}H NMR (Me_2SO-d_6, 400 MHz): δ 4.96 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.43 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 4.40 (dd, 1 H, $J_{5,6a} \approx 10$ Hz, H-6a), 4.02 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 3.83 (dd ~ t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.72 (ddd ~ br t, 1 H, H-5), 3.55 (dd ~ t, 1 H, $J_{5,6b}$ 9.0, $J_{6a,6b}$ 11.0 Hz, H-6b;

assignments of H-4 and H-6b were made according to roof effects), 3.27 (s, 3 H, 1-OCH_3). Anal. Calcd for $C_7H_{10}O_{18}S_4Na_4$: S, 21.29. Found: S, 20.85.

Octa-O-sulfonato-β,β-trehalose octasodium salt (**14**).—Sulfation of **3** (342 mg, 1 mmol) according to the general procedure furnished **14** (215 mg, 20%) as a colourless solid; $[\alpha]_D^{20}$ −11.0° (*c* 0.2, water); ^{1}H NMR (D_2O, 400 MHz): δ 5.24 (d, 1 H, $J_{1,2}$ 5.8 Hz, H-1), 4.81 (dd, 1 H, $J_{3,4}$ 6.1 Hz, H-3), 4.62 (dd ~ t, 1 H, $J_{4,5}$ 6.2 Hz, H-4), 4.41 (dd ~ t, 1 H, $J_{2,3}$ 4.8 Hz, H-2), 4.47 (dd, 1 H, $J_{6a,6b}$ 9.5 Hz, H-6a), 4.23 (dd, 1 H, $J_{5,6b} \approx 7$ Hz, H-6a), 4.20 (ddd ~ dt, 1 H, $J_{5,6a}$ 2.5 Hz, H-5). Anal. Calcd for $C_{12}H_{14}O_{35}S_8Na_8$: S, 22.14. Found: S, 21.74.

Octa-O-sulfonato-α,α-trehalose octasodium salt (**16**).—Sulfation of trehalose dihydrate (**15**, 2.0 g, 5.29 mmol) according to the general procedure furnished **16** (3.27 g, 53%) as a colourless solid; $[\alpha]_D^{20}$ +70.2° (*c* 0.2, water); ^{1}H NMR (D_2O, 400 MHz): δ 5.58 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.77 (dd ~ t, 1 H, $J_{3,4}$ 8.7 Hz, H-3), 4.50 (dd ~ t, 1 H, $J_{4,5}$ 10.2 Hz, H-5), 4.45 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.37 (dd, 1 H, $J_{5,6a}$ 2.6 Hz, H-6a), 4.33 (dd, 1 H, $J_{5,6b}$ 2.5, $J_{6a,6b}$ 11.1 Hz, H-6b), 4.30 (ddd ~ dt, 1 H, H-5). Anal. Calcd for $C_{12}H_{14}O_{35}S_8Na_8$: S, 22.14. Found: S, 22.08.

Methyl hepta-O-sulfonato-β-maltoside heptapotassium salt (**18**).—Methyl β-maltoside [19] (**17**, 490 mg, 1.38 mmol) was sulfated according to the general procedure but using potassium acetate instead of sodium acetate. Rechromatography of product fractions on Sephadex LH 20 and then on Sepharose C25 (K^+) furnished **18** (1.06 g, 65%) as a colourless solid; $[\alpha]_D^{20}$ +36.0° (*c* 0.2, water); MS (ionspray): *m/z* 1183 (100%, reconstructed M); ^{1}H NMR (D_2O, 400 MHz; 1D TOCSY, H,H-COSY, ROESY): δ 5.61 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.91 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1'), 4.814 (dd ~ t, 1 H, H-3'), 4.81 (dd ~ t, 1 H, H-3), 4.57 (dd ~ t, 1 H, $J_{2,3}$ 3.9 Hz, H-2), 4.53 (dd, 1 H, $J_{2',3'}$ 8.8 Hz, H-2'), 4.466 (dd, 1 H, $J_{5',6a'}$ 2.5 Hz, H-6a'), 4.465 (dd, 1 H, $J_{3',4'}$ 8.2 Hz, H-4'), 4.37 (dd, 1 H, $J_{5,6a} \approx 4$ Hz, H-6a), 4.34 (dd, 1 H, H-6b), 4.32 (dd, 1 H, $J_{5',6b'}$ 5.2, $J_{6a',6b'}$ 11.0 Hz, H-6b'), 4.27 (dd, 1 H, $J_{3,4}$ 5.0, $J_{4,5}$ 7.0 Hz, H-4, discrimination of coupling constants by selective irradiation on H-3/H-3'), 4.22 (ddd, 1 H, $J_{4',5'}$ 8.5 Hz, H-5'), 4.19 (ddd ~ dt, 1 H, $J_{5,6b} \approx 7$ Hz, H-5), 3.57 (s, 3 H, OCH_3). Anal. Calcd for $C_{13}H_{17}O_{32}S_7K_7$: S, 18.96. Found: S, 18.53.

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