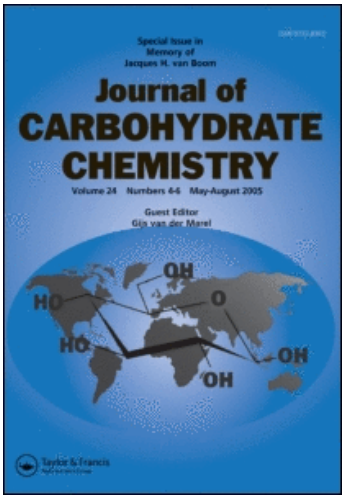

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SYNTHESIS AND CONFORMATIONAL INVESTIGATIONS OF SULFATED CARBOHYDRATES[1]

Katrin C. Probst[a]; Hans Peter Wessel[a]
[a] Pharmaceuticals Division, Discovery Chemistry, Dept. PRBC, Building 92/7.92, F.Hoffmann-La Roche, Ltd., Basel, Switzerland

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SYNTHESIS AND CONFORMATIONAL INVESTIGATIONS OF SULFATED CARBOHYDRATES[1]

Katrin C. Probst and Hans Peter Wessel*

F.Hoffmann-La Roche, Ltd. Pharmaceuticals Division, Discovery Chemistry, Dept. PRBC, Building 92/7.92 CH-4070 Basel, Switzerland

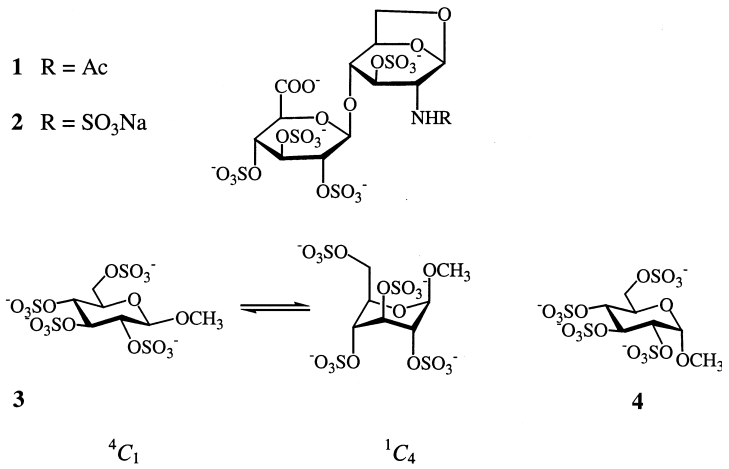
ABSTRACT

Starting from the finding that methyl 2,3,4,6-tetra-*O*-sulfonato-β-D-glucopyranoside (**3**) existed in a conformational equilibrium of the two chair conformers, the effect of sulfation on conformational equilibria was further investigated using a number of sulfated saccharides. Three sulfate groups on positions 3,4, and 6 or two on positions 2 and 3 were not sufficient to induce the conformational change as shown with methyl 2-amino-2-deoxy-3,4,6-tri-*O*-sulfonato-β-D-glucopyranoside. *N*-Sulfation of the amino group of the latter compound furnished an equilibrium of chair conformers with less $^1C_4$ conformer content than for **3**. The presence of persulfated methyl β-D-galactopyranoside in the usual $^4C_1$ conformation suggested the involvement of the 4-*O*-sulfate in the effect. Methyl 2,3,4-tri-*O*-sulfonato-β-D-xylopyranoside was found to prefer the "all-axial" $^1C_4$ conformation demonstrating that *O*-sulfates facilitate 1,3-*O/O*-diaxial interactions better than ester groups and in particular benzoates. Also, sulfated 1,5-anhydro-D-glucitol occurred as a conformational mixture, the influence of the anomeric effect may thus have been overestimated in the previous discussion of this conformational effect.

INTRODUCTION

Sulfated carbohydrates occur widely in glycopeptides and particularly in the polysaccharide family of glycosaminoglycans.[2] Here, heparin and heparan sulfate have found special attention due to their biological implications–more than a hundred heparin binding proteins have now been identified. Both heparin and heparan

*Corresponding author. E-mail: hans_p.wessel@roche.com



Scheme 1.

sulfate contain L-iduronic acid constituents that occur in a skew (^2S_O)-chair ($^4C_1$) equilibrium in solution.[3] This rather unique conformational flexibility has been postulated to have an impact on the binding and thus the biological properties of heparin, heparan sulfate, and dermatan sulfate.[4] In an investigation on glycosaminoglycan-related saccharides, we had discovered that highly sulfated glucuronic acid derivatives **1** and **2** also occur in unusual conformations in solution as judged by ^{1}H NMR spectroscopy, with the involvement of a $B_{3,O}$ conformation in the glucuronic acid moiety being one possibility.[5] Similar findings were reported later for the glucuronic acid moieties in oversulfated chondroitin sulfate.[6]

In the course of our work on heparinoid mimetics, we found that in some sulfated oligosaccharides[7] and "spaced sulfated oligosaccharides",[8] sulfated β-D-glucopyranosides were more active in a cell proliferation assay than the corresponding α-D-glucopyranosides. Interestingly, a simple glucose model system showed that methyl 2,3,4,6-tetra-*O*-sulfonato-β-D-glucopyranoside (**3**) occurs in a chair-chair equilibrium (Scheme 1).[9] It should be noted, that in the $^1C_4$ conformation of **3**, all substituents are axial.

The analogous persulfated methyl α-D-glucopyranoside **4** was found to exist in the "normal" $^4C_1$ conformation.[9] This had led to the interpretation that the anomeric effect is the determining factor for this conformational effect. Here we describe the synthesis and conformation of further sulfated saccharides to shed more light onto this conformational effect.

RESULTS AND DISCUSSION

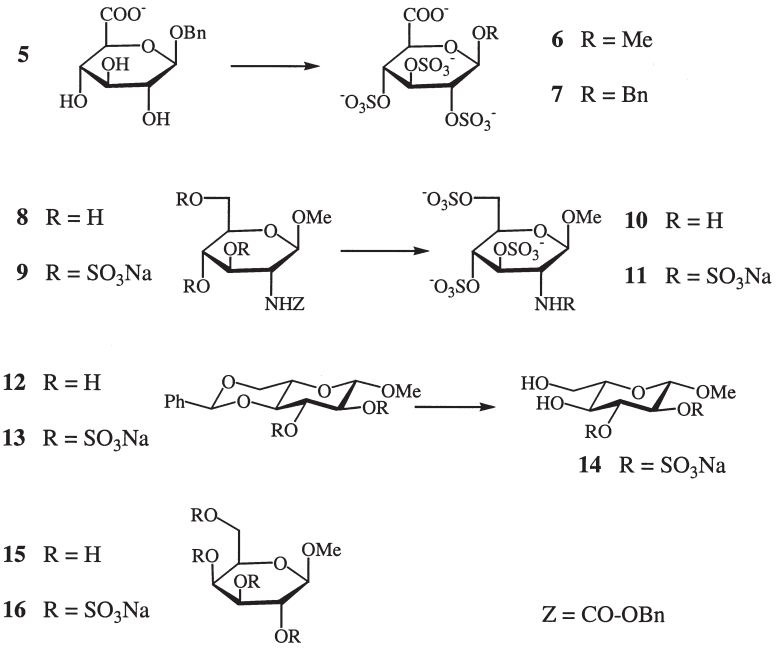
In an attempt to better understand the features governing the conformations of sulfated glycoside derivatives, substituent variations at different positions were investigated. In the β-glucuronide series there is a small dependence of the con-

formational equilibrium on the size of the anomeric substituent, with a variation of $J_{1,2} = 6.7 - 6.4$ Hz for **1** and **2** to $J_{1,2} = 5.8$ Hz for the methyl glucuronide **6**.[9] The sulfated benzyl glucuronide **7**, prepared from known[10] **5** (Scheme 2) by standard sulfation with sulfur trioxide-trimethylamine complex in DMF, showed an intermediate value for $J_{1,2} = 6.0$ Hz (Table 1).

To study the influence of the 2-*O*-sulfate group, methyl 2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside (**8**)[11] was sulfated to afford **9**. Both this compound and the corresponding 2-amino-2-deoxy derivative **10**, prepared by hydrogenolysis of the *N*-benzyloxycarbonyl group protective group, occurred in a conformation close to the standard $^4C_1$ chair conformation.

In the *gluco*-configuration, the $^4C_1$ chair conformation is characterized by *trans*-diaxially positioned ring protons, thus, by large vicinal coupling constants for all ring protons ($J_{2,3} \cong J_{3,4} \cong J_{4,5} \cong 9 \pm 1$ Hz, $J_{1,2} \cong 7.5 \pm 0.5$ Hz) as in derivatives **4**, **13** and **14**. For the $^1C_4$ chair conformation with the ring protons in equatorial positions, diequatorial coupling constants < 2 Hz would be expected, as observed, e.g., in 1,6-anhydro-glucopyranoses.[12] Data that are not compatible with one defined conformer can be interpreted in terms of conformational equilibria. In the gluco series, ring coupling constants of similar size hint at chair-chair equilibria. In cases in which some coupling constants deviate slightly from this pattern, contributions from ring distortions or other conformations, most likely skew conformations, must be considered in addition to the chair-chair equilibria.

N-Sulfation of **10** under basic conditions yielded tetrasulfate **11**. This derivative was found to be present in a conformational equilibrium in solution,



Scheme 2.

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Table 1. Comparison of Coupling Constants for Sulfated Saccharides

Compound	Solvent	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
1	D_2O	6.7	4.1	4.9	4.2
2	D_2O	6.4	3.2	4.0	2.8
7	D_2O	6.0	3.0	4.7	3.3
6	D_2O	5.8	3.0	4.8	6.0
3	D_2O	5.7	5.2	6.8	6.0
4	D_2O	3.6	9.8	8.8	not obs.
4	$(CD_3)_2SO$	3.4	9.8	8.3	9.7
9	D_2O	8.2	10.0	8.9	8.7
10	D_2O	8.1	8.5	9.1	9.3
11	D_2O	6.7	8.0	7.9	7.4
13	D_2O	7.3	8.2	9.3	9.5
14	D_2O	7.7	8.4	9.3	9.0
18	D_2O	$\approx$1.0	2.2	1.8	1.8 (a) 3.4 (b)
18	$(CD_3)_2SO$	$\leq$2.0	4.1	2.9	1.9 (a) 5.0 (b)
22	D_2O	7.3 (a) 3.8 (e)	6.5	6.5	6.8
22	$(CD_3)_2SO$	3.7 (a) 3.8 (e)	3.9	3.4	2.2
24[a]	D_2O	7.1	6.9	8.0	6.7
24[b]	D_2O	6.0	5.1	7.3	6.5

[a] Data for the non-reducing end β-D-glucopyranosyl moiety.
[b] Data for the reducing end β-D-glucopyranosyl moiety.

however, with a lower proportion of the $^1C_4$ conformation than in the glucose analogue **3**. These findings suggest that the 2-*O*-sulfate group has an important impact on the position of the conformational equilibrium. Sulfation of 4,6-*O*-benzylidene-β-D-glucopyranoside (**12**) furnished the disulfate **13**. This derivative was found in the usual $^4C_1$ conformation as expected due to the stabilizing effect of the annulated benzylidene acetal ring. Removal of the benzylidene acetal by hydrogenolysis in the presence of palladium-on-carbon furnished the 2,3-di-*O*-sulfonato derivative **14** in 98% yield. This compound also occurred in the $^4C_1$ conformation in solution indicating that *O*-sulfates in positions 2 and 3 do not destabilize the $^4C_1$ conformation sufficiently to affect the magnitude of the coupling constants.

Next, commercial methyl β-D-galactopyranoside (**15**) was sulfated to afford the tetrasulfate **16** (Scheme 3). This compound differs from the glucopyranoside **3** only in the orientation of the *O*-sulfate in position 4. The ^{1}H NMR spectrum revealed the presence of the classical $^4C_1$ conformation. This suggested that the 4-*O*-sulfate in tetrasulfate **3** in interaction with the other substituents influenced the conformational change observed for **3**.

Sulfation of commercial methyl β-D-xylopyranoside (**17**) in the presence of 4–methylmorpholine yielded the trisulfate **18**. When the sulfation was carried out

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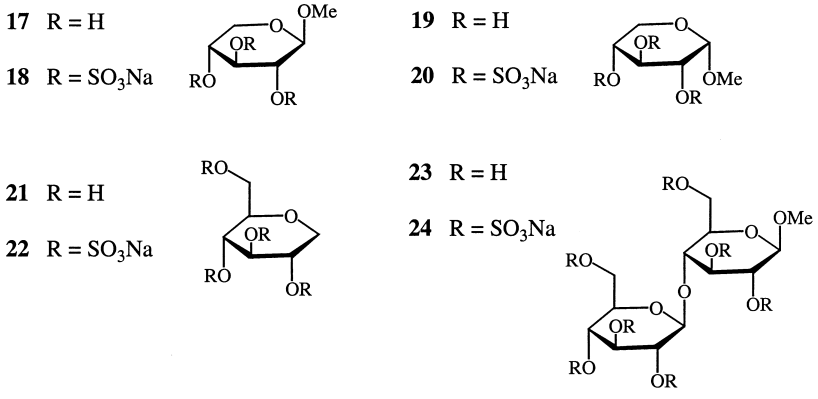
under standard reaction conditions without addition of base anomerization occurred to some degree. To prove the presence of the α-anomer in the reaction mixture the pure compound was synthesized independently. Methyl α-D-xylopyranoside[13] (**19**) was prepared via its 2,4-*O*-boronate as described;[14] standard sulfation furnished **20**. While the persulfated α-D-xylopyranoside **20** was found to exist as the usual $^4C_1$ conformer completely, the analogous β-D-xylopyranoside **18** mainly occurred in the inverted $^1C_4$ conformation (cf. Table 1). A comparison of the conformational equilibria in β-D-xylose and β-D-glucose derivatives confirms the stabilization of the $^4C_1$ conformation by the (substituted) hydroxymethyl group.[15]

There was wide interest in the conformational properties of β-D-xylopyranose derivatives after it was discovered that acetylated or benzoylated β-D-xylopyranosyl chlorides,[16–18] fluorides,[19] and bromides[20] prefer the "all-axial" or slightly distorted[21] conformations in acetone or chloroform solution. This behavior was explained in terms of the strong anomeric effect of the halide atoms. With the anomeric effect decreasing, 1,2,3,4-tetra-*O*-benzoyl-β-D-xylopyranose exists as a 1:1 mixture of the $^4C_1$ and $^1C_4$ conformers in acetone solution,[22] and methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside was found to be present in the normal $^4C_1$ conformation at the 74% level in deuterated acetone.[23] The influence of the anomeric effect is generally diminished in more polar solvents, accordingly, the latter compound had an even lower proportion of the $^1C_4$ conformer in $(CD_3)_2SO$ solution than in acetone.[23] In contrast, methyl 2,3,4-tri-*O*-sulfonato-β-D-xylopyranoside (**18**) studied here mainly exists in the $^1C_4$ conformation in $(CD_3)_2SO$ solution. The relative stability of conformations with 1,3-diaxial substituents has been rationalized with the diminished repulsion of the oxygen atoms induced by the withdrawal of electron density in ester derivatives.[24] Accordingly, solutions of methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside contained a higher proportion of the $^1C_4$ conformer (26% in an acetone solution) than those of methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (19%).[23] Along these lines, the even higher proportion of $^1C_4$ conformer in the sulfated analogue would be explained by the stronger electron-withdrawing properties of the sulfate group.



Scheme 3.

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High proportions of conformations with 1,3-diaxial substituents were found in *myo*-inositol hexaphosphate also, however, only at high pH > 9.5 involving the dianionic species. It was postulated that the diaxial phosphates are stabilized by complexation with metal ions reducing the electrostatic repulsion.[25,26]

To investigate the influence of the anomeric effect on the conformational equilibrium in sulfated carbohydrates, 1,5-anhydro-D-glucitol (**21**) was sulfated to afford the tetrasulfate **22**. The NMR data (cf. Table 1) show that this compound occurs in a conformational equilibrium also with the proportion of the $^1C_4$ conformer higher in $(CD_3)_2SO$ solution than in the more polar deuterated water. A comparison of these data with the methyl glucopyranoside **3** demonstrates that, in water, the proportion of $^1C_4$ conformer is lower in **22** which is not surprising due to the absence of the anomeric effect. Here it is obvious that this conformational effect is not only determined by the anomeric effect but by a summation of factors.

With a view to the presumed biological importance of the conformational equilibria of sulfated poly- or oligosaccharides, we have prepared a simple disaccharide model compound. Standard sulfation of known[27,28] methyl β-cellobioside (**23**) furnished the heptasulfate **24** in 73% yield. Unexpectedly, the non-reducing end β-D-glucopyranosyl moiety contained a markedly lower proportion of the $^1C_4$ conformer than does the methyl β-D-glucopyranoside **3**. This finding is different from the results obtained with the glucuronides discussed above, and the interpretation of this effect will require further data. In contrast, for the reducing end β-D-glucopyranosyl moiety, a lower proportion of the $^4C_1$ conformer was found, although the 4-*O*-sulfate, thought to be of importance as discussed above, is replaced by a glycosyl residue. Thus, both glucopyranosyl moieties occur in conformationally different forms, and it is important to note that in oligosaccharides containing β-D-linked glucosides or glucuronides, conformational flexibility can be induced by sulfation.

In summary, at least three sulfate groups in positions 2, 3, and 4 or 2, 3, and 6 of a gluco or xylo configured sugar are required to induce the presence of a $^1C_4$ conformer. The repulsion of 1,3-diaxially oriented oxygen atoms seems to be reduced by the strongly electron withdrawing sulfate groups.

EXPERIMENTAL

General Methods. Solvents and reagents were bought from Fluka, methyl β-D-galactopyranoside from Aldrich, and methyl β-D-xylopyranoside from Sigma. Solutions were concentrated below 50°C in vacuo on a Büchi rotary evaporator. Qualitative TLC was performed on precoated Silica Gel 60F-254 plates (Merck); compounds were detected by UV light (254 nm) and spraying with a 10% solution of concd sulfuric acid in methanol followed by heating. Medium pressure liquid chromatography (MPLC) was carried out on lobar columns (Merck LiChroprep® Si 60, 40–63 μm) at 2–5 bar (Labomatic MD 80/100 pump). Sulfated compounds were desalted on MCI gel (Mitsubishi Chemical Industries High Porous Polymer CHP 20P, 75~150μ). Gel filtration was carried out on Sephadex® G 25 Na^+

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(Pharmacia Fine Chemicals). 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 at 20°C. ^{1}H NMR spectra were recorded on Bruker AC 250 (250 MHz) and AM 400 (400MHz) spectrometers with an Aspect 3000 and process controller. Chemical shifts are given in ppm relative to tetramethylsilane or sodium 2,2,3,3 tetradeutero-3-(trimethylsilyl)propionate for aqueous solutions as internal standard. Mass spectra were recorded on a MS 9 updated with a Finnigan ZAB console, data system SS 200, VG Altrichem (EI, 70 eV), MS 902 (FAB) with data system DS 2050 (VG), and API III Sciex, Perkin Elmer for negative (ISN) and positive (ISP) electrospray ionization.

General Procedure No. 1 (GP1) for Sulfation. A soln of the carbohydrate compound in dry DMF (6 mL/g) was reacted with SO_3 NMe_3 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 soln and concentrated. The residue was taken up in water and concentrated several times and afterwards desalted by suspending twice in methanol followed by filtration. The crude product was further desalted by gel filtration over an MCI-column and a Sephadex® G 25 Na^+ -column using double-distilled water as eluent. Product fractions were concentrated and lyophilized.

General Procedure No. 2 (GP2) for Sulfation. A soln of the carbohydrate compound in dry DMF (6 mL/g) was reacted with SO_3 NEt_3 complex (1.5 equiv per OH group of the carbohydrate compound) for 72 h at rt. The work-up was performed as described in GP1.

Benzyl 2,3,4-Tri-*O*-sulfonato-β-D-glucopyranosiduronic Acid Tetrasodium Salt (7). Sulfation of **5**[10] (0.4 g, 1.4 mmol) according to GP1 furnished sulfate **7** as a solid (0.15 g, 18%): $[\alpha]_D$ +20.0° (*c* 0.2, water); MS (ISN): *m/z* 612.,5 (23, $[M]^-$), 590.5 (100, $[M\text{-}Na+H]^-$); ^{1}H NMR (D_2O, 250 MHz) δ 7.50 (m, 5H, Ph), 5.05 (ddd∼dt, 1H, H-4), 4.96 (d, J = 12.0 Hz,1H, CH_2Ph), 4.91 (d, 1H, H-1), 4.88 (dd, 1H, H-3), 4.78 (d, 1H, CH_2Ph), 4.52 (ddd, $J_{2,4}$ ≈0.7 Hz, 1H, H-2), 4.33 (d, 1H, H-5).

Anal. Calcd for $C_{13}H_{12}O_{16}S_3Na_4$ (612.4): C, 25.50; H, 1.98; S, 15.71. Found: C, 25.29; H, 1.87; S, 16.06.

Methyl 2-Benzyloxycarbonylamino-2-deoxy-3,4,6-tri-*O*-sulfonato-β-D-gluco-pyranoside Trisodium Salt (9). Sulfation of **8**[11] (0.2 g, 0.64 mmol) according to GP2 furnished **9** as a colorless solid (0.25g, 63%): $[\alpha]_D$ +8.7° (*c* 0.15, water); MS (ISN) *m/z* 610.2 (73, $[M\text{-}Na]^-$), 294.8 (94, $[M\text{-}2Na]^{2-}/2$); ^{1}H NMR (D_2O, 250 MHz) δ 7.45 (m, 5H, aromat), 5.18−5.11 (2 d, 2H, J_{gem} = 12.8 Hz, CH_2Ph), 4.61 (dd, 1H, H-1), 4.61 (dd, 1H, H-6a), 4.55 (dd, 1H, H-3), 4.25 (dd, 1H, H-4), 4.14 (dd, 1H, $J_{5,6b}$ = 7.9 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6b), 3.94 (ddd, 1H, $J_{5,6a}$ ≤2.0 Hz, H-5), 3.65 (dd, 1H, H-2) 3.52 (s, 1H, OCH_3).

Anal. Calcd for $C_{15}H_{18}O_{16}S_3Na_3$ (633.5): C, 28.44; H, 2.86; N, 2.21; S, 15.18; Na, 10.89. Found: C, 27.55; H, 3.11; N, 2.21; S, 15.19; Na, 11.03.

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Methyl 2-Amino-2-deoxy-3,4,6-tri-*O*-sulfonato-β-D-glucopyranoside Trisodium Salt (10). A soln of **9** (0.18 g, 0.28 mmol) in water (3 mL) was hydrogenated in the presence of 10% palladium on carbon (0.09g) for 2 h. Filtration and concentration of the filtrate furnished **10** as a solid (0.148 g, 99%): $[\alpha]_D$ +9.5° (*c* 0.2, water); MS (ISN) *m/z* 476.2 (34, $[M\text{-}Na]^-$); ^1H NMR (D_2O, 250 MHz) δ 4.60 (dd, 1H, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6a), 4.40 (d, 1H, H-1), 4.37 (dd, 1H, H-3), 4.23 (dd, 1H, H-4), 4.14 (dd, 1H, $J_{5,6b}$ = 7.6 Hz, H-6b), 4.23 (dd, 1H, H-4), 3.94 (ddd, 1H, H-5), 3.59 (s, 3H, OCH_3), 2.95 (dd, 1 Hz, H-2).

Anal. Calcd for $C_7H_{12}O_{14}S_3Na_3$ (499.3): C, 16.84; H, 2.42; S, 19.26. Found: C, 16.88; H, 2.44; S, 18.75.

Methyl 2-Amino-2-deoxy-2-*N*-sulfonato-3,4,6-tri-*O*-sulfonato-β-D-glucopyranoside Tetrasodium Salt (11). An aqueous soln of **10** (0.1 g, 0.2 mmol) was titrated with 0.1 N NaOH to pH = 9.1.[5] After the addition of sulfur trioxide-trimethylamine complex (0.111 g, 0.8 mmol) at pH = 8.8–9.3, the soln was kept for 16 h. More sulfur trioxide-trimethylamine complex (0.111 g, 0.8 mmol) was added, and the pH was kept at 9 for 24 h. Then the soln was treated with 1N NaOH to reach pH 12 and was stirred for 2.5 h. Filtration and concentration yielded a crude product which was purified by MPLC on MCI gel furnishing **11** as a colorless solid (0.013 g, 11%): $[\alpha]_D$ -4.1° (*c* 0.2, water); MS (ISN) *m/z* 624.1 (100, $[M+Na]^+$); ^1H NMR (D_2O, 250 MHz) δ 4.56 (d, 1H, H-1), 4.54 (dd, 1H, H-6a), 4.50 (dd, 1H, H-3), 4.38 (dd, 1H, H-4), 4.21 (dd, 1H, $J_{6a,6b}$ = 11.0 Hz, H-6b), 4.04 (ddd, 1H, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 8.5 Hz, H-5), 3.55 (s, 3H, OCH_3), 3.40 (dd, 1H, H-2).

Anal. Calcd for $C_7H_{11}O_{17}S_4Na_4$ (601.4): C, 13.98; H, 1.84; N, 2.34; S, 21.33. Found: C, 13.87; H, 1.92; N, 2.33; S, 21.01.

Methyl 4,6-*O*-Benzylidene-2,3-di-*O*-sulfonato-β-D-glucopyranoside Disodium Salt (13). Sulfation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside[29] (**12**, 1.0 g, 3.54 mmol) according to GP1 furnished **13** as a colorless solid (0.9 g, 52%): $[\alpha]_D$ −63.5° (*c* 0.2, water); MS (ISN): *m/z* 463.1 (52, $[M\text{-}Na]^-$), 441.2 (92, $[M\text{-}2Na+H]^-$); ^1H NMR (D_2O, 250 MHz) δ 7.60 (m, 2H, aromat), 7.60 (m, 3H, aromat), 5.77 (s, 1H, C*H*Ph), 4.79 (d, 1H, H-1), 4.67 (dd, 1H, H-3), 4.42 (dd, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 10.3 Hz, 1H, H-6a), 4.35 (dd, 1H, H-2), 3.96 (dd∼t, 1H, H-4), 3.94 (dd∼t, 1H, H-6b), 3.71 (dd∼t, 1H, H-5), 3.58 (s, 3H, OCH_3);

Anal. Calcd for $C_{14}H_{18}O_{12}S_2Na$ (486.2): C, 34.57; H, 3.32; S, 13.18. Found: C, 34.77; H, 3.45; S, 13.27.

Methyl 2,3-Di-*O*-sulfonato-β-D-glucopyranoside Disodium Salt (14). To a soln of **13** (0.2 g, 0.41 mmol) in water (3 mL) was added 10% palladium on carbon (0.1 g). The reaction was kept under stirring in a hydrogen atmosphere until 25 mL of hydrogen had been taken up. After concentration, **14** was obtained as light yellow amorphous solid (0.18 g, 98%): $[\alpha]_D$ −17.0° (*c* 0.2, water); MS (ISP): *m/z* 421 (100, $[M+Na]^+$); ^1H NMR (D_2O, 250 MHz) δ 4.62 (d, 1H, H-1), 4.44 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.16 (dd, 1H, H-2), 3.94 (dd, 1H, $J_{5,6a}$ = 2.1 Hz,

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$J_{6a,6b}$ = 12.0 Hz, H-6a), 3.77 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, H-6b), 3.72 (dd, 1H, H-4), 3.57 (s, 3H, OCH_3), 3.56 (ddd, 1H, H-5).

Anal. Calcd for $C_7H_{12}O_{12}S_2Na_2$ (398.3): C, 21.11; H, 3.04. Found: C, 20.98; H, 3.23.

Methyl 2,3,4,6-Tetra-*O*-sulfonato-β-D-galactopyranoside Tetrasodium Salt (16). Sulfation of methyl β-D-galactopyranoside (**15**, 1.0 g, 5.15 mmol) according to GP1 furnished **16** as a colorless amorphous solid (0.46 g, 32%): $[\alpha]_D$ +14.5° (*c* 0.2, water); MS (ISN) *m/z* 579.1 (100, $[M\text{-}Na]^-$), 557.0 (2, $[M\text{-}2\ Na]^-$), 378.9 (93, $[M\text{-}2NaO_3SOH]^-$); ^{1}H NMR (D_2O, 400 MHz) δ 5.07 (dd, 1H, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 0 Hz, H-4), 4.78 (s, 1H, OCH_3), 4.64 (dd, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.55 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-3), 4.48 (dd, 1H, H-2), 4.33 (dd, $J_{6a,6b}$ = 11.5 Hz, 1H, H-6a), 4.25 (dd, 1H, H-6b), 4.17 (ddd, 1H, $J_{5,6a}$ = 3.3 Hz, $J_{5,6b}$ = 8.0 Hz, H-5).

Anal. Calcd for $C_7H_{10}O_{18}S_4Na_4$ (602.3): C, 13.96; H, 1.67; S 21.29; Na, 15.27. Found: C, 13.06; H, 2.00; S, 20.52; Na, 15.30.

Methyl 2,3,4-Tri-*O*-sulfonato-β-D-xylopyranoside Trisodium Salt (18). Compound **17** (1.0 g, 6.1 mmol) was sulfated according to GP1 in the presence of 4-methylmorpholine (0.2 mL) to prevent anomerisation and furnished **18** as a colorless solid (1.27 g, 44%): $[\alpha]_D$ −14.5° (*c* 0.2, water); MS (ISN) *m/z* 447.2 (4, $[M\text{-}Na]^-$), 425.0 (3, $[M\text{-}2Na+H]^-$), 403.0 (5, $[M\text{-}3\ Na+2H]^-$), 212.2 (15, $[M/2\text{-}Na]^-$); ^{1}H NMR (D_2O, 250 MHz) δ 4.91(br s, 1H, H-1), 4.83 (br s, 1H, $J_{3,5a}$ ≤1.0 Hz, H-3), 4.54 (m_c, 1H, H-4), 4.43 (dd, 1H, H-2), 4.17 (dd, 1H, $J_{5a,5b}$ = 13.1 Hz, H-5a), 3.88 (dd, 1H, H-5b), 3.45 (s, 3H, OCH_3); ^{1}H NMR ($Me_2SO\text{-}d_6$, 250 MHz): 4.90 (br s, 1H, H-2), 4.79 (m_c, 1H, H-4), 4.43 (br s, 1H, H-3), 4.17 (dd, 1H, $J_{5a,5b}$ = 12.9 Hz, H-5a), 3.88 (dd, 1H, H-5b), 3.45 (s, 3H, OCH_3).

Anal. Calcd for $C_6H_9O_{14}S_3Na_3$ (470.3): S, 20.45; Na, 14.67. Found: S, 21.07; Na, 14.62.

Methyl 2,3,4-Tri-*O*-sulfonato-α-D-xylopyranoside Trisodium Salt (20). Sulfation of **19** (0.33 g, 2.0 mmol) according to GP1 furnished **20** as an amorphous solid (0.352 g, 37%): $[\alpha]_D$ +15.5° (*c* 0.2, water); MS(ISN): *m/z* 470.0 (100, $[M]^-$), 368.0 (54, $[M\text{-}NaSO_3^- + H^+]^-$); ^{1}H NMR ($D_2O$, 250 MHz) δ 5.09 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 4.70 (dd, $J_{3,4}$ = 8.5 Hz, 1H, H-3), 4.45 (ddd, 1H, H-4), 4.40 (dd, $J_{2,3}$ = 7.9 Hz, 1H, H-2), 4.09 (dd, $J_{4,5a}$ =5.0 Hz, $J_{5a,5b}$ = 11.9 Hz, 1H, H-5a), 3.83 (dd, $J_{4,5b}$ = 8.6 Hz, 1H, H-5b), 3.48 (s, 3H, OCH_3).

Anal. Calcd for $C_6H_9O_{14}S_3Na_3$ (470.3): S, 20.45; Na, 14.67. Found: S, 20.67; Na, 15.44.

1,5-Anhydro-2,3,4,6-tetra-*O*-sulfonato-D-glucitol Tetrasodium Salt (22). Sulfation of **21** (0.2 g, 1.22 mmol) according to GP2 furnished **22** as a colorless solid (0.3 g, 44%): $[\alpha]_D$ +25.5° (*c* 0.2, water); MS (ISN): *m/z* 572.0 (100, $[M]^-$), 470.0 (100, $[M\text{-}\ NaSO_3^- + H]^-$); ^{1}H NMR ($D_2O$, 250 MHz) δ 4.66 (dd, 1H, H-4), 4.48 (ddd, 1H, H-2), 4.43 (dd, $J_{5,6a}$ = 2.8 Hz, $J_{6a,6b}$ = 11.5 Hz, 1H, H-6a), 4.35 (dd,

1H, H-3), 4.29 (dd, 1H, H-1e), 4.27 (dd, $J_{5,6b}$ = 4.0 Hz, 1H, H-6b), 4.04 (ddd, 1H, H-5), 3.70 (dd, $J_{1a,1e}$ = 12.2 Hz, 1H, H-1a); ^{1}H NMR (Me_2SO-d_6, 400 MHz) δ 4.35 (dd, 1H, H-3), 4.28 (ddd, 1H, H-2), 4.04 (dd∼t, 1H, H-4), 3.90 (dd, 1H, H-5), 3.86 (dd, 1H, H-1e), 3.81 (dd, $J_{5,6a}$ = 3.1 Hz, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6a), 3.81 (dd, $J_{5,6b}$ = 7.9 Hz, 1H, H-6b), 2.97 (dd, $J_{1a,1e}$ = 12.0 Hz, 1H, H-1a).

Anal. Calcd for $C_6H_8O_{17}S_4Na_4$ (572.3): C, 12.59; H, 1.41; S, 22.41; Na, 16.07. Found: C, 12.47; H, 1.51; S, 21.53; Na, 16.18.

Methyl 4-*O*-(2,3,4,6-tetra-*O*-sulfonato-β-D-glucopyranosyl)-2,3,6-tri-*O*-sulfonato-β-D-glucopyranoside Heptasodium Salt (24). Sulfation of **23**[27,28] (0.4 g, 1.123 mmol) according to GP 1 furnished **39** (0.874 g, 73%) as a colorless solid: $[\alpha]_D$ +2.5 ° (*c* 0.2, water); MS (ISN): *m/z* 1070 (100, $[M]^+$); ^{1}H NMR (D_2O, 250 MHz) δ 4.86 (ddd, $J_{1',2'}$ = 7.0 Hz, 1H, H-1′), 4.84 (dd, $J_{1,2}$ = 6.0 Hz, 1H, H-1), 4.68 (dd, $J_{3,4}$ = 7.3 Hz, 1H, H-3), 4.67 (dd, $J_{3',4'}$ = 7.4 Hz, 1H, H-3′), 4.56 (dd, $J_{5',6a'}$ = 2.8 Hz, $J_{6a',6b'}$ = 11.5 Hz, 1H, H-6a′), 4.48 (dd, $J_{4',5'}$ = 7.3 Hz, 1H, H-4), 4.43 (dd, 1H, H-6a), 4.42 (dd, $J_{2,3}$ = 5.1 Hz, 1H, H-2), 4.36 (dd, $J_{2',3'}$ ≈7.0 Hz, 1H, H-2′), 4.33 (dd, $J_{5,6b}$ = 7.0 Hz, 1H, H-6b), 4.21 (dd, $J_{5',6b'}$ = 7.0 Hz, 1H, H-6b′), 4.18 (dd, $J_{4,5}$ = 6.7 Hz, 1H, H-4), 4.09−4.01 (m, $J_{5,6a}$ = 3.5 Hz, 2H, H-5′, H-5), 3.56 (s, 3H, OCH_3).

Anal. Calcd for $C_{13}H_{17}O_{32}S_7Na_7$ (1071.5): C, 14.58; H, 2.14; S, 20.96; Na, 15.03. Found: C, 13.82; H, 2.07; S, 20.67; Na, 15.28.

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REFERENCES

1. Dedicated to Professor Joachim Thiem on the occasion of his 60th anniversary.
2. Comper, W.D., Ed. *Heparin (and Related Polysaccharides)*, Gordon and Breach Science Publishers: New York, 1981; Vol. 7, 266 pp.
3. Casu, B. Methods of Structural Analysis. In *Heparin - Chemical and Biological Properties, Clinical Applications*; Lane, D.A., Lindahl, U., Eds.; Edward Arnold: London, 1989; 25–49.
4. Casu, B.; Choay, J.; Ferro, D. R.; Gatti, G.; Jacquinet, J.-C.; Petitou, M.; Pravasoli, A.; Ragazzi, M.; Sinaÿ, P.; Torri, G. Controversial Glycosaminoglycan Conformations. Nature (London) **1986**, *322*, 215–216.
5. Wessel, H. P. Sulfated 1,6-Anhydro-4-*O*-(β-D-glucopyranosyluronate)-β -D-glucopyranosyl Derivatives: Syntheses and Conformations. J. Carbohydr. Chem. **1992**, *11*, 1039–1052.
6. Murayama, T.; Toida, T.; Imanari, T.; Yu, G.; Linhardt, R. J. Conformational Changes and Anticoagulant Activity of Chondroitin Sulfate Following its *O*-Sulfonation. Carbohydr. Res. **1998**, *306*, 35–43.

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7. Wessel, H. P.; Tschopp, T. B.; Hosang, M.; Iberg, N. Identification of a Sulfated Tetrasaccharide with Heparin-like Antiproliferative Activity. Bioorg. Med. Chem. Lett. **1994**, *4*, 1419–1422.
8. Wessel, H. P. Heparinoid Mimetics. Topics Curr. Chem. **1997**, *187*, 215–239.
9. Wessel, H. P.; Bartsch, S. Conformational Flexibility in Highly Sulfated β-D-glucopyranoside derivatives. Carbohydr. Res. **1995**, *274*, 1–9.
10. Compernolle, F. Preparation of α-Ethoxyethyl-protected D-Glucopyranuronic Acid Having HO-1 Unsubstituted. Carbohydr. Res. **1980**, *83*, 135–137.
11. Bannister, B. Synthesis and Biological Activities of Some Analogs of Streptozotocin. J. Antibiot. **1972**, *25*, 377–386.
12. Grindley, T. B.; Cude, A.; Kralovec, J.; Thangarasa, R. The Chair-Boat Equilibrium of 1,6-Anhydro-β-D-glucopyranose and Derivatives: An NMR and Molecular Mechanics Study. In *Levoglucosenone and Levoglucosans, Chemistry and Applications*; Wtczak, Z.J., Ed.ATL Press: Mount Prospect, Ill., 1994; pp 147–164.
13. Hudson, C. S. Relations Between Rotatory Power and Structure in the Sugar Group. VI. The Rotatory Powers of the alpha and beta Forms of Methyl D-Xyloside and Methyl L-Arabinoside. J. Am. Chem. Soc. **1925**, *47*, 265–268.
14. Ferrier, R. J.; Prasad, D.; Rudowski, A. Boric Acid Derivatives as Reagents in Carbohydrate Chemistry. Part III. The Interaction of Phenylboronic Acid with Methyl Xylofuranosides and the Isolation of the Four Methyl Xylosides. J. Chem. Soc. **1965**, 858–863.
15. Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: Heidelberg, 1983; p. 7.
16. Durette, P. L.; Horton, D. Conformational Studies on Pyranoid Sugar Derivatives by NMR Spectroscopy. The Conformational Equilibria of some Peracylated Aldopentopyranosyl Halides in Solution. Carbohydr. Res. **1971**, *18*, 57–80.
17. Holland, C. V.; Horton, D.; Jewell, J. S. The Favored Conformation of Tri-*O*-acetyl-β-D-xylopyranosyl Chloride. An All-Axial Tetrasubstituted Six-Membered Ring. J. Org. Chem. **1967**, *32*, 1818–1821.
18. Paulsen, H.; Garrido Espinosa, F.; Trautwein, W.-P.; Heyns, K. Acetoxonium-Umlagerungen der D-Xylose. Chem. Ber. **1968**, *101*, 179–185.
19. Hall, L. D.; Manville, J. F. Studies of Specifically Fluorinated Carbohydrates. Part II. Nuclear Magnetic Resonance Studies of Pentopyranosyl Fluoride Derivatives. Can. J. Chem. **1969**, *47*, 19–30.
20. Bock, K.; Pedersen, C.; Rasmussen, P. Reactions of Acylated Pentoses and Acylated Methyl Pentosides with Dibromomethyl Methyl Ether. Preparation of Bromodeoxypentoses. J. Chem. Soc., Perkin Trans. 1 **1973**, 1456–1461.
21. Lichtenthaler, F. W.; Lindner, H. J. On the Extent of Chair Distortion in "Tetra-axial" Derivatives of 2,3,4-Tri-*O*-benzoyl-β-D-xylopyranose. Carbohydr. Res. **1990**, *200*, 91–99.
22. Durette, P. L.; Horton, D. Conformational Studies on Pyranoid Sugar Derivatives. The Conformational Equilibria of the D-Aldopentopyranose Tetraacetates and Tetrabenzoates. J. Org. Chem. **1971**, *36*, 2658–2669.
23. Durette, P. L.; Horton, D. Conformational Studies on Pyranoid Sugar Derivatives by NMR Spectroscopy. Conformational Equilibria of the Peracetylated and Some Perbenzoylated Methyl D-Aldopentopyranosides in Solution. Carbohydr. Res. **1971**, 403–418.

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24. Lemieux, R. U.; Pavia, A. A. Substitutional and Solvation Effects on Conformational Equilibria. Effects on the Interaction Between Opposing Axial Oxygen Atoms. Can. J. Chem. **1969**, *47*, 4441–4446.
25. Barrientos, L. G.; Murthy, P. P. N. Conformational Studies of *myo*-Inositol Phosphates. Carbohydr. Res. **1996**, *296*, 39–54.
26. Baumann, A. T.; Chateauneuf, G. M.; Boyd, B. R.; Brown, R. E.; Murthy, P. P. N. Conformational Inversion Processes in Phytic Acid: NMR Spectroscopic and Molecular Modeling Studies. Tetrahedron Lett. **1999**, *40*, 4489–4492.
27. Newth, F. H.; Nicholas, S. D.; Smith, F.; Wiggins, L. F. 3:6,3′:6′-Dianhydro-derivatives of β-Methyl Cellobioside and of β-Methyl Maltoside. J. Chem. Soc. **1949**, 2550–2553.
28. Sigurskjold, B. W.; Duus, B.; Bock, K. Hydrolysis of Substrate Analogues Catalysed by β-D-Glucosidase from *Aspergillus niger*. Part II: Deoxy and Deoxyhalo Derivatives of Cellobiose. Acta Chem. Scand. **1991**, *45*, 1032–1041.
29. Evans, M. E. Meth. Carbohydr. Chem. **1980**, *8*, 313.

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