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Synthesis of various sulfoforms of the trisaccharide β-D-Glc*p*A-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→OMP) as probes for the study of the biosynthesis and sorting of proteoglycans

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A straightforward preparation of various sulfoforms of the trisaccharide 4-methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(β-D-galactopyranosyl)-(1⟶3)-β-D-galactopyranoside (**1**), namely its 6a- and 4a-monosulfate, 6b- and 4b-monosulfate and 6a,6b-disulfate derivatives, is reported for the first time. These compounds, which are partial structures of the linkage region of proteoglycans, will serve as probes for the study of the biosynthesis and sorting of these macromolecules. A key trisaccharide derivative, in which the two similar D-Gal units were differentiated at C-4,6 with 4,6-benzylidene and 4,6-di-*tert*-butylsilylene acetals, respectively, was used as a common intermediate. Both acetal groups showed excellent orthogonality, and allowed the preparation of all target compounds in high yield. Noteworthy is the possibility to prepare the 6a- and 6b-monosulfated and the 6a,6b-disulfated species through a one-pot regioselective procedure starting from a tetrol precursor.

Introduction

Proteoglycans (PGs) are macromolecules composed of glycosaminoglycan chains (GAGs) covalently bound to a protein core.[1] They are ubiquitously distributed on the cell surface and in the extracellular matrix. GAGs are increasingly implicated as regulators of many biological processes such as cell growth, adhesion and recognition, blood-coagulation, viral and bacterial infections, and cytokine action, owing to their capacity to interact with protein ligands through specific oligosaccharide sequences.[2] GAG assembly starts with the attachment of D-xylose to one or more L-serine residues within the protein core. The sequential addition of two D-galactose and one D-glucuronic acid units give rise to a tetrasaccharide intermediate that lies at a bifurcation in the biosynthetic pathway (Fig. 1). Attachment of an α-D-GlcNAc residue to this linkage region initiates the formation of *gluco*saminoglycans (heparin, heparan sulfate) whereas those of a β-D-GalNAc unit lead to the assembly of *galacto*saminoglycans (chondroitins sulfate, dermatan sulfate). GAG chains mostly consist of hexosamine and hexuronic acid arranged in alternating sequences, and these repeating units contain various sulfate substituents which create a great degree of structural and functional diversity. The fact that the linkage region should be common to all GAG species contrasts sharply with the structural heterogeneity of the repeating disaccharide region. Hence, the question arises how these different GAGs can be synthesized on this common structure since chain elongation proceeds in a stepwise fashion and is governed by the high substrate specificity of the glycosyltransferases involved.[3]

Interestingly, it has been reported that this common linkage region may be occasionally modified by sulfation at C-4 and/or C-6 of the D-Gal units[4–6] as well as by phosphorylation at C-2 of the D-Xyl unit.[7,8] Although the biological significance of these modifications is still not yet deciphered, it has been suggested[2] that they could act as biosynthetic signals. Recently, it has been demonstrated[9] that phosphorylation at C-2 of the D-Xyl unit should be a transient phenomenon, involved only in the very early steps of the biosynthesis of GAGs. But what is the exact function of the sulfate groups? Investigations of the possible structural variations in this linkage region may help to clarify the biological function of these unique modifying groups.

To address this issue, several syntheses of glycopeptides of various lengths of the linkage region, containing a sulfate group on one or the other D-Gal unit, have been reported,[10–12] but no systematic preparation of all possible sulfoforms has been described. Within the frame of a programme devoted to the study of the biosynthesis of chondroitins sulfate in relation with osteoarthritis, we now report on for the first time a straightforward preparation of various sulfoforms of the trisaccharide 4-methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(β-D-galactopyranosyl)-(1⟶3)-β-D-galactopyranoside (**1**), namely its 6a- and 4a-monosulfated, 6b- and 4b-monosulfated, and 6a,6b-disulfated derivatives (Fig. 2), in which the phenyl group will be useful for the detection of the transfer products and the methoxy group will serve as a marker for NMR studies.

Results and discussion

For the synthesis of the target sulfoforms **2**–**6**, a common key trisaccharide intermediate (**16**) was designed, in which the two

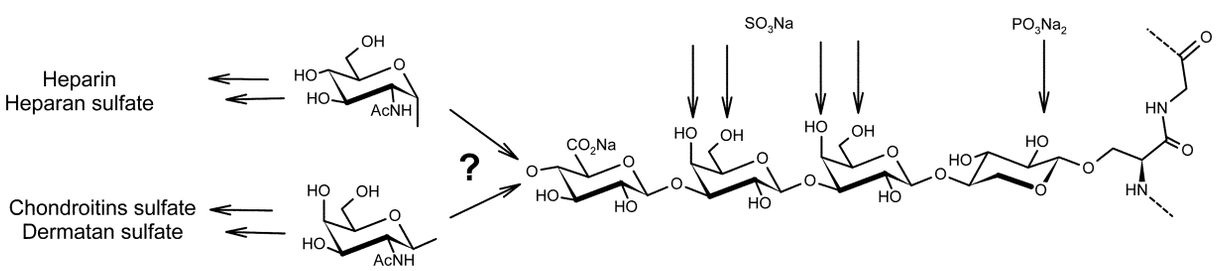

Fig. 1 The linkage region of proteoglycans. The arrows indicate possible substitutions with sulfate and phosphate groups.

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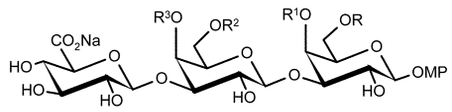


1, R = R¹ = R² = R³ = H

2, R = SO_3Na, R¹ = R² = R³ = H

3, R = R² = R³ = H, R¹ = SO_3Na

4, R = R¹ = R³ = H, R² = SO_3Na

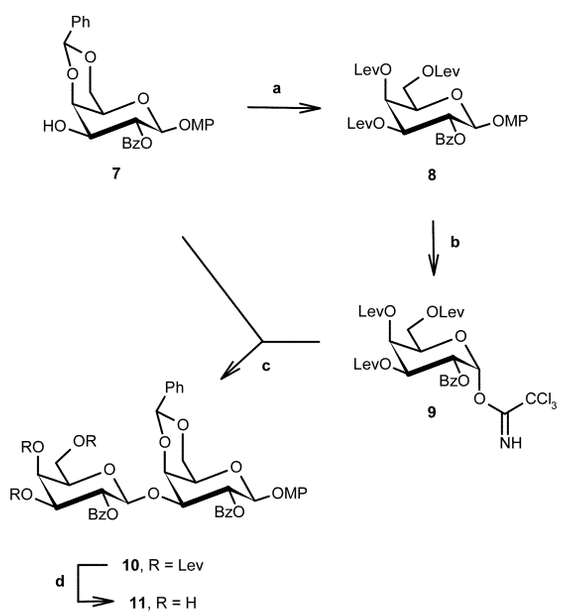
5, R = R¹ = R² = H, R³ = SO_3Na

6, R = R² = SO_3Na, R¹ = R³ = H

Fig. 2 The trisaccharide derivative **1** and its sulfoforms **2**–**6**.

D-Gal units were differentiated at C-4 and C-6 by the use of 4,6-benzylidene and 4,6-di-*tert*-butylsilylene acetals, respectively. The latter may be prepared by stereoselective assembly of a glucuronyl donor (**14**) and a digalactosyl acceptor (**13**) easily available from a digalactosyl precursor (**11**), which may, in turn, be conveniently prepared from a single starting material (**7**). The stereoselective coupling of each unit, ensured by the presence of a stereocontrolling auxiliary (benzoyl group) at C-2, was designed to be performed using the trichloroacetimidate glycosylation procedure.[13]

Preparation of the intermediate disaccharide derivative **11** was achieved as follows (Scheme 1). The common starting material was the known[14] 4-methoxyphenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside **7**, easily obtained from D-galactose in multigram quantities. Acid hydrolysis of **7** with 90% trifluoroacetic acid (TFA) followed by treatment of the resulting triol with 4-oxopentanoic acid (levulinic acid), 1,3-dicyclohexylcarbodiimide and 4-dimethylaminopyridine (DMAP) in dichloromethane afforded the crystalline trilevulinate **8** in 80% overall yield. Introduction of the trichloroacetimidoyl group at C-1 was then achieved through oxidative removal of the anomeric 4-methoxyphenyl group with cerium(IV) ammonium nitrate (CAN),[15] followed by imidoylation of the intermediate free hemiacetal with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the α-imidate **9** in 70% overall yield, the anomeric configuration of which was deduced from its ^{1}H NMR spectrum



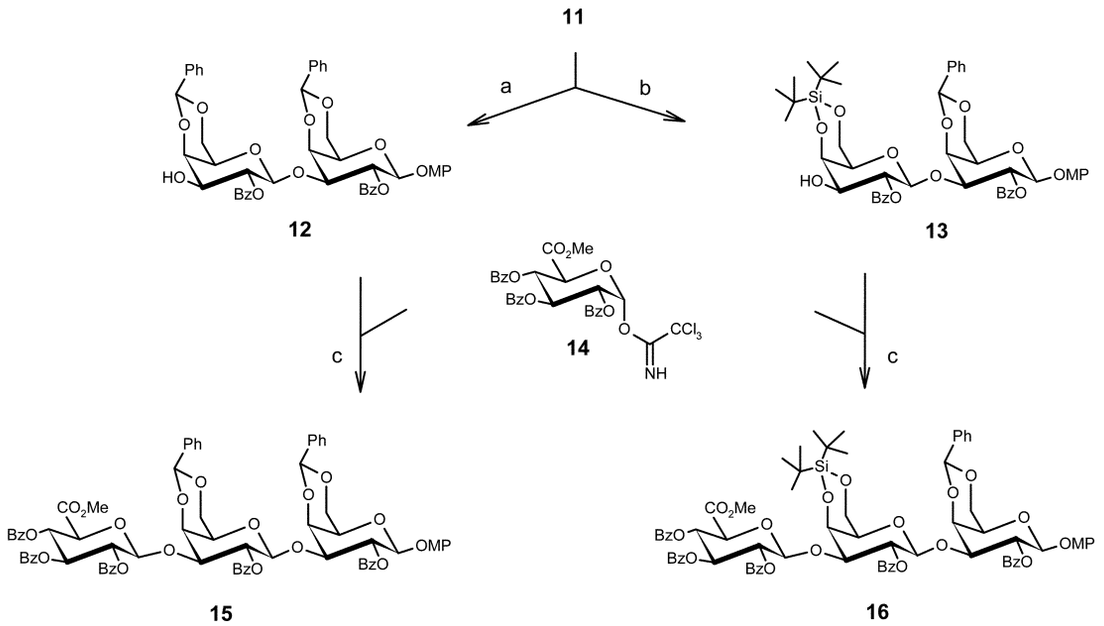
Scheme 1 *Reagents and conditions*: a) 90% TFA, 15 min; then LevOH, DCC, DMAP, CH_2Cl_2, 2 h, 80%; b) CAN, toluene–MeCN–water, 15 min; then CCl_3CN, DBU, CH_2Cl_2, 30 min, 70%; c) TMSOTf, mol. sieves 4 Å, CH_2Cl_2, 30 min, 55%; d) hydrazine acetate, pyridine, 8 min, 90%. MP = 4-methoxyphenyl; Lev = 4-oxopentanoyl (levulinoyl).

($J_{1,2}$ 3.5 Hz). Condensation of the imidate **9** (1.4 mol equiv.) with the alcohol **7** (1 mol equiv.), in dichloromethane at room temperature, and in the presence of trimethylsilyl triflate (TMSOTf, 15% based on **9**) afforded the disaccharide **10** in 55% yield, this moderate yield being essentially due to difficulties encountered in chromatographic separation of the mixture. The anomeric configuration of the newly established interglycosidic linkage was deduced from its ^{1}H NMR spectrum (δ 5.01, $J_{1,2}$ 8.0 Hz, H-1b). Treatment of **10** with hydrazine acetate[16] in pyridine gave the crystalline triol **11** in 90% yield.

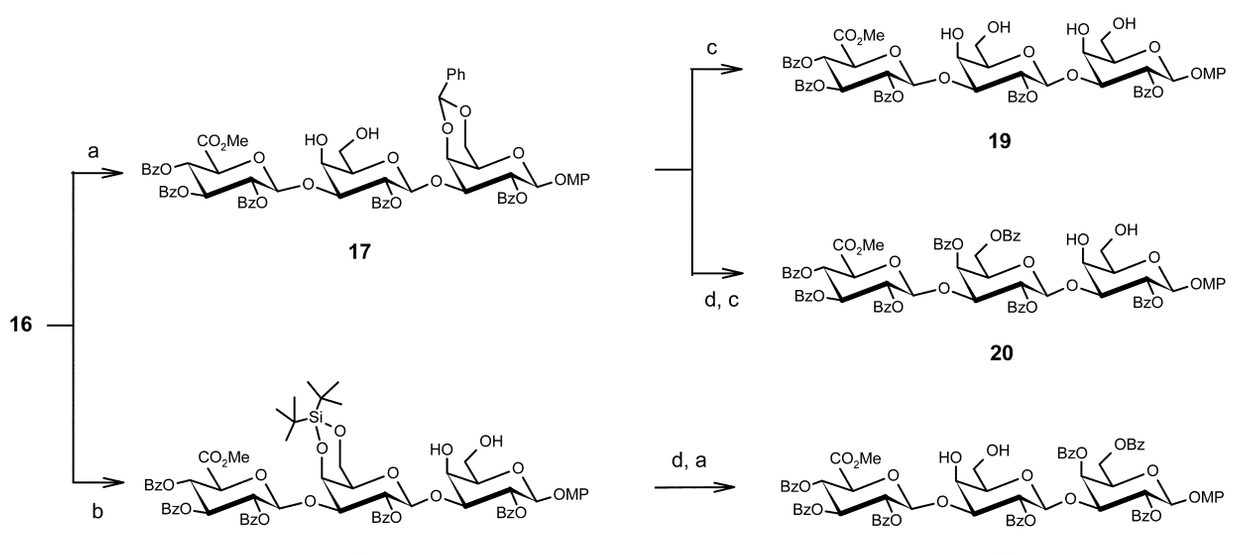
Transformation of **11** into the key trisaccharide derivative **16** was next achieved as follows (Scheme 2). First of all, the triol **11** was treated with benzaldehyde and TFA (5%, v/v) to give the crystalline bis-benzylidene acetal **12**, a symmetrically protected derivative which could serve as a precursor of tetrol **19**, in 88% yield. Treatment of **11** with di-*tert*-butylsilyl ditriflate[17,18] and 2,6-lutidine in dichloromethane gave smoothly the crystalline silylene acetal **13** in 86% yield, the position of which was deduced from its ^{1}H NMR spectrum (δ 2.60, d, J 10.8 Hz, *H*O-3b). The convenience of the 4,6-di-*tert*-butylsilylene acetal (DTBS) in glycoconjugate synthesis was recently highlighted.[19] Coupling reactions that involved the alcohols **12** and **13** and the donor methyl 2,3,4-tri-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate **14**[20] were achieved as described for the preparation of **10** to afford the crystalline trisaccharides **15** and **16** in 25 and 70% yields, respectively, the structures of which were easily deduced from their ^{1}H NMR spectra (δ 4.98, $J_{1,2}$ 7.0 Hz, and δ 4.96, $J_{1,2}$ 7.0 Hz, H-1c, respectively). The low yield obtained in the coupling reaction with **12** was rather unexpected, and changes in the reaction conditions (solvent, catalyst, temperature, details not presented in the Experimental section) did not significantly improve the yield, but this result was in agreement with those reported for glucuronylation reaction of 4,6-*O*-benzylidene-D-*galacto* acceptors.[21] However, the acceptor **13**, in which the bulky silylene acetal was expected to cause steric hindrance at O-3b, gave a satisfactory result probably due to subtle electronic factors, possibly originating from the strong electron-donating effect of the *tert*-butyl groups which should increase the nucleophilic character of the oxygen atom at C-3b.

The protective group pattern in **16** allowed access to the target sulfoforms depending on the partial deprotection sequence (Scheme 3). Attempted deprotection of the DTBS group in **16** under standard conditions (tetra-*n*-butylammonium fluoride–acetic acid in oxolane) failed, but treatment of **16** with triethylamine–trihydrofluoride complex[22] ($Et_3N{\cdot}3HF$) in tetrahydrofuran at 0 °C gave smoothly the crystalline diol **17** in nearly quantitative yield. Careful treatment of **16** with 80% TFA in dichloromethane at 0 °C allowed isolation of the crystalline diol **18** in 80% yield. Acid hydrolysis of **17** with 90% TFA gave the crystalline tetrol **19** in 85% yield, whereas conventional benzoylation (benzoyl chloride in pyridine) of **17** followed by hydrolysis as described above afforded the crystalline diol **20** in 83% overall yield. Benzoylation of **18** followed by desilylation, as described above, provided the diol **21** in 81% overall yield. The ^{1}H NMR spectra for **19**–**21** are in complete agreement with the expected structures and showed high purity for these crystalline intermediates.

Transformation of **19**–**21** into the target molecules **1**–**6** was next achieved as follows (Scheme 4). To avoid an elimination reaction under basic conditions on the sensitive methyl uronate moiety,[20] saponification of the ester groups in **19** was performed first by treatment with lithium hydroperoxide[16] in THF, followed by methanolic sodium hydroxide to afford the target trisaccharide derivative **1**, as its sodium salt, in 92% yield. Tetrol **19** and diols **20** and **21** were regioselectively *O*-sulfonated at C-6 by treatment with the sulfur trioxide–trimethylamine complex ($Me_3N{\cdot}SO_3$, 2 mol equiv. *per* primary hydroxy group) in DMF at 40 °C, followed by ion-exchange chromatography (Na^+ resin) to afford the sodium salts **22**, **23** and **26**, respectively, in 83, 86



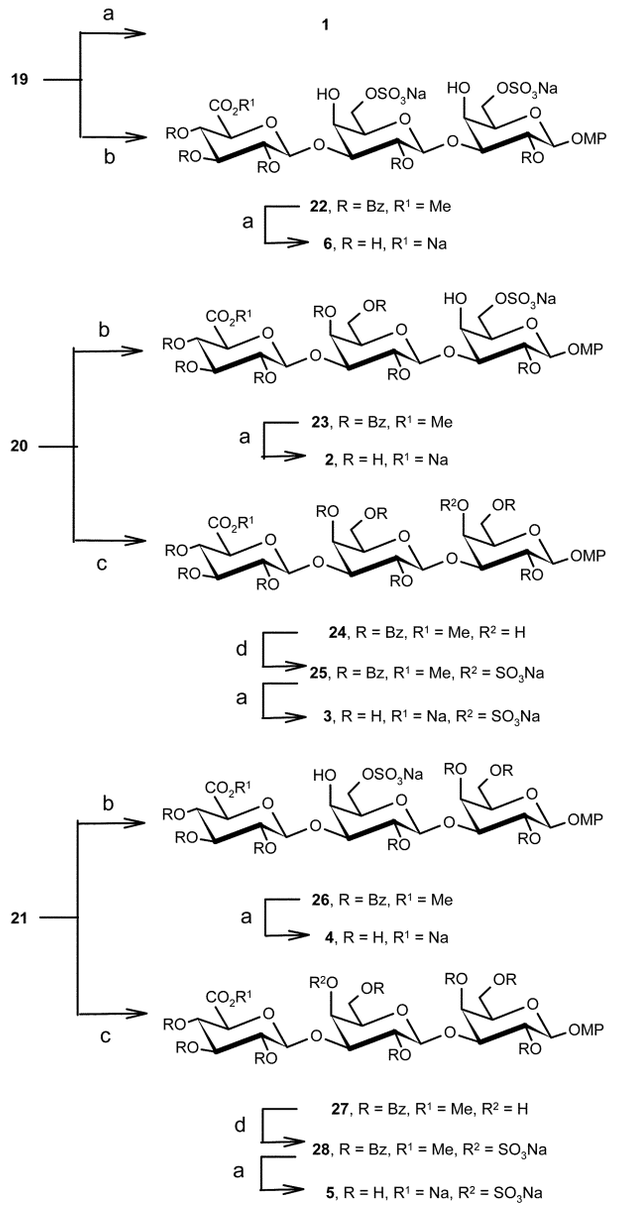
Scheme 2 *Reagents and conditions*: a) PhCHO, TFA, 30 min, 88%; b) DTBS ditriflate, 2,6-lutidine, CH_2Cl_2, 2 h, 86%; c) TMSOTf, mol. sieves 4 Å, CH_2Cl_2, 30 min, **15** : 25%; **16** : 70%.



Scheme 3 *Reagents and conditions*: a) $Et_3N{\cdot}3HF$, THF, 0 °C, 4 h, 95%; b) 80% TFA, CH_2Cl_2, 0 °C, 15 min, 80%; c) 90% TFA, 15 min, 85%; d) PhCOCl, pyridine, 0 °C, 4 h, quantitative.

and 87% yields, respectively. Very small amounts (2–3%) of the corresponding 4,6-disulfate derivatives were also obtained, but easily removed by simple silica chromatography. It is worth noting that tin-mediated regioselective sulfation[23–25] proceeded in lower yields, mainly due to the difficulty in removing completely the tin salts. Comparison of the ^{1}H NMR spectra of **22**, **23** and **26** with those of their non-sulfated precursors, all recorded in 3 : 1 CD_3OD–$CDCl_3$ (a mixture which provided good solubility and resolution for all compounds), showed the expected[26,27] downfield shifts ($\Delta\delta$ ~ 0.4 ppm) of the signals for Gal H-6 in sulfates **22**, **23** and **26**, and no change of the signals for Gal H-4, demonstrating that sulfation occurred exclusively at C-6. For the preparation of the 4-sulfated derivatives, the low reactivity of the axial 4-hydroxy groups in D-*galacto* structures requires first temporary protection at O-6.[28] Thus, treatment of diols **20** and **21** with benzoyl cyanide in pyridine gave the alcohols **24** and **27** in 91 and 90% yields, respectively. These later were sulfated by treatment with a large excess (10 mol equiv.) of $Me_3N{\cdot}SO_3$ in DMF at 60 °C for 4 days, followed by ion-exchange, to give the sodium salts **25** and **28** in 88 and 89% yields, respectively. Comparison of the ^{1}H NMR spectra of **25** and **28** with those of their precursors, recorded as described above, also showed the expected[26] significant downfield shifts ($\Delta\delta$ ~ 0.9 ppm) of the signals for Gal H-4 in sulfates **25** and **28**. Saponification of the esters **22**, **23**, **25**, **26** and **28**, as described for the preparation of **1**, afforded the target sulfoforms **6** and **2**–**5**, respectively, in ~90% yields. The ^{1}H NMR data of the five sulfoforms were compared with those of their non sulfated congener **1** (Table 1). Particularly relevant were the expected[26] downfield shifts ($\Delta\delta$ ~ 0.4 ppm) of the signals for Gal H-6 in the 6-sulfated species, and those ($\Delta\delta$ ~ 0.6 ppm) of the signals for Gal H-4 in the 4-sulfated derivatives. Comparison of the ^{13}C NMR data (Table 2) also showed the expected[26] downfield shifts ($\Delta\delta$ ~ 7 ppm) of the signals for Gal C-6 in the 6-sulfated derivatives and those ($\Delta\delta$ ~ 9 ppm) of the signals for Gal C-4 in the 4-sulfated species. These NMR data are in complete agreement with the expected structures, and in accord with those reported for synthetic derivatives containing D-Gal units bearing sulfate groups at C-4 or C-6[10–12] as well as for fragments isolated from natural proteoglycans.[4–6]

In a continuous effort to try to reduce the number of transformations in a multi-step preparation, we attempted the



Scheme 4 *Reagents and conditions*: a) LiOH–H_2O_2, THF; then NaOH, MeOH, **1**: 92%, **2**–**6**: 90%; b) $Me_3N{\cdot}SO_3$, DMF, 40 °C, 90 min; then ion-exchange (Na^+ resin), **22**: 83%, **23**: 86%; **26**: 87%; c) PhCOCN, pyridine, 16 h, **24**: 91%, **27**: 90%; d) $Me_3N{\cdot}SO_3$ (10 mol equiv.), DMF, 60 °C, 4 d, **25**: 88%, **28**: 89%.

regioselective monosulfation of tetrol **19** (Scheme 5). Thus, carefully controlled treatment of **19** (1 mol equiv.) with $Me_3N{\cdot}SO_3$ (1.5 mol equiv. *per* primary hydroxy group) in DMF at 40 °C for 40 min (optimal reaction time for the major formation of the monosubstituted species determined by TLC control), followed by silica chromatography and ion-exchange, afforded, beside traces of the starting tetrol, the 6a-sulfate **29**, the 6b-sulfate **30** and the 6a,6b-disulfate **22**, respectively, in 27, 20 and 38% yields, respectively. Since mass spectrometry showed that **29** and **30** are monosulfated derivatives, the problem was to determine on which of the two similar D-Gal units was located the sulfate group. Their ^{1}H NMR spectra were recorded under the conditions reported above, and the use of spin decoupling and 2D experiments allowed unambiguous assignment of the Gal-6 protons. The expected downfield shifts ($\Delta\delta \sim 0.4$ ppm) of the signals for Gal H-6 in the sulfated moiety were observed. In addition, saponification of **29** and **30**, as described, afforded the sulfoforms **2** and **4**, respectively, with physical data identical to those of the independently prepared derivatives.

In conclusion, we have reported a stereocontrolled and high-yielding approach for the preparation of a set of sulfoforms of

Table 1 ^{1}H NMR data (500 MHz, D_2O, 25 °C, internal acetone, δ_H 2.225 ppm) for trisaccharide **1** and its sulfoforms **2**–**6**[a]

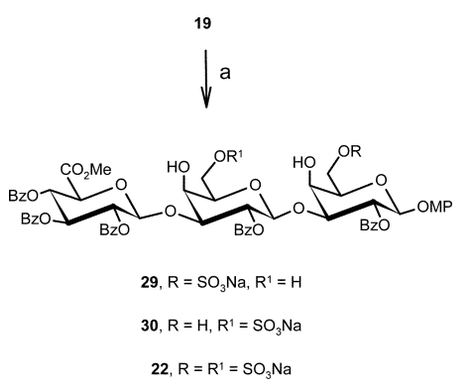
	1	**2**	**3**	**4**	**5**	**6**
H-1a	4.70	4.72	4.75	4.71	4.79	4.74
H-2a	3.70	3.74	3.74	3.76	3.72	3.76
H-3a	3.94	3.97	4.16	3.94	3.96	3.97
H-4a	4.27	4.34	**4.94**	4.36	4.27	4.37
H-5a	3.80	3.97	3.98	3.82	3.82	3.88
H-6a	3.80	**4.14**	3.80	3.82	3.82	**4.24**
		4.24				**4.30**
H-1b	5.05	5.12	5.22	5.18	5.15	5.11
H-2b	3.72	3.74	3.74	3.76	3.72	3.76
H-3b	3.94	3.76	3.98	3.94	4.08	3.97
H-4b	4.20	4.22	4.09	4.24	**4.82**	4.28
H-5b	3.80	3.78	3.86	3.95	3.96	3.95
H-6b	3.80	3.78	3.80	**4.22**	3.82	**4.24**
				4.30		**4.30**
H-1c	4.68	4.70	4.70	4.70	4.74	4.71
H-2c	3.43	3.44	3.44	3.44	3.44	3.45
H-3c	3.52	3.54	3.54	3.53	3.54	3.54
H-4c	3.52	3.54	3.54	3.53	3.54	3.54
H-5c	3.75	3.76	3.80	3.80	3.80	3.82

[a] Bold-type values reflect the positions of sulfation.

Table 2 ^{13}C NMR data (62.8 MHz, D_2O, 25 °C, internal acetone, δ_C 30.35 ppm) for trisaccharide **1** and its sulfoforms **2**–**6**[a]

	1	**2**	**3**	**4**	**5**	**6**
C-1a	101.67	101.56	101.74	101.57	101.65	101.49
C-2a	69.94	69.84	70.37	69.78	69.98	69.77
C-3a	82.26	82.06	78.40	82.48	82.39	82.28
C-4a	68.13	68.24	**77.51**	68.13	68.45	68.02
C-5a	75.01	75.82	74.67	75.42	75.21	72.64
C-6a	60.90	**67.30**	60.95	61.24	60.91	**67.80**
C-1b	104.20	104.25	104.17	104.04	104.35	104.01
C-2b	70.38	70.37	70.44	70.23	71.24	70.14
C-3b	82.61	82.84	82.54	82.75	78.42	82.42
C-4b	68.51	68.32	68.60	68.57	**76.87**	68.59
C-5b	75.18	74.99	75.13	72.75	74.69	73.09
C-6b	61.17	61.12	61.29	**68.03**	61.15	**68.02**
C-1c	103.79	103.80	103.89	103.84	103.89	103.81
C-2c	73.33	73.35	73.24	73.35	73.31	73.34
C-3c	75.49	75.51	75.20	75.54	75.37	75.50
C-4c	71.92	71.97	71.98	71.97	72.06	71.96
C-5c	76.21	76.37	76.41	76.39	76.50	76.38
C-6c	175.79	176.11	176.12	176.14	176.07	176.09

[a] Bold-type values reflect the positions of sulfation.



Scheme 5 Regioselective *O*-sulfonation of tetrol **19**. *Reagents and conditions*: a) $Me_3N{\cdot}SO_3$, DMF, 40 °C, 40 min, **29**: 27%, **30**: 20%, **22**: 38%.

the trisaccharide **1**. The use of common precursors and the possible regioselective preparation of the 6-sulfated species render this route attractive for the further preparation of more

complex structures of the linkage region of proteoglycans. These molecules will be useful to assess the role of the various sulfate groups in the biosynthesis of GAGs. These compounds are currently being evaluated in biological assays, and the results of these studies will be reported elsewhere in due course.

Experimental

General procedures

Melting points were determined with a Büchi apparatus and are uncorrected. Optical rotations were measured at room temperature (22 °C) in a 1 dm cell with a Perkin-Elmer 241 polarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded at 25 °C on Bruker DPX 250 Advance and Varian Unity 500 spectrometers with Me_4Si as internal reference, unless otherwise stated, and *J* values are quoted in Hz. Assignments were based on homo- and heteronuclear correlations using the manufacturers software. In the description of the NMR spectra, a, b, and c refer to the monosaccharide units (a: reducing end) in the oligosaccharides. Low-resolution mass spectra were recorded with a Perkin-Elmer Sciex API 3000 spectrometer in the ion-spray (IS) mode. TLC was performed on Merck 60 F_{254} precoated plates, and compounds were detected by spraying the plates with 5% H_2SO_4 in EtOH, and heating. Flash silica chromatography was performed using Merck silica gel C60 (0.040–0.063 mm). Elemental analyses were carried out at the Service Central de Microanalyse du CNRS (Vernaison, France).

4-Methoxyphenyl 2-*O*-benzoyl-3,4,6-tri-*O*-levulinoyl-β-D-galactopyranoside 8

A solution of 4-methoxyphenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside **7**[14] (4.78 g, 10 mmol) in 90% aq CF_3COOH (40 cm^3) was stirred for 15 min at rt, then was concentrated, evaporated with water (3 × 20 cm^3) and dried *in vacuo*. A mixture of the solid residue, levulinic acid (4.20 g, 36 mmol) and DMAP (0.36 g, 3 mmol) in dry dichloromethane (80 cm^3) was treated portionwise with DCC (7.50 g, 36 mmol), and the mixture was stirred for 2 h. The solids were filtered off, washed with dichloromethane (100 cm^3), and the filtrate was washed with cold 0.1 M hydrochloric acid, saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Crystallization of the residue from EtOAc–petroleum ether gave the *tri-levulinate* **8** (5.48 g, 80% from **7**); mp 94–95 °C; $[\alpha]_D$ +22 (*c* 1 in $CHCl_3$); (Found: C, 61.3; H, 5.9. $C_{35}H_{40}O_{14}$ requires C, 61.4; H, 5.9%); δ_H(250 MHz, $CDCl_3$) 2.04, 2.16, 2.20 (9 H, 3 s, 3 × COCH_3), 2.60 (12 H, m, 6 × CH_2CO), 3.74 (3 H, s, OCH_3), 4.08 (1 H, m, H-5), 4.22 (2 H, m, 2 × H-6), 5.06 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.23 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3), 5.52 (1 H, dd, $J_{4,5}$ 0.8, H-4), 5.69 (1 H, dd, H-2) and 6.70–8.0 (9 H, m, ArH); *m*/*z* 708 [M + Na]$^+$.

2-*O*-Benzoyl-3,4,6-tri-*O*-levulinoyl-1-*O*-trichloroacetimidoyl-α-D-galactopyranose 9

A mixture of **8** (1.77 g, 2.6 mmol) and CAN (7.12 g, 13 mmol) in toluene–acetonitrile–water (1 : 1.5 : 1, 70 cm^3) was stirred for 15 min at rt, then was poured into ice-cold water and extracted with EtOAc (3 × 50 cm^3). The combined extracts were washed with saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Flash silica chromatography (6 : 1 EtOAc–petroleum ether) gave the corresponding hemiacetal (1.35 g, 90%) as a yellow foam. Trichloroacetonitrile (2 cm^3, 20 mmol) was added to a solution of the hemiacetal and DBU (0.06 cm^3, 0.4 mmol) in dry dichloromethane (10 cm^3), and the mixture was stirred for 30 min at rt, then was concentrated. Flash silica chromatography (4 : 1 EtOAc–petroleum ether, containing 0.1% of Et_3N) afforded the *α-imidate* **9** (1.31 g, 70% from **8**) as a colourless glass; $[\alpha]_D$ +96 (*c* 1 in $CHCl_3$); (Found: C, 49.7; H, 4.8; N, 1.8. $C_{30}H_{34}Cl_3NO_{13}$ requires C, 49.8; H, 4.7; N, 1.9%); δ_H(250 MHz, $CDCl_3$) 2.05, 2.15, 2.18 (9 H, 3 s, 3 × COCH_3), 2.62 (12 H, m, 6 × CH_2CO), 4.15 (2 H, m, 2 × H-6), 4.50 (1 H, m, H-5), 5.62 (3 H, m, H-2, H-3, H-4), 6.72 (1 H, d, $J_{1,2}$ 3.5, H-1), 7.20–8.0 (5 H, m, Ph) and 8.60 (1 H, s, C=N*H*); *m*/*z* 795 [M + Na]$^+$, 611 [M − CCl_3CONH]$^+$ for ^{35}Cl.

4-Methoxyphenyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-levulinoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 10

A mixture of imidate **9** (4.92 g, 6.8 mmol), alcohol **7** (2.35 g, 4.9 mmol) and 4 Å powdered molecular sieves (3.0 g) in dry dichloromethane (60 cm^3) was stirred for 1 h at rt under dry argon. A solution of Me_3SiOTf in dry toluene (1 mol dm^{-3}, 1 cm^3) was added, and the mixture was stirred for 30 min, then was quenched with Et_3N (0.4 cm^3), filtered and concentrated. Flash silica chromatography (5 : 2 EtOAc–toluene, containing 0.1% of Et_3N) gave the *disaccharide* **10** (2.80 g, 55%) as a white foam; $[\alpha]_D$ +38 (*c* 1 in $CHCl_3$); (Found: C, 63.4; H, 5.7. $C_{55}H_{58}O_{20}$ requires C, 63.6; H, 5.6%); δ_H(250 MHz, $CDCl_3$) 2.10, 2.15, 2.20 (9 H, 3 s, 3 × COCH_3), 2.55 (12 H, m, 6 × CH_2CO), 3.63 (1 H, m, H-5a), 3.69 (3 H, s, OCH_3), 3.95 (1 H, m, H-5b), 4.10 (2 H, m, 2 × H-6a), 4.22 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.6, H-3a), 4.30 (2 H, m, 2 × H-6b), 4.48 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.91 (1 H, d, $J_{1,2}$ 8.0, H-1a), 5.0 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.02 (1 H, dd, $J_{2,3}$ 10.4, $J_{3,4}$ 3.6, H-3b), 5.40 (2 H, m, H-2b, H-4b), 5.60 (1 H, s, PhC*H*), 5.76 (1 H, dd, H-2a) and 6.70–8.0 (19 H, m, ArH); *m*/*z* 1062 [M + Na]$^+$.

4-Methoxyphenyl *O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 11

A freshly prepared mixture of pyridine–acetic acid–hydrazine hydrate (6 : 4 : 0.5, 42 cm^3) was added to a solution of **10** (2.08 g, 2 mmol) in pyridine (10 cm^3), and the mixture was stirred for 8 min at rt, then was diluted with dichloromethane (250 cm^3), washed with water, saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Crystallization of the residue from MeOH gave the *triol* **11** (1.34 g, 90%); mp 256–258 °C; $[\alpha]_D$ +18 (*c* 1 in $CHCl_3$); (Found: C, 64.4; H, 5.5. $C_{40}H_{40}O_{14}$ requires C, 64.5; H, 5.4%); δ_H(250 MHz, CD_3OD) 3.60 (2 H, m, H-5a, H-3b), 3.68 (3 H, s, OCH_3), 3.75 (1 H, m, H-5b), 3.80 (2 H, m, 2 × H-6b), 3.84 (1 H, dd, $J_{3,4}$ 3.6, $J_{4,5}$ 0.8, H-4b), 4.18 (2 H, m, 2 × H-6a), 4.22 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.54 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.78 (1 H, d, $J_{1,2}$ 8.0, H-1a), 5.01 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.24 (1 H, dd, $J_{2,3}$ 10.2, H-2b), 5.37 (1 H, s, PhC*H*), 5.64 (1 H, dd, H-2a) and 6.70–8.0 (19 H, m, ArH); *m*/*z* 768 [M + Na]$^+$.

4-Methoxyphenyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 12

A mixture of triol **11** (372 mg, 0.5 mmol), benzaldehyde (5 cm^3) and CF_3COOH (0.2 cm^3) was stirred for 30 min at rt, then was cooled to 0 °C. Triethylamine (0.6 cm^3) was added, and the mixture was diluted with dichloromethane (50 cm^3), washed with saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Recrystallization of the jelly-like residue from EtOH afforded the *alcohol* **12** (369 mg, 88%); mp 310–313 °C; $[\alpha]_D$ +50 (*c* 1 in pyridine); (Found: C, 67.7; H, 5.25. $C_{47}H_{44}O_{14}$ requires C, 67.8; H, 5.3%); δ_H(250 MHz, $CDCl_3$) 2.55 (1 H, d, *J* 10.0, *H*O-3b), 3.45 (1 H, m, H-5a), 3.55 (1 H, m, H-5b), 3.70 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3b), 3.72 (3 H, s, OCH_3), 4.10 (4 H, m, 2 × H-6, H-3a, H-4b), 4.35 (3 H, m, 2 × H-6, H-4a), 5.0 (1 H, d, $J_{1,2}$ 8.0, H-1a), 5.02 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.35 (1 H, dd, H-2b),

5.43, 5.53 (2 H, 2 s, 2 × PhC*H*), 5.81 (1 H, dd, $J_{2,3}$ 10.2, H-2a) and 6.70–8.10 (24 H, m, ArH); *m/z* 856 $[M + Na]^+$.

4-Methoxyphenyl *O*-(2-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 13

To a cooled (0 °C) solution of triol **11** (745 mg, 1 mmol) and 2,6-lutidine (0.29 cm³, 2.5 mmol) in dry dichloromethane (10 cm³) was added di-*tert*-butylsilyl ditriflate (0.39 cm³, 1.15 mmol), and the mixture was stirred for 1 h at 0 °C, then for 1 h at rt. The mixture was diluted with dichloromethane (50 cm³), washed with water, cold 0.1 M hydrochloric acid, saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Flash silica chromatography (5 : 2 toluene–EtOAc) and crystallization of the residue from EtOAc–petroleum ether gave the *silylene acetal* **13** (765 mg, 86%); mp 285–287 °C; $[\alpha]_D$ +17 (*c* 1 in $CHCl_3$); (Found: C, 65.1; H, 6.4. $C_{48}H_{56}O_{14}Si$ requires C, 65.1; H, 6.4%); δ_H(250 MHz, $CDCl_3$) 1.01, 1.05 (18 H, 2 s, 2 × C(CH_3)$_3$), 2.60 (1 H, d, *J* 10.8, *HO*-3b), 3.55 (3 H, m, H-5a, H-5b, H-3b), 4.20 (5 H, m, 2 × H-6a, 2 × H-6b, H-3a), 4.40 (2 H, m, H-4a, H-4b), 4.92 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.96 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.34 (1 H, dd, $J_{2,3}$ 10.3, H-2b), 5.43 (1 H, s, PhC*H*), 5.75 (1 H, dd, $J_{2,3}$ 10.4, H-2a) and 6.70–8.0 (19 H, m, ArH); *m/z* 908 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 15

A mixture of alcohol **12** (166 mg, 0.2 mmol) and methyl 2,3,4-tri-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate **14**[20] (200 mg, 0.3 mmol) was treated as described for the preparation of **10**. Flash silica chromatography (15 : 1 dichloromethane–EtOAc, containing 0.1% of Et_3N) and crystallization of the residue from EtOH afforded the *trisaccharide* **15** (67 mg, 25%); mp 305–308 °C; $[\alpha]_D$ +30 (*c* 1 in $CHCl_3$); (Found: C, 67.3; H, 5.1. $C_{75}H_{66}O_{23}$ requires C, 67.5; H, 5.0%); δ_H(250 MHz, $CDCl_3$) 3.33 (1 H, m, H-5a), 3.46 (1 H, m, H-5b), 3.71 (3 H, s, OCH_3), 3.95 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.01 (2 H, m, 2 × H-6), 4.30 (1 H, d, $J_{4,5}$ 10.0, H-5c), 4.32 (3 H, m, 2 × H-6, H-3b), 4.40 (2 H, m, H-4a, H-4b), 4.95 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.96 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.98 (1 H, d, $J_{1,2}$ 7.0 Hz, H-1c), 5.29, 5.44 (2 H, 2 s, 2 × PhC*H*), 5.45 (1 H, dd, $J_{2,3}$ 9 0, H-2c), 5.53 (1 H, dd, $J_{2,3}$ 10.3, H-2b), 5.70 (3 H, m, H-2a, H-3c, H-4c) and 6.70–8.0 (39 H, m, ArH); *m/z* 1358 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 16

A mixture of alcohol **13** (885 mg, 1 mmol) and imidate **14** (930 mg, 1.4 mmol) was treated as described for the preparation of **10**. Flash silica chromatography (18 : 1 dichloromethane–EtOAc, containing 0.1% of Et_3N) and crystallization of the residue from EtOH gave the *trisaccharide* **16** (972 mg, 70%); mp 263–265 °C; $[\alpha]_D$ +16 (*c* 1 in $CHCl_3$); (Found: C, 65.7; H, 5.7. $C_{76}H_{78}O_{23}Si$ requires C, 65.8; H, 5.7%); δ_H(250 MHz, $CDCl_3$) 1.01, 1.15 (18 H, 2 s, 2 × C(CH_3)$_3$), 3.45 (1 H, m, H-5b), 3.50 (1 H, m, H-5a), 3.65 (3 H, s, OCH_3), 3.69 (3 H, s, COOCH_3), 3.93 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 4.10 (2 H, m, H-6a, H-6b), 4.17 (1 H, dd, $J_{4,5}$ 10.0, H-5c), 4.21 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3a), 4.30 (3 H, m, H-4a, H-6a, H-6b), 4.70 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.92 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.93 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.96 (1 H, d, $J_{1,2}$ 7.0, H-1c), 5.34 (1 H, s, PhC*H*), 5.45 (2 H, m, H-2c, H-2b), 5.59 (1 H, dd, $J_{3,4}$ 9.0, H-4c), 5.65 (1 H, dd, H-2a), 5.66 (1 H, t, $J_{2,3}$ 9.0, H-3c) and 6.70–8.10 (34 H, m, ArH); *m/z* 1411 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 17

To a cooled (0 °C) solution of **16** (1.0 g, 0.72 mmol) in dry THF (15 cm³) was added $Et_3N{\cdot}3HF$ (0.25 cm³, 1.5 mmol), and the mixture was stirred for 4 h at 0 °C, then was diluted with dichloromethane (100 cm³) and pyridine (10 cm³, to avoid precipitation of the diol), washed with saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Recrystallization of the solid residue from MeOH–pyridine gave the *diol* **17** (856 mg, 95%); mp 320–323 °C; $[\alpha]_D$ +28 (*c* 0.5 in pyridine); (Found: C, 65.3; H, 5.15. $C_{68}H_{62}O_{23}$ requires C, 65.5; H, 5.0%); δ_H(250 MHz, $(CD_3)_2SO$ + D_2O) 3.50 (1 H, m, H-5a), 3.65 (3 H, s, OCH_3), 3.70 (3 H, s, COOCH_3), 3.90 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.8, H-3b), 4.0 (4 H, m, H-3a, H-5b, H-6a, H-6b), 4.15 (1 H, d, $J_{4,5}$ 10.0, H-5c), 4.25 (4 H, m, H-4a, H-4b, H-6a, H-6b), 4.80 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.90 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.0 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.45 (3 H, m, H-2b, H-2c, H-4c), 5.65 (2 H, m, H-2a, H-3c) and 6.70–8.10 (24 H, m, ArH); *m/z* 1270 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-β-D-galactopyranoside 18

To a cooled (0 °C) solution of **16** (0.5 g, 0.36 mmol) in dichloromethane (12 cm³) was added dropwise 80% aq CF_3COOH (1.2 cm³), and the mixture was stirred for 15 min at 0 °C, then was diluted with dichloromethane (50 cm³), washed with water, saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Flash silica chromatography (8 : 1 dichloromethane–acetone) and recrystallization of the residue from EtOH afforded the *diol* **18** (0.42 g, 80%); mp 265–268 °C; $[\alpha]_D$ +33 (*c* 1 in $CHCl_3$); (Found: C, 63.6; H, 5.8. $C_{69}H_{74}O_{23}Si$ requires C, 63.8; H, 5.75%); δ_H(250 MHz, $CDCl_3$) 1.01, 1.10 (18 H, 2 s, 2 × C(CH_3)$_3$), 2.28, 2.84 (2 H, 2 br s, 2 × O*H*), 3.49 (1 H, m, H-5b), 3.64 (3 H, s, OCH_3), 3.68 (3 H, s, COOCH_3), 3.85 (2 H, m, 2 × H-6a), 3.92 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 4.01 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.6, H-3a), 4.16 (1 H, d, $J_{4,5}$ 10.0, H-5c), 4.25 (2 H, m, 2 × H-6b), 4.69 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.75 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.88 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.0 (1 H, d, $J_{1,2}$ 7.0, H-1c), 5.43 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.50 (2 H, m, H-2b, H-4c), 5.63 (1 H, dd, H-2a), 5.67 (1 H, t, $J_{3,4}$ 9.0, H-3c) and 6.70–8.0 (29 H, m, ArH); *m/z* 1323 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-β-D-galactopyranoside 19

A solution of **17** (0.44 g, 0.35 mmol) in 90% aq CF_3COOH (10 cm³) was stirred for 15 min at rt, then was concentrated, evaporated with water (3 × 10 cm³) and dried *in vacuo*. Recrystallization of the solid residue from MeOH gave the *tetrol* **19** (345 mg, 85%); mp 205–207 °C; $[\alpha]_D$ +20 (*c* 1 in $CHCl_3$); (Found: C, 63.1; H, 5.2. $C_{61}H_{58}O_{23}$ requires C, 63.2; H, 5.05%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.63 (3 H, s, OCH_3), 3.65 (3 H, s, COOCH_3), 3.66 (1 H, m, H-5a), 3.80 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3a), 3.85 (5 H, m, H-5b, 2 × H-6a, 2 × H-6b), 3.92 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 4.17 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.22 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.32 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.69 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.82 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.96 (1H, d, $J_{1,2}$ 7.5, H-1c), 5.35 (2 H, m, H-2b, H-2c), 5.46 (1 H, dd, H-2a), 5.48 (1 H, t, $J_{3,4}$ 9.5, H-4c), 5.71 (1 H, t, $J_{2,3}$ 9.5, H-3c) and 6.70–8.0 (29 H, m, ArH); *m/z* 1160 $[M + H]^+$, 1182 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-β-D-galactopyranoside 20

A mixture of diol **17** (0.4 g, 0.32 mmol) and benzoyl chloride (0.15 cm³, 1.3 mmol) in dry pyridine (6 cm³) was stirred for

4 h at 0 °C, then was quenched with MeOH (0.5 cm^3), diluted with dichloromethane (50 cm^3), washed with water, saturated aq $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. A solution of the crude solid residue in 90% aq CF_3COOH (10 cm^3) was stirred for 15 min at rt, then was concentrated, evaporated with water (3 × 10 cm^3) and dried *in vacuo*. Flash silica chromatography (8 : 1 dichloromethane–acetone) and crystallization of the residue from MeOH afforded the *diol* **20** (363 mg, 83% from **17**); mp 233–235 °C; $[a]_D$ +42 (*c* 1 in $CHCl_3$); (Found: C, 65.75; H, 5.0. $C_{75}H_{66}O_{25}$ requires C, 65.9; H, 4.85%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.49 (1 H, m, H-5a), 3.60 (3 H, s, OCH_3), 3.61 (1 H, dd, *J* 5,6 and 12.0, H-6a), 3.64 (3 H, s, $COOCH_3$), 3.79 (1 H, dd, $J_{5,6}$ 7.0, H-6a), 3.88 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.12 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.24 (1 H, m, H-5b), 4.28 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.36 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 4.50 (2 H, m, 2 × H-6b), 4.76 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.82 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.94 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.21 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.50 (3 H, m, H-2a, H-2b, H-4c), 5.58 (1 H, dd, $J_{3,4}$ 9.5, H-3c), 5.88 (1 H, dd, $J_{4,5}$ 0.8, H-4b) and 6.70–8.10 (39 H, m, ArH); *m*/*z* 1368 $[M + H]^+$, 1390 $[M + Na]^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside 21

Compound **18** (585 mg, 0.45 mmol) was benzoylated as described for the preparation of **20**, then was desilylated as described for the preparation of **17**. Crystallization of the solid residue from EtOH gave the *diol* **21** (0.50 g, 81% from **18**); mp 255–257 °C; $[a]_D$ +23 (*c* 1 in $CHCl_3$); (Found: C, 65.8; H, 5.0. $C_{75}H_{66}O_{25}$ requires C, 65.9; H, 4.85%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.59 (1 H, m, H-5b), 3.60 (3 H, s, OCH_3), 3.63 (3 H, s, $COOCH_3$), 3.68 (1 H, dd, *J* 5.0 and 12.0, H-6b), 3.81 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 3.86 (1 H, dd, $J_{5,6}$ 7.0, H-6b), 4.08 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.20 (1 H, m, H-5a), 4.31 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.39 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3a), 4.50 (2 H, m, 2 × H-6a), 4.73 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.91 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.98 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.23 (1 H, dd, H-2b), 5.29 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.47 (1 H, t, $J_{3,4}$ 9.5, H-4c), 5.66 (2 H, m, H-2a, H-3c), 5.84 (1 H, dd, $J_{4,5}$ 0.8, H-4a) and 6.70–8.10 (39 H, m, ArH); *m*/*z* 1390 $[M + Na]^+$.

4-Methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(β-D-galactopyranosyl)-(1⟶3)-β-D-galactopyranoside 1

A solution of the ester **19** (208 mg, 0.18 mmol) in THF (5 cm^3) was treated at 0 °C with a freshly prepared solution of hydrogen peroxide (30 wt% solution in water, 0.6 cm^3) and lithium hydroxide (1 mol dm^{-3}, 1 cm^3), and the mixture was stirred for 1 h at 0 °C and 5 h at rt, then was cooled to 0 °C. Methanol (2 cm^3) and sodium hydroxide (4 mol dm^{-3}, 2 cm^3) were added, and the mixture was stirred for 4 h at rt. Water (10 cm^3) was added, and the mixture was treated with Amberlite IR-120 $[H^+]$ resin to pH 3 (pHmeter control), then was filtered, concentrated and dried *in vacuo*. Benzoic acid was extracted from the glassy residue with cold abs EtOH (2 × 5 cm^3). The remaining solid was dissolved in water (5 cm^3), and the pH of the solution was brought to 7 (pHmeter control) with diluted aq sodium hydroxide. The solution was eluted from a column (2 × 80 cm) of Sephadex LH-20 with water and freeze-dried to give the *target trisaccharide* **1** (107 mg, 92%) as a white powder; $[a]_D$ −18 (*c* 1 in water); (Found: C; 46.25; H, 5.6. $C_{25}H_{35}NaO_{18}$ requires C, 46.45; H, 5.45%); δ_H(500 MHz, D_2O, internal acetone) data for ring protons are reported in Table 1, 3.70 (3 H, s, OCH_3) and 6.90 (4 H, m, ArH); δ_C(62.8 MHz, D_2O, internal acetone) data for ring carbons are reported in Table 2, 55.98 (OCH_3), 115.22, 118.42, 151.15, and 154.87 (6 C, ArC).

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranoside 22

A solution of the tetrol **19** (116 mg, 0.16 mmol) and the sulfur trioxide–trimethylamine complex (84 mg, 0.64 mmol) in dry DMF (4 cm^3) was stirred for 90 min at 40 °C under dry argon, then was cooled, quenched with MeOH (1 cm^3) and concentrated. Flash silica chromatography (4 : 1 dichloromethane–MeOH, containing 2% of Et_3N) gave a fraction that was eluted from a column (1.5 × 20 cm) of Sephadex SP C25 $[Na^+]$ resin with 9 : 5 : 1 dichloromethane–MeOH–water to give the *disodium salt* **22** (184 mg, 83%) as a white powder; $[a]_D$ +37 (*c* 1 in MeOH); (Found: C, 53.45; H, 4.3. $C_{61}H_{56}Na_2O_{29}S_2$ requires C, 53.75; H, 4.15%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.58 (3 H, s, OCH_3), 3.64 (3 H, s, $COOCH_3$), 3.83 (2 H, m, H-5a, H-5b), 3.92 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.6, H-3a), 3.93 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 4.20 (2 H, m, H-4a, H-4b), 4.25 (4 H, m, 2 × H-6a, 2 × H-6b), 4.34 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.74 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.82 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.01 (1 H, d, $J_{1,2}$ 7.0, H-1c), 5.25 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.48 (2 H, m, H-2b, H-4c), 5.59 (2 H, m, H-2a, H-4c) and 6.70–8.0 (29 H, m, ArH); *m*/*z* 1386 $[M + Na]^+$.

4-Methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-6-*O*-sodium sulfonato-β-D-galactopyranoside 6

The ester **22** (177 mg, 0.13 mmol) was saponified as described for the preparation of **1** to give the *target 6a,6b-disulfate* **6** (100 mg, 91%) as a white hygroscopic powder; $[a]_D$ −13 (*c* 1 in water); (Found: C, 35.0; H, 4.15. $C_{25}H_{33}Na_3O_{24}S_2$ requires C, 35.3; H, 3.9%); δ_H(500 MHz, D_2O, internal acetone) data for ring protons are reported in Table 1, 3.72 (3 H, s, OCH_3) and 6.90 (4 H, m, ArH); δ_C(62.8 MHz, D_2O, internal acetone) data for ring carbons are reported in Table 2, 55.99 (OCH_3), 115.24, 118.31, 151.24, and 154.82 (6 C, ArC).

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranoside 23

A solution of the diol **20** (205 mg, 0.15 mmol) and the sulfur trioxide–trimethylamine complex (40 mg, 0.3 mmol) in dry DMF (4 cm^3) was stirred for 7 h at 40 °C, then was cooled, quenched with MeOH (0.5 cm^3) and concentrated. Flash silica chromatography (10 : 1 dichloromethane–MeOH, containing 2% of Et_3N) gave a fraction that was submitted to ion-exchange as described for the preparation of **22** to afford the *6a-sulfate* **23** (190 mg, 86%) as a white powder; $[a]_D$ +36 (*c* 1 in $CHCl_3$); (Found: C, 60.8; H, 4.7. $C_{75}H_{65}NaO_{28}S$ requires C, 61.0; H, 4.45%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.57 (3 H, s, OCH_3), 3.64 (3 H, s, $COOCH_3$), 3.84 (1 H, m, H-5a), 3.93 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.20 (3 H, m, 2 × H-6a, H-4a), 4.30 (1 H, m, H-5b), 4.34 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.39 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3b), 4.50 (2 H, m, 2 × H-6b), 4.77 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.89 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.04 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.21 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.50 (3 H, m, H-2a, H-2b, H-4c), 5.61 (1 H, dd, $J_{3,4}$ 9.5, H-3c), 5.92 (1 H, dd, $J_{4,5}$ 0.8, H-4b) and 6.70–8.10 (39 H, m, ArH); *m*/*z* 1499 $[M + Na]^+$.

4-Methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(β-D-galactopyranosyl)-(1⟶3)-6-*O*-sodium sulfonato-β-D-galactopyranoside 2

The ester **23** (177 mg, 0.12 mmol) was saponified as described for the preparation of **1** to give the *target 6a-sulfate* **2** (80 mg, 89%) as a white hygroscopic powder; $[a]_D$ −16 (*c* 1 in water); (Found: C, 39.9; H, 4.8. $C_{25}H_{34}Na_2O_{21}S$ requires C, 40.1; H, 4.6%); δ_H(500 MHz, D_2O, internal acetone) data for ring

protons are reported in Table 1, 3.71 (3 H, s, OCH_3) and 6.90 (4 H, m, ArH); δ_C(62.8 MHz, D_2O, internal acetone) data for ring carbons are reported in Table 2, 55.99 (O*C*H$_3$), 115.24, 118.34, 151.20, and 154.87 (6 C, ArC).

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-gluco-pyranosyluronate)-(1⟶3)-(2,4,6-tri-*O*-benzoyl-β-D-galacto-pyranosyl)-(1⟶3)-2,6-di-*O*-benzoyl-β-D-galactopyranoside 24

A mixture of diol **20** (178 mg, 0.13 mmol) and benzoyl cyanide (35 mg, 0.26 mmol) in dry pyridine (4 cm^3) was stirred overnight at rt, then was quenched with MeOH (0.5 cm^3) and concentrated. Flash silica chromatography (9 : 1 dichloromethane–EtOAc) and crystallization of the residue from MeOH gave the *alcohol* **24** (174 mg, 91%); mp 222–224 °C; $[\alpha]_D$ +55 (*c* 1 in $CHCl_3$); (Found: C, 66.8; H, 4.95. $C_{82}H_{70}O_{26}$ requires C, 66.9; H, 4.8%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.63 (3 H, s, OCH_3), 3.67 (3 H, s, COOCH_3), 3.80 (2 H, m, H-5a, H-5b), 3.91 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.17 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.19 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.32 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 4.50 (4 H, m, 2 × H-6a, 2 × H-6b), 4.74 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.83 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.94 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.25 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.58 (4 H, m, H-2a, H-2b, H-3c, H-4c), 5.88 (1 H, dd, $J_{4,5}$ 0.8, H-4b) and 6.70–8.10 (44 H, m, ArH); *m*/*z* 1494 [M + Na]$^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-gluco-pyranosyluronate)-(1⟶3)-(2,4,6-tri-*O*-benzoyl-β-D-galacto-pyranosyl)-(1⟶3)-2,6-di-*O*-benzoyl-4-*O*-sodium sulfonato-β-D-galactopyranoside 25

A mixture of alcohol **24** (147 mg, 0.1 mmol) and the sulfur trioxide–trimethylamine complex (132 mg, 1 mmol) in dry DMF (3 cm^3) was stirred for 4 d at 60 °C under dry argon, then was treated as described for the preparation of **23** to give the *sodium salt* **25** (138 mg, 88%) as a white powder; $[\alpha]_D$ +44 (*c* 1 in $CHCl_3$); (Found: C, 62.4; H, 4.6. $C_{82}H_{69}NaO_{29}S$ requires C, 62.6; H, 4.4%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.59 (3 H, s, OCH_3), 3.64 (3 H, s, COOCH_3), 3.91 (1 H, m, H-5a), 4.11 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.21 (1 H, m, H-5b), 4.36 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.38 (1H, dd, $J_{2,3}$ 10.1, $J_{3,4}$ 3.6, H-3b), 4.60 (4 H, m, 2 × H-6a, 2 × H-6b), 4.81 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.91 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.04 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.14 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 5.23 (1 H, dd, $J_{2,3}$ 9.5, H-2c), 5.43 (1 H, dd, H-2b), 5.56 (2 H, m, H-2a, H-4c), 5.64 (1 H, t, $J_{3,4}$ 9.5, H-3c), 5.97 (1 H, dd, $J_{4,5}$ 0.8, H-4b) and 6.70–8.10 (44 H, m, ArH); *m*/*z* 1597 [M + Na]$^+$.

4-Methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(β-D-galactopyranosyl)-(1⟶3)-4-*O*-sodium sulfonato-β-D-galactopyranoside 3

The ester **25** (127 mg, 80 μmol) was saponified as described for the preparation of **1** to give the *target 4a-sulfate* **3** (54 mg, 90%) as a white powder; $[\alpha]_D$ −15 (*c* 1 in water); (Found: C, 39.9; H, 4.7. $C_{25}H_{34}Na_2O_{21}S$ requires C, 40.1; H, 4.6%); δ_H(500 MHz, D_2O, internal acetone) data for ring protons are reported in Table 1, 3.71 (3 H, s, OCH_3) and 6.90 (4 H, m, ArH); δ_C(62.8 MHz, D_2O, internal acetone) data for ring carbons are reported in Table 2, 55.99 (O*C*H$_3$), 115.24, 118.49, 151.11, and 154.95 (6 C, ArC).

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-gluco-pyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-2,4,6-tri-*O*-benzoyl-β-D-galacto-pyranoside 26

The diol **21** (205 mg, 0.15 mmol) was sulfated as described for the preparation of **23** to give the *sodium salt* **26** (192 mg, 87%) as a white powder; $[\alpha]_D$ +28 (*c* 1 in $CHCl_3$); (Found: C, 60.8; H, 4.6. $C_{75}H_{65}NaO_{28}S$ requires C, 61.0; H, 4.45%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.61 (3 H, s, OCH_3), 3.65 (3 H, s, COOCH_3), 3.85 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 3.91 (1 H, m, H-5b), 4.21 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.28 (3 H, m, H-5b, 2 × H-6b), 4.37 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.41 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3a), 4.50 (2 H, m, 2 × H-6a), 4.74 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.94 (1 H, d, $J_{1,2}$ 8.0, H-1b), 5.05 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.17 (1 H, dd, $J_{2,3}$ 9.5, H-2c), 5.31 (1 H, dd, H-2b), 5.45 (1 H, t, $J_{3,4}$ 9.5, H-4c), 5.59 (1 H, dd, H-2a), 5.71 (1 H, dd, H-3c), 5.94 (1 H, dd, $J_{4,5}$ 0.8, H-4a) and 6.70–8.10 (39 H, m, ArH); *m*/*z* 1496 [M + Na]$^+$.

4-Methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-β-D-galactopyranoside 4

The ester **26** (160 mg, 108 μmol) was saponified as described for the preparation of **1** to give the target *6b-sulfate* **4** (73 mg, 90%) as a white powder; $[\alpha]_D$ −11 (*c* 1 in water); (Found: C, 39.8; H, 4.85. $C_{25}H_{34}Na_2O_{21}S$ requires C, 40.1; H, 4.6%); δ_H(500 MHz, D_2O, internal acetone) data for ring protons are reported in Table 1, 3.72 (3 H, s, OCH_3) and 6.90 (4 H, m, ArH); δ_C(62.8 MHz, D_2O, internal acetone) data for ring carbons are reported in Table 2, 56.00 (O*C*H$_3$), 115.24, 118.38, 151.13, and 154.86 (6 C, ArC).

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-gluco-pyranosyluronate)-(1⟶3)-(2,6-di-*O*-benzoyl-β-D-galactopyrano-syl)-(1⟶3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside 27

The diol **21** (205 mg, 0.15 mmol) was treated as described for the preparation of **24**. Crystallization of the residue from EtOH gave the *alcohol* **27** (198 mg, 90%); mp 196–198 °C; $[\alpha]_D$ +33 (*c* 1 in $CHCl_3$); (Found: C, 66.8; H, 4.9. $C_{82}H_{70}O_{26}$ requires C, 66.9; H, 4.8%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.57 (3 H, s, OCH_3), 3.65 (3 H, s, COOCH_3), 3.86 (2 H, m, H-3b, H-5a), 4.09 (1 H, m, H-5b), 4.22 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.25 (1 H, dd, $J_{3,4}$ 3.6, $J_{4,5}$ 0.8, H-4b), 4.27 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.55 (4 H, m, 2 × H-6a, 2 × H-6b), 4.74 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.85 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.91 (1 H, d, $J_{1,2}$ 7.0, H-1c), 5.32 (2 H, m, H-2b, H-2c), 5.58 (1 H, t, $J_{3,4}$ 9.5, H-4c), 5.68 (2 H, m, H-2a, H-3c), 5.85 (1 H, dd, $J_{4,5}$ 0.8, H-4a) and 6.70–8.10 (44 H, m, ArH): *m*/*z* 1494 [M + Na]$^+$.

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-gluco-pyranosyluronate)-(1⟶3)-(2,6-di-*O*-benzoyl-4-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside 28

The alcohol **27** (147 mg, 0.1 mmol) was sulfated as described for the preparation of **24** to give the *sodium salt* **28** (140 mg, 89%) as a white powder; $[\alpha]_D$ +34 (*c* 1 in $CHCl_3$); (Found: C, 62.4; H, 4.65. $C_{82}H_{69}NaO_{29}S$ requires C, 62.6; H, 4.4%); δ_H(250 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.57 (3 H, s, OCH_3), 3.64 (3 H, s, CO-OCH_3), 4.0 (3 H, m, H-3b, H-5a, H-5b), 4.27 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 4.34 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.50 (4 H, m, 2 × H-6a, 2 × H-6b), 4.74 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.78 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.90 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.11 (1 H, dd, $J_{3,4}$ 3.6, $J_{4,5}$ 0.8, H-4b), 5.14 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.45 (1 H, dd, $J_{2,3}$ 10.2, H-2b), 5.62 (1 H, dd, H-2a), 5.58 (2 H, m, H-3c, H-4c), 5.78 (1 H, dd, $J_{4,5}$ 0.8, H-4a) and 6.70–8.10 (44 H, m, ArH); *m*/*z* 1597 [M + Na]$^+$.

4-Methoxyphenyl *O*-(sodium β-D-glucopyranosyluronate)-(1⟶3)-(4-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-β-D-galactopyranoside 5

The ester **28** (110 mg, 70 μmol) was saponified as described for the preparation of **1** to give the *target 4b-sulfate* **5** (48 mg, 90%) as a white powder; $[\alpha]_D$ −17 (*c* 1 in water); (Found: C, 39.8; H, 4.75. $C_{25}H_{34}Na_2O_{21}S$ requires C, 40.1; H, 4.6%); δ_H(500 MHz, D_2O, internal acetone) data for ring protons are reported in Table 1, 3.72 (3 H, s, OCH_3) and 6.90 (4 H, m, ArH); δ_C(62.8 MHz, D_2O, internal acetone) data for ring carbons are reported in Table 2, 56.00 (O*C*H$_3$), 115.24, 118.41, 151.15, and 154.89 (6 C, ArC).

4-Methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-gluco-pyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyrano-side 29 and 4-methoxyphenyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1⟶3)-(2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1⟶3)-2-*O*-benzoyl-β-D-galactopyranoside 30

A mixture of tetrol **19** (116 mg, 0.1 mmol) and the sulfur trioxide–trimethylamine complex (40 mg, 0.3 mmol) in dry DMF (4 cm^3) was stirred for 40 min at 40 °C under dry argon, then was directly quenched with MeOH (0.5 cm^3) and concentrated. Flash silica chromatography (8 : 1 dichloromethane–MeOH, containing 2% of Et_3N) gave first the starting *tetrol* **19** (6 mg, 5%). Next eluted was a fraction that was submitted to ion-exchange as described previously to give the *6a-sulfated derivative* **29** (34 mg, 27%) as a white powder; $[a]_D$ +32 (*c* 1 in MeOH); δ_H(500 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.57 (3 H, s, OCH_3), 3.64 (3 H, s, COOCH_3), 3.74 (1 H, m, H-5a), 3.84 (1 H, m, H-5b), 3.87 (2 H, m, 2 × H-6b), 3.93 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3a), 3.99 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.6, H-3b), 4.15 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.17 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.28 (2 H, m, 2 × H-6a), 4.32 (1 H d, $J_{4,5}$ 9.5, H-5c), 4.71 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.80 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.95 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.21 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.45 (3 H, m, H-2b, H-3c, H-4c), 5.51 (1 H, dd, H-2a) and 6.70–8.0 (29 H, m, ArH); *m*/*z* 1284 $[M + Na]^+$.

Further elution gave a second fraction that was treated similarly to give the *6b-sulfated derivative* **30** (26 mg, 20%) as a colourless glass; $[a]_D$ +26 (*c* 1 in MeOH); δ_H(500 MHz, 3 : 1 CD_3OD–$CDCl_3$) 3.55 (3 H, s, OCH_3), 3.63 (3 H, s, COOCH_3), 3.68 (1 H, m, H-5a), 3.82 (1 H, m, H-5b), 3.85 (2 H, m, 2 × H-6a), 3.94 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6, H-3b), 3.99 (1 H, dd, $J_{2,3}$ 10.1, $J_{3,4}$ 3.6, H-3a), 4.16 (1 H, dd, $J_{4,5}$ 0.8, H-4a), 4.18 (1 H, dd, $J_{4,5}$ 0.8, H-4b), 4.24 (2 H, m, 2 × H-6b), 4.34 (1 H, d, $J_{4,5}$ 9.5, H-5c), 4.69 (1 H, d, $J_{1,2}$ 8.0, H-1a), 4.76 (1 H, d, $J_{1,2}$ 8.0, H-1b), 4.99 (1 H, d, $J_{1,2}$ 7.5, H-1c), 5.17 (1 H, dd, $J_{2,3}$ 9.0, H-2c), 5.45 (1 H, dd, H-2b), 5.51 (2 H, m, H-3c, H-4c), 5.56 (1 H, dd, H-2a) and 6.70–8.0 (29 H, m, ArH); *m*/*z* 1284 $[M + Na]^+$.

Elution with 4 : 1 dichloromethane–MeOH gave a last fraction that was submitted to ion exchange as described above to give the *6a,6b-disulfated derivative* **22** (52 mg, 38%), with identical physical data as for those previously prepared.

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