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An expeditious preparation of various sulfoforms of the disaccharide β-D-Gal*p*-(1 → 3)-D-Gal*p*, a partial structure of the linkage region of proteoglycans, as their 4-methoxyphenyl β-D-glycosides

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Abstract—An expeditious preparation of various sulfoforms of the disaccharide 4-methoxyphenyl *O*-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside, namely the 4^{I}- and 6^{I}-sulfate, the 4^{II}- and 6^{II}-sulfate, and the $6^{I},6^{II}$-disulfate derivatives, is reported for the first time. These molecules will be useful for the study of the early steps of the biosynthesis and sorting of proteoglycans. All target compounds were readily obtained from the common key intermediate 4-methoxyphenyl *O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-levulinoyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside, easily prepared from the common starting material 4-methoxyphenyl 4,6-*O*-benzylidene-β-D-galactopyranoside. Noticeable is the possible preparation of the different 6-*O*-sulfonated species through a one-pot procedure starting from a tetrol precursor.

Keywords: Proteoglycan; Linkage region; Digalactoside; Sulfoforms

1. Introduction

Proteoglycans (PGs) are complex macromolecular glycoproteins that contain a core protein to which side chains of glycosaminoglycans (GAGs) are covalently attached to L-serine residues. Although GAGs have different repetitive disaccharide units, they are, with the exception of keratan sulfate, covalently linked to the protein core through a common tetrasaccharide sequence,[1] namely β-D-Glc*p*A-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Xyl*p* (Fig. 1). However, it has been demonstrated that this linkage region may be occasionally modified by sulfation at C-4 and/or C-6 of the D-Gal units[2–4] as well as by phosphorylation at C-2 of the D-Xyl residue.[5,6] Recently, it has been established that phosphorylation should be a transient phenomenon,[7] and that the phosphate content decreases after introduction of the D-GlcA residue. Thus, the presence of this phosphate group should be related to the very early steps of the biosynthesis of GAGs. The biological significance of the possible sulfations is however still unknown, but these modifications are possibly related to GAG chain elongation and sorting. The question is what determines whether the GAG chain becomes a glucosaminoglycan (heparin, heparan sulfate) or a galactosaminoglycan (chondroitin sulfates, dermatan sulfate) starting from a common precursor. Investigations of the structural variations in the GAG–protein linkage region may clarify the biological functions of these unique modifying groups. Since the human galactose β-1,3-glucuronyltransferase (GlcAT-1), the key enzyme in the biosynthesis of GAGs, is now available,[8,9] it should be relevant to gain insight into its specificity, particularly toward variously sulfated oligosaccharides of the linkage region. Several syntheses of glycopeptides of various length containing a sulfate group on one or the other D-Gal units have been reported[10–12] in the last decade, but no preparation of all the possible sulfoforms has been described.

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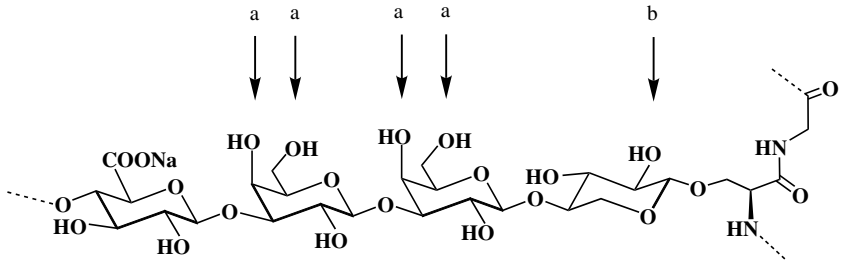


Figure 1. The carbohydrate–protein linkage region of proteoglycans. The arrows indicate possible substitution with a sulfate (a) or a phosphate (b) group.

As a preliminary study devoted to the elucidation of the role of these sulfate groups in the biosynthesis of chondroitin sulfates in relation with osteoarthritis, we now report on for the first time an expeditious preparation of various sulfoforms of the disaccharide 4-methoxyphenyl *O*-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside, namely its 6^{I}-, 6^{II}-, 4^{I}-, and 4^{II}-monosulfate and $6^{I},6^{II}$-disulfate derivatives. The phenyl group will be useful for the detection of the oligosaccharides and the methoxy group will serve as a marker to check the purity of the products by NMR spectroscopy.

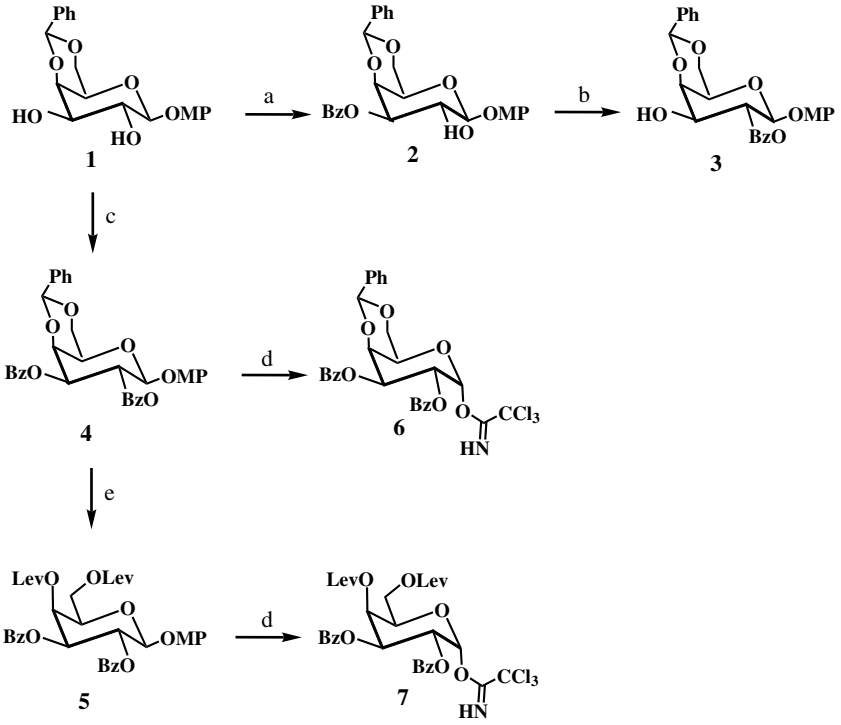
2. Results and discussion

For the synthesis of the target sulfoforms **16**, **19**, **22**, **24**, and **27**, a common disaccharide intermediate (**9**) was designed, which possesses at C-4 and C-6 a pair of orthogonal protecting groups, that is, benzylidene acetal and 4-oxopentanoyl (levulinoyl) esters, thus allowing selective removal and regioselective introduction of the sulfate groups. This later may, in turn, be prepared from a glycosyl acceptor (**3**) and a glycosyl donor (**7**), both readily available from a common starting material (**1**).

Preparation of the glycosyl acceptor **3** and donor **7** was achieved as follows (Scheme 1). Treatment of known 4-methoxyphenyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**1**)[13] with benzoyl cyanide and triethylamine in acetonitrile gave the crystalline 3-*O*-benzoyl derivative **2** in 75% yield. This later was submitted to 3 → 2 *O*-acyl migration by treatment[14] with dilute sodium hydroxide in acetone at 0 °C to afford crystalline **3** in 82% yield, the structure of which was evident from its ^{1}H NMR spectrum (Table 1). For the preparation of the glycosyl donor **7**, diol **1** was benzoylated to give crystalline **4** in 92% yield. This provides a stereocontrolling auxiliary at C-2, which would induce 1,2-*trans* linkage formation after activation at the anomeric center. Acid hydrolysis of **4** with 90% trifluoroacetic acid gave the corresponding diol, which was directly treated with 4-oxopentanoic acid (levulinic acid), 1,3-dicyclohexylcarbodiimide, and 4-dimethylaminopyridine in dichloromethane to give crystalline **5** in 87% yield. At this point, transformation of **4** into the α-imidate **6** was first examined. This later should be useful for the preparation of a symmetrically protected disaccharide derivative, potential precursor of tetrol **12**. Oxidative removal of the anomeric 4-methoxyphenyl group in **4** with ceric ammonium nitrate (CAN) was troublesome and gave the corresponding hemiacetal in low yield with extensive degradation. This intermediate was treated with trichloroacetonitrile and 1,8-diazabicyclo-[5,4,0]-undec-7-ene (DBU) in dichloromethane to afford the α-imidate **6** in 48% overall yield, the structure of which was deduced from its ^{1}H NMR spectrum (Table 1). However, similar treatment of **5** gave readily the crystalline α-imidate **7** in 69% overall yield.

Coupling reactions that involved acceptor **3** and donors **6** and **7** was next studied (Scheme 2). Condensation of imidate **6** (1.3 equiv) and alcohol **3** in dichloromethane, in the presence of trimethylsilyl triflate, afforded a ~1:1 mixture of disaccharides **8a** and **8b**. Changes in the temperature, the nature of the solvent or the catalyst (not described in Section 3) did not significantly modify the product distribution. Such a lack of stereoselectivity in glycosylation reactions with activated D-*galacto* structures bearing 4,6-acetal protection (4,6-*O*-benzylidene[15] or 4,6-*O*-di-*tert*-butylsilylene[16]), despite the presence of a C-2 participating group, has still been observed. However, coupling of imidate **7** and acceptor **3** under the same conditions afforded the crystalline key disaccharide intermediate **9** in 70% yield, the structure of which was easily deduced from its ^{1}H NMR spectrum (Table 2).

Transformation of **9** into the intermediate diols **11** and **14** and tetrol **12** was then achieved as follows (Scheme 3). Acid hydrolysis of **9** with 90% trifluoroacetic acid followed by conventional benzoylation gave **10** in 87% yield. Treatment of **10** with in situ prepared hydrazine acetate[17] afforded crystalline **11** in 90% yield. Selective *O*-delevulinoylation of **9** followed by acid hydrolysis as described above gave the crystalline tetrol **12** in 81% overall yield, whereas *O*-delevulinoylation of **9** followed by benzoylation gave crystalline **13** in 88% yield. Acid hydrolysis of **13** afforded the crystalline diol **14** in 92% yield. The structure of these intermediates were easily assigned through their ^{1}H NMR spectra (Table 2).



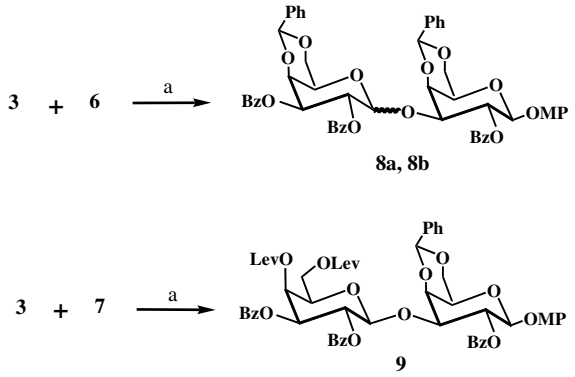
Scheme 1. Reagents and conditions: (a) PhCOCN, Et_3N, MeCN, rt, 30 min; (b) 0.05 M NaOH, acetone, 0 °C, 3 min; (c) PhCOCl, pyridine, 0 °C, 1 h; (d) CAN, toluene–MeCN–water, rt, 15 min; then CCl_3CN, DBU, CH_2Cl_2, rt, 30 min; (e) 90% TFA, rt, 15 min; then levulinic acid, DCC, DMAP, CH_2Cl_2, rt, 2 h.

Table 1. ^{1}H NMR data: carbohydrate ring protons for monosaccharide derivatives **2**–**7**[a]

	2	3	4	5	6	7
H-1	4.90	5.05	5.19	5.17	6.89	6.79
$J_{1,2}$	7.8	8.0	8.0	8.0	3.6	3.8
H-2	4.40	5.60	6.09	5.89	6.07	5.90
$J_{2,3}$	10.2	10.0	10.0	10.2	10.0	10.0
H-3	5.20	3.95	5.42	5.47	5.88	5.81
$J_{3,4}$	3.8	3.9	3.8	3.8	3.8	3.8
H-4	4.50	4.35	4.65	5.68	4.77	5.78
$J_{4,5}$	1.0	0.9	0.9	1.0	0.8	1.0
H-5	3.65	3.65	3.77	4.17	4.15	4.60
$J_{5,6a}$	2.5	2.8	2.5	5.0		6.0
$J_{5,6b}$	2.5	2.8	2.5	6.5		6.5
H-6a	4.35	4.45	4.46	4.35	4.42	4.20
$J_{6a,6b}$	−12.4	−11.0	−12.0	−11.0	−12.0	−11.0
H-6b	4.05	4.10	4.18	4.24	4.15	4.20

[a]Chemical shifts (δ, in ppm) and coupling constants (J, in Hz) for solns in $CDCl_3$.

Having in hand the intermediates **11**, **12**, and **14**, their transformation into the target sulfoforms **16**, **19**, **22**, **24**, and **27** was readily achieved as follows (Scheme 4). Treatment of diols **11** and **14** and tetrol **12** with sulfur trioxide–trimethylamine complex (1.5 equiv per primary hydroxyl group) in *N,N*-dimethylformamide at 40 °C for 90 min followed by ion-exchange chromatography (Na^+ resin) afforded the sodium salts **15**, **21**, and **23**, respectively, in 82%, 80%, and 83% yields, respectively. Comparison of the ^{1}H NMR spectra of **15**, **21**, and **23** with those of their nonsulfated precursors (Table 2), all recorded in the same mixture of solvents (3:1 CD_3OD–$CDCl_3$, for solubility reason) showed the expected[18] downfield shifts ($\Delta\delta \sim 0.3$–0.5 ppm) of the signals for H-6a and H-6b in the 6-*O*-sulfonated-D-*galacto* species. For the preparation of the 4-*O*-sulfonated-D-*galacto* derivatives, protection at O-6 was first required.[19] Thus, treatment of diols **11** and **14** with benzoyl cyanide in pyridine afforded alcohols **17** and **25** in 87% and 88% yields, respectively. These later were *O*-sulfonated by treatment with an excess (10 equiv) of sulfur trioxide–trimethylamine complex in *N,N*-dimethylformamide at



Scheme 2. Reagents and conditions: (a) donor (1.3 equiv), TMSOTf, mol. sieves 4 Å, CH_2Cl_2, rt, 30 min.

60 °C for 3 days followed by ion-exchange to give the sodium salts **18** and **26** in 93% and 90% yields, respectively. Comparison of the ^{1}H NMR spectra of **18** and **26** (Table 2) with those of their precursors also showed the expected[19] downfield shifts ($\Delta\delta \sim 0.8$–0.9 ppm) of the signals for H-4 in the 4-*O*-sulfonated derivatives. Final saponification of **15**, **18**, **21**, **23**, and **26** with sodium hydroxide in methanol gave the 6^{II}-sulfate **16**, the 4^{II}-sulfate **19**, the $6^{I},6^{II}$-sulfate **22**, the 6^{I}-sulfate **24**, and the 4^{I}-sulfate **27**, respectively, in 89–91% yields. Similarly, the crystalline nonsulfated derivative **20** was prepared by saponification of tetrol **12** in 91% yield. The ^{1}H NMR spectra of the five sulfoforms were compared with those of their nonsulfated congener **20** (Table 2). Particularly relevant were the downfield shifts ($\Delta\delta \sim 0.3$–0.6 ppm) of the signals for H-6a and H-6b in the 6-sulfated species, as well as the downfield shifts ($\Delta\delta$ 0.7–1.0 ppm) of the signals for H-4 in the 4-sulfated derivatives. Comparison of the ^{13}C NMR spectra (Table 3) also showed the expected[19] downfield shifts ($\Delta\delta \sim 6$–7 ppm) of the signals for C-6 and the downfield shifts ($\Delta\delta \sim 7$–8 ppm) of the signals for C-4 in the sulfated species. These NMR data are in complete agreement with the expected structures, and accord well with those reported for synthetic derivatives having D-Gal units *O*-sulfonated at C-4 or C-6,[10–12] as well as for fragments isolated from natural sources.[2–4]

Noteworthy is the possibility to prepare the 6^{I}-, 6^{II}-sulfated and the $6^{I},6^{II}$-disulfated derivatives through a one-pot procedure starting from tetrol **12** (Scheme 5). Careful *O*-sulfonation of **12** (1 equiv) with the sulfur trioxide–trimethylamine complex (2.5 equiv) in *N,N*-dimethylformamide at 40 °C for 40 min (optimal formation of the monosubstituted derivatives determined by TLC analysis), followed by ion-exchange, afforded, besides traces of tetrol **12** (5%), the 6^{I}-sulfate **28**, the 6^{II}-sulfate **29**, and the $6^{I},6^{II}$-disulfate **21**, respectively, as their sodium salts, in 26%, 24%, and 40% yields, respectively. The structures of **28** and **29** were easily assigned through spin-decoupling and 2D NMR experiments, and their ^{1}H NMR spectra (Table 2) also showed the expected downfields shifts of the signals for H-6a and H-6b in **28** and **29**.

In conclusion, we have reported a stereocontrolled and high-yielding approach for the preparation of various sulfoforms of the digalactoside **20**. The use of common precursors and the possible one-pot preparation of the 6-*O*-sulfonated derivatives considerably reduce the number of transformations, and render this route attractive for the preparation of larger molecules from the glycosaminoglycan–protein linkage region of GAGs. These sulfoforms are currently being evaluated in biological assays, and the results of these studies will be reported elsewhere in due course.

3. Experimental

3.1. General methods

Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. ^{1}H NMR spectra were recorded at 25 °C with Bruker DPX 250 Advance and Varian Unity 500 spectrometers operating at 250 and 500 MHz, respectively, with Me_4Si as internal standard, unless otherwise stated. ^{13}C NMR spectra were recorded with a Bruker Advance DPX 250 spectrometer operating at 62.8 MHz. Assignments were based on homo- and heteronuclear correlations using the supplier's sofware. Low resolution mass spectra were obtained on a Perkin–Elmer Sciex API 3000 spectrometer operating in the ion-spray (IS) mode. Flash-column chromatography was performed on silica gel (E. Merck, 40–63 μm). Elemental analyses were performed by the Service Central de Microanalyse du CNRS (Vernaison, France).

3.2. 4-Methoxyphenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (2)

Triethylamine (0.5 mL) was added to a suspension of 4-methoxyphenyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**1**) (3.0 g, 8 mmol) and benzoyl cyanide (1.38 g, 10.4 mmol) in anhyd MeCN (40 mL), and the mixture was stirred for 30 min at rt. Methanol (1 mL) was added, and the mixture was concentrated. The solid residue was recrystallized from hot EtOH to give **2** (2.88 g, 75%); mp 202–203 °C; $[\alpha]_D^{22}$ +55° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 1); δ 8.10–6.70 (m, 14H, aromatic H), 5.50 (s, 1H, PhC*H*), 3.75 (s, 3H, OCH_3), 2.45 (d, 1H, *J* 3.0 Hz, *H*O-2); ISMS: *m/z* 501 $[M+Na]^+$. Anal. Calcd for $C_{27}H_{26}O_8$: C, 67.77; H, 5.48. Found: C, 67.82; H, 5.41.

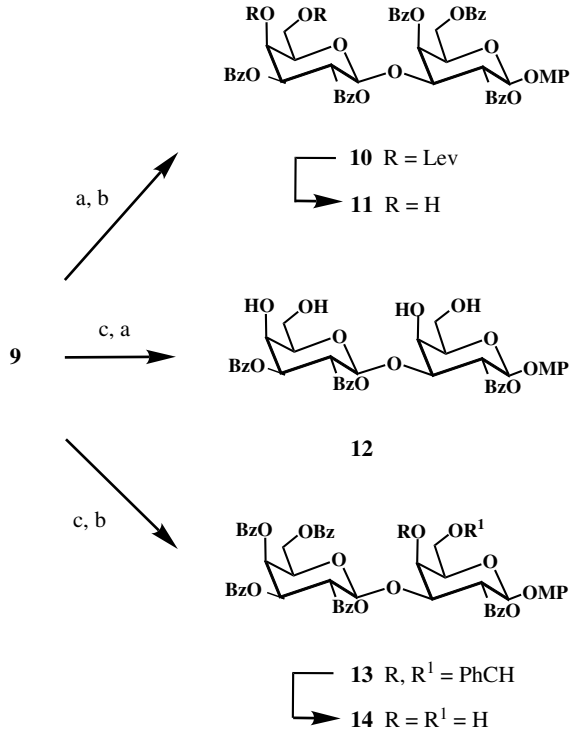
Table 2. ^{1}H NMR data: carbohydrate ring protons for disaccharide derivatives **8**–**29**[a]

	H-1^I	H-2^I	H-3^I	H-4^I	H-5^I	H-6a^I	H-6b^I	H-1^{II}	H-2^{II}	H-3^{II}	H4^{II}	H-5^{II}	H-6a^{II}	H-6b^{II}
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$				$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$			
8a	5.07	5.90	4.10	4.12	3.52	4.29	3.65	5.71	5.70	5.59	4.38	3.72	3.94	3.40
	7.5	10.0	3.8	0.8				3.8	10.2	3.8	1.0			
8b	5.03	5.85	4.41	4.47	3.57	4.37	4.08	5.15	5.78	5.17	4.53	3.58	4.19	4.04
	8.0	10.1	3.8	0.8				8.0	10.0	3.8	0.8			
9	5.02	5.76	4.28	4.53	3.66	4.15	4.15	5.05	5.64	5.23	5.57	4.05	4.40	4.30
	8.0	10.0	3.9	0.8				8.0	10.1	3.8	0.8			
10	4.93	5.85	4.49	5.95	4.30	4.68	4.45	5.07	5.43	5.17	5.49	4.02	4.27	4.08
	8.0	10.0	3.8	0.9				8.0	10.1	3.9	0.8			
11[b]	4.95	5.83	4.24	6.10	4.28	4.61	4.40	5.05	5.61	5.12	4.28	3.70	3.90	3.90
	8.0	10.2	3.9	0.8				8.0	10.0	3.8	0.8			
12[b]	4.96	5.73	4.10	4.28	3.85	3.91	3.81	4.99	5.64	5.20	4.38	3.85	3.91	3.81
	8.0	8.0	3.8	0.8				8.0	10.0	3.8	0.8			
13	4.94	5.82	4.17	4.47	3.39	4.30	3.90	5.15	5.78	5.47	5.94	4.34	4.74	4.37
	8.0	10.1	3.9	0.8				8.0	10.2	3.9	0.9			
14[b]	4.89	5.79	4.01	4.24	3.65	3.90	3.80	5.03	5.32	5.54	5.94	4.39	4.64	4.54
	8.0	10.0	3.8	0.9				8.0	10.1	3.9	0.9			
15[b]	4.94	5.74	4.60	5.99	4.51	4.48	4.48	5.14	5.50	5.07	4.23	4.04	**4.37**[d]	**4.21**
	8.0	10.0	3.9	0.8				8.0	10.2	3.8	0.9			
16[c]	4.53	3.58	3.86	4.21	3.70	3.75	3.54	4.91	3.59	3.60	3.87	3.87	**4.11**	**4.09**
	8.0	10.1	3.8	1.0				8.0	10.2	3.7	0.9			
17[b]	4.92	5.78	4.32	5.95	4.01	4.65	4.55	4.95	5.56	5.11	4.25	4.14	4.65	4.55
	8.0	10.0	3.8	0.8				8.0	10.1	3.8	0.9			
18[b]	4.86	5.71	4.32	5.78	4.21	4.85	4.60	4.95	5.48	5.21	**5.04**	3.92	4.30	4.30
	8.0	10.1	3.8	0.8				8.0	10.2	3.9	0.9			
19[c]	4.60	3.56	4.01	4.13	3.80	3.80	3.52	4.92	3.56	3.69	**4.82**	3.80	3.80	3.52
	8.0	10.1	3.9	1.0				8.0	10.2	3.8	0.8			
20[c]	4.53	3.58	3.82	4.15	3.65	3.80	3.52	4.88	3.55	3.66	3.80	3.65	3.78	3.54
	8.0	10.2	3.8	0.8				8.0	10.1	3.6	0.9			
21[b]	4.96	5.55	4.13	4.41	4.10	**4.30**	**4.30**	4.97	5.67	5.21	4.28	4.10	**4.30**	**4.30**
	8.0	10.0	3.8	0.8				8.0	10.1	3.9	0.8			
22[c]	4.57	3.59	3.84	4.23	4.01	**4.18**	**4.10**	4.89	3.55	3.61	3.88	3.87	**4.18**	**4.10**
	8.0	10.2	3.7	0.8				8.0	10.2	3.6	0.9			
23[b]	4.91	5.62	4.11	4.37	4.07	**4.28**	**4.28**	5.13	5.69	5.55	5.93	4.46	4.64	4.51
	8.0	10.0	3.9	0.9				8.0	10.2	3.8	0.8			
24[c]	4.53	3.57	3.83	4.21	4.00	**4.14**	**4.11**	4.89	3.55	3.48	3.83	3.67	3.80	3.53
	8.0	10.0	3.6	0.9				8.0	10.1	3.8	0.8			
25[b]	4.87	5.74	4.05	4.29	3.92	4.60	4.60	5.03	5.81	5.51	5.96	4.38	4.60	4.60
	8.0	10.0	3.8	0.8				8.0	10.2	3.9	0.9			
26[b]	4.93	5.55	4.25	**5.26**	4.05	4.70	4.70	5.15	5.77	5.54	5.91	4.43	4.70	4.70
	8.0	10.1	3.9	0.9				8.0	10.2	3.8	0.8			
27[b]	4.61	3.56	4.01	**4.82**	3.85	3.70	3.52	4.91	3.56	3.68	4.14	3.79	3.80	3.52
	8.0	10.1	3.8	0.8				8.0	10.1	3.6	0.9			
28[b]	4.93	5.55	4.05	4.47	4.10	**4.34**	**4.18**	4.95	5.65	5.19	4.26	3.73	3.93	3.82
	8.0	10.0	3.8	0.9				8.0	10.2	3.7	0.8			
29[b]	4.85	5.47	4.02	4.32	3.71	3.80	3.70	4.88	5.58	5.07	4.15	4.00	**4.25**	**4.18**
	8.0	10.0	3.8	0.9				8.0	10.2	3.8	0.9			

[a] For solns in $CDCl_3$, unless otherwise stated.
[b] 3:1 CD_3OD–$CDCl_3$.
[c] D_2O.
[d] Values in bold type reflect the locations of the sulfate groups.

3.3. 4-Methoxyphenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (3)

A soln of **2** (3.83 g, 8 mmol) in acetone (80 mL) was treated at 0 °C with NaOH (0.05 M, 80 mL), and the mixture was vigorously stirred for 3 min at this temperature. The copious precipitate was collected by filtration, washed well with cold water, and recrystallized from MeOH–pyridine to give **3** (3.14 g, 82%); mp 254–256 °C; $[\alpha]_D^{22}$ −3° (*c* 1, pyridine); ^{1}H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 1); δ 8.10–6.70 (m, 14H, aromatic H), 5.65 (s, 1H, PhC*H*), 3.75 (s, 3H, OCH_3), 2.70 (d, 1H, *J* 10.0 Hz, *H*O-3); ISMS: *m*/*z* 501 $[M+Na]^+$, 479 $[M+H]^+$. Anal. Calcd for $C_{27}H_{26}O_8$: C, 67.77; H, 5.48. Found: C, 67.70; H, 5.39.



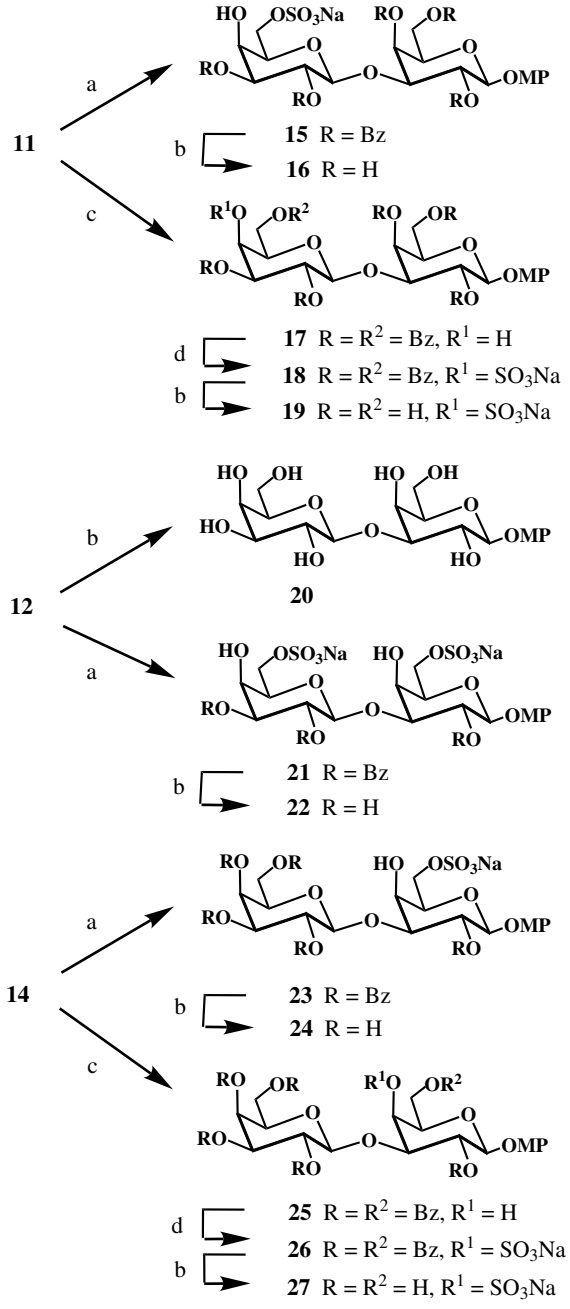
Scheme 3. Reagents and conditions: (a) 90% TFA, rt, 15 min; (b) PhCOCl, pyridine, rt, 16 h; (c) hydrazine acetate, pyridine, rt, 8 min.

3.4. 4-Methoxyphenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (4)

A mixture of **1** (1.82 g, 4.9 mmol) and benzoyl chloride (1.82 mL, 15.7 mmol) in anhyd pyridine (20 mL) was stirred for 1 h at 0 °C. Methanol (2 mL) was added, and the mixture was diluted with CH_2Cl_2 (100 mL), washed with water, satd aq $NaHCO_3$, and water, dried ($MgSO_4$) and concentrated. The residue was crystallized from EtOAc–petroleum ether to afford **4** (2.64 g, 92%); mp 196–197 °C; $[\alpha]_D^{22}$ +121° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 1); δ 8.10–6.70 (m, 19H, aromatic H), 5.58 (s, 1H, PhC*H*), 3.75 (s, 3H, OCH_3); ISMS: *m*/*z* 605 $[M+Na]^+$. Anal. Calcd for $C_{34}H_{30}O_9$: C, 70.09; H, 5.08. Found: C, 70.15; H, 5.08.

3.5. 4-Methoxyphenyl 2,3-di-*O*-benzoyl-4,6-di-*O*-levulinoyl-β-D-galactopyranoside (5)

A soln of **4** (2.65 g, 4.55 mmol) in 9:1 TFA–water (15 mL) was stirred for 15 min at rt, then was concentrated, evaporated with water (3×10 mL), and dried under diminished pressure. A soln of the residue, levulinic acid (1.31 g, 11.2 mmol) and DMAP (122 mg, 1 mmol) in anhyd CH_2Cl_2 (40 mL) was treated portionwise with DCC (2.32 g, 11.2 mmol), and the mixture was stirred for 2 h at rt. The precipitated DCU was filtered off, washed with CH_2Cl_2, and the filtrate was washed with cold 0.1 M HCl, satd aq $NaHCO_3$, and water, dried ($MgSO_4$) and concentrated. A soln of the residue in 1:1 toluene–EtOAc was filtered through a pad of Celite, and the clear filtrate was concentrated. The residue was crystallized from EtOAc–petroleum ether to give **5** (2.74 g, 87% from **4**); mp 128–129 °C; $[\alpha]_D^{22}$ +72° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 1); δ 8.0–6.70 (m, 14H, aromatic

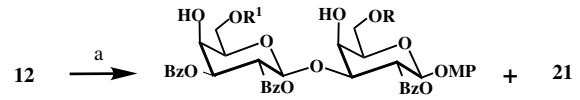


Scheme 4. Reagents and conditions: (a) $Me_3N{\cdot}SO_3$ (1.5 equiv), DMF, 40 °C, 90 min; then ion-exchange resin $[Na]^+$; (b) 4 M NaOH, MeOH, rt, 2 h; (c) PhCOCN, pyridine, rt, 16 h; (d) $Me_3N{\cdot}SO_3$ (10 equiv), DMF, 60 °C, 72 h.

Table 3. ^{13}C NMR data (62.8 MHz, D_2O, 25 °C) for sulfoforms **16**, **19**, **22**, **24**, **27**, and their nonsulfated congener **20**[a]

	16	19	20	22	24	27
C-1^{I}	101.62	101.69	101.66	101.53	101.62	101.78
C-2^{I}	69.77	71.31	69.97	69.74	69.82	72.81
C-3^{I}	82.76	82.39	82.30	82.32	82.15	78.46
C-4^{I}	68.46	68.45	68.50	68.42	68.13	**75.57**
C-5^{I}	75.35	75.16	75.21	72.73	72.77	74.64
C-6^{I}	61.19	60.88	60.91	**67.29**	**67.19**	60.96
C-1^{II}	104.38	104.47	104.58	104.39	104.59	104.29
C-2^{II}	71.09	71.74	71.28	71.10	71.25	70.42
C-3^{II}	72.60	69.96	72.75	72.55	71.73	71.24
C-4^{II}	68.55	**76.92**	68.80	68.42	68.78	69.10
C-5^{II}	72.83	74.55	75.32	73.05	75.24	75.35
C-6^{II}	**67.65**	61.13	61.13	**67.93**	61.12	61.30
Ar_C	154.82	154.85	154.91	154.81	154.84	154.93
	151.14	151.17	151.16	151.24	151.21	151.11
	118.38	118.42	118.42	118.31	118.34	118.50
	115.21	115.21	115.25	115.22	115.22	115.21
OCH_3	55.96	55.96	55.96	55.96	55.96	55.96

[a]Bold type values reflect the positions of sulfations.



28 R = SO_3Na, R^1 = H
29 R = H, R^1 = SO_3Na

Scheme 5. Reagents and conditions: (a) $Me_3N{\cdot}SO_3$ (2.5 equiv), DMF, 40 °C, 40 min; then ion-exchange resin $[Na]^+$.

H), 3.75 (s, 3H, OC*H*$_3$), 2.60 (m, 8H, C*H*$_2$CO), 2.20, 2.10 (2s, 6H, COC*H*$_3$); ISMS: *m*/*z* 714 $[M+Na]^+$. Anal. Calcd for $C_{37}H_{38}O_{13}$: C, 64.34; H, 5.54. Found: C, 64.31; H, 5.52.

3.6. 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-1-*O*-trichloroacetimidoyl-α-D-galactopyranose (6)

A mixture of **4** (0.58 g, 1 mmol) and CAN (1.65 g, 3 mmol) in 1:1.5:1 toluene–MeCN–water (17.5 mL) was stirred for 15 min at 0 °C, then was diluted with EtOAc (50 mL), washed with water, satd aq $NaHCO_3$, and water, dried ($MgSO_4$) and concentrated. Flash chromatography of the residue on a column of silica gel with 9:1 CH_2Cl_2–EtOAc gave the corresponding free hemiacetal (250 mg).

A mixture of the above isolated hemiacetal, CCl_3CN (0.5 mL, 5 mmol), and DBU (20 μL, 0.13 mmol) in anhyd CH_2Cl_2 (3 mL) was stirred for 30 min at rt, then was concentrated. Flash chromatography of the residue on a column of silica gel with 2:1 petroleum ether–EtOAc containing 0.2% of Et_3N gave **6** (303 mg, 48% from **4**) as a colorless syrup; $[\alpha]_D^{22}$ +165° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 1); δ 8.60 (s, 1H, C=N*H*), 8.0–7.20 (m, 15H, Ph), 5.60 (s, 1H, PhC*H*); ISMS: *m*/*z* 644 $[M+Na]^+$ for ^{35}Cl. Anal. Calcd for $C_{29}H_{24}Cl_3NO_8$: C, 56.10; H, 3.90; N, 2.26. Found: C, 56.01; H, 3.98; N, 2.15.

3.7. 2,3-Di-*O*-benzoyl-4,6-di-*O*-levulinoyl-1-*O*-trichloroacetimidoyl-α-D-galactopyranose (7)

A mixture of **5** (1.38 g, 2 mmol) and CAN (5.48 g, 10 mmol) was treated for 15 min at rt as described for the preparation of **6**. Flash chromatography of the residue on a column of silica gel with 1:1 toluene–EtOAc gave the corresponding free hemiacetal (950 mg).

The above isolated hemiacetal was treated as described for the preparation of **5**. Flash chromatography of the residue on a column of silica gel with 2:1 toluene–EtOAc containing 0.2% of Et_3N followed by crystallization of the residue from diethyl ether afforded **7** (1.01 g, 69% from **6**); mp 106–107 °C; $[\alpha]_D^{22}$ +110° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 1); δ 8.60 (s, 1H, C=N*H*), 8.0–7.30 (m, 10H, Ph), 2.60 (m, 8 H, C*H*$_2$CO), 2.20, 2.10 (2s, 6H, COC*H*$_3$); ISMS: *m*/*z* 752 $[M+Na]^+$ for ^{35}Cl. Anal. Calcd for $C_{32}H_{32}Cl_3NO_{12}$: C, 52.72; H, 4.42; N, 1.92. Found: C, 52.63; H, 4.51; N, 1.78.

3.8. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α- (8a) and β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (8b)

A mixture of imidate **5** (250 mg, 0.4 mmol), alcohol **3** (143 mg, 0.3 mmol), and 4 Å powdered molecular sieves (200 mg) in anhyd CH_2Cl_2 (4 mL) was stirred for 30 min under dry Ar. A soln of Me_3SiOTf in toluene (1 M, 64 μL) was added, and the mixture was stirred for 30 min. Triethylamine (0.1 mL) was added, and the

mixture was filtered and concentrated. Flash chromatography of the residue on a column of silica gel with 20:1 CH_2Cl_2–EtOAc containing 0.1% of Et_3N gave first **8a** (87 mg, 31%); mp 255–257 °C (from EtOAc–petroleum ether); $[\alpha]_D^{22}$ +200° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 5.33, 5.03 (2s, 2H, PhC*H*), 3.72 (s, 3H, OCH_3); ISMS: *m*/*z* 960 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{48}O_{15}$: C, 69.22; H, 5.16. Found: C, 69.12; H, 5.26.

Next eluted was **8b** (90 mg, 32%); mp 310–312 °C (from MeOH–CH_2Cl_2); $[\alpha]_D^{22}$ +94° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 5.50, 5.44 (2s, 2H, PhC*H*), 3.72 (s, 3H, OCH_3); ISMS: *m*/*z* 960 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{48}O_{15}$: C, 69.22; H, 5.16. Found: C, 69.28; H, 5.21.

3.9. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-levulinoyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-4,6-*O*-benzylidene- β-D-galactopyranoside (9)

A mixture of imidate **7** (520 mg, 0.7 mmol) and alcohol **3** (263 mg, 0.55 mmol) was treated as described for the preparation of **8a**,**b**. Flash chromatography of the residue on a column of silica gel with 1:1 toluene–EtOAc containing 0.1% of Et_3N and crystallization from EtOAc–petroleum ether gave **9** (403 mg, 70%); mp 177–178 °C; $[\alpha]_D^{22}$ +54° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 24H, aromatic H), 5.62 (s, 1H, PhC*H*), 3.70 (s, 3H, OCH_3), 2.70 (m, 8H, CH_2CO), 2.20, 2.05 (2s, 6H, COCH_3); ISMS: *m*/*z* 1063 $[M+Na]^+$. Anal. Calcd for $C_{57}H_{56}O_{19}$: C, 65.51; H, 5.40. Found: C, 65.62; H, 5.31.

3.10. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-levulinoyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (10)

A soln of **9** (1.40 g, 1.34 mmol) in 9:1 TFA–water (15 mL) was stirred for 15 min at rt, then was concentrated, evaporated with water (3 × 10 mL), and dried under diminished pressure. A mixture of the residue and benzoyl chloride (0.6 mL, 5 mmol) in anhyd pyridine (10 mL) was stirred overnight at rt. Methanol (1 mL) was added, and the mixture was diluted with CH_2Cl_2 (100 mL), washed with water, satd aq $NaHCO_3$, and water, dried ($MgSO_4$) and concentrated. Flash chromatography of the residue on a column of silica gel with 3:2 toluene–EtOAc gave **10** (1.36 g, 87%) as a white foam; $[\alpha]_D^{22}$ +81° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 3.67 (s, 3H, OCH_3), 2.70 (m, 8H, CH_2CO), 2.20, 2.05 (2s, 6H, COCH_3); ISMS: *m*/*z* 1188 $[M+Na]^+$. Anal. Calcd for $C_{71}H_{64}O_{22}$: C, 65.97; H, 5.19. Found: C, 65.91; H, 5.17.

3.11. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (11)

A soln of **10** (1.07 g, 0.92 mmol) in pyridine (10 mL) was treated for 8 min at rt with a freshly prepared mixture of pyridine (12 mL), HOAc (8 mL), and hydrazine hydrate (1 mL), then was diluted with CH_2Cl_2 (100 mL), washed with water, brine, and water, dried ($MgSO_4$) and concentrated. Crystallization of the residue from hot EtOH gave **11** (802 mg, 90%); mp 218–219 °C; $[\alpha]_D^{22}$ +91° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 3.67 (s, 3H, OCH_3); ISMS: *m*/*z* 992 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{48}O_{17}$: C, 66.94; H, 4.99. Found: C, 66.81; H, 5.04.

3.12. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-β-D-galactopyranoside (12)

Compound **9** (1.05 g, 1 mmol) was treated as described for the preparation of **11** to give the intermediate diol as a sparingly soluble solid. A suspension of this solid in 9:1 TFA–water (15 mL) was stirred for 15 min at rt, and the resulting clear soln was then concentrated, and evaporated with water (3 × 10 mL). The solid residue was recrystallized from MeOH–pyridine to give **12** (617 mg, 81%); mp 244–246 °C; $[\alpha]_D^{22}$ +40° (*c* 1, pyridine); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 19H, aromatic H), 3.70 (s, 3H, OCH_3); ISMS: *m*/*z* 783 $[M+Na]^+$. Anal. Calcd for $C_{40}H_{40}O_{15}$: C, 63.15; H, 5.30. Found: C, 62.98; H, 5.41.

3.13. 4-Methoxyphenyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (13)

Compound **9** (1.05 g, 1 mmol) was treated as described for the preparation of **11**. Conventional benzoylation (benzoyl chloride in pyridine) of the resulting diol followed by flash chromatography on a column of silica gel with 5:1 toluene–EtOAc and crystallization of the residue from hot EtOH afforded **13** (930 mg, 88%); mp 158–160 °C; $[\alpha]_D^{22}$ +92° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 34H, aromatic H), 5.53 (s, 1H, PhC*H*), 3.71 (s, 3H, OCH_3); ISMS: *m*/*z* 1070 $[M+Na]^+$. Anal. Calcd for $C_{61}H_{52}O_{17}$: C, 69.31; H, 4.96. Found: C, 69.40; H, 5.02.

3.14. 4-Methoxyphenyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-β-D-galactopyranoside (14)

A soln of **13** (2.11 g, 2 mmol) in 9:1 TFA–water (20 mL) was stirred for 20 min at rt, then was concentrated, and evaporated with water (2 × 10 mL). Recrystallization of the residue from hot EtOH gave **14** (1.77 g, 92%); mp 230–232 °C; $[\alpha]_D^{22}$ +13° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 3.68 (s, 3H, OCH_3); ISMS: *m*/*z* 992 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{48}O_{17}$: C, 66.94; H, 4.99. Found: C, 66.82, H, 5.06.

3.15. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (15)

A mixture of **11** (388 mg, 0.4 mmol) and sulfur trioxide–trimethylamine complex (84 mg, 0.6 mmol) in anhyd DMF (10 mL) was stirred for 90 min at 40 °C, then was cooled. Methanol (0.5 mL) was added, and the mixture was concentrated. Flash chromatography of the residue on a column of silica gel with 9:1 CH_2Cl_2–MeOH containing 2% of Et_3N followed by elution from a column (1.5 × 25 cm) of Sephadex SP C25 $[Na^+]$ with 9:5:1 CH_2Cl_2–MeOH–water afforded **15** (352 mg, 82%) as a white powder; $[\alpha]_D^{22}$ +80° (*c* 1, MeOH); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 3.63 (s, 3H, OCH_3); ISMS: *m*/*z* 1093 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{47}NaO_{20}S$: C, 60.56; H, 4.05; S, 2.99. Found: C, 60.30; H, 4.21; S, 2.80.

3.16. 4-Methoxyphenyl *O*-(6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside (16)

A soln of **15** (320 mg, 0.3 mmol) in MeOH (8 mL) was treated for 2 h at rt with NaOH (4 M, 1.5 mL), then was diluted with water (8 mL). The pH of the soln was brought to 3.5 (pH meter control) with Amberlite IR-120 $[H^+]$ resin, and the mixture was filtered, concentrated and dried under diminished pressure. Benzoic acid was extracted with cold abs EtOH (2 × 5 mL), and the residue was dissolved in water (5 mL). The soln was brought to pH 7 with 0.1 M NaOH, then was eluted from a column (1.5 × 80 cm) of Sephadex LH-20 with water to give **16** (148 mg, 90%) as a white hygroscopic powder; $[\alpha]_D^{22}$ −7° (*c* 1, water); ^1H NMR (500 MHz, D_2O, internal acetone, δ_H 2.225): carbohydrate ring protons (see Table 2); δ 6.95 (m, 4H, aromatic H), 3.70 (s, 3H, OCH_3); ^{13}C NMR (D_2O, internal acetone, δ_C 30.45): see Table 3. Anal. Calcd for $C_{19}H_{27}NaO_{15}S$: C, 41.46; H, 4.94; S, 5.82. Found: C, 41.21; H, 5.11; S, 5.70.

3.17. 4-Methoxyphenyl *O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (17)

A mixture of **11** (388 mg, 0.4 mmol) and benzoyl cyanide (106 mg, 0.8 mmol) in anhyd pyridine (8 mL) was stirred overnight at rt. Methanol (0.5 mL) was added, and the mixture was concentrated. Flash chromatography of the residue on a column of silica gel with 6:1 toluene–EtOAc gave **17** (374 mg, 87%) as a white foam; $[\alpha]_D^{22}$ +74° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 34H, aromatic H), 3.67 (s, 3H, OCH_3); ISMS: *m*/*z* 1096 $[M+Na]^+$. Anal. Calcd for $C_{61}H_{52}O_{18}$: C, 68.28; H, 4.88. Found: C, 68.32; H, 4.80.

3.18. 4-Methoxyphenyl *O*-(2,3,6-tri-*O*-benzoyl-4-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (18)

A mixture of **17** (280 mg, 0.26 mmol) and sulfur trioxide–trimethylamine complex (350 mg, 2.6 mmol) in anhyd DMF (5 mL) was stirred for 72 h at 60 °C, then was cooled and treated as described for the preparation of **15** to give **18** (284 mg, 93%) as a white powder; $[\alpha]_D^{22}$ +78° (*c* 1, MeOH); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 34H, aromatic H), 3.63 (s, 3H, OCH_3); ISMS: *m*/*z* 1198 $[M+Na]^+$. Anal. Calcd for $C_{61}H_{51}NaO_{21}S$: C, 62.35; H, 4.37; S, 2.73. Found: C, 62.12; H, 4.51; S, 2.54.

3.19. 4-Methoxyphenyl *O*-(4-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside (19)

Compound **18** (240 mg, 0.2 mmol) was treated as described for the preparation of **16** to give **19** (100 mg, 89%) as a white powder; $[\alpha]_D^{22}$ −5.5° (*c* 1, water); ^1H NMR (500 MHz, D_2O, internal acetone, δ_H 2.225): carbohydrate ring protons (see Table 2); δ 6.95 (m, 4H, aromatic H), 3.70 (s, 3H, OCH_3); ^{13}C NMR (D_2O, internal acetone, δ_C 30.45): see Table 3. Anal. Calcd for $C_{19}H_{27}NaO_{15}S$: C, 41.46; H, 4.94; S, 5.82. Found: C, 41.18; H, 5.15; S, 5.61.

3.20. 4-Methoxyphenyl *O*-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside (20)

A soln of **12** (228 mg, 0.3 mmol) in MeOH (10 mL) was treated for 2 h with methanolic MeONa (1 M, 1 mL), then was deionized with Amberlite IR-120 $[H^+]$ resin, filtered, and concentrated. Crystallization of the residue from MeOH gave **20** (122 mg, 91%); mp 192–193 °C; $[\alpha]_D^{22}$ −6° (*c* 1, water); ^1H NMR (500 MHz, D_2O, internal acetone, δ_H 2.225): carbohydrate ring protons (see Table 2); δ 6.95 (m, 4H, aromatic H), 3,69 (s, 3H, OCH_3); ^{13}C

NMR (D_2O, internal acetone, δ_C 30.45): see Table 3. Anal. Calcd for $C_{19}H_{28}O_{12}$: C, 50.89; H, 6.29. Found: C, 50.77; H, 6.33.

3.21. 4-Methoxyphenyl *O*-(2,3-di-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranoside (21)

A mixture of **12** (228 mg, 0.3 mmol) and sulfur trioxide–trimethylamine complex (120 mg, 0.9 mmol) in anhyd DMF (8 mL) was stirred for 90 min at 40 °C, then was treated as described for the preparation of **15**. Recrystallization of the solid residue from aq MeOH gave **21** (232 mg, 80%); mp 232–234 °C; $[\alpha]_D^{22}$ +70° (*c* 0.5, MeOH); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 19H, aromatic H), 3.63 (s, 3H, OC*H*$_3$); ISMS: *m*/*z* 988 $[M+Na]^+$. Anal. Calcd for $C_{40}H_{38}Na_2O_{21}S_2$: C, 49.80; H, 3.57; S, 6.65. Found: C, 49.52; H, 3.74; S, 6.41.

3.22. 4-Methoxyphenyl *O*-(6-*O*-sodium sulfonato-β-D-galactopyranosyl)-(1 → 3)-6-*O*-sodium sulfonato-β-D-galactopyranoside (22)

Compound **21** (410 mg, 0.42 mmol) was treated as described for the preparation of **16** to give **22** (252 mg, 91%) as a white hygroscopic powder; $[\alpha]_D^{22}$ −11° (*c* 1, water); ^1H NMR (500 MHz, D_2O, internal acetone, δ_H 2.225): carbohydrate ring protons (see Table 2); δ 6.95 (m, 4H, aromatic H), 3.71 (s, 3H, OC*H*$_3$); ^{13}C NMR (D_2O, internal acetone, δ_C 30.45): see Table 3. Anal. Calcd for $C_{19}H_{26}Na_2O_{18}S_2$: C, 34.97; H, 4.02; S, 9.83. Found: C, 34.70; H, 4.21; S, 9.58.

3.23. 4-Methoxyphenyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzoyl-6-*O*-sodium sulfonato-β-D-galactopyranoside (23)

Compound **14** (388 mg, 0.4 mmol) was treated as described for the preparation of **16**. Crystallization of the residue from EtOH gave **23** (356 mg, 83%); mp 190–192 °C; $[\alpha]_D^{22}$ +108° (*c* 1, MeOH); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 29H, aromatic H), 3.66 (s, 3H, OC*H*$_3$); ISMS: *m*/*z* 1093 $[M+Na]^+$. Anal. Calcd for $C_{54}H_{47}NaO_{20}S$: C, 60.56; H, 4.05; S, 2.99. Found: C, 60.48; H, 4.12; S, 2.79.

3.24. 4-Methoxyphenyl *O*-(β-D-galactopyranosyl)-(1 → 3)-6-*O*-sodium sulfonato-β-D-galactopyranoside (24)

Compound **23** (321 mg, 0.3 mmol) was treated as described for the preparation of **16**. Crystallization of the residue from aq MeOH gave **24** (150 mg, 91%); mp 226–228 °C; $[\alpha]_D^{22}$ −9° (*c* 1, water); ^1H NMR (500 MHz, D_2O, internal acetone, δ_H 2.225): carbohydrate ring protons (see Table 2); δ 6.95 (m, 4H, aromatic H), 3.70 (s, 3H, OC*H*$_3$); ^{13}C NMR (D_2O, internal acetone, δ_C 30.45): see Table 3. Anal. Calcd for $C_{19}H_{27}NaO_{15}S$: C, 41.46; H, 4.94; S, 5.82. Found: C, 41.25; H, 5.07; S, 9.60.

3.25. 4-Methoxyphenyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2,6-di-*O*-benzoyl-β-D-galactopyranoside (25)

Compound **14** (436 mg, 0.45 mmol) was treated as described for the preparation of **17**. Crystallization of the residue from EtOAc–petroleum ether gave **25** (426 mg, 88%); mp 240–242 °C; $[\alpha]_D^{22}$ +119° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 34H, aromatic H), 3.67 (s, 3H, OC*H*$_3$); ISMS: *m*/*z* 1096 $[M+Na]^+$. Anal. Calcd for $C_{62}H_{52}O_{18}$: C, 68.28; H, 4.88. Found: C, 68.21; H, 4.82.

3.26. 4-Methoxyphenyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2,6-di-*O*-benzoyl-4-O-sodium sulfonato-β-D-galactopyranoside (26)

Compound **25** (322 mg, 0.3 mmol) was treated as described for the preparation of **18** to give **26** (317 mg, 90%) as a white powder; $[\alpha]_D^{22}$ +93° (*c* 1, MeOH); ^1H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 34H, aromatic H), 3.66 (s, 3H, OC*H*$_3$); ISMS: *m*/*z* 1198 $[M+Na]^+$. Anal. Calcd for $C_{61}H_{51}NaO_{21}S$: C, 62.35; H, 4.37; S, 2.73. Found: C, 62.15; H, 4.51; S, 2.52.

3.27. 4-Methoxyphenyl *O*-(β-D-galactopyranosyl)-(1 → 3)-4-*O*-sodium sulfonato-β-D-galactopyranoside (27)

Compound **26** (258 mg, 0.22 mmol) was treated as described for the preparation of **16** to give **27** (109 mg, 90%) as a white hygroscopic powder; $[\alpha]_D^{22}$ −5° (*c* 1, water); ^1H NMR (500 MHz, D_2O, internal acetone, δ_H 2.225): carbohydrate ring protons (see Table 2); δ 6.95 (m, 4H, aromatic H), 3.70 (s, 3H, OC*H*$_3$); ^{13}C NMR (D_2O, internal acetone, δ_C 30.45): see Table 3. Anal. Calcd for $C_{19}H_{27}NaO_{15}S$: C, 41.46; H, 4.94; S, 5.82. Found: C, 41.21; H, 5.12; S, 5.59.

3.28. Controlled *O*-sulfonation of tetrol 12

A mixture of **12** (152 mg, 0.2 mmol) and sulfur trioxide–trimethylamine complex (66 mg, 0.5 mmol) in anhyd DMF (6 mL) was stirred at 40 °C. After 40 min, TLC (5:1 CH_2Cl_2–MeOH) showed optimal formation of intermediate monosulfates. The mixture was then cooled, quenched with MeOH (0.5 mL), and concentrated. Flash chromatography of the residue on a column of silica gel with 7:1 CH_2Cl_2–MeOH containing 2% of Et_3N gave first the starting material (8 mg, 5%).

Next eluted was a second fraction that was submitted to ion-exchange as described previously. Crystallization of the residue from MeOH gave the 6^{I}-sulfate **28** (45 mg, 26%); mp 225–228 °C; $[\alpha]_D^{22}$ +91° (*c* 1, MeOH); ^{1}H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.70 (m, 19H, aromatic H), 3.66 (s, 3H, OCH_3); ISMS: *m*/*z* 886 $[M+Na]^+$. Anal. Calcd for $C_{40}H_{39}NaO_{18}S$: C, 55.68; H, 4.56. Found: C, 55.39; H, 4.72.

Further elution gave a third fraction that was treated similarly to give the 6^{II}-sulfate **29** (41 mg, 24%) as a white powder; $[\alpha]_D^{22}$ +78° (*c* 1, MeOH); ^{1}H NMR (250 MHz, 3:1 CD_3OD–$CDCl_3$): carbohydrate ring protons (see Table 2); δ 8.10–6.80 (m, 19H, aromatic H), 3.67 (s, 3H, OCH_3); ISMS: *m*/*z* 886 $[M+Na]^+$. Anal. Calcd for $C_{40}H_{39}NaO_{18}S$: C, 55.68; H, 4.56. Found: C, 55.42; H, 4.75.

Elution with 4:1 CH_2Cl_2–MeOH gave a fourth fraction that was submitted to the same treatment to give the disulfate **21** (78 mg, 40%), mp 231–233 °C; $[\alpha]_D^{22}$ +69° (*c* 0.8, MeOH).

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