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Synthesis of β-D-GlcA-(1→3)-β-D-Gal disaccharides with 4- and 6-sulfate groups and 4,6-disulfate groups

Marianne Zsiška* and Bernd Meyer†

*Complex Carbohydrate Research Center and Department of Biochemistry, University of Georgia, 220 Riverbend Road, Athens, GA 30602 (U.S.A.) and *Department of Chemistry, University of Oldenburg, P.O. Box 2503, D-2900 Oldenburg (F.R.G.)*

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ABSTRACT

The sodium salts of the 6-sulfate **7**, the 4-sulfate **10**, and the 4,6-disulfate **12** of benzyl 3-*O*-(β-D-glucopyranosyl uronate)-β-D-galactopyranoside (**5**) have been synthesized. Methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyran)uronate (**1**) was coupled with benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**2**) to yield **3**. The benzylidene acetal of **3** was hydrolyzed to give benzyl 2-*O*-benzoyl-3-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-β-D-galactopyranoside (**4**). Compound **4** was utilized as a key intermediate to prepare the sulfated disaccharides **7**, **10**, and **12**. Direct sulfation of **4** with sulfur trioxide–trimethylamine for 2 days yielded the 6-sulfate **6**. The 4,6-disulfate **11** was accessible by running the reaction under the same conditions for 14 days. The 4-sulfate **9** was obtained after protecting the 6-OH group of **4** by reaction with benzoyl imidazole to give the 6-benzoate **8**, followed by sulfation under vigorous conditions. Treatment of the protected compounds **4**, **6**, **9**, and **11** with aqueous sodium hydroxide in tetrahydrofuran gave the unprotected **5**, **7**, **10**, and **12**, respectively.

INTRODUCTION

Sulfated oligo- and poly-saccharides like heparin or chondroitin sulfates exhibit a number of physiological functions[1–6]. The interaction of sulfate groups with protein receptors or their influence on the conformation of oligosaccharides has been the focus of recent research[7–9]. To avoid the problems created by isolation of the oligosaccharides from natural sources[3,10,11], synthetic strategies have been used[12–14]. The syntheses of the two repeating units of dermatansulfate[15] and of oligosaccharide fragments of keratan sulfate[16] and, recently, chondroitin sulfates[17] have been published.

Several studies have focused on the influence of sulfate groups on the conformation of oligosaccharides related to heparin[8]. It has been shown that the conformation of the iduronic acid ring is dependent on the pattern of sulfation on that residue and that it adopts conformations between the $^1C_4$ and the $^2S_0$ conformations[8].

In the preceding paper, we have reported the synthesis of sulfated disaccharides that contain the disaccharide β-D-Gal-(1→4)-β-D-GlcA as a common structural element[18]. These compounds carry a uronic acid group and one or two sulfate groups in proximity to the glycosidic bond. The positions that are occupied by charged groups

† To whom correspondence should be addressed.

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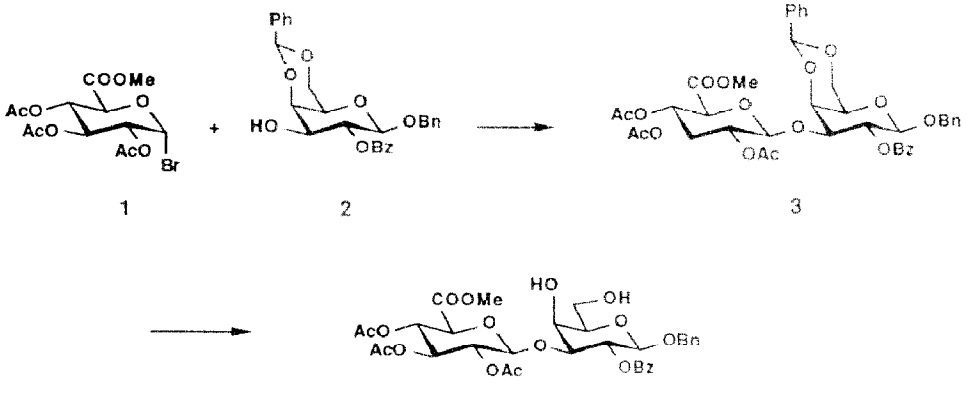
would be on opposite sides of the molecule if it were in the energetically favored conformation of an uncharged β-(1→4)-linked disaccharide[19]. Herein we present the synthesis of sulfated disaccharides with a common β-D-GlcA-(1→3)-β-D-Gal structural element which serve as models for the complementary repeating unit in chondroitin sulfates. In these cases the positions which carry the charged groups should be located on the same side of the molecule as deduced from the conformation of uncharged β-(1→3)-linked disaccharides[20]. These two sets of ionic disaccharides were used for an analysis of the conformational influences of charged groups[21].

DISCUSSION

We synthesized the sulfated derivatives **7**, **10**, and **12** of β-D-GlcA-(1→3)-β-D-Gal, as well as the unsulfated derivative **5**, in order to study the conformational changes of oligosaccharides induced by sulfate groups[21]. We synthesized **5**, **7**, **10**, and **12** as the benzyl β-D-glycosides in order to anchor the reducing end in the equatorial position. The key intermediate is benzyl 2-*O*-benzoyl-3-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-β-D-galactopyranoside (**4**). From **4**, the sulfated disaccharides **7**, **10**, **12**, and the unsulfated **5** can be synthesized in a few steps. The monosaccharide building blocks used in the glycoside synthesis are the D-glucopyranosyl uronate bromide **1** (ref. 22) and the 3-OH (unprotected) benzyl β-D-galactoside **2** (refs. 23, 24). Benzyl 3-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**3**) could be obtained in a reasonable yield of 79% by the silver carbonate-catalyzed glycosylation under activation *in situ* with iodine[25]. Only the β-linked disaccharide **3** is formed, and no products with an α-linkage could be detected. Cleavage of the 4,6-*O*-benzylidene group of **3** with 90% trifluoroacetic acid in dichloromethane led to the formation of **4** in 92% yield.

Sulfation of **4** with the trimethylamine–sulfur trioxide complex in *N*,*N*-dimethylformamide at room temperature led to the 6-sulfate **6** which was obtained in 75% yield. 4,6-Disulfate **11** was obtained as a byproduct in 7% yield. The selectivity of 6-sulfation



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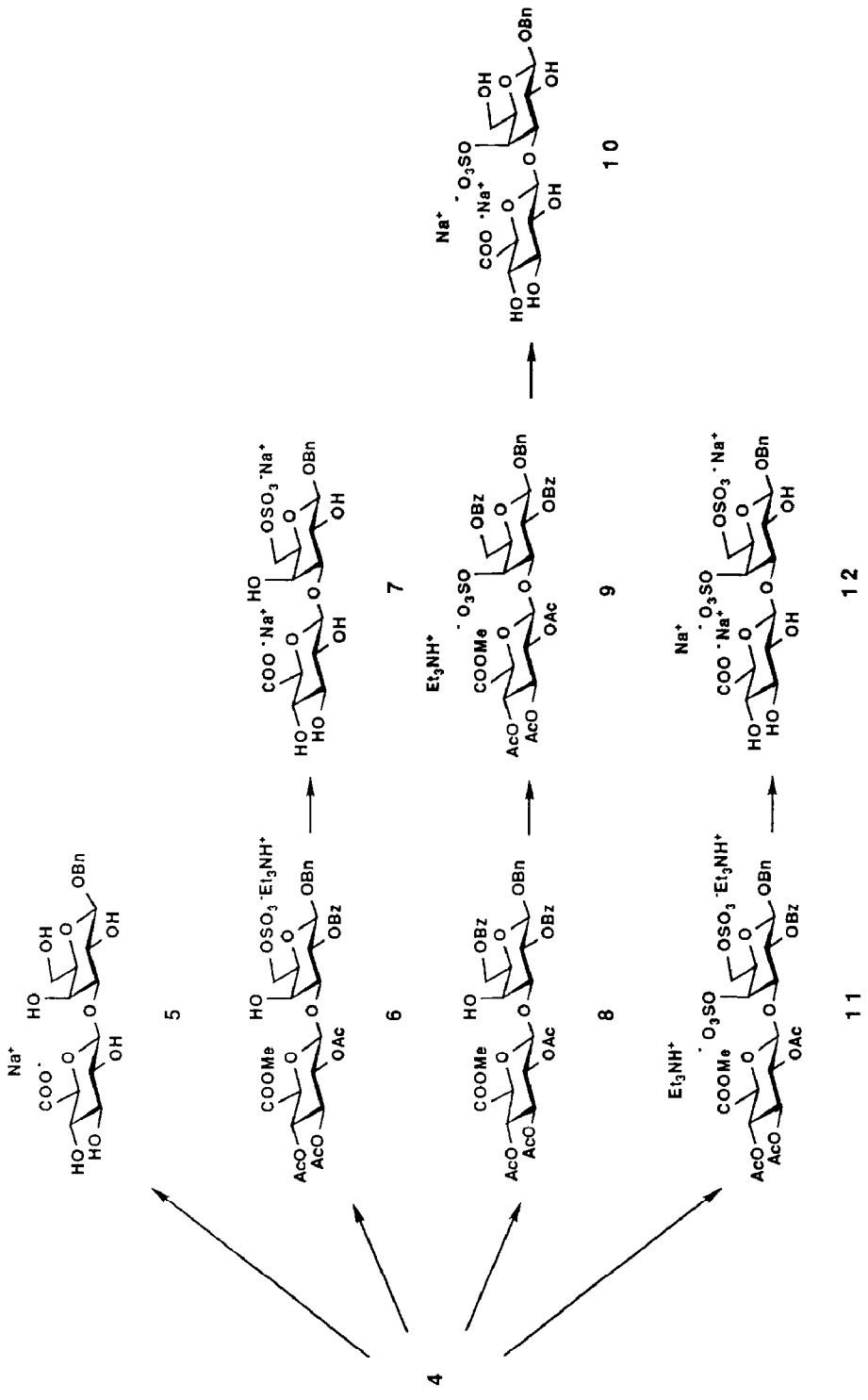
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forming **6** *vs.* 4,6-di-sulfation forming **11** is 11:1, significantly lower than that observed in the monosulfation of β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl uronate, which led to the exclusive formation of the 6′-sulfate[18]. A prolonged reaction time of 14 days gave the 4,6-disulfate **11** in almost quantitative yield. Monosulfation of the 4-position is only possible with prior protection of the 6-OH group. The 6-benzoate **8** was obtained by regioselective benzoylation of **4** with *N*-benzoylimidazole. Sulfation of **8** at the 4-position was achieved at 60° to give **9** in 87% yield. The sulfated compounds **6**, **9**, and **11** were easily purified after conversion into the triethylammonium salts. The triethylammonium salts can be chromatographed on silica gel with a triethylamine-containing eluent[26].

Sulfates **6**, **9**, and **11** were identified by characteristic ^{1}H- and ^{13}C-n.m.r. chemical shifts relative to the unsulfated precursors **4** and **8**, respectively. The H-6a and H-6b signals of the 6-monosulfate **6** and the 4,6-disulfate **11** are shifted 0.4–0.5 p.p.m. to lower field compared to the analogous signals for **4** (Table I). The H-5 signals of **6** and **11** are shifted downfield by 0.3 and 0.4 p.p.m., respectively. The 4-sulfate groups of the 4,6-disulfate **11** and of the 4-sulfate **9** cause downfield shifts of the H-4 signals of 0.7–0.8 p.p.m., while H-3 and H-5 of **9** and H-3 of **11** experience only a small downfield shift of 0.1 p.p.m.

The ^{13}C-n.m.r. spectra of the 6-sulfated **6** and **11** show a downfield shift of the C-6 signal of 3.9 and 4.9 p.p.m. (Table II). The deshielding α-effect of the 4-sulfate group is stronger than the α-effect of the 6-sulfate group, as indicated by the downfield shift of the C-4 signal of **9** and **11** of 5.7 and 6.5 p.p.m., respectively. The primary sulfate group in **6** causes an upfield shift of the C-5 signal of −2.0 p.p.m., whereas the 4-sulfate group in **9** unexpectedly causes a downfield shift of the C-5 signal of 0.7 p.p.m. The C-5 signal of the disulfate **11** is shifted upfield only by −1.4 p.p.m. Obviously, the combined effects of the primary and secondary sulfate groups are additive. In the presence of a 4-sulfate group the C-3 signal of **9** and **11** is shifted by −3.0 −3.2 p.p.m. to higher field.

The deprotection of **4**, **6**, **9**, and **11** was expectedly difficult because 4-*O*-acyl-D-glucuronic acids show an increased sensitivity to *Δ*-4′,5′ elimination reactions[15a]. Deacylation under Zemplén conditions, with triethylamine in water–methanol, with sodium carbonate–methanol, or with potassium carbonate–methanol, gave considerable amounts of *Δ*-4′,5′-elimination products. The best results were obtained when deacylating **4**, **6**, **9**, and **11** with aq. sodium hydroxide in tetrahydrofuran to produce **5**, **7**, **10**, and **12** in 76–94% yield. The disaccharides **4**, **6**, **9**, and **11** that carry a 4-*O*-acyl-D-glucuronate residue turned out to be more base labile than the corresponding disaccharides which carry a 4-*O*-glycosyl residue[18]. The charged disaccharides **5**, **7**, **10**, and **12** were purified using ion-exchange chromatography on S-Sepharose, followed by size-exclusion chromatography on Fractogel HW40.

The ^{1}H- and ^{13}C-n.m.r. spectra of **5**, **7**, **10**, and **12** could be assigned completely by 2D (^{1}H,^{1}H) COSY n.m.r. spectra, by 1D decoupling experiments, and by 2D (^{13}C,^{1}H) COSY n.m.r. spectra. Generally, the shift effects caused by sulfation are stronger in the unprotected sulfates **5**, **7**, and **12** than in their protected precursors **6**, **9**, and **11**. The signals of the α-protons are shifted downfield 0.45–0.55 p.p.m. by a primary sulfate

TABLE I

^{1}H-N.m.r. chemical shifts [p.p.m.], coupling constants [Hz][a] of **4**, **6**, **8**, **9**, and **11** and shift differences [p.p.m.] of the sulfated compounds **6**, **9**, and **11** with the unsulfated **4** or **8**, respectively, measured in $CDCl_3$

	δ					$\Delta\delta$		
	4	**6**	**11**	**8**	**9**	**6 − 4**	**11 − 4**	**9 − 8**
H-1	4.534 (8.1)	4.519 (8.1)	4.526 (7.8)	4.472 (8.1)	4.436 (7.6)	−0.015	−0.008	−0.036
H-2	5.528 (9.8)	5.508 (9.9)	5.352 (10.1)	5.562 (9.8)	5.434 (9.7)	−0.020	−0.176	−0.128
H-3	3.847 (3.4)	3.865 (3.3)	3.916 (3.4)	3.841 (3.2)	3.932 (3.1)	0.018	0.069	0.091
H-4	4.185 (1.2)	4.241 (1.1)	4.908 (1.2)	4.181 (1.2)	5.004 (1.2)	0.056	0.723	0.823
H-5	3.613 (4.8, 6.8)	3.909 (6.3, 6.5)	4.062 (6.5, 5.6)	3.843 (6.5, 6.2)	3.940 (7.5, 4.2)	0.296	0.449	0.097
H-6a	3.832 (11.6)	4.290 (10.9)	4.368 (11.5)	4.650 (12.0)	4.747 (12.1)	0.458	0.536	0.097
H-6b	3.981	4.357	4.473	4.690	4.844	0.376	0.492	0.154
H-1′	4.702 (7.7)	4.688 (7.7)	4.624 (7.1)	4.660 (7.5)	4.697 (8.0)	−0.014	−0.078	0.037
H-2′	4.954 (9.2)	4.921 (9.1)	4.931 (9.2)	4.935 (9.1)	4.970[b]	−0.033	−0.023	0.015
H-3′	5.068 (9.2)	5.043 (9.2)	4.973 (9.3)	5.047 (9.2)	4.970 (9.9)	−0.025	−0.095	−0.057
H-4′	5.154 (9.5)	5.123 (9.5)	5.199 (10.0)	5.139 (9.5)	5.262 (9.9)	−0.031	0.045	0.123
H-5′	4.042	4.007	3.924	3.956	3.956	−0.035	−0.118	0.000

[a] Coupling constants are in parentheses. [b] Coupling constant could not be determined.

TABLE II

^{13}C-N.m.r. chemical shifts [p.p.m.] of **4**, **6**, **8**, **9**, and **11** and shift differences of the sulfated compounds **6**, **9**, and **11** with **4** or **8**, respectively, measured in $CDCl_3$

	δ					$\Delta\delta$		
	4	**6**	**11**	**8**	**9**	**6 − 4**	**11 − 4**	**9 − 8**
C-1	99.27	99.40	99.33	98.73	98.39	0.13	0.06	−0.44
C-2	70.86	70.75	71.24	70.67	71.29	−0.11	0.38	0.67
C-3	80.89	80.64	77.65	80.67	77.72	−0.25	−3.24	−2.95
C-4	68.66	68.06	75.12	68.51	74.17	−0.60	6.46	5.66
C-5	74.40	72.45	73.04	72.07	72.80	−1.95	−1.36	0.73
C-6	61.82	65.73	66.75	63.43	64.33	3.91	4.93	0.90
C-1′	101.11	101.10	101.49	101.22	101.37	−0.01	0.38	0.15
C-2′	70.62	70.61	71.08	70.73	71.29	−0.01	0.46	0.56
C-3′	71.77	71.87	72.53	71.64	72.65	0.10	0.76	1.01
C-4′	69.12	69.14	68.89	69.14	68.85	0.02	−0.23	−0.29
C-5′	71.97	72.03	71.90	72.28	72.29	0.06	−0.07	0.01

TABLE III

^{1}H-N.m.r. chemical shifts [p.p.m.] and coupling constants [Hz][a] of **5**, **7**, **10**, and **12** and chemical shift differences [p.p.m.] of the sulfated compounds **7**, **10**, and **12** with the unsulfated **5**, measured in D_2O

	δ				$\Delta\delta$		
	5	**7**	**10**	**12**	**7 − 5**	**10 − 5**	**12 − 5**
H-1	4.381 (7.3)	4.399 (7.6)	4.423 (8.0)	4.416 (8.0)	0.018	0.042	0.035
H-2	3.587 (9.8)	3.594 (9.8)	3.624 (10.0)	3.636 (10.0)	0.007	0.037	0.049
H-3	3.636 (2.4)	3.662 (3.3)	3.880 (3.2)	3.906 (3.1)	0.026	0.244	0.270
H-4	4.061 (0.5)	4.113 (1.1)	4.680 (1.1)	4.714 (1.0)	0.052	0.619	0.653
H-5	3.567 (4.2, 7.8)	3.805 (5.3, 7.3)	3.691[b]	3.952 (8.6, 3.2)	0.238	0.124	0.385
H-6a	3.650[b]	4.110[b]	3.691[b]	4.105 (−11.3)	0.460	0.041	0.455
H-6b	3.650	4.110	3.691	4.205	0.460	0.041	0.555
H-1′	4.538 (7.7)	4.549 (7.7)	4.624 (7.8)	4.637 (7.9)	0.011	0.104	0.099
H-2′	3.295 (9.6)	3.297 (9.5)	3.289 (9.5)	3.294 (9.4)	0.002	−0.006	−0.001
H-3′	3.409[b]	3.402[b]	3.387 (8.8)	3.391 (8.3)	−0.007	−0.022	−0.018
H-4′	3.409 (9.7)	3.402 (8.7)	3.430 (9.7)	3.433 (9.6)	−0.007	0.021	0.024
H-5′	3.604	3.607	3.578	3.581	0.003	−0.026	−0.023

[a] Coupling constants were determined by first order analysis. [b] Coupling constant not assigned because of spectral overlap.

group (**7** and **12**) and 0.6 p.p.m. by a secondary sulfate group (**10** and **12**) (Table III). The H-5 signal of the 6-sulfate **7** is shifted downfield 0.2 p.p.m., and the H-5 signal of the 4-sulfate **10** is shifted downfield 0.1 p.p.m. The *β*-effect of a primary sulfate group on H-5 is stronger than the *β*-effect of a secondary sulfate group. An additive downfield shift of the H-5 signal of 0.4 p.p.m. is observed in the 4,6-disulfate **12**. The H-3 signals of **10** and **12** are shifted downfield by 0.25 p.p.m. The presence of the 4-sulfate groups is also noticeable across the glycosidic bond. The H-1′ signal of 4,6-disulfate **12**, as well as that of the 4-sulfate **10**, are shifted downfield by 0.1 p.p.m.

In the ^{13}C-n.m.r. spectra, sulfate groups cause a downfield shift of the α-carbon of 6.7–7.0 p.p.m. at C-6 (**7** and **12**) and of 8.8–9.1 p.p.m. at C-4 (**10** and **12**) (Table IV). 6-Sulfation causes a *β*-effect of −2.2 p.p.m. at C-5 (**7**), whereas 4-sulfation causes a *β*-effect on C-5 of only −0.4 p.p.m. (**10**). 4,6-Disulfation leads to the additive effect on C-5 of −2.5 p.p.m. (**12**). In contrast to the relatively small high-field shift on C-5 that is observed upon 4-sulfation, C-3 exhibits an extremely large *β*-effect of −5.7 p.p.m. (**10** and **12**). A similar effect was already observed for the protected compounds **9** and **11** (*cf.* above). The *β*-effect of a secondary sulfate group is reported to be typically −0.6–−1.0 p.p.m.[27]. Also, unexpectedly, the 4-sulfate group causes a shift effect on both C-2 and C-1′ of **10** and **12**, with C-2 being shifted downfield by ~0.8 p.p.m. and C-1′ being shifted upfield by ~0.7 p.p.m.

TABLE IV

^{13}C-N.m.r. chemical shifts [p.p.m.] of **5**, **7**, **10**, and **12** and shift differences [p.p.m.] of the sulfated compounds **7**, **10**, and **12** with the unsulfated **5**, measured in D_2O

	δ				*Δδ*		
	5	**7**	**10**	**12**	7 − 5	10 − 5	12 − 5
C-1	101.55	101.40	101.70	101.46	−0.15	0.15	−0.09
C-2	70.04	70.01	70.93	70.88	−0.03	0.89	0.84
C-3	82.95	82.74	77.23	77.24	−0.21	−5.72	−5.71
C-4	68.35	68.29	77.40	77.13	−0.06	9.05	8.78
C-5	75.09	72.89	74.72	72.61	−2.20	−0.37	−2.48
C-6	61.17	67.86	61.17	68.16	6.69	0.00	6.99
C-1′	103.83	103.88	103.12	103.10	0.05	−0.71	−0.73
C-2′	73.36	73.41	73.31	73.33	0.05	−0.05	−0.03
C-3′	75.50	75.55	75.55	75.59	0.05	0.05	0.09
C-4′	71.97	72.03	72.07	72.10	0.06	0.10	0.13
C-5′	76.37	76.39	76.39	76.39	0.02	0.02	0.02

EXPERIMENTAL

General methods. — All reagents and solvents used in reactions were anhydrous. Column chromatography was carried out on Silica Gel 60 (E. Merck). ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Bruker AM 300 spectrometer operating at 300 MHz or 75.5 MHz, respectively. Mass spectra were recorded on a MAT 212 instrument.

Benzyl 2-O-benzoyl-4,6-O-benzylidene-3-O-[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-β-D-galactopyranoside (**3**). — Silver carbonate (2.76 g) and freshly powdered activated molecular sieves 4Å (3–5 g) were added to a solution of benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside[24] (**2**, 590 mg, 1.28 mmol) in dichloromethane (20 mL). After stirring for 1 h at room temperature with the exclusion of light, iodine (450 mg, 3.5 mmol) was added. A solution of methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranosyl)uronate[22] (**1**, 1.46 g, 3.7 mmol) in dichloromethane (20 mL) was added dropwise over 2 h to the reaction mixture. The mixture was stirred in the dark for 3 days. Dilution with dichloromethane and filtration over Celite, followed by evaporation of the filtrate, gave a residue which was chromatographed on silica gel (5:1 dichloromethane–ethyl acetate). The educt **2** (106 mg, 18%) was recovered as the first fraction. Further elution gave **3** (790 mg, 79%) as colorless crystals: m.p. 252–253°; $[\alpha]_D^{20}$ −3.6° (*c* 0.7, CH_2Cl_2); m.s. (d.c.i.–ammonia): *m/z* 796 $(M + NH_4)^+$; 736 $(M - HOAc + NH_4)^+$; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 1.502, 1.908, 1.980 (3 s, CH_3CO), 3.457 (m, H-5), 3.675 (s, CO_2CH_3), 3.943 (d, H-5′), 3.970 (dd, H-3), 4.098 (dd, H-6a), 4.365 (dd, H-4), 4.381 (dd, H-6b), 4.557 (d, H-1), 4.641 [d, Ha(Bn)], 4.737 (d, H-1′), 4.853 [d, Hb(Bn)], 4.927 (dd, H-2′), 5.018 (dd, H-3′), 5.151 (dd, H-4′), 5.570 (s, PhC*H*), 5.666 (dd, H-2), 7.0–7.6, 7.95 (2 m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 10.2, $J_{3,4}$ 3.5, $J_{4,5}$ 1.2, $J_{5,6a}$ 1.6, $J_{5,6b}$ 2.1, $J_{6a,6b}$ −12.3, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.1, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.8, $J_{H(Bn)}$ −12.6 Hz; ^{13}C-n.m.r. (75.5 MHz, $CDCl_3$): δ 19.93, 20.38, 20.51 (3 × *C*H_3CO), 52.80 (CO_2CH_3), 66.77 (C-5), 68.88 (C-6), 69.29 (C-4′), 69.55 [*C*H_2(Bn)], 70.56 (C-2), 70.96 (C-2′), 72.09 (C-3′), 72.34 (C-5′), 75.81 (C-4), 77.98 (C-3), 99.23 (C-1), 101.00 [*C*H(Bn)], 101.24 (C-1′), 126.32, 127.54, 127.80, 128.03, 128.15, 128.41, 128.76, 129.71, 133.15, 137.10 (Ar), 167.08, 2 × 168.99, 2 × 169.22 (4 × C=O and C-6′).

Anal. Calc. for $C_{40}H_{42}O_{16}$: C, 61.69; H, 5.44. Found: C, 61.46; H, 5.49.

Benzyl 2-O-benzoyl-3-O-[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-β-D-galactopyranoside (**4**). — Aq. trifluoroacetic acid (90%, 2.5 mL) was added at 0° to a stirred solution of **3** (510 mg, 0.65 mmol) in dichloromethane (25 mL). After stirring for 30 min, the reaction was quenched with satd. sodium hydrogencarbonate (20 mL). The layers were separated, and the aq. layer was repeatedly extracted with dichloromethane. The combined organic phases were evaporated *in vacuo*. Column chromatography (2:1 ethyl acetate–petroleum ether) and subsequent crystallization from dichloromethane–hexane yielded colorless crystals of **4** (415 mg, 92%): m.p. 175° (dec.); $[\alpha]_D^{20}$ −24.1° (*c* 0.6, CH_2Cl_2); m.s. (d.c.i.–ammonia): *m/z* 708 $(M + NH_4)^+$; 648 $(M - HOAc + NH_4)^+$; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 1.510, 1.889, 1.954 (3 s, CH_3CO), 2.950, 3.232 (2 s, OH-4, OH-6), 3.613 (ddd, H-5), 3.705 (s, CO_2CH_3), 3.832 (dd, H-6a), 3.847 (dd, H-3), 3.981 (dd, H-6b), 4.042 (d, H-5′), 4.185 (dd, H-4), 4.534 (d, H-1), 4.617 [d, Ha(Bn)], 4.702 (d, H-1′), 4.799 [d, Hb(Bn)], 4.954 (dd, H-2′), 5.068 (dd, H-3′), 5.154 (dd, H-4′), 5.528 (dd, H-2), 7.0–7.15 (m, Bn), 7.4–8.0 (m, Bz); $J_{1,2}$ 8.1, $J_{2,3}$ 9.8, $J_{3,4}$ 3.4, $J_{4,5}$ 1.2, $J_{5,6a}$ 4.8, $J_{5,6b}$ 6.75, $J_{6a,6b}$ −11.6, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.2, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5, $J_{H(Bn)}$ −12.8 Hz; ^{13}C-n.m.r. (75.5 MHz, $CDCl_3$): δ 19.68, 20.33, 20.36 (3 × *C*H_3CO), 52.97 (CO_2CH_3), 61.82 (C-6), 68.66 (C-4), 69.12 (C-4′), 69.91 [*C*H_2(Bn)], 70.62 (C-2′), 70.86 (C-2), 71.77 (C-3′), 71.97 (C-5′), 74.40 (C-5), 80.89 (C-3), 99.27 (C-1), 101.11 (C-1′), 127.11, 127.53,

127.75, 128.38, 129.69, 133.16, 137.00 (Ar), 164.95, 167.07, 169.18, 169.27, 169.91 (4 × C=O and C-6').

Anal. Calc. for $C_{33}H_{38}O_{16}$: C, 57.39; H, 5.55. Found: C, 56.97; H, 5.51.

Benzyl 2-O-benzoyl-3-O-[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-6-O-sulfo-β-D-galactopyranoside, triethylammonium salt (**6**). — Trimethylamine sulfur trioxide (139 mg, 1.0 mmol) was added to a solution of **4** (110 mg, 0.16 mmol) in *N,N*-dimethylformamide (2 mL). The solution was stirred at room temperature under the exclusion of light. After 2 days t.l.c. control revealed complete conversion of the educt **4**. The solution was diluted with triethylamine (1 mL) and toluene (5 mL) and subsequently filtered. The filtrate was evaporated *in vacuo*, and the residue was purified by column chromatography (6:4:0.3 toluene–ethanol–triethylamine) to yield **6** as first fraction and **11** as second fraction. Evaporation of the appropriate fractions yielded colorless syrups which were dissolved in dichloromethane and extracted once with a small amount of water. Filtration over a filter paper, followed by washing with dichloromethane and evaporation *in vacuo*, yielded **11** (12 mg, 7%) and **6** (104 mg, 75%), respectively. Compound **11** was identified by its n.m.r. spectra and by physical data (*cf.* below). Physical data of **6**: $[\alpha]_D^{20}$ −13.9° (*c* 1.0, CH_2Cl_2); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 1.326 [t, $N(CH_2CH_3)_3$], 1.503, 1.899, 1.998 (3 s, CH_3CO), 3.15 [q, $N(CH_2CH_3)_3$], 3.738 (s, CO_2CH_3), 3.865 (dd, H-3), 3.909 (ddd, H-5), 4.007 (d, H-5'), 4.241 (dd, H-4), 4.290 (dd, H-6a), 4.357 (dd, H-6b), 4.519 (d, H-1), 4.601 [d, Ha(Bn)], 4.688 (d, H-1'), 4.814 [d, Hb(Bn)], 4.921 (dd, H-2'), 5.043 (dd, H-3'), 5.123 (dd, H-4'), 5.508 (dd, H-2), 7.0–7.15 (Bn), 7.4–7.65, 7.9–8.0 (2 m, Bz), 9.45 (s, NH^+); $J_{1,2}$ 8.1, $J_{2,3}$ 9.9, $J_{3,4}$ 3.3, $J_{4,5}$ 1.1, $J_{5,6a}$ 6.3, $J_{5,6b}$ 6.5, $J_{6a,6b}$ −10.9, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.1, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5, J_{HaHb} −12.6 Hz; ^{13}C-n.m.r. (75.5 MHz, $CDCl_3$): δ 8.69 [$N(CH_2CH_3)_3$], 19.69, 20.41, 20.59 (3 × CH_3CO), 46.59 [$N(CH_2CH_3)_3$], 52.98 (CO_2CH_3), 65.73 (C-6), 68.06 (C-4), 69.14 (C-4'), 69.95 [CH_2(Bn)], 70.61 (C-2'), 70.75 (C-2), 71.87 (C-3'), 72.03 (C-5'), 72.45 (C-5), 80.64 (C-3), 99.40 (C-1), 101.10 (C-1'), 127.54, 128.04, 128.42, 128.87, 129.48, 133.26, 136.99 (Ar), 164.85, 165.10, 166.87, 169.27, 169.83 (4 × C=O and C-6').

Anal. Calc. for $C_{39}H_{51}NO_{19}S$: C, 53.73; H, 6.13; N, 1.61. Found: C, 53.93; H, 6.09; N, 1.72.

Benzyl 2-O-benzoyl-3-O-[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-4,6-di-O-sulfo-β-D-galactopyranoside, bis(triethylammonium) salt (**11**). — Trimethylamine–sulfur trioxide complex (180 mg, 1.26 mmol) was added to a solution of **4** (100 mg, 0.145 mmol) in *N,N*-dimethylformamide (0.3 mL). The solution was stirred at room temperature with the exclusion of light. After 14 days t.l.c. control revealed complete conversion of **4** to **11**. Purification as described for **6** yielded a syrup which was crystallized from dichoromethane–carbon tetrachloride to give colorless **11** (147 mg, 96%): m.p. 107–108°; $[\alpha]_D^{20}$ −29.2° (*c* 0.5, CH_2Cl_2); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 1.350 [t, $N(CH_2CH_3)_3$], 1.604, 1.871, 1.940 (3 s, CH_3CO), 3.183 [q, $N(CH_2CH_3)_3$], 3.744 (s, CO_2CH_3), 3.916 (dd, H-3), 3.924 (d, H-5'), 4.062 (ddd, H-5), 4.368 (dd, H-6a), 4.473 (dd, H-6b), 4.526 (d, H-1), 4.607 [d, Ha(Bn)], 4.624 (d, H-1'), 4.802 [d, Hb(Bn)], 4.908 (dd, H-4), 4.931 (dd, H-2'), 4.973 (dd, H-3'), 5.199 (dd, H-4'), 5.352 (dd, H-2), 7.0–7.2 (m, Bn), 7.4–8.0 (m, Bz), 9.39 (s, NH^+); $J_{1,2}$ 7.8, $J_{2,3}$ 10.1, $J_{3,4}$ 3.4, $J_{4,5}$ 1.2, $J_{5,6a}$ 6.5, $J_{5,6b}$ 5.6,

$J_{6a,6b}$ −11.5, $J_{1',2'}$ 7.1, $J_{2',3'}$ 9.2, $J_{3',4'}$ 9.3, $J_{4',5'}$ 10.0, $J_{H(Bn)}$ −12.7 Hz; ^{13}C-n.m.r. (75.5 MHz, $CDCl_3$): δ 14.97 [$N(CH_2CH_3)_3$], 52.71 (CO_2CH_3), 63.59 [$N(CH_2CH_3)_3$], 66.75 (C-6), 68.89 (C-4'), 70.09 [CH_2(Bn)], 71.08 (C-2'), 71.24 (C-2), 71.90 (C-5'), 72.53 (C-3'), 73.04 (C-5), 75.12 (C-4), 77.65 (C-3), 99.33 (C-1), 101.49 (C-1'), 127.54, 129.49, 128.06, 128.44, 128.87, 129.49, 133.26, 136.93 (Ar), 165.08, 166.90, 2 × 169.10, 170.00 (4 × C=O and C-6').

Anal. Calc. for $C_{45}H_{68}N_2O_{22}S_2$: C, 51.32; H, 6.51; N, 2.66. Found: C, 50.98; H, 6.46; N, 2.58.

*Benzyl 2,6-di-*O*-benzoyl-3-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)uronate]-β-*D*-galactopyranoside* (**8**). — A 0.04M solution of freshly prepared *N*-benzoylimidazole in dichloromethane (0.46 mL, 18 μmol) was added to a stirred solution of **4** (116 mg, 16.8 μmol) in dichloromethane (3 mL). After stirring at room temperature for 10 days, the solution was diluted with dichloromethane and washed with satd. sodium hydrogencarbonate and water. The organic layer was dried over sodium sulfate, concentrated under reduced pressure, adsorbed on silica gel, and purified by column chromatography (1:1 ethyl acetate–petroleum ether). Evaporation and crystallization from dichloromethane–carbon tetrachloride yielded colorless **8** (96 mg, 72%): m.p. 168–172°; $[\alpha]_D^{20}$ −24.0° (*c* 0.7, CH_2Cl_2); m.s. (d.c.i.–ammonia): *m/z* 812 $(M + NH_4)^+$; 752 $(M - HOAc + NH_4)^+$; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 1.58, 1.95, 1.98 (3 s, 3 × CH_3CO), 2.79 (s, OH-4), 3.660 (s, CO_2CH_3), 3.841 (dd, H-3), 3.843 (ddd, H-5), 3.956 (d, H-5'), 4.181 (dd, H-4), 4.472 (d, H-1), 4.621 [d, Ha(Bn)], 4.650 (dd, H-6a), 4.660 (d, H-1'), 4.690 (dd, H-6b), 4.813 [d, Hb(Bn)], 4.935 (dd, H-2'), 5.047 (dd, H-3'), 5.139 (dd, H-4'), 5.562 (dd, H-2), 7.0–7.12 (m, Bn), 7.4–8.1 (m, Bz); $J_{1,2}$ 8.1, $J_{2,3}$ 9.8, $J_{3,4}$ 3.2, $J_{4,5}$ 1.2, $J_{5,6a}$ 6.5, $J_{5,6b}$ 6.2, $J_{6a,6b}$ −12.0, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.1, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5, $J_{H(Bn)}$ −12.6 Hz; ^{13}C-n.m.r. (75.5 MHz, $CDCl_3$): δ 19.78, 20.04, 20.45 (3 × CH_3CO), 52.94 (CO_2CH_3), 63.43 (C-6), 68.51 (C-4), 69.14 (C-4'), 69.63 [CH_2(Bn)], 70.67 (C-2), 70.73 (C-2'), 71.64 (C-3'), 72.07 (C-5), 72.28 (C-5'), 80.67 (C-3), 98.73 (C-1), 101.22 (C-1'), 127.59, 127.81, 128.11, 128.37, 128.86, 129.56, 129.65, 130.20, 130.76, 133.11, 133.18, 133.49, 136.67, 138.11 (Ar), 164.76, 166.38, 166.78, 169.10, 169.22, 169.96 (5 × C=O and C-6').

Anal. Calc. for $C_{40}H_{42}O_{17}$: C, 60.45; H, 5.33. Found: C, 60.01; H, 5.28.

*Benzyl 2,6-di-*O*-benzoyl-3-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)uronate]-4-*O*-sulfo-β-*D*-galactopyranoside, triethylammonium salt* (**9**). — Trimethylamine–sulfur trioxide (70 mg, 710 μmol) was added to a solution of **8** (32 mg, 40 μmol) in *N,N*-dimethylformamide (0.3 mL). The suspension was stirred at 60–70° for 7 days under the exclusion of light. Purification as described for **6** yielded a syrup which was crystallized from dichloromethane–carbon tetrachloride to give colorless **9** (34 mg, 87%): m.p. 102°; $[\alpha]_D^{20}$ −30.0° (*c* 2.0, CH_2Cl_2); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 1.392 [t, $N(CH_2CH_3)_3$], 1.614, 1.895, 1.939 (3 s, 3 × CH_3CO), 3.20 [q, $N(CH_2CH_3)_3$], 3.694 (s, CO_2CH_3), 3.932 (dd, H-3), 3.940 (m, H-5), 3.956 (d, H-5'), 4.436 (d, H-1), 4.560 [d, Ha(Bn)], 4.697 (d, H-1'), 4.734 [d, Hb(Bn)], 4.747 (dd, H-6a), 4.844 (dd, H-6b), 4.970 (m, H-2', H-3'), 5.004 (dd, H-4), 5.262 (dd, H-4'), 5.434 (dd, H-2), 7.00–7.09 (m, Bn), 7.40–7.62, 7.95, 8.10 (3 m, Bz), 9.69 (s, NH^+); $J_{1,2}$ 7.6, $J_{2,3}$ 9.7, $J_{3,4}$ 3.1, $J_{4,5}$ 1.2, $J_{5,6a}$ 7.5, $J_{5,6b}$ 4.2, $J_{6a,6b}$ −12.1, $J_{1',2'}$ 8.0, $J_{3',4'}$ 9.9, $J_{4',5'}$ 9.9, $J_{H(Bn)}$ −12.5 Hz; ^{13}C-n.m.r. (75.5 MHz,

$CDCl_3$): δ 8.63 [$N(CH_2CH_3)_3$], 46.21 [$N(CH_2CH_3)_3$], 52.93 (CO_2CH_3), 64.33 (C-6), 68.85 (C-4'), 69.52 [CH_2(Bn)], 71.29 (C-2), 71.29 (C-2'), 72.65 (C-3'), 72.29 (C-5'), 72.80 (C-5), 74.17 (C-4), 77.72 (C-3), 98.39 (C-1), 101.37 (C-1'), 127.69, 127.98, 128.19, 128.33, 128.53, 129.67, 129.77, 130.40, 132.89, 133.36, 136.68 (Ar), 165.08, 166.35, 166.97, 169.12, 169.31, 170.13 (5 × C=O and C-6').

Anal. Calc. for $C_{42}H_{55}NO_{30}S$: C, 56.61; H, 5.89; N, 1.44. Found: C, 56.48; H, 5.93; N, 1.47.

Benzyl 3-O-(β-D-glucopyranosyl uronate)-6-O-sulfo-β-D-galactopyranoside, disodium salt (**7**). — A M aq. solution of sodium hydroxide (138 μL) was added at 0° to a solution of **6** (20 mg, 23 μmol) in tetrahydrofuran (0.6 mL). After stirring overnight at 0°, the solution was neutralized with dilute acetic acid (10%) and lyophilized. The crude mixture was purified by ion-exchange chromatography with S-Sepharose (Na^+) using water as eluent. Concentration of the eluate and two subsequent runs on a size-exclusion gel (Fractogel HW40) with water as eluent yielded after lyophilization **7** (10 mg, 76%) as an amorphous solid: $[\alpha]_D^{20}$ = −16.4° (*c* 0.9, H_2O); ^{1}H-n.m.r. (300 MHz, D_2O): δ 3.297 (dd, H-2'), 3.402 (m, H-3', H-4'), 3.594 (dd, H-2), 3.607 (d, H-5'), 3.662 (dd, H-3), 3.805 (ddd, H-5), 4.110 (m, H-6a, H-6b), 4.113 (dd, H-4), 4.399 (d, H-1), 4.549 (d, H-1'), 4.670 [d, Ha(Bn)], 4.839 [d, Hb(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 7.6, $J_{2,3}$ 9.8, $J_{3,4}$ 3.3, $J_{4,5}$ 1.1, $J_{5,6a}$ 5.3, $J_{5,6b}$ 7.3, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.5, $J_{3',4'}$ 8.7, J_{HBnH} = 11.6 Hz; ^{13}C-n.m.r. (75.5 MHz, D_2O): δ 67.86 (C-6), 68.29 (C-4), 70.01 (C-2), 71.65 [CH_2(Bn)], 72.03 (C-4'), 72.89 (C-5), 73.41 (C-2'), 75.55 (C-3'), 76.39 (C-5'), 82.74 (C-3), 101.40 (C-1), 103.88 (C-1'), 128.55, 128.72, 128.96, 129.23, 131.49 (Ar), 176.15 (C-6').

Anal. Calc. for $C_{19}H_{24}Na_2O_{12}S$: C, 40.01; H, 4.24. Found: C, 39.69; H, 4.27.

Benzyl 3-O-(β-D-glucopyranosyl uronate)-4-O-sulfo-β-D-galactopyranoside, disodium salt (**10**). — A solution of **9** (17 mg, 17 μmol) in tetrahydrofuran (0.25 mL) was treated with M sodium hydroxide (130 μL) as described for **7** to yield amorphous **10** (9.0 mg, 90%): $[\alpha]_D^{20}$ = −25.0 (*c* 0.4, H_2O); ^{1}H-n.m.r. (300 MHz, D_2O): δ 3.289 (dd, H-2'), 3.387 (dd, H-3'), 3.430 (dd, H-4'), 3.578 (d, H-5'), 3.624 (dd, H-2), 3.691 (m, H-5, H-6a, H-6b), 3.880 (dd, H-3), 4.423 (d, H-1), 4.642 (d, H-1'), 4.650 [d, Hb(Bn)], 4.680 (dd, H-4), 4.844 [d, Ha(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 10.0, $J_{3,4}$ 3.2, $J_{4,5}$ 1.1, $J_{5,6a}$ 7.8, $J_{5,6b}$ 9.5, $J_{1',2'}$ 8.8, $J_{4,5}$ 9.7, J_{HBnH} = 12.7 Hz; ^{13}C-n.m.r. (75.5 MHz, D_2O): δ 61.17 (C-6), 70.93 (C-2), 71.74 [CH_2(Bn)], 72.07 (C-4'), 73.31 (C-2'), 74.72 (C-5), 75.55 (C-3'), 76.39 (C-5'), 77.23 (C-3), 77.40 (C-4), 101.70 (C-1), 103.12 (C-1'), 128.53, 128.68, 128.94, 129.02, 131.56 (Ar), 176.13 (C-6').

Anal. Calc. for $C_{19}H_{24}Na_2O_{15}S$: C, 40.01; H, 4.24. Found: C, 39.68; H, 4.10.

Benzyl 3-O-(β-D-glucopyranosyl uronate)-4,6-di-O-sulfo-β-D-galactopyranoside, trisodium salt (**12**). — Treatment of a solution of **11** (20 mg, 19 μmol) in tetrahydrofuran (0.6 mL) with M sodium hydroxide (133 μL) as described for **7** yielded colorless **12** (12 mg, 94%): $[\alpha]_D^{20}$ = −34.8° (*c* 0.6, H_2O); ^{1}H-n.m.r. (300 MHz, D_2O): δ 3.294 (dd, H-2'), 3.391 (dd, H-3'), 3.433 (dd, H-4'), 3.581 (d, H-5'), 3.636 (dd, H-2), 3.906 (dd, H-3), 3.952 (dd, H-5), 4.105 (dd, H-6a), 4.205 (dd, H-6b), 4.416 (d, H-1), 4.637 (d, H-1'), 4.663 [d, Ha(Bn)], 4.714 (dd, H-4), 4.851 [d, Hb(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 10.0, $J_{3,4}$ 3.1, $J_{4,5}$ 1.0, $J_{5,6a}$ 8.6, $J_{5,6b}$ 3.2, $J_{6a,6b}$ = 11.3, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.4, $J_{3',4'}$ 8.3, $J_{4',5'}$ 9.6, J_{HBnH} = 11.3 Hz;

^{13}C-n.m.r. (75.5 MHz, D_2O): δ 68.16 (C-6), 70.88 (C-2), 71.81 [CH_2(Bn)], 72.10 (C-4'), 72.61 (C-5), 73.33 (C-2'), 75.59 (C-3'), 76.39 (C-5'), 77.13 (C-4), 77.24 (C-3), 101.46 (C-1), 103.10 (C-1'), 128.53, 128.73, 128.96, 129.01, 131.47 (Ar), 176.10 (C-6').

Anal. Calc. for $C_{19}H_{23}Na_3O_{18}S_2$: C, 33.94; H, 3.45. Found: C, 34.22; H, 3.51.

Benzyl 3-O-(β-D-glucopyranosyl uronate)-β-D-galactopyranoside, sodium salt (**5**). — A solution of **4** (20 mg, 29 μmol) in tetrahydrofuran (0.3 mL) was treated with M sodium hydroxide (145 μL) as described for **7** to yield amorphous **5** (11 mg, 81%): $[\alpha]_D^{20}$ −41.5° (*c* 0.3, H_2O); ^{1}H-n.m.r. (300 MHz, D_2O): δ 3.295 (dd, H-2'), 3.409 (m, H-3', H-4'), 3.567 (ddd, H-5), 3.587 (dd, H-2), 3.604 (d, H-5'), 3.636 (dd, H-3), 3.650 (m, H-6a, H-6b), 4.061 (dd, H-4), 4.381 (d, H-1), 4.538 (d, H-1'), 4.644 [d, Hb(Bn)], 4.834 [d, Ha(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 7.3, $J_{2,3}$ 9.8, $J_{3,4}$ 2.4, $J_{4,5}$ 0.5, $J_{5,6a}$ 4.2, $J_{5,6b}$ 7.8, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.6, $J_{4',5'}$ 9.7, $J_{H(Bn)}$ −11.6 Hz; ^{13}C-n.m.r. (75.5 MHz, D_2O): δ 61.17 (C-6), 68.35 (C-4), 70.04 (C-2), 71.52 [CH_2(Bn)], 71.97 (C-4'), 73.36 (C-2'), 75.09 (C-5), 75.50 (C-3'), 76.37 (C-5'), 82.95 (C-3), 101.55 (C-1), 103.83 (C-1'), 128.53, 128.67, 128.93, 129.00, 131.54 (Ar), 176.13 (C-6').

Anal. Calc. for $C_{19}H_{25}NaO_{12}$: C, 48.72; H, 5.38. Found: C, 48.45; H, 5.43.

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