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Syntheses of disaccharides with (1→4)-β glycosidic linkages related to the 4- and 6-sulfates and the 4,6-disulfates of chondroitin

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ABSTRACT

The sodium salts of the 6′-sulfate **12**, the 4′-sulfate **15** and the 4′,6′-disulfate **17** of benzyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosiduronate **10** have been synthesized. Methyl [benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosid]uronate (**9**) has been prepared as a key intermediate from benzyl 4′,6′-*O*-benzylidene-β-D-lactopyranoside (**2**). Protection of **2** at C-6 with the *tert*-butyldimethylsilyl group, followed by *O*-perbenzoylation and disilylation, gave benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**7**). Oxidation of the 6-position of **7** proved to be difficult. However, **7** could be converted into the *tert*-butyl glucuronate **8** using chromium trioxide–pyridine and *tert*-butanol. Simultaneous hydrolysis of the benzylidene acetal and the *tert*-butyl ester groups, followed by esterification of the resulting free acid with diazomethane, yielded **9**. Compound **9** was directly sulfated with sulfur trioxide–trimethylamine within 12 h to give the 6′-sulfate **11**. The 4′,6′-disulfate **16** was accessible by running the reaction under the same conditions for 14 days. The 4′-sulfate **14** was obtained after protecting the 6′-OH group of **9** with benzoyl cyanide to give the 6′-benzoate **13** followed by sulfation under more vigorous reaction conditions. Deesterification of **9**, **11**, **14**, and **16** was achieved by treatment with aqueous sodium hydroxide in tetrahydrofuran to give **10**, **12**, **15**, and **17**, respectively.

INTRODUCTION

Sulfated oligo- and poly-saccharides like heparin or chondroitin sulfates exhibit a number of physiological functions[1,2,3]. Heparin is, for example, involved in the blood clotting cascade by interacting with antithrombin III and factor Xa[1,2,4]. Furthermore, it is involved in the release of lipids by interacting with lipoprotein lipase[2]. A similar activity is found for other sulfated oligosaccharides like dermatan sulfate and heparan sulfate[5]. Sulfate groups are also found in many oligosaccharides that are constituents of glycoproteins and glycolipids[6]. The interaction of sulfate groups with protein receptors or their influence on the conformation of oligosaccharides has been the focus of recent research[7,8]. Some specific sulfate groups of the antithrombin III-binding pentasaccha-

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ride of heparin are involved in the interaction with the protein, while others are not[9].

In recent years it was shown that the synthesis of oligosaccharides related to glycosaminoglycans can have advantages over the isolation of fragments from biological material[10]. Synthesis avoids the problems created by microheterogenity and modifications introduced at the enzymatic or chemical-cleavage step[5,11,12]. This fact was most strikingly demonstrated by the synthesis of the pentasaccharide that is responsible for binding to antithrombin III and factor Xa[10,13,14]. The syntheses of the two repeating units of dermatan sulfate[15] and of oligosaccharide fragments of keratan sulfate[16] were published. Furthermore, the syntheses of monosulfated oligosaccharides have been published[17].

Several studies have focused on the influence of sulfate groups on the conformation of oligosaccharides related to heparin[5]. It was shown that the conformation of the iduronic acid ring is dependent on the pattern of sulfation around that residue and adopts conformations between the $^1C_4$ and the $^2S_0$ conformations[5].

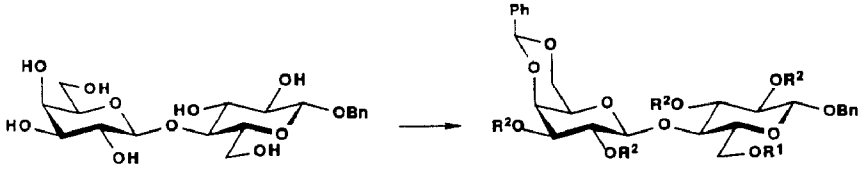
Glycosaminoglycans often contain densely clustered, negatively charged groups, *i.e.*, sulfate and carboxylate groups. The interactions between negatively charged groups that are located in close proximity within one molecule would normally result in a repulsive interaction between these groups. However, attractive interactions would result if cations were complexed by the two negatively charged groups. Differences in the nature of the interactions may be observed between sulfate–sulfate and carboxylate–sulfate interactions because the ability of a carboxylate to complex cations is significantly higher than that of a sulfate group. Thus, a carboxylate and a sulfate group may exhibit attractive interactions, whereas two sulfate groups in the same relative position may exhibit repulsive forces. The position of cations relative to the anionic groups would determine the net effect of these charged substituents on the conformation of the oligosaccharides.

Chondroitin sulfates form a family of polysaccharides that differ only in the position and degree of sulfation. Thus, they contain a set of model disaccharides for a systematic study of the effects of charged groups on the conformation of the glycosidic bonds. Chondroitin-4-sulfate, -6-sulfate and -4,6-disulfate all contain both sulfate and carboxylate groups in close proximity. Furthermore, the conformation of the glycosyl rings should not be affected by the charged groups. Thus, it should be possible to derive general rules for conformational changes induced by these charged groups.

DISCUSSION

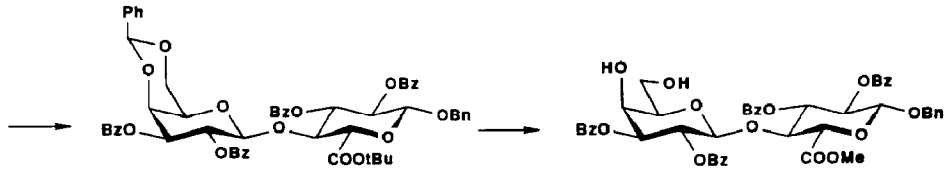
We synthesized the sulfated derivatives of β-D-Gal-(1→4)-β-D-GlcA **12**, **15**, and **17**, as well as the unsulfated derivative **10**, in order to study the conformational changes of oligosaccharides induced by sulfate groups[18]. These disaccharides are closely related to disaccharide fragments of chondroitin sulfates. We synthesized **10**, **12**, **15**, and **17** as the benzyl β-D-glycosides in order to anchor the reducing end in the equatorial position. Our synthetic strategy was based on the modification of lactose. Thus, we avoided the glycosylation of the extremely unreactive 4-OH group of a glucuronic acid derivative[19].

The analogous sulfated oligosaccharides derived from β-D-GlcA-(1→3)-β-D-Gal have been prepared by using a glycoside synthesis to build the key intermediate[20].



1

2 $R^1 = R^2 = H$
3 $R^1 = tBuPh_2Si$, $R^2 = H$
4 $R^1 = tBuPh_2Si$, $R^2 = Bz$
5 $R^1 = tBuMe_2Si$, $R^2 = H$
6 $R^1 = tBuMe_2Si$, $R^2 = Bz$
7 $R^1 = H$, $R^2 = Bz$



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9

Benzylidenation of benzyl β-D-lactoside (**1**) yields the 4′,6′-*O*-benzylidene-β-D-lactopyranoside (**2**) whose yield could be improved by 21% compared to earlier results[21] using a modified work-up procedure. Protection of the 6-OH group with the *tert*-butyldiphenylsilyl group, using the procedure described by Hanessian[22], proceeded very slowly, and a yield of only 46% of **3** could be obtained. 2,3,2′,3′-Tetra-*O*-benzoylation of **3** gave **4** in 94% yield. Cleavage of the silyl ether of **4** gave **7** in only 53% yield. The yields of this silylation–desilylation step could be improved by using the sterically less demanding *tert*-butyldimethylsilyl protecting group. Under proper reaction control, **5** could be obtained in 93% yield. Immediate quenching of the reaction after complete monosilylation was necessary to avoid oversilylation. *O*-Perbenzoylation of **5** gave **6** which was desilylated by tetrabutylammonium fluoride in almost quantitative yield to give **7**.

Oxidation of **7** requires essentially neutral conditions, because the benzylidene acetal is labile with acids. Compound **7** was stable against Fetizon's reagent[23] or pyridinium dichromate[24], while it decomposed with pyridinium chlorochromate-based reagents[25,26]. However, oxidation could be achieved by using the Corey–Samuelsson procedure[27]. Under these conditions, oxidation with chromium trioxide–pyridine in *N,N*-dimethylformamide leads initially to an aldehyde group at C-6 that is converted *in situ* to the *tert*-butyl hemiacetal. Further oxidation yielded the *tert*-butyl ester **8** in a total yield of 46%. Cleavage of both the benzylidene acetal and the *tert*-butyl ester function was achieved with 90% trifluoroacetic acid in dichloromethane after a reaction time of several hours. The hydrolysis product was directly esterified with diazomethane to give the methyl ester **9**.

The 6′-OH function of **9** could be selectively sulfated with sulfur trioxide trimethylamine in *N*,*N*-dimethylformamide to give **11**. The rate of the reaction was strongly dependent on the concentration of the sugar. Compound **11** was purified as the triethylammonium salt, which could easily be chromatographed on a silica gel column with a triethylamine-containing eluent[28] to give a yield of 83%.

The disulfation of **9** proved to be more difficult because of the low reactivity of the axial 4′-OH group[29]. However, disulfation at both 4′-OH and the 6′-OH groups could be achieved by a prolonged reaction time of 14 days. The 4′,6′-disulfate **16** was isolated in 83% yield. The 6′-sulfate **11** was obtained as a byproduct in 14% yield. Photolytic decomposition of the solvent *N*,*N*-dimethylformamide was found to hinder the reaction. Thus, exclusion of light was important to obtain a high yield in the sulfation reaction. The two sulfates **11** and **16** were easily separated and purified by column chromatography on silica gel.

It was necessary to protect the 6′-OH group of **9** in order to achieve a monosulfation at the 4′-position. Reaction of **9** with benzoyl cyanide[30] gave the selectively 6′-benzoylated **13** in nearly quantitative yield. As expected, monosulfation of the secondary 4′-OH group was significantly slower than that observed for the primary 6′-OH group. Reaction with sulfur trioxide trimethylamine at 60° for 7 days gave the 4′-sulfated ester **14** in 97% yield.

Compounds **11**, **14**, and **16** were characterized by their ^{1}H- and ^{13}C-n.m.r. spectra (Tables I and II). The ^{1}H-n.m.r. spectrum of the 6′-sulfate **11** shows the signals of H-6′a and H-6′b shifted downfield by 0.4 p.p.m. A much stronger downfield shift of 0.7 p.p.m. is observed for the signals of both H-6′ protons of the 4′,6′-disulfate **16**. The 4′-sulfate group causes a downfield shift of the signal of H-4′ of 0.8–1.0 p.p.m. in both the 4′-sulfate **14** and the 4′,6′-disulfate **16**. The ^{13}C-n.m.r. spectra of the sulfates **11**, **14**, and **16** show that the 6′-sulfate group has a deshielding α-effect of 2.5–3.0 p.p.m. and a shielding β-effect of −1.8 p.p.m. (*cf.* Table II). The 4′-sulfate **14** shows a downfield shift of 4.8 p.p.m. on the signal of C-4′ compared to the unsulfated **13**. The signal of C-4′ of the disulfate **16** is shifted 6.0 p.p.m. downfield compared to its precursor **9**. Thus, the shift effects of the 4′-sulfate group seem to be more pronounced than those of the 6′-sulfate.

The ionic target compounds **10**, **12**, **15**, and **17** are accessible from the methyl esters **9**, **11**, **14**, and **16** by saponification. By using aqueous sodium hydroxide in tetrahydrofuran, we were able to avoid β-elimination reactions in the glucuronic ester residue. The deesterification reaction of the protected compounds was virtually quantitative. Complete exchange of triethylammonium ions against sodium cations was achieved by chromatography on S-Sepharose (Na^+ form). The unprotected sulfated disaccharides were purified from accompanying salts by size-exclusion chromatography. The isolated yields of analytically pure samples were reduced to 60–80% by losses in the final purification step. The ionic disaccharides **10**, **12**, **15**, and **17** have been synthesized in overall yields of 5–7%, starting from lactose.

Complete assignments of the ^{1}H-n.m.r. spectra of **10**, **12**, **15**, and **17** were possible by 2D (^{1}H,^{1}H) COSY n.m.r. spectra, 2D (^{13}C, ^{1}H) COSY n.m.r. spectra, and 1D

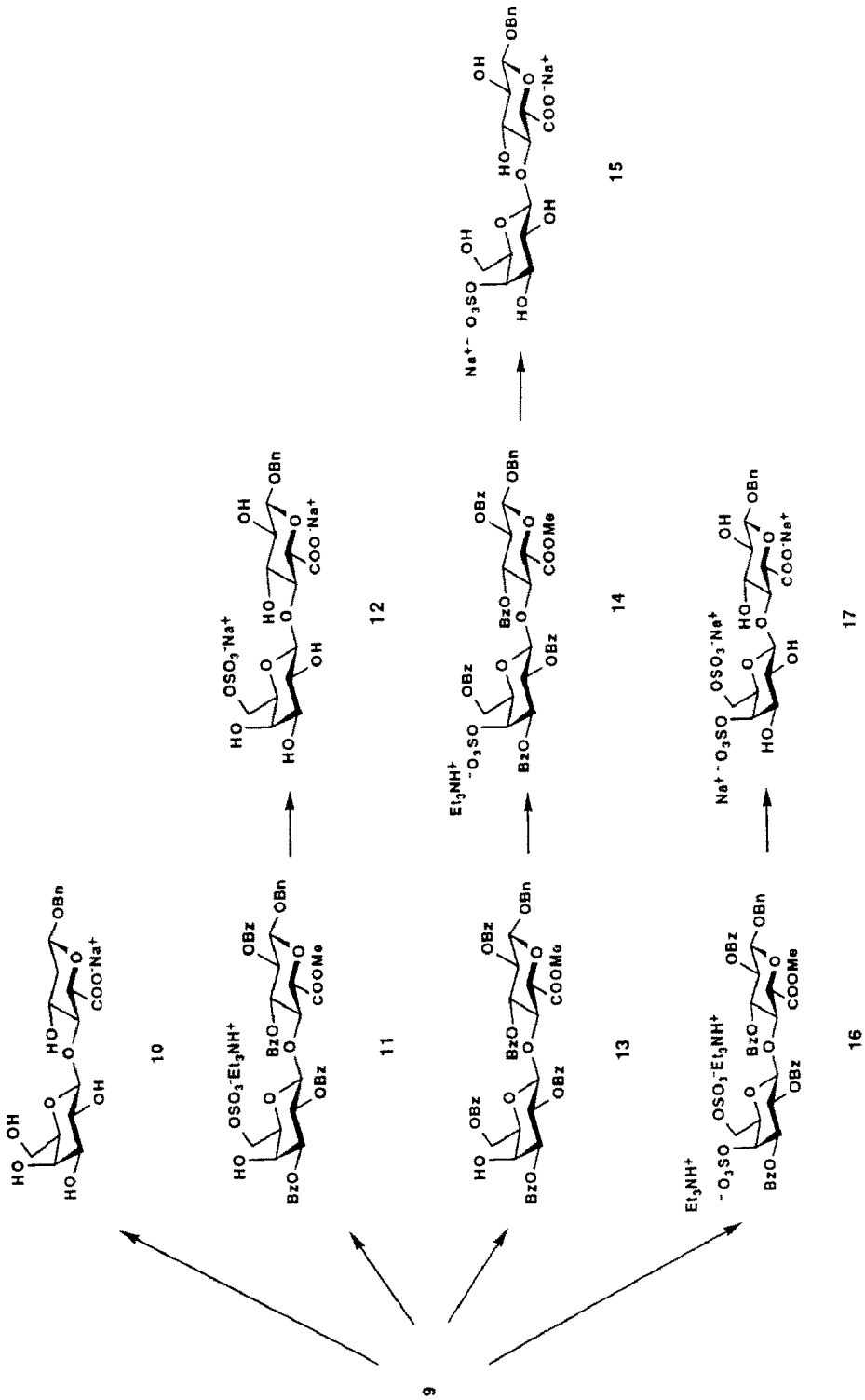

TABLE I

^{1}H-N.m.r. chemical shifts (p.p.m.) and coupling constants (Hz) of **9**, **11**, **16**, **13**, and **14** and shift differences (p.p.m.) of the sulfated compounds **11**, **16**, and **14** relative to the unsulfated **9** or **13**, respectively[a]

	δ (p.p.m.)[b]					$\Delta\delta$		
	9	**11**	**16**	**13**	**14**	**11 − 9**	**16 − 9**	**14 − 13**
H-1	4.795 (7.0)	4.712 (7.3)	4.729 (7.1)	4.747 (7.3)	4.712 (7.3)	−0.083	−0.066	−0.035
H-2	5.427 (8.7)	5.332 (9.1)	5.306 (9.1)	5.461 (9.2)	5.365 (9.1)	−0.095	−0.121	0.996
H-3	5.681 (8.5)	5.577 (9.0)	5.561 (9.1)	5.668 (9.0)	5.584 (9.0)	−0.104	0.120	−0.083
H-4	4.451 (8.8)	4.409 (9.2)	4.517 (9.2)	4.432 (9.2)	4.416 (9.1)	−0.042	0.066	−0.016
H-5	4.067	3.967	3.956	4.020	3.967	−0.100	−0.111	−0.053
H-1′	4.882 (7.8)	4.805 (7.8)	4.930 (7.9)	4.905 (7.8)	4.886 (7.8)	0.078	0.048	−0.019
H-2′	5.590 (10.3)	5.489 (10.5)	5.388 (10.5)	5.574 (10.4)	5.541 (10.5)	−0.101	0.202	−0.033
H-3′	5.150 (3.1)	5.115 (3.3)	5.209 (3.2)	5.222 (3.3)	5.234 (3.3)	−0.035	0.059	0.012
H-4′	4.161 (1.0)	4.228 (1.0)	4.989 (1.3)	4.082 (1.3)	5.015 (1.3)	0.067	0.827	0.033
H-5′	3.510 (5.7, 4.2)	3.660 (—)[c]	4.181 (5.7, 6.2)	3.786 (6.1, 6.7)	3.903 (6.9, 5.3)	0.150	0.671	0.117
H-6′a	3.206 (−12.5)	3.660 (—)[c]	3.880 (−11.5)	3.581 (−11.2)	3.791 (−11.5)	0.454	0.674	0.210
H-6′b	3.291	3.660	3.988	3.946	4.388	0.369	0.697	0.442

[a] Measured in $CDCl_3$. [b] Coupling constants (Hz) are in parentheses. [c] Assignment not possible because of spectral overlap.

TABLE II

^{13}C-N.m.r. chemical shifts (p.p.m.) of **9**, **11**, **16**, **13**, and **14** and shift differences (p.p.m.) of the sulfated compounds **11**, **16**, and **14** relative to the unsulfated **9** or **13**, respectively[a]

	δ (p.p.m.)					*Δδ*		
	9	**11**	**16**	**13**	**14**	**11−9**	**16−9**	**14−13**
C-1	99.43	99.56	99.67	99.73	99.62	0.13	0.24	−0.11
C-2	71.27	71.67	72.19	71.37	71.59[b]	0.40	0.82	0.22
C-3	72.52	72.40	72.33	72.30[b]	72.29	−0.12	−0.19	0.01
C-4	76.53	76.08	75.92	76.44	76.25	−0.45	−0.61	−0.19
C-5	73.96	74.08	74.61	74.04	74.29	0.12	0.65	0.25
C-1′	101.28	100.93	100.59	101.03	100.84	−0.35	−0.69	−0.19
C-2′	69.90	69.82	70.08	69.78	69.99	−0.08	0.18	0.21
C-3′	74.22	73.53	72.46	73.80	72.29	−0.69	−1.76	−1.51
C-4′	67.80	65.67	71.89	66.90	71.79[b]	−2.13	4.09	1.89
C-5′	74.30	72.51	72.96	72.35[b]	72.70	−1.79	−1.34	0.35
C-6′	61.97	63.47	65.16	61.56	62.85	1.50	3.19	1.29

[a] Measured in $CDCl_3$. [b] Assignments uncertain.

decoupling experiments. Comparison of the ^{1}H-n.m.r. chemical shifts of the sulfated disaccharides **12**, **15**, and **17** with those of the corresponding unsulfated reference compound **10** shows the influence of deshielding by a sulfate group on the geminal and vicinal protons (*cf.* Table III). The signals of the α-protons of the 6′-sulfate **12** show a downfield shift of 0.4–0.5 p.p.m. The 4′-sulfate **15** causes a larger downfield shift of the signal of the α-proton H-4′ of 0.8 p.p.m. However, the deshielding β-effect of the primary sulfate group in **12** appears to be stronger than that of the secondary sulfate group in **15**. The H-5′ signal of **12** is shifted downfield by 0.3 p.p.m., whereas the H-3′ and H-5′ signals of **15** are shifted downfield by approximately 0.15 p.p.m. Interestingly, the disulfated **17** exhibits a downfield shift at H-5′ of 0.5 p.p.m., which appears to be the sum of the effects observed for the two monosulfated disaccharides **12** and **15**. This chemical shift would seem to indicate that there is no strong interaction between the two sulfate groups.

The ^{13}C-n.m.r. chemical shifts of **10**, **12**, **15**, and **17** were assigned by 2D (^{13}C,^{1}H)-correlated spectra. The differences of the chemical shifts (*Δδ*) between the sulfated disaccharides **12**, **15**, and **17** and the unsulfated reference compound **10** are shown in Table IV. The presence of a 6′-sulfate group causes a downfield shift of the C-6′ signal of 6.2 p.p.m. and an upfield shift of the C-5′ signal of 2.5 p.p.m. The 4′-monosulfate **15** has an α-effect of 7.9 p.p.m. and β-effects of −0.7 p.p.m. on the signal of C-5′ and of −1.0 p.p.m. on the signal of C-3′, respectively. The 4′,6′-disulfate **17** shows downfield shifts of the C-6′ signal of 6.8 p.p.m. and of the C-4′ signal of 7.6 p.p.m. The C-1′ signals of **12** and **17** show a shift of 0.7 to 0.8 p.p.m. to lower field, whereas the signal of C-2′ is shifted upfield by 0.15 p.p.m. These effects indicate a change of the conformation[31] of the glycosidic linkage, which is also supported by the downfield shift of the C-4 signals in **12** and **17** of 1.4 and 2.1 p.p.m. respectively. In contrast, the signals of C-1′, C-2′, and C-4 of

TABLE III

^{1}H-N.m.r. data of the β-D-Gal-(1→4)-β-D-GlcA-linked compounds **10**, **12**, **15**, and **17**. Chemical shifts (p.p.m.), coupling constants (Hz) and shift differences (p.p.m.) of the sulfated compounds relative to **10**[a].

	δ (p.p.m.)[b]				Δδ		
	10	**12**	**15**	**17**	**12 − 10**	**15 − 10**	**17 − 10**
H-1	4.433 (7.9)	4.447 (8.0)	4.444 (8.0)	4.457 (8.0)	0.014	0.011	0.024
H-2	3.288 (9.4)	3.303 (9.2)	3.288 (9.3)	3.304 (9.0)	0.015	0.000	0.016
H-3	3.494 (8.6)	3.507 (8.7)	3.508 (8.5)	3.518 (9.0)	0.013	0.014	0.024
H-4	3.647 (9.8)	3.605 (9.6)	3.611 (9.8)	3.611 (9.7)	−0.042	−0.036	−0.036
H-5	3.729	3.746	3.734	3.750	0.017	0.005	0.021
H-1′	4.336 (7.7)	4.356 (7.7)	4.396 (7.8)	4.411 (7.8)	0.020	0.078	0.060
H-2′	3.417 (9.9)	3.446 (10.0)	3.436 (10.1)	3.465 (10.0)	0.029	0.019	0.048
H-3′	3.537 (3.4)	3.574 (3.4)	3.687 (3.3)	3.721 (3.4)	0.037	0.150	0.184
H-4′	3.781 (1.3)	3.849 (1.3)	4.543 (1.3)	4.582 (1.3)	0.068	0.762	0.801
H-5′	3.583 (4.4, 8.5)	3.853 (8.0, 4.5)	3.718 (4.2, 8.2)	4.008 (8.5, 3.5)	0.270	0.135	0.425
H-6′a	3.600 (−10.7)	4.095 (−)	3.695 (−)	4.115 (−11.2)	0.495	0.095	0.515
H-6′b	3.660	4.095	3.695	4.191	0.435	0.035	0.531

[a] Measured in D_2O. [b] Coupling constants (Hz, in parentheses) were determined by first-order analysis. [c] Coupling constants could not be determined.

TABLE IV

^{13}C-N.m.r. chemical shifts (p.p.m.) of **10**, **12**, **15**, and **17** and shift differences (p.p.m.) of the sulfated compounds compared to **10**[a].

	δ				Δδ		
	10	**12**	**15**	**17**	**12 − 10**	**15 − 10**	**17 − 10**
C-1	101.62	101.08	101.14	101.03	−0.54	−0.48	−0.59
C-2	72.88	72.74	72.85	72.68	−0.14	−0.03	−0.20
C-3	74.69	74.89	74.71	75.03	0.20	0.02	0.34
C-4	80.49	81.92	80.78	82.59	1.43	0.29	2.10
C-5	75.99	75.68	75.95	75.57	−0.31	−0.04	−0.42
C-1′	103.02	103.68	103.04	103.83	0.66	0.02	0.81
C-2′	71.25	71.10	71.27	71.10	−0.15	0.02	−0.15
C-3′	72.77	72.49	71.80	71.56	−0.28	−0.97	−1.21
C-4′	68.78	68.45	76.70	76.34	−0.33	7.92	7.56
C-5′	75.61	73.15	74.88	72.68	−2.46	−0.73	−2.93
C-6′	61.24	67.46	61.24	68.01	6.22	0.00	6.77

[a] Measured in D_2O.

the 4′-sulfate **15** have chemical shifts similar to the unsulfated **10**. The changes of the chemical shifts observed for the monosulfated **12** and **15** are in agreement with those observed for monosulfated galactose derivatives[32].

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-n.m.r. spectra were recorded with a Bruker WP 80 (80 MHz) or AM 300 (300 MHz) spectrometer, and all ^{13}C-n.m.r. spectra were recorded with a Bruker AM 300 (^{13}C 75.5 MHz) spectrometer. Mass spectra were recorded on an MAT 212 instrument.

Benzyl 4',6'-O-benzylidene-β-lactopyranoside (**2**) (ref. 21). — Benzyl β-D-lactopyranoside[33] (**1**, 4.0 g, 9.3 mmol) was added in one portion to a stirred solution of dry zinc chloride (1.8 g) in benzaldehyde (25 mL). After 2 days a hot saturated solution of sodium carbonate was added under vigorous stirring. After filtration and repeated washing with methanol, the combined organic solutions were evaporated to dryness. Dissolving the residue in methanol, followed by filtration over silica gel, removed residual zinc salts. The eluate was evaporated and chromatographed on silica gel (9:1 dichloromethane–methanol) to yield 3.20 g of **2** (66%; lit.[21]: 45%) as crystals: m.p. 187° [lit.[21]: 167°]; $[\alpha]_D^{20}$ −47.6° (*c* 0.7, MeOH) [lit.[21]: −51° in pyridine].

Benzyl 4',6'-O-benzylidene-6-O-tert-butyldiphenylsilyl-β-lactopyranoside (**3**). — Imidazole (0.27 g, 4 mmol) was added to a stirred solution of **2** (1.00 g, 1.9 mmol) in *N*,*N*-dimethylformamide (10 mL), followed by addition of *tert*-butyldiphenylsilyl chloride (0.6 mL, 2.1 mmol). After 20 h at room temperature, a new portion of the silyl chloride (0.5 mL, 1.7 mmol) was added, and stirring was continued for additional 24 h. The solution was hydrolyzed in ice-water, and the aq. phase was repeatedly extracted with dichloromethane. The combined organic phases were washed with saturated aq. ammonium sulfate and water, dried with sodium sulfate, and the solvent was evaporated. Column chromatography of the resulting colorless syrup with 9:1 dichloromethane–methanol gave pure **3**, which was crystallized from toluene to yield **3** (680 mg, 46%): m.p. 91–92°; $[\alpha]_D^{20}$ −32.7° (*c* 0.8, CH_2Cl_2); ^{1}H-n.m.r. data (80 MHz, $CDCl_3$): δ 1.07 [s, $C(CH_3)_3$], 3.43–4.28 (m, 12 H), 4.38, 4.46 (2d, H-1 and H-1'), 4.58 [d, Ha(Bn)], 4.92 [d, Hb(Bn)], 5.49 (s, PhC*H*), 6.90–8.05 (m, 20 H, Ar); $J_{1,2} = J_{1',2'}$ 8.0, $J_{H(Bn)}$ −11.5 Hz.

Anal. Calc. for $C_{42}H_{50}O_{11}Si$: C, 66.47; H, 6.64. Found: C, 66.76; H, 6.58.

Benzyl 2,2',3,3'-tetra-O-benzoyl-4',6'-O-benzylidene-6-O-tert-butyldiphenylsilyl-β-lactopyranoside (**4**). — Compound **3** (0.77 g, 1 mmol) was dissolved in pyridine (5 mL), cooled in an ice-bath and treated with benzoyl chloride (0.4 mL, 5.5 mmol). After stirring overnight at room temperature, the solution was poured into ice-water, and the aq. layer was extracted five times with dichloromethane. The combined organic phases were washed with a saturated solution of sodium hydrogencarbonate and evaporated to dryness after the addition of toluene. The resulting yellow syrup was dissolved in dichloromethane and evaporated in the presence of silica gel (5 g). Column chromatography with 1:1 ethyl acetate–petroleum ether and subsequent crystallization with ethyl acetate–petroleum ether yielded **4** as colorless crystals (1.12 g, 94%): m.p. 116.5°; $[\alpha]_D^{20}$ +14.4° (*c* 1.0, CH_2Cl_2); ^{1}H-n.m.r. data (80 MHz, $CDCl_3$): δ 1.15 [s, $C(CH_3)_3$], 3.26–3.43 (m, 2 H, H-5, H-5'), 3.68–4.10 (m, 4 H, H-6, H-6'), 4.37 (dd, H-4'), 4.45 (dd, H-4), 4.61 [d, Ha(Bn)], 4.86 [d, Hb(Bn)], 5.07 (d, H-1'), 5.19 (dd, H-3'), 5.29 (s, PhC*H*), 5.44–5.77 (m, 2

H, H-2, H-3),5.72 (dd, H-2'), 6.86–8.15 (45 H, Ar); $J_{1,2}$ 7.6, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0, $J_{4,5}$ 8.0, $J_{5,6a}$ 10.2 $J_{5,6b}$ 3.5, $J_{4,5'}$ 1.5, J_{HaHb} −12.1 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$), δ 19.53 [$C(CH_3)_3$], 26.95 [$C(CH_3)_3$], 61.48 (C-6), 66.46 (C-5'), 68.19 (C-6'), 69.38 (C-2'), 69.80 [CH_2(Bn)], 72.29 (C-2), 72.86 (C-3'), 73.07 (C-3), 73.24 (C-4'), 74.00 (C-4'), 75.43 (C-5), 99.05 (C-1), 100.30 (C-1'), 100.76 (PhCH), 126.47, 127.45, 127.55, 127.63, 127.73, 127.83, 127.96, 128.04, 128.15, 128.22, 128.29, 128.60, 129.50, 129.70, 129.80, 129.84, 129.88, 130.04, 132.44, 132.89, 132.95, 133.23, 135.45, 135.56, 135.95, 136.01, 136.87, 137.50 (Ar), 164.51, 165.35, 165.70, 166.02 (4 × C=O).

Anal. Calc. for $C_{76}H_{68}O_{15}Si$: C, 71.53; H, 5.66. Found: C, 71.04; H, 5.75.

Benzyl 4',6'-O-benzylidene-6-O-tert-butyldimethylsilyl-β-lactopyranoside (**5**). — Imidazole (0.42 g, 7.3 mmol) and *tert*-butyldimethylsilyl chloride (0.58 g, 4.0 mmol) were added to a stirred solution of **2** (1.90 g, 3.7 mmol) in *N,N*-dimethylformamide (15 mL). After 24 h at room temperature, a new portion of the silyl chloride (0.29 g, 2.0 mmol) was added, and stirring was continued until the educt was no longer detectable by t.l.c. After working up as described for **3**, compound **5** was obtained as colorless plates (2.17 g, 93%): m.p. 202°; $[\alpha]_D^{20}$ −31.2° (*c* 0.8, CH_2Cl_2); m.s. (d.c.i., ammonia): *m/z* 652, $(M + NH_4)^+$; ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 0.008, 0.042 [2s, $Si(CH_3)_2$], 0.931 [s, $Si\text{-}C(CH_3)_3$], 3.327 (ddd, H-5), 3.403 (dd, H-2), 3.494 (ddd, H-5'), 3.530, 3.604 (2dd, H-3 and H-4), 3.582 (dd, H-3'), 3.682 (dd, H-2'), 3.895 (m, H-6a, H-6b), 4.028 (dd, H-6'a), 4.148 (dd, H-4'), 4.231 (dd, H-6'b), 4.338 (d, H-1), 4.350 (d, H-1'), 4.561 [d, Ha(Bn)], 4.828 [d, Hb(Bn)], 5.485 (s, PhC*H*), 7.05–7.42 (m, Ar); $J_{1,2}$ 7.8, $J_{2,3}$ 8.9, $J_{3,4}$ 9.0, $J_{4,5}$ 9.0, $J_{5,6a}$ 2.9, $J_{5,6b}$ 3.0, $J_{1,2'}$ 7.6, $J_{2',3'}$ 9.5, $J_{3',4'}$ 3.8, $J_{4',5'}$ 0.9, $J_{5',6'a}$ 1.6, $J_{5',6'b}$ 1.0, $J_{6a,6b}$ −12.8, J_{HaHb} −11.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ −5.06, −5.24 [$Si(CH_3)_2$], 18.38 [$Si\text{-}C(CH_3)_3$], 25.92 [$Si\text{-}C(CH_3)_3$], 62.60 (C-6), 66.92 (C-5'), 68.91 (C-6'), 70.86 [CH_2(Bn)], 71.20 (C-2'), 72.71 (C-2), 73.64 (C-3), 75.00 (C-4'), 75.03 (C-4), 75.03 (C-5), 75.57 (C-3'), 101.29, 101.32 (C-1', C-1), 103.68 (PhCH), 126.35, 127.94, 128.24, 128.26, 128.45, 129.23, 137.10, 137.38 (Ar).

Anal. Calc. for $C_{53}H_{62}O_{11}Si$: C, 66.55; H, 7.30. Found: C, 66.13; H, 7.23.

Benzyl 2',2',3,3'-tetra-O-benzoyl-4',6'-O-benzylidene-6-O-tert-butyldimethylsilyl-β-lactopyranoside (**6**). — Compound **5** (1.87 g, 2.95 mmol) was benzoylated as described for **4** using pyridine (20 mL) and benzoyl chloride (2.00 mL, 2.95 mmol) to yield colorless crystals of **6** (2.62 g, 85%): m.p. 211–212°; $[\alpha]_D^{20}$ +40.1° (*c* 0.8, CH_2Cl_2); m.s. (d.c.i., ammonia): *m/z* 1068 $(M + NH_4)^+$; ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ −0.032, 0.010 [2s, $Si(CH_3)_2$], 0.862 [s, $Si\text{-}C(CH_3)_3$], 3.050 (m, H-5'), 3.254 (ddd, H-5), 3.586 (dd, H-6'a), 3.659 (dd, H-6a), 3.721 (dd, H-6b), 3.741 (dd, H-6'b), 4.054 (dd, H-4), 4.279 (dd, H-4'), 4.503 [d, Ha(Bn)], 4.552 (d, H-1), 4.743 [d, Hb(Bn)], 4.863 (d, H-1'), 5.066 (dd, H-3'), 5.210 (s, PhC*H*), 5.280 (dd, H-2), 5.613 (dd, H-3), 5.660 (dd, H-2'), 7.05–7.55, 7.80–8.05 (2m, Ar); $J_{1,2}$ 7.9, $J_{2,3}$ 9.5, $J_{3,4}$ 9.3, $J_{4,5}$ 9.6, $J_{5,6a}$ 4.0, $J_{5,6b}$ 2.1, $J_{6a,6b}$ −12.8, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.3 $J_{3',4'}$ 3.6, $J_{4',5'}$ 0.8, $J_{5',6'a}$ 1.7, $J_{5',6'b}$ 1.7, $J_{6'a,6'b}$ −12.3, J_{HaHb} −12.5 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ −5.34, −5.04 [$Si(CH_3)_2$], 18.29 [$Si\text{-}C(CH_3)_3$], 25.92 [$Si\text{-}C(CH_3)_3$], 61.43 (C-6), 66.40 (C-5'), 68.09 (C-6'), 69.60 (C-2'), 70.03 [CH_2(Bn)], 72.40 (C-2), 73.01 (C-3'), 73.12 (C-4'), 73.73 (C-3), 75.51 (C-5), 75.87 (C-4), 99.00 (C-1), 100.62 (PhCH), 101.02 (C-1'), 126.41, 127.64, 127.82, 128.22, 128.31, 128.43, 128.66, 129.03,

129.41, 129.58, 129.66, 129.75, 129.86, 132.72, 132.94, 133.19, 133.27, 136.87, 137.50 (Ar), 164.74, 165.19, 165.36, 166.14 (4 × C=O).

Anal. Calc. for $C_{60}H_{62}O_{15}Si$: C, 68.55; H, 5.95. Found: C, 68.49; H, 6.04.

*Benzyl 2,2',3,3'-tetra-*O*-benzoyl-4',6'-*O*-benzylidene-β-lactopyranoside* (**7**). — A M solution of anhydrous tetrabutylammonium fluoride in tetrahydrofuran (2.9 mL) was added to crystalline **6** (1.50 g, 1.43 mmol) under an argon atmosphere. After stirring at room temperature for 30 min, dichloromethane (50 mL) was added to the solution. The organic phase was shaken immediately with a saturated aq. ammonium sulfate solution, separated, washed with water, and dried over sodium sulfate. After evaporation to dryness, the crude product was purified by column chromatography with 19:1 dichloromethane–methanol to yield **7** (1.27 g, 95%) as crystals: m.p. 247°; $[\alpha]_D^{20}$ +51.0° (*c* 0.8, CH_2Cl_2); m.s. (d.c.i.–ammonia): *m/z* 954, $(M + NH_4)^+$; ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 1.716 (s, OH-6), 3.019 (m, H-5'), 3.426 (ddd, H-5), 3.520 (dd, H-6'a), 3.589 (dd, H-6a), 3.660 (dd, H-6'b), 3.674 (dd, H-6b), 4.140 (dd, H-4), 4.252 (dd, H-4'), 4.522 [d, Ha(Bn)], 4.630 (d, H-1), 4.736 [d, Hb(Bn)], 4.813 (d, H-1'), 5.117 (dd, H-3'), 5.209 (s, PhC*H*), 5.293 (dd, H-2), 5.653 (dd, H-3), 5.676 (dd, H-2'), 7.05–8.00 (m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 9.6, $J_{3,4}$ 9.0, $J_{4,5}$ 9.5, $J_{5,6a}$ 3.4, $J_{5,6b}$ 2.4, $J_{6a,6b}$ −12.1, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.6, $J_{4',5'}$ 0.8, $J_{5',6'a}$ 1.7, $J_{5',6'b}$ 1.3, $J_{6'a,6'b}$ −12.4, $J_{H(Bn)}$ −12.5 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 60.83 (C-6), 66.40 (C-5'), 68.02 (C-6'), 69.60 (C-2'), 70.93 [CH_2(Bn)], 72.28 (C-2), 72.76 (C-3'), 73.15 (C-4'), 73.81 (C-3), 74.89 (C-5), 76.01 (C-4), 99.49 (C-1), 100.61 (PhCH), 101.41 (C-1'), 126.38, 127.61, 127.87, 128.25, 128.32, 128.43, 128.72, 129.03, 129.38, 129.70, 129.88, 132.94, 133.05, 133.29, 133.30, 136.68, 137.51 (Ar), 2 × 164.96, 165.31, 166.10 (4 × C=O).

Anal. Calc. for $C_{54}H_{48}O_{15}$: C, 69.22; H, 5.16. Found: C, 68.78; H, 5.08.

tert-*Butyl [benzyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid]uronate* (**8**). — Pyridine (0.65 mL, 8.0 mmol) and chromium(VI) oxide (400 mg, 4.00 mmol) were added sequentially to a mixture of dichloromethane and *N,N*-dimethylformamide (4:1, 10 mL). After stirring the dark mixture for 15 min at room temperature, a solution of **7** (830 mg, 0.9 mmol) in dichloromethane–*N,N*-dimethylformamide (4:1, 2 mL) was added, and stirring was continued for an additional 10 min. Acetic anhydride (0.75 mL, 8.0 mmol) and molten *tert*-butanol (1.9 mL, 20 mmol) were added. After 18 h the reaction was quenched with ethanol (0.5 mL), and after further 10 min, ethyl acetate (50 mL) was added under vigorous stirring. Immediate filtration over a short silica gel column with ethyl acetate as eluent, followed by evaporation, yielded a black syrup. Column chromatography with 1:1 ethyl acetate–hexane, followed by coevaporation with toluene, yielded colorless crystals of **8** (413 mg, 46%): m.p. 81°; $[\alpha]_D^{20}$ +28.0° (*c* 1.0, CH_2Cl_2); m.s. (d.c.i.–ammonia): *m/z* 1024, $(M + NH_4)^+$; 968, $(M - C_4H_8 + NH_4)^+$; ^{1}H-n.m.r data (300 MHz, $CDCl_3$): δ 1.355 [s, $C(CH_3)_3$], 3.300 (m, H-5'), 3.778 (dd, H-6'a), 3.980 (d, H-5), 4.013 (dd, H-6'b), 4.394 (dd, H-4'), 4.570 (dd, H-4), 4.618 [d, Ha(Bn)], 4.747 (d, H-1), 4.857 [d, Hb(Bn)], 5.013 (d, H-1'), 5.179 (dd, H-3'), 5.328 (s, PhC*H*),5.413 (dd, H-2), 5.700 (dd, H-2'), 5.734 (dd, H-3), 7.08–7.55, 7.88–8.05 (2m, Ar); $J_{1,2}$ 7.4, $J_{2,3}$ 8.8, $J_{3,4}$ 8.8, $J_{4,5}$ 9.0, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.7, $J_{4',5'}$ 0.6, $J_{5',6'a}$ 2.3, $J_{5',6'b}$ 1.1, $J_{6'a,6'b}$ −12.4, $J_{H(Bn)}$ −12.4

Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 27.80 [$C(CH_3)_3$], 66.46 (C-5'), 68.23 (C-6'), 69.36 (C-2'), 70.62 [CH_2(Bn)], 72.06 (C-2), 72.93 (C-3), 72.98 (C-3'), 73.22 (C-4'), 75.20 (C-5), 75.66 (C-4), [82.63 $C(CH_3)_3$], 99.41 (C-1), 100.14 (C-1'), 100.61 (PhCH), 126.38, 127.83, 128.11, 128.24, 128.30, 128.64, 129.07, 129.43, 129.54, 129.79, 129.89, 2 × 132.85, 133.03, 133.26, 136.63, 137.51 (Ar), 165.24, 165.24, 165.25, 166.15, 166.54 (4 × C=O and C-6).

Anal. Calc. for $C_{58}H_{54}O_{17}$: C, 69.18; H, 5.40. Found: C, 68.69; H, 5.42.

*Methyl (benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid)uronate* (**9**). — Trifluoroacetic acid (90%, 6 mL) was added dropwise under stirring to a solution of **8** (300 mg, 0.3 mmol) in dichloromethane (50 mL) at 0°. Stirring was continued for 5–10 h at 0° until t.l.c. analysis showed a complete reaction. Ice–water was added to the solution, the layers were separated, and the organic layer was washed with aq. sodium hydrogencarbonate and water. After evaporation of the extract to dryness and coevaporation of the residual solvents with toluene several times, the crude product was dissolved in tetrahydrofuran (5 mL) and esterified with a solution of diazomethane in diethyl ether (5 mL, approx. 0.2M). After stirring for 10 min at room temperature, the reaction was quenched with a small amount of acetic acid. The solution was taken up in dichloromethane and washed with water, saturated aq. sodium hydrogencarbonate, water, and evaporated to dryness. The resulting syrup was purified by column chromatography with 1:1 ethyl acetate–petroleum ether. After coevaporation of the residual solvents with toluene, **9** was obtained as colorless crystals (180 mg, 68%); m.p. 239–241°; $[\alpha]_D^{20}$ −4.7° (*c* 0.5, CH_2Cl_2); m.s. (d.c.i., ammonia): *m/z* 894, ($M + NH_4$)$^+$; ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 2.634 (d, 4-OH), 3.206 (dd, H-6'a), 3.291 (dd, H-6'b), 3.338 (s, CO_2CH_3), 3.510 (ddd, H-5'), 4.067 (d, H-5), 4.161 (ddd, H-4'), 4.451 (d, H-4), 4.582 [d, Ha(Bn)], 4.795 (d, H-1), 4.855 [d, Hb(Bn)], 4.882 (d, H-1'), 5.150 (dd, H-3'), 5.427 (dd, H-2), 5.590 (dd, H-2'), 5.681 (dd, H-3), 7.15–7.58, 7.85–8.03 (2 m, Ar); $J_{1,2}$ 7.0, $J_{2,3}$ 8.7, $J_{3,4}$ 8.5, $J_{4,5}$ 8.8, $J_{5,6'}$ 7.8, $J_{5,6''}$ 10.3, $J_{1',2'}$ 3.1, $J_{2',3'}$ 7.0, $J_{3',4'}$ 5.7, $J_{4',5'}$ 4.2, $J_{6'a,6'b}$ −12.5, $J_{HBn,HBn}$ −12.5, $J_{4',OH}$ 4.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 52.36 (CO_2CH_3), 61.97 (C-6'), 67.80 (C-4'), 69.90 (C-2'), 70.82 [CH_2(Bn)], 71.27 (C-2), 72.52 (C-3), 73.96 (C-5), 74.22 (C-3'), 74.30 (C-5'), 76.53 (C-4), 99.43 (C-1), 101.28 (C-1'), 127.73, 127.84, 128.19, 128.31, 128.54, 129.16, 129.60, 129.65, 129.74, 129.82, 132.97, 133.21, 136.40 (Ar), 165.16, 165.16, 165.74, 165.74, 167.96 (4 × C=O and C-6).

Anal. Calc. for $C_{48}H_{44}O_{17}$: C, 65.75; H, 5.06. Found: C, 65.36; H, 5.00.

*Methyl (benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-6-*O*-sulfo-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside)uronate, triethylammonium salt* (**11**). — A solution of **9** (29 mg, 33 μmol) in *N*,*N*-dimethylformamide (0.3 mL) was treated with the trimethylamine–sulfur trioxide complex ($Me_3N{\cdot}SO_3$, 30 mg). After stirring at room temperature under the exclusion of light for 24 h, triethylamine (1 mL) and toluene (5 mL) were added, and the mixture was filtered to separate the precipitated alkylamine–sulfur trioxide complex. The filtrate was concentrated *in vacuo* and purified by column chromatography (6:4:0.3 toluene–ethanol–triethylamine). After evaporation of the solvents, a colorless syrup was obtained which was dissolved in a small amount of dichloromethane. Washing with water, followed by filtration over filter paper and

evaporation, yielded **11** (29 mg, 83%) as colorless syrup: $[\alpha]_D^{20}$ +0.5° (*c* 1.6, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 1.391 [t, $N(CH_2CH_3)_3$], 3.104 [q, $N(CH_2CH_3)_3$], 3.407 (s, CO_2CH_3), 3.660 (m, H-5'; H-6'a,6'b), 3.967 (d, H-5), 4.228 (dd, H-4'), 4.409 (dd, H-4), 4.526 [d, Ha(Bn)], 4.712 (d, H-1), 4.793 [d, Hb(Bn)], 4.805 (d, H-1'), 5.115 (dd, H-3'), 5.332 (dd, H-2), 5.489 (dd, H-2'), 5.577 (dd, H-3), 7.10–7.55, 7.85–7.98 (2m, Ar), 9.41 (s, NH^+); $J_{1,2}$ 7.3, $J_{2,3}$ 9.1, $J_{3,4}$ 9.0, $J_{4,5}$ 9.2, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.3, $J_{4',5'}$ 1.0, $J_{H(Bn)}$ −12.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 8.61 [$N(CH_2CH_3)_3$], 46.51 [$N(CH_2CH_3)_3$], 52.42 (CO_2CH_3), 63.47 (C-6'), 65.67 (C-4'), 69.82 (C-2'), 70.73 [CH_2(Bn)], 71.67 (C-2), 72.40 (C-3), 72.51 (C-5'), 73.53 (C-3'), 74.08 (C-5), 76.08 (C-4), 99.56 (C-1), 100.93 (C-1'), 127.61, 127.78, 128.15, 128.22, 128.42, 128.90, 129.22, 129.59, 129.73, 132.92, 133.10, 133.35, 136.31 (Ar), 165.00, 165.08, 165.27, 165.63, 167.90 (4 × C=O and C-6).

Anal. Calc. for $C_{54}H_{59}NO_{19}S$: C, 61.30; H, 5.62; N, 1.32. Found: C, 61.04; H, 5.72; N, 1.42.

*Methyl [benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-sulfo-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid]uronate, bis(triethylammonium) salt* (**16**). — A solution of **9** (76 mg, 87 μmol) in *N,N*-dimethylformamide (1.0 mL) was reacted with Me_3N–SO_3 (100 mg) for 14 days as described for **11** to yield the 6'-sulfate **11** (13 mg, 14%) as a byproduct and the 4',6'-di-sulfate **16** which was crystallized from dichloromethane and toluene to yield **16** (89 mg, 83%): m.p. 105–106°; $[\alpha]_D^{20}$ +2.3° (*c* 1.9, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 1.256 [t, $N(CH_2CH_3)_3$], 3.05 [q, $N(CH_2CH_3)_3$], 3.494 (s, CO_2CH_3), 3.880 (dd, H-6'a), 3.956 (d, H-5), 3.988 (dd, H-6'b), 4.181 (ddd, H-5'), 4.517 (dd, H-4), 4.541 [d, Ha(Bn)], 4.729 (d, H-1), 4.808 [d, Hb(Bn)], 4.930 (d, H-1'), 4.989 (dd, H-4'), 5.209 (dd, H-3'), 5.306 (dd, H-2), 5.388 (dd, H-2'), 5.561 (dd, H-3), 7.05–7.58, 7.83–8.10 (2 m, Ar), 9.33, (s, NH^+); $J_{1,2}$ 7.1, $J_{2,3}$ 9.1, $J_{3,4}$ 9.1, $J_{4,5}$ 9.2, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.2, $J_{4',5'}$ 1.3, $J_{5',6'a}$ 5.7, $J_{5',6'b}$ 6.2, $J_{6'a,6'b}$ −11.5, $J_{H(Bn)}$ −12.5 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 8.56 [$N(CH_2CH_3)_3$], 46.39 [$N(CH_2CH_3)_3$], 52.57 (CO_2CH_3), 65.16 (C-6'), 70.08 (C-2'), 70.72 [CH_2(Bn)], 71.89 (C-4'), 72.19 (C-2), 72.33 (C-3), 72.46 (C-3'), 72.96 (C-5'), 74.61 (C-5), 75.92 (C-4), 99.67 (C-1), 100.59 (C-1'), 127.69, 127.78, 127.98, 128.27, 128.63, 129.67, 129.79, 129.86, 132.50, 133.06, 133.22, 136.50 (Ar), 165.00, 165.80, 166.98, 166.98, 167.77 (4 × C=O and C-6).

Anal. Calc. for $C_{60}H_{74}N_2O_{22}S_2$: C, 58.16; H, 6.02; N, 2.26. Found: C, 58.17; H, 5.95; N, 2.52.

*Methyl [benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid]uronate* (**13**). — A solution of **9** (20 mg, 23 μmol) in dichloromethane (0.5 mL) was treated with pyridine (20 μL) and benzoyl cyanide (3.4 mg, 26 μmol) and was stirred for 3 days at room temperature. After dilution with dichloromethane, the organic phase was washed with saturated aq. sodium hydrogencarbonate and water, filtered through a small filter paper, and concentrated *in vacuo*. Crystallization from diethyl ether–petroleum ether yielded white platelets of **13** (21 mg, 94%): m.p. 102–103°; $[\alpha]_D^{20}$ +15.9° (*c* 0.9, CH_2Cl_2); m.s. (d.c.i.–ammonia): *m/z* 998, $(M + NH_4)^+$; ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 3.401 (s, CO_2CH_3), 3.581 (dd, H-6'a), 3.786 (ddd, H-5'), 3.946 (dd, H-6'b), 4.020 (d, H-5), 4.082 (dd, H-4'), 4.432 (dd, H-4), 4.582 [d,

Ha(Bn)], 4.747 (d, H-1), 4.847 [d, Hb(Bn)], 4.905 (d, H-1'), 5.222 (dd, H-3'), 5.461 (dd, H-2), 5.574 (dd, H-2'), 5.668 (dd, H-3), 7.10–8.05 (m, Ar); $J_{1,2}$ 7.3, $J_{1',2'}$ 9.2, $J_{2',3'}$ 9.0, $J_{3',4'}$ 9.2, $J_{1,2}$ 7.8, $J_{2,3}$ 10.4, $J_{3,4}$ 3.3, $J_{4,5}$ 1.3, $J_{5',6'a}$ 6.1, $J_{5',6'b}$ 6.7, $J_{6'a,6'b}$ −11.2, $J_{(HBn)}$ −12.4 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 52.48 (CO_2CH_3), 61.56 (C-6'), 66.90 (C-4'), 69.78 (C-2'), 70.85 [CH_2(Bn)], 71.37 (C-2), 72.30, 72.35 (C-3, C-5'), 73.80 (C-3'), 74.04 (C-5), 76.44 (C-4), 99.73 (C-1), 101.03 (C-1'), 127.23, 127.78, 127.91, 128.31, 128.36, 128.52, 129.02, 129.26, 129.51, 129.71, 129.82, 129.87, 130.01, 133.08, 133.20, 2 × 133.32, 133.39, 136.38 (Ar), 165.06, 165.06, 165.46, 165.73, 166.03, 168.01 (5 × C=O and C-6).

Anal. Calc. for $C_{55}H_{52}O_{17}$: C, 67.34; H, 4.93. Found: C, 66.85; H, 4.84.

*Methyl [benzyl 2,3-di-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-*O*-sulfo-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid]uronate, triethylammonium salt* (**14**). — Compound **13** (22 mg, 22 μmol) was dissolved in *N*,*N*-dimethylformamide (0.25 mL) and $Me_3N{\cdot}SO_3$ (100 mg, 0.72 mmol) was added. After stirring of the suspension for 7 days at 60–70°, the mixture was worked up as described for **11**. Crystallizing from dichloromethane–diethyl ether and drying in high vacuum at 30° yielded **14** (25 mg, 97%) as colorless platelets: m.p. 105–106°; $[\alpha]_D^{20}$ −2.10° (*c* 1.0, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 1.30 [t, $N(CH_2CH_3)_3$], 3.05 [q, $N(CH_2CH_3)_3$], 3.458 (s, CO_2CH_3), 3.791 (dd, H-6'a), 3.903 (ddd, H-5'), 3.967 (d, H-5), 4.388 (dd, H-6'b), 4.416 (dd, H-4), 4.521 [d, Ha(Bn)], 4.712 (d, H-1), 4.800 [d, Hb(Bn)], 4.886 (d, H-1'), 5.015 (dd, H-4'), 5.234 (dd, H-3'), 5.365 (dd, H-2), 5.451 (dd, H-2'), 5.584 (dd, H-3), 7.10–7.55, 7.85–8.05 (2m, Ar), 10.21 (b, NH⁺); $J_{1,2}$ 7.3, $J_{1',2'}$ 9.1, $J_{2',3'}$ 9.0, $J_{3',4'}$ 9.1, $J_{1,2}$ 7.8, $J_{2,3}$ 10.5, $J_{3,4}$ 3.3, $J_{4,5}$ 1.3, $J_{5',6'a}$ 6.9, $J_{5',6'b}$ 5.3, $J_{6'a,6'b}$ −11.5, $J_{(HBn)}$ −12.8 Hz; ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 8.57 [$N(CH_2CH_3)_3$], 46.23 [$N(CH_2CH_3)_3$], 52.65 (CO_2CH_3), 62.85 (C-6'), 69.99 (C-2'), 70.76 [CH_2(Bn)], 71.59, 71.79 (C-2, C-4'), 72.29 (C-3), 72.29 (C-3'), 72.70 (C-5'), 74.29 (C-5), 76.25 (C-4), 99.62 (C-1), 100.84 (C-1'), 127.69, 127.85, 128.00, 128.30, 128.42, 129.70, 129.83, 129.89, 132.62, 132.95, 2 × 133.17, 136.39 (Ar), 165.04, 165.39, 165.88, 166.00, 166.17, 167.88 (5 × C=O and C-6).

Anal. Calc. for $C_{61}H_{61}NO_{20}S$: C, 63.04; H, 5.46; N, 1.21. Found: C, 62.68; H, 5.54; N, 1.29.

*Benzyl [4-*O*-(6-*O*-sulfo-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid]uronate, disodium salt* (**12**). — A M aq. solution of sodium hydroxide (115 μL) was added at 0° to a solution of **11** (20 mg, 19 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, followed by concentration of the eluate and two subsequent runs on a size-exclusion gel (Fractogel HW40) with water as eluent. Lyophilization yielded **12** (8 mg, 74%) as an amorphous solid: $[\alpha]_D^{20}$ −19.5° (*c* 0.3, H_2O); ^{1}H-n.m.r. data (300 MHz, D_2O): δ 3.303 (dd, H-2), 3.446 (dd, H-2'), 3.507 (dd, H-3), 3.574 (dd, H-3'), 3.605 (dd, H-4), 3.746 (d, H-5), 3.849 (dd, H-4'), 3.853 (ddd, H-5'), 4.095 (m, H-6'a, H-6'b), 4.356 (d, H-1'), 4.447 (d, H-1), 4.610 [d, Ha(Bn)], 4.823 [d, Hb(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 9.2, $J_{3,4}$ 8.7, $J_{4,5}$ 9.6, $J_{1',2'}$ 7.7, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.3, $J_{5',6'a}$ 5.0, $J_{5',6'b}$ 4.5, $J_{(HBn)}$ −11.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, D_2O): δ 67.46 (C-6'), 68.45 (C-4'), 71.10 (C-2'), 71.65 [CH_2(Bn)], 72.49 (C-3'), 72.74 (C-2), 73.15 (C-5'), 74.89 (C-3), 75.68 (C-5), 81.92 (C-4), 101.08 (C-1), 103.68 (C-1'), 128.56, 128.69, 128.96, 129.98, 131.62 (Ar), 175.28 (C-6).

Anal. Calc for $C_{19}H_{24}Na_2O_{15}S$: C, 40.00; H, 4.24. Found: C, 39.75; H, 4.33.

Benzyl [4-O-(4-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosid]uronate, disodium salt (**15**). — A solution of **14** (18 mg, 15 μmol) in tetrahydrofuran (0.3 mL) was de-esterified with M aq. sodium hydroxide (97 μL) and purified as described for **12**. Lyophilization yielded colorless, amorphous **15** (7 mg, 79%): $[\alpha]_D^{20}$ −20.6° (*c* 0.7, H_2O); ^{1}H-n.m.r. data (300 MHz, D_2O): δ 3.288 (dd, H-2), 3.436 (dd, H-2′), 3.508 (dd, H-3), 3.611 (dd, H-4), 3.687 (dd, H-3′), 3.695 (m, H-6′a, H-6′b), 3.718 (ddd, H-5′), 3.734 (d, H-5), 4.396 (d, H-1′), 4.444 (d, H-1), 4.543 (dd, H-4′), 4.612 [d, Ha(Bn)], 4.823 [d, Hb(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 9.3, $J_{3,4}$ 8.5, $J_{4,5}$ 9.8, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.3, $J_{4',5'}$ 1.3, $J_{5',6'a}$ 4.2, $J_{5',6'b}$ 8.2, $J_{H(Bn)}$ −11.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, D_2O): δ 61.24 (C-6′), 71.27 (C-2′), 71.65 [CH_2(Bn)], 71.80 (C-3′), 72.85 (C-2), 74.71 (C-3), 74.88 (C-5′), 75.95 (C-5), 76.70 (C-4′), 80.78 (C-4), 101.14 (C-1), 103.04 (C-1′), 128.53, 128.70, 129.02, 131.48 (Ar), 175.58 (C-6).

Anal. Calc. for $C_{19}H_{24}Na_2O_{15}S$: C, 40.00; H, 4.24. Found: C, 40.38; H, 4.35.

Benzyl 4-O-[(4,6-di-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosid]uronate, trisodium salt (**17**). — Deesterification of a solution of **16** (13 mg, 10 μmol) in tetrahydrofuran (0.4 mL) with M aq. sodium hydroxide (76 μL) as described for **12** yielded after lyophilization colorless, amorphous **17** (5 mg, 71%): $[\alpha]_D^{20}$ −13.8° (*c* 0.3, H_2O); ^{1}H-n.m.r. data (300 MHz, D_2O): δ 3.304 (dd, H-2), 3.465 (dd, H-2′), 3.518 (dd, H-3), 3.611 (dd, H-4), 3.721 (dd, H-3′), 3.750 (dd, H-5), 4.008 (ddd, H-5′), 4.115 (dd, H-6′a), 4.191 (dd, H-6′b), 4.411 (d, H-1′), 4.457 (d, H-1), 4.582 (dd, H-4′), 4.617 [d, Ha(Bn)], 4.830 [d, Hb(Bn)], 7.28–7.40 (m, Ar); $J_{1,2}$ 8.0, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0, $J_{4,5}$ 9.7, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.3, $J_{5',6'a}$ 8.5, $J_{5',6'b}$ 3.5, $J_{6'a,6'b}$ −11.2, $J_{H(Bn)}$ −11.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, D_2O): δ 68.01 (C-6′), 71.10 (C-2′), 71.56 (C-3′), 71.65 [CH_2(Bn)], 72.68 (C-5′), 72.68 (C-2), 75.03 (C-3), 75.57 (C-5), 76.34 (C-4′), 82.59 (C-4), 101.03 (C-1), 103.83 (C-1′), 128.68, 128.82, 129.11, 129.19, 131.59 (Ar), 175.36 (C-6).

Anal. Calc. for $C_{19}H_{23}Na_3O_{18}S_2$: C, 33.94; H, 3.45. Found: C, 34.35; H, 3.52.

Benzyl [4-O-(β-D-galactopyranosyl)-β-D-glucopyranosid]uronate, sodium salt (**10**). — A solution of **9** (19 mg, 22 μmol) in tetrahydrofuran (0.3 mL) was treated with M sodium hydroxide (115 μL) as described for **12** to yield **10** (6 mg, 59%) after lyophilization: $[\alpha]_D^{20}$ +5.9° (*c* 0.3, H_2O); ^{1}H-n.m.r. data (300 MHz, D_2O): δ 3.288 (dd, H-2), 3.417 (dd, H-2′), 3.494 (dd, H-3); 3.537 (dd, H-3′), 3.583 (ddd, H-5′), 3.600 (dd, H-6′a), 3.660 (dd, H-6′b), 3.647 (dd, H-4), 3.729 (d, H-5), 3.781 (dd, H-4′), 4.336 (d, H-1′), 4.433 (d, H-1), 4.604 [d, Ha(Bn)], 4.816 [d, Hb(Bn)], 7.25–7.40 (m, Ar); $J_{1,2}$ 7.9, $J_{2,3}$ 9.4, $J_{3,4}$ 8.6, $J_{4,5}$ 9.8, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.3, $J_{5',6'a}$ 4.4, $J_{5',6'b}$ 8.5, $J_{6'a,6'b}$ −10.7, $J_{H(Bn)}$ −11.6 Hz; ^{13}C-n.m.r. data (75.5 MHz, D_2O): δ 61.24 (C-6′), 68.78 (C-4′), 71.25 (C-2′), 71.67 [CH_2(Bn)], 72.77 (C-3′), 72.88 (C-2), 74.69 (C-3), 75.61 (C-5′), 75.99 (C-5), 80.49 (C-4), 101.62 (C-1), 103.02 (C-1′), 128.57, 128.70, 128.95, 129.09, 131.75 (Ar), 175.48 (C-6).

Anal. Calc. for $C_{19}H_{25}NaO_{12}$: C, 48.72; H, 5.38. Found: C, 49.01; H, 5.45.

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REFERENCES

1 U. Lindahl and M. Höök, *Ann. Rev. Biochem.*, 47 (1978) 385–417.
2 B. Casu, *Adv. Carbohydr. Chem. Biochem.*, 43 (1985) 51–134.
3 G. A. Maresh, *Arch. Biochem. Biophys.*, 233 (1984) 428–437; W. D. Comper and T. C. Laurent, *Physiol. Rev.*, 58 (1978) 255–315.
4 L. Thunberg, G. Bäckström, and U. Lindahl, *Carbohydr. Res.*, 100 (1982) 393–410; P. Casu, P. Oreste, G. Torri, G. Zoppetti, J. Choay, J. C. Lormeau, M. Petitou, and P. Sinaÿ, *Biochem. J.*, 197 (1981) 599–609.
5 G. Bengtsson, T. Olivecrona, M. Höök, J. Riesenfeld, and U. Lindahl, *Biochem. J.*, 189 (1980) 625–633; P. Avogaro and F. Belussi, *Pharmacol. Res. Commun.*, 9 (1977) 501–506.
6 D. D. Roberts, *Methods Enzymol.*, 138 (1987) 437–483; E. D. Green, H. van Halbeek, J. Boime, and J. U. Baenziger, *J. Biol. Chem.*, 260 (1985) 15623–15630; G. S. Bedi, W. C. French, and O. P. Bahl, *J. Biol. Chem.*, 257 (1982) 4345–4355; E. F. Neufeld and G. Ashwell, in W. J. Lennarz (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum Press, New York, 1980, pp. 241–266.
7 M. Ragazzi, D. R. Ferro, B. Perly, P. Sinaÿ, M. Petitou, and J. Choay, *Carbohydr. Res.*, 195 (1990) 169–188.
8 D. R. Ferro, A. Provasoli, M. Ragazzi, G. Torri, B. Casu, G. Gatti, J.-C. Jacquinet, P. Sinaÿ, M. Petitou, and J. Choay, *J. Am. Chem. Soc.*, 108 (1986) 6773–6778; D. R. Ferro, A. Provasoli, M. Ragazzi, B. Casu, G. Torri, V. Bossennec, B. Perly, P. Sinaÿ, M. Petitou, and J. Choay, *Carbohydr. Res.*, 195 (1990) 157–167; C. A. A. van Boeckel, S. F. van Aelst, G. N. Wagenaars, J.-R. Mellema, H. Paulsen, I. Peters, A. Polies, and V. Sinnwell, *Recl. Trav. Chim. Pays-Bas*, 106 (1987) 19–20; H. Paulsen, A. Polies, V. Sinnwell, C. A. A. van Boeckel, *Justus Liebigs Ann. Chem.*, (1988) 411–418.
9 M. Petitou, J.-C. Lormeau, and J. Choay, *Eur. J. Biochem.*, 176 (1988) 637–640.
10 J. Choay, M. Petitou, J.-C. Lormeau, P. Sinaÿ, B. Casu, and G. Gatti, *Biochem. Biophys. Res. Commun.*, 116 (1983) 492–499; M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, P. Sinaÿ, J.-C. Jacquinet, and G. Torri, *Carbohydr. Res.*, 167 (1987) 67–75; C. A. A. van Boeckel, T. Beetz, J. N. Vos, A. J. M. de Jong, S. F. van Aelst, R. H. van den Bosch, J. M. R. Mertens, and F. A. van der Vlugt, *J. Carbohydr. Chem.*, 4 (1985) 293–321; Y. Ichikawa, R. Monden, and H. Kuzuhara, *Tetrahedron Lett.*, (1986) 611–614.
11 R. W. Jeanloz in W. Pigman and D. Horton (Eds.), *The Carbohydrates*, Vol. IIB, Academic Press, New York, 1970, pp. 589–625.
12 D. A. Rees, *Ann. Rep. Prog. Chem.*, 62 (1965) 469–487; K.-G. Jacobsson, J. Riesenfeld, and U. Lindahl, *J. Biol. Chem.*, 260 (1985) 15354–15359; K. Sugasawa, H. Uchiyama, and N. Wajima, *Carbohydr. Res.*, 158 (1986) 183–196.
13 Y. Ichikawa, R. Monden, and H. Kuzuhara, *Carbohydr. Res.*, 172 (1988) 37–64; M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, J.-C. Jacquinet, P. Sinaÿ, and G. Torri, *Carbohydr. Res.*, 167 (1987) 253–264.
14 H. Paulsen, A. Hußinger, and C. A. A. van Boeckel, *Justus Liebigs Ann. Chem.*, (1988) 419–426; T. Chiba, J.-C. Jacquinet, P. Sinaÿ, M. Petitou, and J. Choay, *Carbohydr. Res.*, 174 (1988) 253–264; C. A. A. van Boeckel, J. E. M. Basten, H. Lucas, and S. F. van Aelst, *Angew. Chem.*, 100 (1988) 1217–1218; H. P. Wessel, T. Labler, and T. B. Schopp, *Helv. Chim. Acta*, 72 (1989) 1268–1277.
15 A. Maira, X. Dong, M. Petitou, and P. Sinaÿ, *Carbohydr. Res.*, 195 (1990) 29–50; J.-C. Jacquinet and P. Sinaÿ, *Carbohydr. Res.*, 159 (1987) 229–253.
16 M. Kobayashi, T. Yamazaki, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 51–67.
17 Vandana, O. Hindsgaul, and J. U. Baenziger, *Can. J. Chem.*, 65 (1987) 1645–1652; V. Srivastava and O. Hindsgaul, *Carbohydr. Res.*, 185 (1989) 163–169; C. A. A. van Boeckel, P. Westerduin, and J. H. van Boom, *Carbohydr. Res.*, 153 (1984) 219–234.
18 M. Zsiška and B. Meyer, unpublished observations.
19 H. Paulsen, *Angew. Chem.*, 94 (1982) 184–201.
20 M. Zsiška and B. Meyer, unpublished observations.
21 A. Liptak, I. Jodal, and P. Nanasi, *Carbohydr. Res.*, 52 (1976) 17–32.
22 S. Hanessian and P. Lavallé, *Can. J. Chem.*, 53 (1975) 2975–2977.
23 M. Fétizon, Y. Henry, N. Morcan, G. Moreau, M. Golfier, and T. Prange, *Tetrahedron*, 29 (1973) 3013–3014.
24 E. J. Corey and G. Schmidt, *Tetrahedron Lett.*, (1979) 399–402.
25 B. B. Bissember and R. H. Wightman, *Carbohydr. Res.*, 81 (1980) 187–191; E. J. Corey and J. Suggs, *Tetrahedron Lett.*, (1975) 2647–2650.

26 F. Anderson and B. Samuelsson, *Carbohydr. Res.*, 129 (1984) c1–c3.
27 E. J. Corey and B. Samuelsson, *J. Org. Chem.*, 49 (1984) 4735.
28 B. Meyer and R. Stuike-Prill, *J. Org. Chem.*, 55 (1990) 906–910.
29 J. R. Turvey, *Adv. Carbohydr. Chem.*, 20 (1965) 183–218.
30 H. Paulsen and A. Bünsch, *Carbohydr. Res.*, 100 (1982) 143–167.
31 K. Bock, A. Brignole, and B. W. Sigurjskold, *J. Chem. Soc., Perkin Trans. 2*, (1986) 1711–1713.
32 P. J. Archbald, M. D. Fenn, and A. B. Roy, *Carbohydr. Res.*, 93 (1981) 177–190; R. R. Contreras, J. P. Kamerling, J. Breg, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 179 (1988) 411–418.
33 D. Beith-Halami, H. M. Flowers, and D. Shapiro, *Carbohydr. Res.*, 5 (1967) 25–30.