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Sulfation of *O*-isopropylidenated lactose derivatives at the 2′-position induces an unusual $^{3,0}B$-boat conformation of the D-galactosyl residue

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ABSTRACT

2,3:5,6:3′,4′-Tri-*O*-isopropylidenelactose dimethyl acetal was converted into the 2′-sulfate derivatives **9** and **10** and the 2′,6′-di-sulfate derivative **2**. A 2′-sulfate group was shown to induce a change of the ring conformation of the galactosyl residue to a $^{3,0}B$ conformation. 2′-Sulfated lactose derivatives were synthesized which differed only in the group linked to O-6′, namely the 6′-*O*-*tert*-butyldimethylsilyl **9**, the 6′-*O*-triphenylmethyl **10**, and the 6′-sulfate **2**, all of which adopt a conformation close to a $^{3,0}B$ boat. The conformation of the galactosyl ring is slightly influenced by the group at O-6′, with **2** showing the most pronounced effect, and **9** showing the smallest changes. It was proved by various n.m.r. spectroscopic parameters, which included coupling constants, n.O.e.s, and T_1 values, that the galactosyl residues of **9**, **10**, and **2** adopt a $^{3,0}B$ conformation. However, other protective groups at O-2′, such as an *O*-acetyl group in **7**, an *O*-benzyl group in **8**, or the hydroxy group itself in **1**, **4**, **5**, and **3**, do not cause a change of the ring conformation. Using the GESA program, we found that the observed change of the conformation cannot be explained by unfavorable sterical interactions between the 2′-sulfate group and other parts of the molecule. However, the conformational change observed here could be attributable to electronic effects that are unique to the sulfate group.

INTRODUCTION

Sulfate groups have been shown to have unique effects on the conformation of the glycosidic bond and the ring conformation in 2-sulfated iduronic acids[1–5]. A detailed knowledge of the influence of sulfate groups on the conformation is important for an understanding of molecular recognition phenomena and for the prediction of conformational changes induced by sulfate groups. The most important experimental method used to analyze oligosaccharide conformations is n.m.r. spectroscopy. In order to obtain reliable information on conformation from n.m.r. spectroscopy, correct assignments of the chemical shifts of all proton resonances are essential[6]. Application of 2D n.m.r. spectroscopic techniques has made it possible to solve this assignment problem, even for complex molecules[7–10]. Information on the three-dimensional structure of

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oligosaccharides is derived mainly from n.O.e. measurements[11–13], T_1 measurements [14–16], changes in chemical shifts[17,18], and coupling constants[19,20].

A variety of different methods to introduce sulfate groups into oligosaccharides have been described[21–23]. A solution of sulfur trioxide–amine complexes in *N*,*N*-dimethylformamide has commonly been employed. Many of the purifications of sugar sulfates described in the literature[24–27] involve tedious work-up procedures which often result in decreased yields; however, converting sulfates into triethylammonium salts allows ready purification by using silica gel chromatography with triethylamine-containing eluents[28].

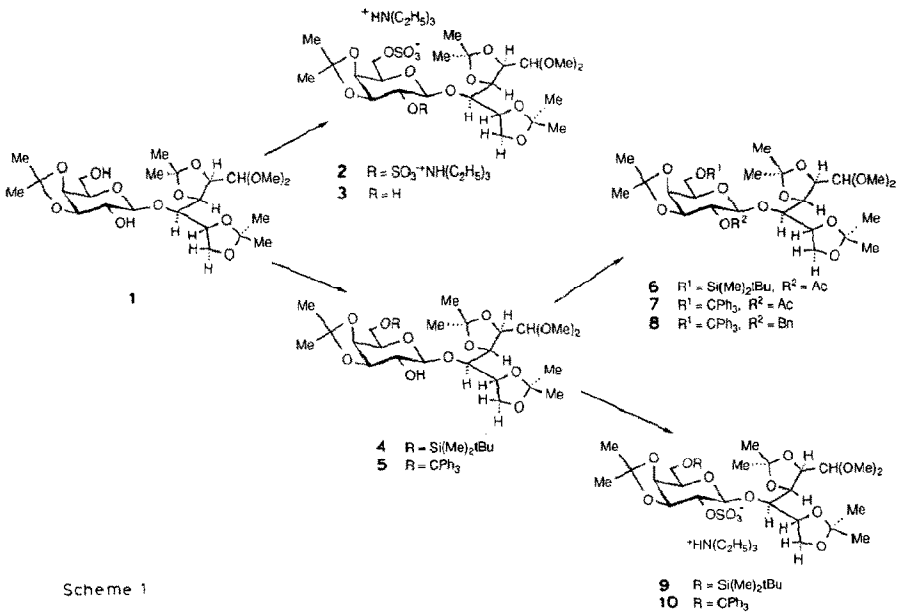
As part of a larger effort to understand the influence of sulfate groups on the conformation of sulfated oligo- and poly-saccharides, we are presently engaged in synthesizing small, model oligosaccharides. Herein are presented the syntheses and conformational analyses of the selectively sulfated derivatives of lactose.

RESULTS AND DISCUSSION

2,3:5,6:3′,4′-Tri-*O*-isopropylidenelactose dimethyl acetal[29,30] (**1**) (Scheme 1) was used as starting compound for the syntheses of differently protected sulfates of lactose. In order to obtain 2′-*O*-sulfo lactose derivatives from **1**, the O-6′ position must be protected. Compound **1** was converted into **4** using *tert*-butyldimethyl chloro silane and imidazole in *N*,*N*-dimethylformamide (DMF) solution[31,32]. Reaction of **4** with the pyridine–sulfur trioxide complex in DMF at room temperature under inert gas atmosphere for 12 h resulted in the formation of **9** in excellent yield. Under the same reaction conditions, **1** was sulfated using twice the amount of sulfating reagent to give the 2′,6′-disulfate **2**. Triethylamine converted the pyridinium salts of the sulfates into the corresponding triethylammonium salts, which were easily purified by silica gel chromatography with triethylamine–containing eluents[28].

We synthesized the 6′-*O*-trityl derivative **10** after having obtained unusual n.m.r. parameters for **9** and **2** (see as follows). Treatment of **1** with chlorotriphenylsilane in pyridine and heating of the solution resulted in the formation of **5** (ref. 35) which was subsequently sulfated as described above to give **10**.

The n.m.r. spectra of the products were fully interpreted using extensive 2D n.m.r. techniques. The n.m.r. analyses of the sulfated lactose derivatives **9**, **10**, and **2** revealed substantial changes of the spectral parameters of the galactosyl residue when compared to their respective unsulfated parent compounds **4**, **5**, and **1**. Most strikingly, the coupling constant $J_{1',2'}$ showed a much smaller value in the sulfated molecules than that observed in the unsulfated ones. Table I shows the n.m.r. parameters of those carbon and hydrogen atoms that exhibit a significant change upon sulfation. The pyranose rings of the unsulfated galactosyl residues of **4**, **5**, and **3** exhibit the expected coupling constants and chemical shifts compatible with a ring having a slightly distorted $^4C_1$ conformation. In contrast, the sulfates **9**, **10**, and **2** show very small values for the coupling constants $J_{1',2'}$ and $J_{2',3'}$ (see Table I), a fact that indicates that the conformation



Scheme 1

of the D-galactopyranosyl ring may have changed (see Fig. 1). Interestingly, the coupling constants of the D-galactopyranosyl ring differ significantly with changes of the O-6′ substituent. Both $J_{1',2'}$ and $J_{2',3'}$ become smaller from the 6′-*O*-silyl **9** to the 6′-*O*-trityl **10** to the 6′-O-sulfo derivative **2**, a trend that in turn indicated that the conformation of the D-galactose ring is not only dependent upon the sulfation at O-2′, but is also dependent upon the substituent at O-6′. An analysis of the coupling constants $J_{1',2'}$ and $J_{2',3'}$ led to the conclusion that the conformation of the D-galactosyl residue had changed to a $^{3,0}B$ boat conformation. As indicated by their $J_{1',2'}$ and $J_{2',3'}$ coupling constants, other protecting groups at C-2′, such as an acetyl group in **6** and **7**, a benzyl group in **8** (ref. 33), or the 2′-OH group itself in the 6′-sulfated derivative **3** do not change the conformation of the pyranosyl ring. The observed coupling constants $J_{1',2'}$ and $J_{2',3'}$ of **6**, **7**, **3**, and **8** are in agreement with a slightly disturbed $^4C_1$ chair.

Further indication of an unusual conformation was derived from the analysis of the ^{1}H-n.m.r. and ^{13}C-n.m.r. chemical shifts. H-2′ is shifted downfield by 0.9 p.p.m., whereas the protons H-1′ and H-3′ in a β-position to the sulfate group are shifted downfield by 0.5 to 0.6 p.p.m., respectively. This shift is much larger than that normally observed[34,35]. On the other hand, the downfield shift for C-2′ is very small compared to the normally observed downfield shift for the sulfated carbon in comparable compounds[34]. Obviously the effects on the chemical shifts in **9**, **10**, and **2** are not only caused by the sulfation, but are also due to a change of the ring conformation. Furthermore, the signal of C-6′ of **2** gives only a relatively small downfield shift of 3 p.p.m. compared to

the unsulfated parent molecule[33]. Normally, upon sulfation, C-6 is shifted downfield by about 6 p.p.m[24,34,36,37].

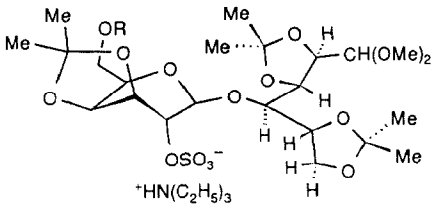


Fig. 1. The $^{3,0}B$ conformation of **2**.

TABLE I

Characteristic n.m.r. parameters of compounds **2**–**7**, **9**, and **10**

	(a) Selected ^{1}H-n.m.r. chemical shifts (δ, p.p.m.)						
δ	*H-1′*	*H-2′*	*H-3′*	*H-4′*	*H-5′*	*H-6a′*	*H-6b′*
2	4.91	4.37	4.52	4.15	3.85	4.02	3.97
3	4.41	3.46	3.99	4.16	4.08	4.21	4.17
4	4.39	3.54	4.04	4.21	3.77	3.90	3.77
5	4.30	3.46	4.00	4.32	3.74	3.50	3.24
6	4.73	5.01	4.10	4.25	3.77	3.87	3.77
7	4.70	4.93	4.14	4.46	3.82	3.58	3.20
9	4.86	4.43	4.52	4.21	3.65	3.77	3.65
10	4.90	4.50	4.64	4.44	3.75	3.45	3.18
	(b) Selected $J_{H,H}$ coupling constants (Hz)						
Hz	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{1,2}$		
2	2.4	3.2	7.2	2.0	6.1		
3	8.1	7.1	5.6	2.5	5.6		
4	8.1	7.6	5.3	1.8	6.0		
5	8.3	7.6	5.4	2.2	6.1		
6	8.4	7.6	5.3	1.8	6.0		
7	8.4	7.7	5.2	2.0	6.0		
9	4.1	4.1	6.8	—	6.3		
10	3.7	3.8	6.8	1.9	6.3		
	(c) Selected ^{13}C-n.m.r. chemical shifts (δ, p.p.m.)						
δ	*C-1′*	*C-2′*	*C-3′*	*C-6′*	*C-1*	*C-6*	
2	104.02	74.03	77.00	65.21	102.46	65.37	
3	103.42	74.07	78.86	66.13	104.31	64.50	
4	103.92	74.37	78.80	61.43	105.03	64.60	
5	104.03	74.40	78.99	61.97	104.61	64.43	
6	100.34	72.89	77.18	61.17	104.99	64.54	
7	100.58	72.91	77.38	61.82	104.66	64.48	
9	103.07	76.79	76.00	61.54	105.16	66.46	
10	103.02	76.36	75.83	61.82	104.56	66.21	

The changes of the chemical shifts of the *O*-methyl groups at C-1 can be used to obtain information about the conformation of the glucosyl residues. A comparison of the chemical shifts of the *O*-methyl groups in **7** to those of **4** and **6** shows a marked downfield shift of one of the *O*-methyl groups, whereas the other is shifted upfield. The 2′-sulfated analog **10** has both *O*-methyl groups shifted upfield by 0.3 p.p.m., and the disulfate **2** has the resonances of the methyl groups shifted downfield by 0.2 p.p.m. Thus, we conlcude that the functional group linked to O-6′ is close to the *O*-methyl groups of the acetal at C-1. The distance between the *O*-methyl groups and the group linked to O-6′ seems to be somewhat decreased in the $^{3,0}B$ conformation, a fact which becomes evident by comparing the chemical shifts of the methyl groups of **3** and **2**.

We used n.O.e. data, relaxation times (T_1), and $^1J_{C\text{-}1',H\text{-}1'}$ coupling constants to verify that the change of the n.m.r. parameters discussed above are due to a change of the conformation of the D-galactosyl ring and not due to the formation of derivatives that differ from the D-*galacto* stereochemistry. In a $^4C_1$ conformation with a β-anomeric center, the protons H-1′, H-3′, and H-5′ have a *syn*–diaxial orientation. Upon irradiation of H-1′, the relatively short distance between these protons usually gives rise to n.O.e.s at H-3′ and H-5′ (ref. 38). However, in **10** there is only an n.O.e. detectable from H-1′ to H-5′, but none from H-1′ to H-3′ (see Table II). Compared to a typical $^4C_1$ conformation, the distance between H-1′ and H-5′ has not changed significantly, whereas H-1′ and H-3′ are further apart from each other. Additionally, an n.O.e. of 4.5% is found from H-1′ to H-2′ which would not be expected for a $^4C_1$ conformation. These findings are only compatible with a $^{3,0}B$ conformation of the pyranose ring. Furthermore, in agreement with a $^{3,0}B$ conformation, the coupling constant $^1J_{C\text{-}1',H\text{-}1'}$ 162.5 Hz for **10** indicates an almost axial orientation of H-1′, proving that no inversion at the anomeric center had taken place[39].

The protons H-1′ and H-4 are relatively close in space as shown by the n.O.e.s from H-1′ to H-4 and from H-4 to H-1′ (see Table II). Thus, the glycosidic bond adopts a conformation that is similar to those reported for normal β-glycosides[38,40]. Because of the uncertainty in determining reference distances[10], we are not able to quantify our analysis of the conformation of the glycosidic bond, but can merely state that the n.O.e.s agree with the calculations (see the following).

The longitudinal relaxation time (T_1) of H-2′ is a significant feature for assigning the ring conformation of the galactosyl residue. Both a $^4C_1$ and a $^{3,0}B$ conformation should have a relatively long T_1 value for H-2′. However, T_1(H-2′) should be shorter for a $^{3,0}B$ than for a $^4C_1$ conformation because, in a $^{3,0}B$ conformation, the protons H-1′ and H-3′ move closer to H-2′. A calculation of theoretical T_1 values for a $^{3,0}B$ conformation and a $^4C_1$ conformation that is slightly distorted by a 3′,4′-*O*-isopropylidene ring revealed that our experimental T_1 values fit only with the calculated data for a $^{3,0}B$ conformation (see Table III). The experimental ratio of the T_1 values of H-2′ and H-3′ is 1.33, a value in agreement with T_1(H-2′) / T_1(H-3′) = 1.41 calculated for a $^{3,0}B$ conformation compared to T_1(H-2′) / T_1(H-3′) = 3.00 for a $^4C_1$ conformation. Experimentally the ratio of the relaxation times of T_1(H-2) / T_1(H-3) of methyl β-D-galactopyranoside was determined to be 4.2 (ref. 40).

TABLE II

{H,H} n.O.e. values of **10**

Saturation of H-1'				*Saturation of H-4*			
H-2'	*H-3'*	*H-5'*	*H-4*	*H-2*	*H-3*	*H-5*	*H-1'*
4.5%	0%	6.5%	5.0%	1.3%	4.1%	1.9%	4.4%

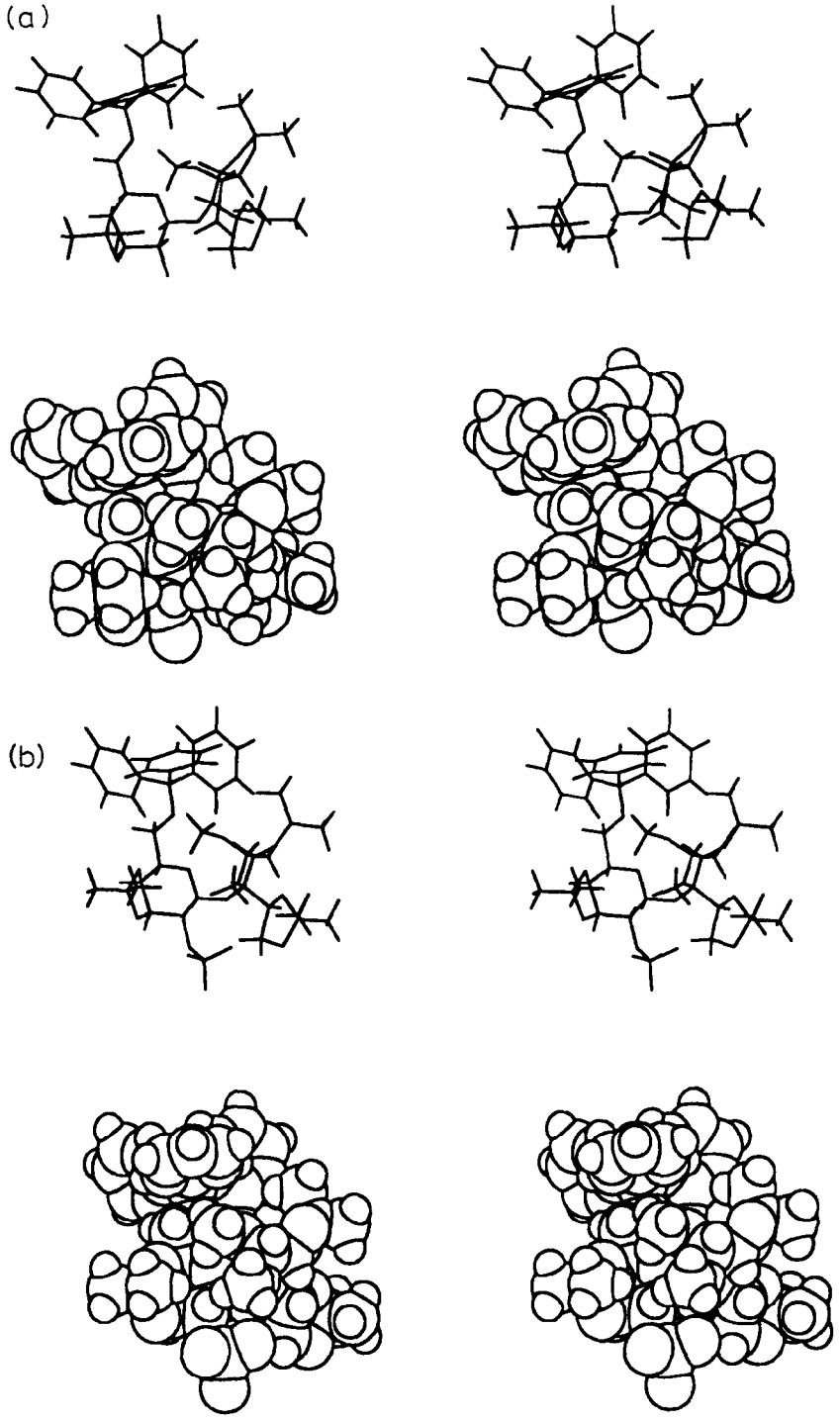
We used the GESA program[41] to calculate the favored conformations of the molecules **5**, **10** and **2** (see Fig. 2 and Table IV). During this procedure we kept the ring of the galactose residue fixed in a slightly distorted $^4C_1$ chair conformation as found in the X-ray analysis of methyl 3,4-*O*-ethylidene-β-D-galactopyranoside[42]. These calculations were intended to investigate the influence of steric interactions that may have caused the change in the conformation of the pyranose ring. The results are summarized in Table IV and indicate that no strong repulsive interactions are expected from the 2'-sulfated molecules with the galactose in the $^4C_1$ conformation. Thus, steric interactions seem not to be responsible for the experimentally observed change in the ring conformation. Possibly, electronic effects in the neighborhood of the glycosidic bond, acting in concert with the strain introduced by the 3',4'-*O*-isopropylidene ring, may have caused the change of the conformation of the galactopyranose ring. We find in these calculations that the dimethyl acetal group should be close to the substituent at O-6' (see Fig. 2). This theoretical finding agrees excellently with the experimental results from n.m.r. spectroscopy that indicate a close proximity of these groups (see in the foregoing). The calculation shows the two protons H-1' and H-4 to be in close proximity which agrees nicely with the experimental results from n.O.e. n.m.r. spectroscopy.

TABLE III

Experimental and calculated T_1-values for **10**[a]

	H-1'	*H-2'*	*H-3'*	*H-4'*	*H-5'*	*H-6a'*
T_1(exp)	0.38	0.93	0.70	0.54	0.41	0.24
T_1(calc for $^{3,0}B$)	0.31	0.93	0.66	0.51	0.35	
T_1(calc for $^4C_1$)	0.24	0.93	0.31	0.31	0.20	
	H-1	H-3	H-4	H-5	H-6a	
T_1 (exp)	0.64	0.51	0.40	0.52	0.30	

[a] The calculations were carried out using a slightly distorted $^4C_1$ or a $^{3,0}B$ conformation, respectively, and were normalized on the experimental value of H-2'.


(a)
(b)

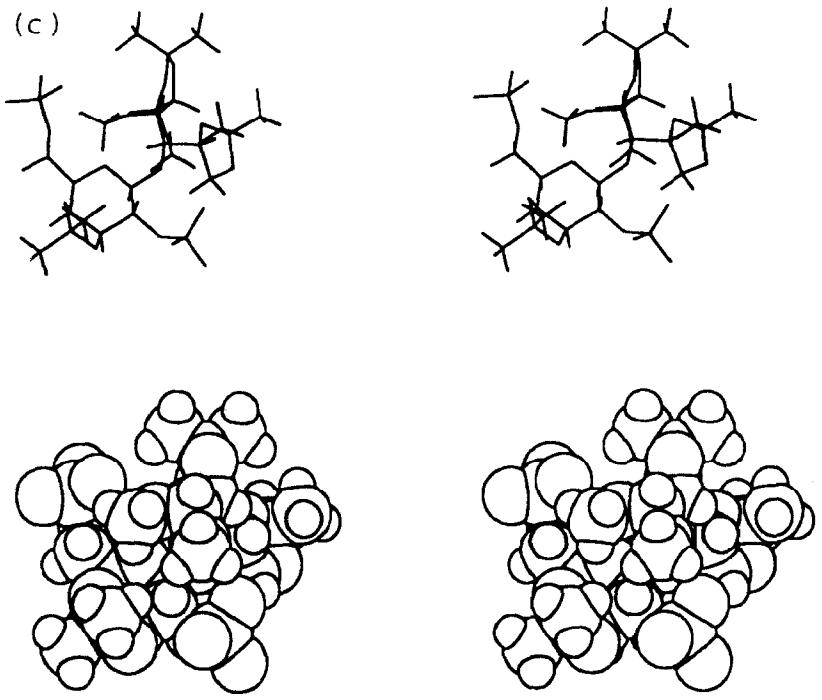


Fig. 2. Stereo representation of the stick and CPK models of the preferred conformations of (a) **5***gt*, (b) **10***gt*, and (c) **2***gt* as calculated by the GESA program. View from top on the D-galactose ring which is located at the lower left corner of the figures.

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi 510 apparatus and are uncorrected. T.l.c. was performed on Silica Gel F-254 (Merck). The compounds were detected by dipping the plates in 10% aq. sulfuric acid and heating at 150°. Preparative chromatography was done on Silica Gel-60 (E. Merck, 63–200 mesh). Pyridine and dimethylformamide (DMF) were dried with $LiAlH_4$ and distilled. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter. N.m.r. spectra were obtained with Bruker (300- and 500-MHz for ^1H and 62.9 and 75.5 MHz for ^{13}C) spectrometers. Spin-lattice relaxation times were measured by standard inversion recovery method utilizing ten different τ values. The n.O.e. values were obtained by one-dimensional difference spectroscopy with saturation times of 0.5 s. The homonuclear {H,H}-COSY spectra were obtained with a 45° detection pulse. GESA calculations were performed on an IBM 3090–200 at the UCNS, University of Georgia.

*6′-*O*-tert-Butyldimethylsilyl-2,3:5,6:3′,4′-tri-*O*-isopropylidenelactose dimethyl acetal* (**4**). — To a solution of 2,3:5,6:3′,4′-tri-*O*-isopropylidenelactose dimethyl acetal

TABLE IV

Calculated preferred conformations of compounds **2**, **5**, and **10**

	2gt	**2tg**	**5gt**	**5tg**	**10gt**	**10tg**
φ,ψ OMe_1	32/101	32/98	28/121	34/91	28/121	33/91
φ,ψ OMe_2	−52/32	−52/31	−54/45	−48/26	−54/45	−48/26
$\Theta_{H\text{-}1\text{-}C\text{-}1\text{-}C\text{-}2\text{-}C\text{-}3}$	−152	−149	−151	−158	−151	−156
$\Theta_{C\text{-}2\text{-}C\text{-}3\text{-}C\text{-}4\text{-}H\text{-}4}$	175	172	174	177	174	175
$\Theta_{H\text{-}4\text{-}C\text{-}4\text{-}C\text{-}5\text{-}C\text{-}6}$	−65	−70	−67	−60	−67	−66
$\varphi,\psi_{glycosidic}$	56/−18	54/−15	56/−18	50/−23	56/−18	50/−20
χ_1,χ_2 (2′-O-SO_3)	−1/−70	−1/−70	—	—	0/−69	−1/−67
χ_1,χ_2 (6′-O-SO_3)	−56/−145	−73/149	—	—	—	—
χ_1,χ_2 Trityl	—	—	61/178	56/171	61/178	56/177
$\omega_{C\text{-}5',C\text{-}6'}$	62	−175	54	180	54	−180
E [kcal.mol^{-1}]	5.8	6.6	0.1	2.2	−1.0	1.2

(**1**, 1 g, 1.97 mmol) and imidazole (0.3 g) in dry *N*,*N*-dimethylformamide (10 mL) was added *tert*-butyldimethylsilyl chloride (0.36 g, 5.2 mmol). The mixture was stirred for several hours at room temperature in an inert gas atmosphere. The progress of the reaction was monitored by t.l.c. (1:2 toluene–ethyl acetate). After the reaction was completed (t.l.c.), the mixture was poured into ice water and was extracted with chloroform (500 mL). The organic layer was washed successively with 0.5M HCl, and water. The solvent was evaporated, and the remaining water in the crude product was coevaporated with toluene. The resulting syrup was subjected to column chromatography (2:1 toluene–ethyl acetate) to give **4** (0.88 g, 73%) as a syrup: $[\alpha]_D^{21}$ +11.21° (*c* 0.8, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 4.372 (d, H-1), 4.466 (dd, H-2), 3.895 (t, H-3), 4.041 (t, H-4), 4.285 (ddd, H-5), 4.152 (dd, H-6a), 4.004 (dd, H-6b), 4.392 (d, H-1′), 3.544 (t, H-2′), 4.041 (dd, H-3′), 4.211 (dd, H-4′), 3.895 (t, H-6a′), 3.794–3.743 (m, H-5′, H-6b′) 3.435, 3.414 (s, OMe), 1.510, 1.492, 2 × 1.379, 2 × 1.331 (s, Me), 0.891 (s, Si-C-Me), and 0.063 (s, SiMe); $J_{1,2}$ 6.0, $J_{2,3}$ 7.5, $J_{3,4}$ 1.5, $J_{4,5}$ 2.5, $J_{5,6a}$ 6.6, $J_{5,6b}$ 6.6, $J_{6a,6b}$ −8.8, $J_{1',2'}$ 8.1, $J_{2',3'}$ 7.6, $J_{3',4'}$ = 5.3, $J_{4',5'}$ 1.8, $J_{5',6a'}$ 8.5, and $J_{6a',6b'}$ −8.6 Hz. ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 109.97, 109.62, 108.15 (CMe_2), 105.03 (C-1), 74.72 (C-2), 77.84 (C-3), 76.35 (C-4), 77.66 (C-5), 64.60 (C-6), 103.92 (C-1′), 74.37 (C-2′), 78.80 (C-3′), 72.54 (C-4′), 73.58 (C-5′), 61.43 (C-6′), 56.04, 52.82 (OMe), 2 × 28.13, 27.07, 26.30, 26.12, 25.57, 24.33 (CMe_2 Si-*C*-Me), 3 × 25.69 (Si-C-*Me*) and −5.83 (Si-Me).

Anal. Calc for $C_{29}H_{54}O_{12}Si$: C,55.93; H, 8.74. Found: C, 56.00; H, 8.65.

2′-O-Acetyl-6′-O-tert-butyldimethylsilyl-2,3:5,6:3′,4′-tri-O-isopropylidenelactose dimethyl acetal (**6**). — Compound **4** (60 mg, 0.096 mmol) was acetylated with acetic anhydride–pyridine. After evaporation of the solvents, **6** was obtained in quantitative yield: $[\alpha]_D^{21}$ +5.68° (*c* 0.5, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 4.356 (d, H-1), 4.447 (t, H-2), 3.953 (d, H-3), 4.039 (t, H-4), 4.289 (ddd, H-5), 3.953 (d, H-6a,b), 4.731 (d, H-1′), 5.007 (t, H-2′), 4.095 (dd, H-3′), 4.247 (dd, H-4′), 3.872 (d, H-6a′), 3.801–3.734 (m, H-5′), H-6b′), 3.409 (s, OMe), 1.552, 1.471, 2 × 1.358, 1.326, 1.311 (s, Me), 0.894 (s,

Si-C-Me), and 0.066 (s, SiMe); $J_{1,2}$ 6.0, $J_{2,3}$ 6.9, $J_{3,4}$ 1.5, $J_{4,5}$ 2.4, $J_{5,6a}$ 6.9, $J_{5,6b}$ 6.9, $J_{1',2'}$ 8.4, $J_{2',3'}$ 7.6, $J_{3',4'}$ 5.3, $J_{4',5'}$ 1.8, and $J_{6a',6b'}$ −11.0 Hz. ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 169.31 (C=O), 110.02 (CMe_2), 104.99 (C-1), 74.82 (C-2), 77.92 (C-3), 73.66 (C-4), 78.08 (C-5), 64.54 (C-6), 100.34 (C-1'), 72.89 (C-2'), 77.18 (C-3'), 73.00 (C-4'), 72.89 (C-5'), 61.17 (C-6'), 55.54, 53.01 (OMe), 2 × 27.71, 27.37, 26.22, 25.99, 25.68, 24.54 (CMe_2, Si-*C*-Me), 3 × 26.09 (Si-C-*Me*), and −5.74 (Si-Me).

Anal. Calc. for $C_{31}H_{56}O_{13}Si$: C, 56.00; H, 8.49. Found: C, 56.30; H, 8.40.

6'-O-tert-*Butyldimethylsilyl-2'*-O-*sulfo-2,3:5,6:3',4'-tri*-O-*isopropylidenelactose dimethyl acetal, triethylammonium salt* (**9**). — Compound **4** (1.5 g, 2.4 mmol) was dissolved in dry DMF (140 mL) and treated with an excess of SO_3–trimethylamine (2.6 g, 14.4 mmol, a 6 mol. equiv. excess) at room temperature under inert gas atmosphere. The mixture was stirred and monitored by t.l.c. (3:1:0.1 toluene–ethanol–triethylamine). After the educt was no longer detectable, triethylamine (5 mL) was added to the mixture, and the solution was concentrated under reduced pressure. Purification of the mixture by column chromatography (3:1:0.1 toluene–ethanol–triethylamine) yielded **9** (1.85 g, 95%): $[\alpha]_D^{21}$ −10.20° (*c* 0.7, CH_2Cl_2); ^{1}H-n.m.r. data (500 MHz, $CDCl_3$): δ 4.321 (d, H-1), 4.534 (t, H-2), 4.056 (dd, H-3), 3.802 (dd, H-4), 4.229–4.193 (m, H-5), 4.134 (dd, H-6a), 4.042 (dd, H-6b), 4.861 (d, H-1'), 4.426 (t, H-2'), 4.524 (dd, H-3'), 4.212 (dd, H-4'), 3.767 (dd, H-6a'), 3.673–3.618 (m, H-5', H-6b'), 3.352 (s, OMe), 3.120 [q, $(CH_3CH_2)_3N$], 1.438, 1.380, 1.360, 1.343, 1.282, 1.248 (s, Me), 1.319 [t, $(CH_3CH_2)_3N$], 0.840 (s, Si-C-*Me*), and 0.007 (s, SiMe); $J_{1,2}$ 6.3, $J_{2,3}$ 7.4, $J_{3,4}$ 1.2, $J_{4,5}$ 6.6, $J_{5,6a}$ 5.8, $J_{5,6b}$ 5.9, $J_{6a,6b}$ −9.0, $J_{1',2'}$ 4.1, $J_{2',3'}$ 4.1, $J_{3',4'}$ 6.8, $J_{5',6a'}$ 7.2, $J_{6a',6b'}$ −8.3, and J_{NCH_2,CH_3} 7.4 Hz. ^{13}C-n.m.r. data (62.9 MHz, $CDCl_3$): δ 110.11, 109.35, 108.57 (CMe_2), 105.16 (C-1), 74.45 (C-2), 77.85 (C-3), 76.45 (C-4), 76.79 (C-5), 66.46 (C-6), 103.07 (C-1'), 76.79 (C-2'), 76.00 (C-3'), 71.52 (C-4'), 72.42 (C-5'), 61.54 (C-6'), 55.52, 52.41 (OMe), 46.60 [$(CH_3CH_2)_3N$], 2 × 27.59, 2 × 26.92, 26.73, 25.87, 25.39 (CMe_2, Si-*C*-Me), 25.87 (Si-C-*Me*), 8.64 [$(CH_3CH_2)_3N$], and −5.65 (Si-Me).

Anal. Calc. for $C_{35}H_{69}NO_{15}SSi$: C, 52.28; H, 8.65. Found: C, 51.95; H, 8.55.

2'-O-*Acetyl-2,3:5,6:3',4'-tri*-O-*isopropylidene-6'*-O-*trityllactose dimethyl acetal* (**7**). — Compound **5** (60 mg, 0.096 mmol) was acetylated with acetic anhydride–pyridine. After evaporation of the solvents and multiple coevaporations of the remaining solvents with toluene *in vacuo*, **7** was obtained in quantitative yield: $[\alpha]_D^{21}$ + 38.87° (*c* 0.8, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 7.500–7.033 (m, ArH), 4.224 (d, H-1), 4.300 (dd, H-2), 3.855 (dd, H-3), 3.970 (t, H-4), 4.24 (m, H-5), 3.910 (d, H-6a, H-6b), 4.698 (d, H-1'), 4.933 (t, H-2'), 4.143 (dd, H-3'), 4.462 (dd, H-4'), 3.816 (m, H-5'), 3.576 (t, H-6a'), 3.196 (dd, H-6b'), 3.100, 3.666 (s, OMe), 2.107 (s, OAc), 1.600, 1.491, 1.451, 1.333, and 2 × 1.300 (s, Me); $J_{1,2}$ 6.0, $J_{2,3}$ 7.1, $J_{3,4}$ 1.4, $J_{4,5}$ 2.2, $J_{5,6a}$ 7.1, $J_{5,6b}$ 7.1, $J_{1',2'}$ 8.4, $J_{2',3'}$ 7.7, $J_{3',4'}$ 5.2, $J_{4',5'}$ 2.0, $J_{5',6a'}$ 8.5, $J_{5'6b'}$ 5.0, and $J_{6a',6b'}$ −8.6 Hz. ^{13}C-n.m.r. data (62.9 MHz, $CDCl_3$): δ 169.41 (C=O), 143.86 (CAr_3), 128.68, 128.19, 125.28 (Ar), 110.67, 110.19, 107.84 (CMe_2), 104.66 (C-1), 74.44 (C-2), 77.97 (C-3), 73.42 (C-4), 78.40 (C-5), 64.48 (C-6), 100.58 (C-1'), 72.91 (C-2'), 77.38 (C-3'), 73.69 (C-4'), 71.87 (C-5'), 61.82 (C-6'), 55.21, 52.67 (OMe), 27.81, 27.41, 26.11 2 × 26.00, 24.55 (CMe_2).

Anal. Calc. for $C_{44}H_{56}O_{13}$: C, 66.65; H, 7.12. Found: C, 66.80; H, 7.03.

2,3:5,6:3′,4′-Tri-O-*isopropylidene-2′*-O-*sulfo-6′*-O-*trityllactose dimethyl acetal, triethylammonium salt* (**10**). — Compound **5** (1.1 g, 1.47 mmol) in abs. DMF (105 mL) was treated under stirring with an excess of SO_3–trimethylamine (1.23 g, 8.8 mmol, an 6 mequiv. excess) at room temperature. The reaction was monitored by t.l.c. (3:1:0.1 toluene–ethanol–triethylamine). After completion of the reaction, triethylamine (3 mL) was added, and the solution was concentrated under diminished pressure. Purification of the mixture by column chromatography (3:1:0.1 toluene–ethanol–triethylamine) yielded **10** (1.30 g, 93%): $[\alpha]_D^{21}$ −29.54° (*c* 0.7, CH_2Cl_2); ^{1}H-n.m.r. data (300 MHz, $CDCl_3$): δ 7.316–7.140 (m, ArH), 4.245 (d, H-1), 4.454 (dd, H-2), 4.019 (dd, H-3), 3.817 (dd, H-4), 4.227 (dd, H-5), 4.170 (dd, H-6a), 4.058 (dd, H-6b), 4.906 (d, H-1′), 4.498 (t, H-2′), 4.641 (dd, H-3′), 4.440 (dd, H-4′), 3.766–3.716 (m, H-5′), 3.451 (t, H-6a′), 3.179 (dd, H-6b′), 3.151, 3.060 (s, OMe), 3.135 [q, $(CH_3CH_2)_3N$], 2.430 (s, NH^+), 1.432, 1.385, 2 × 1.361, 1.336, 1.320 (s, Me), and 1.361 [t, $(CH_3CH_2)_3N$]; $J_{1,2}$ 6.3, $J_{2,3}$ 7.6, $J_{3,4}$ 1.1, $J_{4,5}$ 6.0, $J_{5,6a}$ 5.9, $J_{5,6b}$ 5.6, $J_{6a,6b}$ −8.5, $J_{1',2'}$ 3.7, $J_{2',3'}$ 3.8, $J_{3',4'}$ 6.8, $J_{4',5'}$ 1.9, $J_{5',6a'}$ 7.7, $J_{5',6b'}$ 5.4, $J_{6a',6b'}$ −8.4, and J_{NCH_2,CH_3} 7.2 Hz. ^{13}C-n.m.r. data (75.5 MHz, $CDCl_3$): δ 143.92 (CAr_3), 128.68, 127.60, 126.85 (Ar), 109.98, 109.24, 108.35 (CMe_2), 104.56 (C-1), 73.83 (C-2), 77.77 (C-3), 75.83 (C-4), 76.87 (C-5), 66.21 (C-6), 103.02 (C-1′), 76.36 (C-2′), 75.77 (C-3′), 71.87 (C-4′), 71.01 (C-5′), 61.82 (C-6′), 55.00, 51.68 (OMe), and 46.44 [$(CH_3CH_2)_3N$], 27.47, 26.75, 26.73, 26.55, 25.71, 25.35 (CMe_2), and 8.64 [$(CH_3CH_2)_3N$].

Anal. Calc for $C_{48}H_{69}NO_{15}S$: C, 61.85; H, 7.46. Found: C, 61.55; H, 7.34.

2,3:5,6:3′,4′-Tri-O-*isopropylidene-2′,6′-di*-O-*sulfolactose dimethyl acetal, triethylammonium salt* (**2**) *and 2,3:5,6:3′,4′-tri*-O-*isopropylidene-6′*-O-*sulfolactose dimethyl acetal, triethylammonium salt* (**3**). — Compound **1** (0.5 g, 0.38 mmol) was dissolved in absolute DMF (50 mL). Under stirring and inert gas atmosphere, an excess of SO_3–triethylamine complex (3.4 g, 18.9 mmol, 12 mequiv. excess) was added. The reaction mixture was stirred under an inert gas atmosphere for 12 h and monitored by t.l.c. After completion of the reaction, triethylamine (5 mL) was added. The solution was evaporated *in vacuo*, and the remaining syrup was chromatographed on a silica gel column with (1:2:0.1 toluene–ethanol–triethylamine). If either the solvent or the sulfur trioxide complex were contaminated with traces of water, two fractions could be separated. In this case the triethylammonium salt of 2,3:5,6:3′,4′-tri-*O*-isopropylidene-6′-*O*-sulfolactose dimethyl acetal (**3**) was eluted from the column as the first component, and **2** was obtained as the second component. Under completely dry reaction conditions, **2** (0.81 g, 95%) could be isolated: $[\alpha]_D^{21}$ −25.20° (*c* 0.6, CH_2Cl_2); ^{1}H-n.m.r. data (500 MHz, $CDCl_3$): δ 4.227 (d, H-1), 4.428 (dd, H-2), 3.993 (d, H-3), 3.731 (d, H-4), 4.094 (dd, H-5), 4.040–4.036 (m, H-6a), 3.916 (dd, H-6b), 4.914 (d, H-1′), 4.374 (t, H-2′), 4.520 (dd, H-3′), 4.145 (dd, H-4′), 3.849 (ddd, H-5′), 4.019 (dd, H-6a′), 3.970 (dd, H-6b′), 3.571, 3.569 (s, OMe), 3.058 [q, $(CH_3CH_2)_3N$], 2.724, 2.722 (s, NH^+), 1.327, 1.275, 1.243, 1.214, 1.170, 1.131 (s, Me), and 1.229 [t, $(CH_3CH_2)_3N$]; $J_{1,2}$ 6.1, $J_{2,3}$ 7.0, $J_{3,4}$ 1.5, $J_{4,5}$ 6.0, $J_{5,6a}$ 6.0, $J_{5,6b}$ 6.0, $J_{6a,6b}$ −8.8, $J_{1',2'}$ 2.4, $J_{2',3'}$ 3.2, $J_{3',4'}$ 7.2, $J_{4',5'}$ 2.0, $J_{5',6a'}$ 6.9, $J_{5',6b'}$ 7.2, $J_{6a',6b'}$ −11.0, and J_{NCH_2,CH_3} 7.3 Hz. ^{13}C-n.m.r. data (62.9 MHz, $CDCl_3$): δ 109.28, 108.78, 107.71 (CMe_2), 102.46 (C-1), 74.23 (C-2), 73.91 (C-3), 70.62 (C-4), 75.26 (C-5), 65.37 (C-6), 104.02 (C-1′), 74.03 (C-2′), 77.00 (C-3′), 69.27 (C-4′), 70.15 (C-5′), 65.21 (C-6′), 54.54, 52.24

(OMe), 46.07 [$(CH_3CH_2)_3N$], 26.82, 26.10, 25.98, 25.77, 24.98, 24.31 (CMe_2), and 8.15 [$(CH_3CH_2)_3N$].

Anal. Calc. for $C_{35}H_{70}N_2O_{18}S_2$: C, 48.26; H, 8.10. Found: C, 48.03; H, 7.99.

Physical data for **3**: $[\alpha]_D^{21}$ +22.73° (*c* 0.4, CH_2Cl_2); ^{1}H-n.m.r. data (500 MHz, $CDCl_3$): δ 4.358 (d, H-1), 4.422 (dd, H-2), 3.894 (dd, H-3), 4.003 (t, H-4), 4.223 (dd, H-5), 4.075 (dd, H-6a), 3.936 (dd, H-6b), 4.405 (d, H-1′), 3.464 (t, H-2′), 3.986 (dd, H-3′), 4.160 (dd, H-4′), 4.080 (ddd, H-5′), 4.210 (dd, H-6a′), 4.174 (dd, H-6b′), 3.382 (s, OMe), 3.109 [q,$(CH_3CH_2)_3N$], 1.424, 1.411, 1.326, 1.311, 1.253, 1.244 (s, Me), and 1.311 [t, $(CH_3CH_2)_3N$]; $J_{1,2}$ 5.6, $J_{2,3}$ 7.6, $J_{3,4}$ 1.5, $J_{4,5}$ 2.3, $J_{5,6a}$ 6.1, $J_{5,6b}$ 7.0, $J_{6a,6b}$ −8.7, $J_{1',2'}$ 8.1, $J_{2',3'}$ 7.1, $J_{3',4'}$ 5.6, $J_{4',5'}$ 2.5, $J_{5',6a'}$ 6.1, $J_{5',6b'}$ 8.4, $J_{6a',6b'}$ −11.4, and J_{NCH_2,CH_3} 7.3 Hz. ^{13}C-n.m.r. data (62.9 MHz, $CDCl_3$): δ 109.84, 109.79, 108.04 (CMe_2), 104.31 (C-1), 74.82 (C-2), 77.57 (C-3), 76.00 (C-4), 77.53 (C-5), 64.50 (C-6), 103.42 (C-1′), 74.07 (C-2′), 78.86 (C-3′), 73.04 (C-4′), 71.51 (C-5′), 66.13 (C-6′), 55.68, 53.37 (OMe), 46.43 [$(CH_3CH_2)_3N$], 27.98, 27.00, 26.29, 25.18, 25.49, 24.15 (CMe_2), and 8.54 [$(CH_3CH_2)_3N$].

Anal. Calc. for $C_{29}H_{55}NO_{15}S$: C, 50.49; H, 8.04. Found: C, 50.25; H, 8.08.

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