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Synthesis of a set of di- and tri-sulfated galabioses

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Abstract

Among cell-adhesion molecules, L-selectin recognizes sulfated sLe^x with relatively low affinity. Here, we aimed at artificial mimics by synthesizing a set of di- and tri-sulfated galabioses, which may surpass the affinity of sulfated sLe^x. As a strategy to obtain 3′,6′,6-tri-*O*-sulfogalabioses, regioselective reductive cleavage of 4,6- and 4′,6′-di-*O*-benzylidenegalabioses was employed. Two suitably protected galactose precursors were conjugated to yield α and β anomers (48 and 18%, respectively) by using a pentenyl galactoside donor and iodinium di-*sym*-collidine perchlorate as the catalyst. For synthesizing the 3′,6-di-*O*-sulfogalabiose, however, a trichloroacetimidate donor was superior (52%) to the pentenyl one (30%).

Keywords: Galabiose; α Anomer; β Anomer; Regio-selective sulfation; 3-(2-Aminoethylthio)propyl sugars

1. Introduction

Increasing numbers of biomolecules have been shown to recognize anionic sugar molecules. Among them, the selectin family of cell-adhesion molecules is known to bind sialylated oligosaccharides on the cell surface,[1–3] to mediate the primary weak interactions of lymphocytes, monocytes or granulocytes to vascular endothelial cells, such that the rolling phenomenon takes place.[4] While the intrinsic ligands for all three members of the selectin family share the core structure of sialyl Le^x,[5] the L-selectin requires additional O-sulfation on the O-6 of the galactose or *N*-acetylglucosamine residue.[6–10] The monovalent 6′-O-sulfated sLe^x is reported to bind L-selectin at 250 μM of IC_{50},[11] which is relatively weaker than the intrinsic ligand GlyCAM-1, 108 μM.[12] To circumvent such low affinity in monovalent form, L-selectin, whose expression is more concentrated on the pili of the cell surface,[13] apparently exploits the clustered presence of O-glycosylation sites on the natural ligands, GlyCAM-1[14] or CD34.[15] Indeed, previous studies in vitro have shown remarkable enhancement of the affinity by using polyvalent oligosaccharide structures.[16,17]

When the regioselectively monosulfated galactoses were clustered on polyglutamic acid, they showed barely detectable affinities (mM as galactose), whereas the individual monosaccharide sulfates showed no detectable affinity (>100 mM).[18] Since the overall affinities of the clustered galactose sulfates were far below the practical value, we next considered

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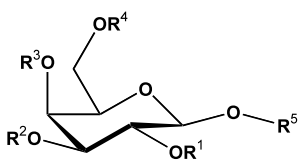
sulfated disaccharide structures that possess a primary amino group on the aglycone for subsequent formation of clusters. In our experience with sulfated glucose polymers, the affinity of the (1→3) linkage was superior to that of (1→4) or (1→6) linkages.[17] The well known unnatural ligand for L-selectin, fucoidan, also consists of α-(1→3) linked fucose.[19] Thus we constructed a series of α- and β-(1→3) linked galabioses as possible artificial mimics, having selective sulfation at the 3′, 6 or 6′ positions. The 3′-sulfate here acts as a substitute for the 3′-sialic acid residue.[20] The rationale for the 6- and 6′-sulfates is based on the fact that the monosaccharide components of the natural ligand oligosaccharides consist of 6-O-sulfated galactose, fucose, sialic acid, *N*-acetylglucosamine, and 6-O-sulfated *N*-acetylglucosamine.[14]



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	$(CH_2)_3CH=CH_2$
2	H	H	PhCH<		$(CH_2)_3CH=CH_2$
3	H	MBn	PhCH<		$(CH_2)_3CH=CH_2$
4	Bn	MBn	PhCH<		$(CH_2)_3CH=CH_2$
5	Bn	H	Bn	H	$(CH_2)_3CH=CH_2$
6	Bn	Ac	Bn	Ac	$(CH_2)_3CH=CH_2$
7	H	H	H	H	$CH_2CH=CH_2$
8	H	H	PhCH<		$CH_2CH=CH_2$
9	H	MBn	PhCH<		$CH_2CH=CH_2$
10	Bn	MBn	PhCH<		$CH_2CH=CH_2$
11	Bn	H	PhCH<		$CH_2CH=CH_2$
12	H	MBn	H	H	$CH_2CH=CH_2$
13	H	MBn	H	$C(Ph)_3$	$CH_2CH=CH_2$
14	Bn	MBn	Bn	H	$CH_2CH=CH_2$
15	Bn	H	Bn	Ac	$CH_2CH=CH_2$
16	Bn	MBn	Bn	Bn	$CH_2CH=CH_2$
17	Bn	Ac	Bn	Bn	$CH_2CH=CH_2$
18	Bn	Ac	Bn	Bn	H
19	Bn	Ac	Bn	Bn	$C(=NH)CCl_3$

MBn = 4-methoxybenzyl

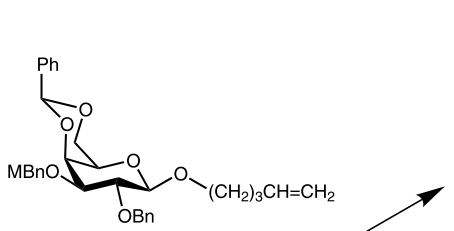
Scheme 1.

2. Results and discussion

Throughout this work, all galactose precursors were suitably protected prior to glycosidation to enable precise regioselective sulfation to optimize the glycosidation step. Two different methods were employed according to the stability of the donor and the efficacy of the reaction.

To obtain the 3′,6,6′-tri-O-sulfated compounds **23**, **27**, a combination of pentenyl donor, iodinium di-*sym*-collidine perchlorate as the catalyst, and 1:4 CH_2Cl_2–Et_2O as the solvent was optimal in terms of glycosidation yield, giving the anomeric 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methoxybenzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside (**20** (α) 48%; **21** (β) 18%).[21] To increase the ratio of β anomer, a switch of the solvent to acetonitrile only decreased the efficacy of glycosidation to 24%, and did not materially affect the anomeric ratio (**20**:**21** = 1.5:1).[22,23] Use of trichloroacetimidate as the donor, with the same protecting groups as **4**, gave less than 30% of glycosidation product. Thus, the α/β mixture obtained by using the pentenyl donor **4** in 1:4 CH_2Cl_2–Et_2O was purified by repeated chromatography and crystalization from EtOH. The position of glycosidation on the acceptor was confirmed by the downfield shift of C-3 (72.4 to 74.5 (α) or 75.1 (β) ppm).[24] In the next step, regioselective reductive cleavage of two 4,6-*O*-benzylidene groups by $LiAlH_4$–$AlCl_3$ afforded an acceptable yield of 6,6′-dihydroxy derivatives (**22**, 33%; **27**, 35%) from **20** and **21**[25] (Schemes 1–3).

The strategy for 3′,6-dihydroxygalabiose, the reductive cleavage of 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-methoxybenzyl-α-D-galactopyranosyl)-β-D-galactopyranoside, obtained by pentenyl glycosidation, gave exclusively 3′,4-dihydroxy derivative. Thus, avoiding cleavage of the benzylidene group, compound **15** was obtained as the selectively protected acceptor. When the pentenyl 2,4,6-tri-*O*-benzyl-3-*O*-methoxybenzyl-α-D-galactopyranosyl donor reacted with **15**, the glycosidation yield was less than 30%. The trichloroacetimidate donor having the same protective groups gave an unsatisfactory



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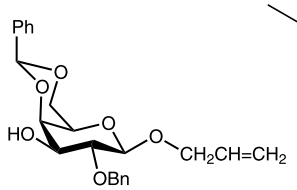

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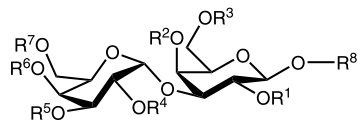
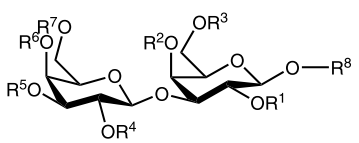
R^8= **20-24, 27,28** : $CH_2CH=CH_2$

25, 26, 29, 30 : $(CH_2)_3S(CH_2)_2NH_2$

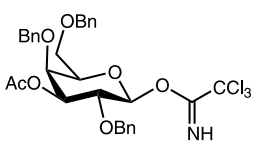


	R^1	R^2	R^3	R^4	R^5	R^6	R^7
20	Bn	PhCH<		Bn	MBn	PhCH<	
22	Bn	Bn	H	Bn	MBn	Bn	H
23	Bn	Bn	H	Bn	H	Bn	H
24	Bn	Bn	SO_3Na	Bn	SO_3Na	Bn	SO_3Na
25	Bn	Bn	SO_3Na	Bn	SO_3Na	Bn	SO_3Na
26	H	H	SO_3Na	H	SO_3Na	H	SO_3Na



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
21	Bn	PhCH<		Bn	MBn	PhCH<	
27	Bn	Bn	H	Bn	H	Bn	H
28	Bn	Bn	SO_3Na	Bn	SO_3Na	Bn	SO_3Na
29	Bn	Bn	SO_3Na	Bn	SO_3Na	Bn	SO_3Na
30	H	H	SO_3Na	H	SO_3Na	H	SO_3Na

Scheme 2.



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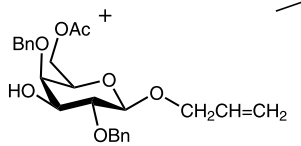

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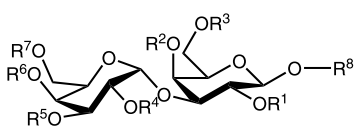
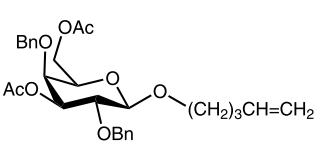


	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
31	Bn	Bn	Ac	Bn	Ac	Bn	Bn	$CH_2CH=CH_2$
32	Bn	Bn	H	Bn	H	Bn	Bn	$CH_2CH=CH_2$
33	Bn	Bn	SO_3Na	Bn	SO_3Na	Bn	Bn	$CH_2CH=CH_2$
34	Bn	Bn	SO_3Na	Bn	SO_3Na	Bn	Bn	$(CH_2)_3S(CH_2)_2NH_2$
35	H	H	SO_3Na	H	SO_3Na	H	H	$(CH_2)_3S(CH_2)_2NH_2$



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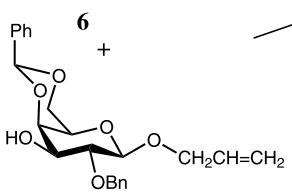

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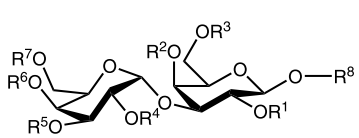

	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
36	Bn	PhCH<		Bn	Ac	Bn	Ac	$CH_2CH=CH_2$
37	Bn	PhCH<		Bn	H	Bn	H	$CH_2CH=CH_2$
38	Bn	PhCH<		Bn	SO_3Na	Bn	SO_3Na	$CH_2CH=CH_2$
39	Bn	PhCH<		Bn	SO_3Na	Bn	SO_3Na	$(CH_2)_3S(CH_2)_2NH_2$
40	H	H	H	H	SO_3Na	H	SO_3Na	$(CH_2)_3S(CH_2)_2NH_2$

Scheme 3.

result because of its extreme lability. Finally, 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl trichloroacetimidate (**19**),[26] which is reasonably stable, in reaction with **15** improved the glycosidation yield to 52%. The downfield chemical shift of the C-3 acceptor was from 74.2 to 77.7 ppm.[24]

To access the 3′,6′-dihydroxy compound, **37**, compounds **6** and **11** were coupled in the presence of iodinium di-*sym*-collidine perchlorate as described for **20** and **21**, followed by transesterification with NaOMe, to give the desired product, **37**.

The primary spacer arm attached to galabiose was the 2-propenyl group so that it could be substituted with cysteamine[27] after sulfation at the open hydroxy groups. There was a possible option of starting with another spacer that has a latent amino group at the end. However, when we tried the *N*-benzyloxycarbonylaminohexyl group, the protection of the O-2 position of the acceptor galactoside, leaving OH-3 open was extremely difficult, presumably because of steric hindrance.[18] The random glycosidation of the 2,3-dihydroxy acceptor was unsuccessful because of its poor solubility in the reaction solvent 1:4 CH_2Cl_2–Et_2O.

Although cysteamine could be linked at the double bond of the 2-propenyl group almost quantitatively with a 10-fold excess under UV irradiation, the subsequent steps of purification and deprotection required improvement. Separation on a Sephadex LH-20 column, which is a good alternative to the conventional silica gel chromatography to avoid loss of sulfated sugars,[18] was poor unless 0.5% Et_3N was added to the eluant. This amine, however, was bound tightly to sulfate esters and resisted removal by a cation-exchange resin in either the Na^+ or H^+ form. Thorough evaporation in the presence of a 2-fold excess of $NaHCO_3$ was required to replace the Et_3N counter-ion. The sodium form is required because the Et_3N or pyridine forms substantially destabilize the sulfate esters.[18] For the final deprotection, the thioether introduced by cysteamine made hydrogenolysis over palladium impossible. We therefore used trifluoroacetic acid–bromotrimethylsilane–thioanisole for final removal of the benzyl protective groups.[28]

NMR analyses of the sulfated products showed noteworthy downfield shifts of the geminal protons of the 6-sulfate groups and the carbon atoms connecting to the sulfate groups (Tables 1–5), indicating that the sulfate groups had been introduced at the desired positions, and no desulfation had taken place during the deprotection step.[29–31]

In this way, we constructed anionic galabioses to be tested as artificial ligands for L-selectin. The rationale for galabiose was that the (1→3) linkage, which is most commonly observed in Gal-Gal or Gal-GalNAc structures in mammals, showed the highest affinity among glucose polymers of various configurations.[17] Interestingly, the binding affinities of the trisulfated galabioses, **26** and **30**, were 120 μM and 160 μM kD, respectively, toward L-selectin in our liquid phase system (data not shown), which are reasonably high compared with the affinity of the intrinsic ligand.

3. Experimental

General methods.—Melting points were determined by a Yanagimoto micro melting point apparatus and uncorrected. Optical rotations were measured with a Jasco automatic digital polarimeter, Model DIP-140, in a 0.5 dm tube. All solvents were dried over 4 Å molecular sieves except for MeOH (3 Å molecular sieves) and $CHCl_3$ (Na_2SO_4) before use. Aqueous solutions of H_2SO_4 and $NaHCO_3$ used for washing the organic solution were 1 M and saturated, respectively. ^1H and ^{13}C NMR spectra were obtained with a Jeol Model JNM-GSX400 or JNM-α 500 spectrometer at 22 °C. The internal standards for ^1H NMR and ^{13}C NMR measurement were Me_4Si for $CDCl_3$ and CD_3OD, or acetone (2.200 ppm) for measurements in D_2O. All evaporations were done with a rotary evaporator. TLC was performed on Silica Gel 60 F_{254}-precoated aluminum sheets (EM Industries, Inc., Gibbstown, NJ) and compounds were detected by UV absorbance (254 nm), ninhydrin reaction or charring (after spraying with 15% H_2SO_4 in 50% EtOH). Column chromatography was performed on Silica Gel 60 (EM Industries, Inc) or Sephadex LH-20 (Pharmacia Biotech. Inc. Uppsala, Sweden). All solvent compositions are given as v/v.

Table 1
^{1}H NMR data for pentenyl galactosides **2**, **3**, **4**, and **6** [a]

	H-1 (d, $J_{1,2}$)	H-2 (dd, $J_{2,3}$)	H-3 (dd, $J_{3,4}$)	H-4 (bd)	H-5	H-6 (dd, $J_{6,6}$)	H-6 (dd)	O-(CH_2)$_2$ (m)	CH_2-CH (m)	CH (m)	CH_2 (dt)	Ph-CH (s)	Ph-CH_2 (d)	CH_3O (s)
2	4.28	3.75	3.70	4.22	3.49	4.34	4.09	2.16	3.99	5.83	5.08–4.96	5.56		
	(7.4)		(3.6)			(12.5)		1.77	3.53					
3	4.28	3.97	3.46	4.10	3.34	4.30	4.02	2.15	3.96	5.82	5.03	5.46	4.69/4.69	3.80
	(7.7)	(9.5)	(3.7)			(12.5)		1.75	3.52		4.96			
4	4.37	3.82	3.53	4.07	3.30	4.29	4.01	2.17	3.99	5.82	5.01	5.49	4.92/4.78	3.80
	(7.7)	(9.5)	(3.7)			(12.5)		1.76	3.51		4.96		4.70/4.69	
6	4.40	3.78	3.89	3.87	3.65	4.27	4.05	2.16	3.94	5.81	5.01		4.88/4.65	
	(7.7)	(10.3)	(3.3)			(11.0)		1.74	3.78		4.96		4.66/4.54	

[a] Values in parentheses are the associated coupling constants reported in Hz.
The solvent employed was $CDCl_3$.

*4-Pentenyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**3**).*—A mixture of **1** (8.02 g, 32.3 mmol),[32] benzaldehyde (17.5 mL, 167 mmol) and formic acid (17.5 mL, 464 mmol) was allowed to react at 22 °C for 1 h and then neutralized with aqueous $NaHCO_3$. By the addition of petroleum ether to the mixture, amorphous solid **2**[33] was isolated and crystallized from EtOH.[34] The mother liquor was chromatographed on a column of silica gel, eluted with 1:1 toluene–EtOAc. The fractions were concentrated in vacuo and crystallized from EtOH to give additional **2**. The total yield was 5.85 g (52%); m.p. 160–162 °C; R_f 0.26 (1:4 toluene–EtOAc). Compound **2** (6.12 g, 18.2 mmol) was dissolved in toluene (70 mL) and azeotropically distilled in the presence of Bu_2SnO (4.75 g, 1.05 fold excess) for 50 min. Tetrabutylammonium iodide (9.40 g, 1.4 fold excess) and 4-methoxybenzyl chloride (3.52 mL, 1.4 fold excess) were added and the mixture was stirred at 90 °C overnight, diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$. The organic layer was dried with Na_2SO_4, concentrated in vacuo and crystallized from EtOH to afford the title compound **3**. The mother liquor was chromatographed on a column of silica gel, eluted with 2:1 hexane–$CHCl_3$ to yield additional **3**; total yield 4.40 g (53%); mp 135.5–136.5 °C; $[\alpha]_D^{27}$ + 42.1° ($CHCl_3$); R_f 0.38 (2:1 toluene–EtOAc). Anal. Calcd for $C_{26}H_{32}O_7$: C, 68.40; H, 7.06. Found: C, 68.23; H, 7.13.

*4-Pentenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**4**).*—To a stirred solution of **3** (2.67 g, 5.86 mmol) and NaH (60%, 1.4 g) in 26 mL dry THF, benzyl bromide (4.18 mL, 35.1 mmol) was added dropwise and allowed to react at 24 °C overnight. After quenching with MeOH, the suspension was evaporated in vacuo, diluted with toluene, and washed twice with ice-cold water. The toluene layer was dried with Na_2SO_4, concentrated, and subjected to column chromatography on silica gel using 2:1 hexane–EtOAc as eluant. Crystallization from EtOH afforded **4** (2.93g, 92%); mp 117–118 °C; $[\alpha]_D^{28}$ + 32.8° ($CHCl_3$). Anal. Calcd for $C_{33}H_{38}O_7$: C, 72.51; H, 7.01. Found: C, 72.32; H, 6.78.

Table 2
^{1}H NMR data for propenyl galactosides **8-12** and **14-17**, and galactosyl imidate **19** [a]

	H-1 (d, $J_{1,2}$)	H-2 (dd, $J_{2,3}$)	H-3 (dd, $J_{3,4}$)	H-4 (bd)	H-5	H-6 (dd, $J_{6,6}$)	H-6 (dd)	O–CH_2 (m)	CH (m)	CH_2 (m)	CH_3O (s)	Ph–CH (s)	Ph–CH_2 (d)
8	4.35	3.79	3.71	4.22	3.49	4.35	4.09	4.45	5.97	5.33		5.56	
	(7.3)	(9.5)	(3.7)			(12.5)		4.14		5.24			
9	4.36	4.01	3.47	4.12	3.35	4.31	4.04	4.42	5.97	5.32	3.80	5.46	4.70/4.68
	(8.1)	(9.5)	(3.7)			(12.8)		4.14		5.21			
10	4.43	3.85	3.53	4.06	3.29	4.29	3.99	4.45	5.96	5.33	3.78	5.48	4.93/4.77, 4.70/4.68
	(7.7)	(9.9)	(3.7)			(12.5)		4.13		5.18			
11	4.46	3.67	3.74	4.23	3.45	4.34	4.09	4.47	5.96	5.35		5.56	5.00/4.73
	(7.3)	(9.5)	(3.7)			(12.5)		4.15		5.21			
12	4.27	3.67	3.34	3.99	3.42	3.75	3.72	4.36	5.96	5.32	3.77		4.68/4.59
	(7.7)	(9.5)	(3.3)		(m)	(12.1)		4.14		5.15			
14	4.41	3.84	3.50	3.73	3.35	3.76	3.48	4.40	5.94	5.31	3.81		4.95/4.65, 4.94/4.77,
	(7.7)	(9.9)	(2.9)		(bt)	(11.5)		4.13		5.17			4.73/4.67
15	4.40	3.65	3.66	3.78	3.60	4.26	4.09	4.41	5.94	5.32			4.99/4.67, 4.85/4.66
	(7.7)					(11.4)		4.12		5.20			
16	4.40	3.81	3.49	3.85	3.51	3.58	3.58	4.40	5.92	5.30	3.78		4.93/4.60, 4.92/4.76
	(8.0)	(9.6)	(3.0)					4.10		5.16			4.66/4.65, 4.43/4.41
17	4.47	3.79	4.89	3.94	3.67	3.61	3.61	4.41	5.93	5.31			4.88/4.64, 4.59/4.50
	(7.7)	(10.3)						4.12		5.18			4.49/4.44
19	6.52	4.20	5.32	4.18	4.28	3.60	3.56						4.67/4.63, 4.61/4.52
	(3.6)	(10.6)	(3.3)			(9.5)							4.50/4.42

[a] Values in parentheses are the associated coupling constants reported in Hz.
The solvent employed was $CDCl_3$, except for **12**, which was measured in CD_3OD.

Table 3
^{1}H NMR data for the unsulfated and sulfated galabioses **20–21**, **23–25** and **27–40** [a]

	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4	H-5	H-6 ($J_{6,6}$)	H-6	H-1′ ($J_{1',2'}$)	H-2′ ($J_{2',3'}$)	H-3′ ($J_{3',4'}$)	H-4′	H-5′	H-6′ ($J_{6',6'}$)	H-6′
20	4.48 (7.5)	3.90 (9.9)	3.80 (3.5)	4.29	3.56	4.34 (12.5)	4.06	5.22 (3.1)	4.06 (10.1)	4.00 (3.5)	3.85	3.73	3.97 (12.5)	3.51
21	4.43 (7.3)	3.93 (9.8)	3.99 (3.7)	4.36	3.37	4.30	4.05	4.99 (7.9)	3.85 (9.8)	3.42 (3.6)	4.01	3.11	4.19	3.94
23	4.41 (7.3)	3.83 (10.4)	3.76	3.80	3.38	3.75 (11.0)	3.51	5.20 (3.1)	3.86 (10.4)	4.02 (3.1)	3.49	4.04	3.39 (11.0)	3.15
24	4.45 (7.9)	3.68 (9.8)	3.85 (2.4)	4.03	3.73	4.09	4.08	5.25 (3.6)	4.06 (10.4)	5.10 (3.1)	4.26	4.42	4.00 (9.2)	3.86
25	4.41 (7.9)	3.66 (10.4)	3.88 (2.4)	4.04	3.75	4.10	4.08	5.26 (3.1)	4.03 (10.4)	5.09	4.26	4.41	4.04 (9.2)	3.86
26	4.67 (7.9)	3.62	3.78	4.20–4.14		4.20–4.14	4.20–4.14	5.04 (3.1)	4.02–3.99	4.62	4.39		4.20–4.14	4.20–4.14
27	4.40 (7.0)	3.91–3.86	3.91–3.86	3.86	3.40	3.74 (11.4)	3.49	4.86 (7.3)	3.66 (11.0)	4.63	3.82	3.40	3.75 (11.4)	3.63
28	4.42 (7.7)	3.68 (9.5)	3.92 (3.3)	3.99	3.81	4.14 (10.3)	4.10	4.87 (7.9)	3.73 (9.9)	4.55 (3.3)	4.39	3.76	4.14	4.14
29	4.37 (7.9)	3.64 (9.8)	3.92	4.0	3.82	4.16 (10.7)	4.07	4.85 (7.3)	3.75 (9.8)	4.55	4.39	3.78	4.17	4.16
30	4.64	3.64	3.82			4.22–4.16	4.22–4.16	4.72		4.36			4.22–4.16	4.22–4.16
31	4.29 (7.3)	3.84 (10.3)	3.75	3.80	3.41	4.18 (11.0)	3.99	5.28 (3.6)	4.19 (10.3)	5.48	3.90	4.42	3.46 (9.9)	3.24
32	4.32 (7.3)	3.82 (9.8)	3.73	3.80	3.30	3.71 (11.6)	3.44	5.26 (3.7)	3.85 (10.4)	4.07	3.65	4.37	3.51 (9.8)	3.37
33	4.23 (7.9)	3.68 (10.4)	3.83	4.04	3.65	4.09	4.08	5.29 (3.6)	4.10 (10.4)	5.21	4.27	4.40	3.41 (11.0)	2.88
34	4.16 (7.3)	3.62	3.82	4.01	3.64	4.08	4.07	5.28 (3.7)	4.10 (10.4)	5.18	4.27	4.38	3.41 (11.0)	2.91
35	4.47 (7.9)	3.64	3.78	4.23–4.16	3.76–3.71	4.23–4.16	4.23–4.16	5.20 (3.7)	4.01	4.59	4.35	4.23–4.16	3.76–3.71	3.76–3.71
36	5.45 (7.7)	3.79 (9.9)	3.97	~3.85	3.35	4.34	4.07	5.39 (3.6)	4.06	5.39	4.30	4.17	~3.86	~3.84
37	4.48 (7.3)	3.90 (9.9)	3.85	4.27	3.39	4.34 (12.1)	4.07	5.22 (3.7)	3.85 (9.9)	4.06	3.55	3.88	3.41 (11.4)	3.14
38	4.56 (7.3)	3.78 (10.4)	3.94	4.50	3.52	4.19	4.14	5.27 (3.6)	3.97 (10.4)	5.03	4.30	4.23	4.06 (9.8)	3.89
39	4.53 (7.9)	3.76 (9.8)	~3.98	4.51	3.54	4.19	4.16	5.30 (3.7)	4.00 (9.8)	5.03	4.31	4.28	4.08 (9.8)	3.90
40	4.64 (7.9)	3.61 (9.8)	3.77–3.71	4.21–4.13	3.67	3.77–3.71	3.77–3.71	5.20 (3.7)	4.01 (10.4)	4.62	4.39	4.42	4.21–4.13	4.21–4.13

[a] Values in parentheses are the associated coupling constants reported in Hz.
The solvents employed were $CDCl_3$ for **20–21**, **23**, **27**, **32** and **37**, D_2O for **26**, **30**, **35** and **40**, and CD_3OD for the others.

Table 4
^{13}C NMR data for **3**, **6**, **10**–**12**, **14**, **15**, **17**, and **19** [a]

	C-1	C-2	C-3	C-4	C-5	C-6	O-CH_2	CH_2CH_2	CH_2-CH	CH	CH_2	Ph-CH	Ph-CH_2	CH_3O	CH_3CO
3	103.0	70.0	78.7	73.2	66.7	69.3	30.2	28.6	69.1	138.2	114.8	101.1	71.1	55.3	
6	103.9	76.7	75.0	73.8	71.7	62.2	30.2	28.9	69.5	138.0	115.0		74.9, 74.7		
10	102.7	78.5	78.9	74.1	66.5	69.2	70.2			134.3	117.1	101.4	75.3, 71.7	55.2	
11	102.5	79.3	72.4	75.5	66.5	69.1	70.2			134.1	117.3	101.4	74.9		
12	104.0	71.8	82.1	67.2	76.5	62.5	71.0			135.9	117.4		72.3	55.7	
14	103.2	79.7	82.0	73.0	74.6	62.0	70.4			134.3	117.1		75.3, 74.1, 73.1	55.3	
15	102.8	79.3	74.2	74.7	72.2	62.8	70.1			134.0	117.3		74.9, 74.7		20.8
17	102.8	77.0	75.0	74.5	73.1	68.3	70.2			134.0	117.2		75.0, 74.7, 73.5		20.8
19	94.6	73.2	72.2	74.9	71.3	67.7							75.3, 73.4, 72.8		21.0

[a] The solvent employed was $CDCl_3$, except for **12**, which was measured in CD_3OD.

Table 5
^{13}C NMR data for the unsulfated and sulfated galabioses **20**–**21**, **23**–**25**, **27**–**29**, **32**–**34** and **37**–**39** [a]

	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	O-CH_2	CH (CH_2) [b]	CH_2 (CH_2-S) [b]	Ph-CH	Ph-CH_2	CH_3O
20	102.8	77.3	74.5	71.8	66.4	69.3	94.0	75.2	75.0	74.9	62.5	69.2	70.4	134.2	117.2	101.3, 101.0	75.3, 72.5, 71.7	55.2
21	102.7	79.5	75.1	76.1	66.7	69.0	103.2	78.6	78.3	74.3	66.4	69.2	70.2	134.2	117.2	101.1, 100.6	75.1, 74.8, 71.6	55.3
23	103.3	78.5	77.7	72.4	70.1	62.3	94.9	77.4	70.6	77.3	74.4	62.0	70.4	134.1	117.3		75.4, 75.1, 74.4, 74.1	
24	104.6	79.2	78.8	74.3	74.1	67.5	97.3	75.8	79.3	77.3	69.9	67.6	71.4	135.7	117.4		76.4, 76.2, 76.1, 75.5	
25	105.2	79.1	78.4	74.0	73.9	67.5	96.6	75.6	79.3	77.2	69.7	67.6	69.2	31.1	29.1		76.4, 76.1, 76.0, 75.5	
27	103.1	80.0	79.6	75.0	74.6	62.0	104.1	79.7	74.2	75.3	74.5	61.8	70.4	134.1	117.3		75.0, 74.8, 74.6, 74.2	
28	104.0	81.2	81.1	78.0	74.1	67.9	105.1	79.2	81.8	76.1	73.7	67.1	71.3	135.5	117.4		76.4, 76.2, 76.0, 75.9	
29	105.1	81.1	81.4	78.2	74.2	68.4	105.2	79.2	81.8	76.1	73.8	67.3	69.5	31.4	29.1		76.3, 76.1, 76.0, 75.9	
32	103.4	78.4	77.9	72.0	74.4	62.0	95.2	77.6	70.3	77.3	68.9	68.8	70.4	134.2	117.1		75.2, 75.2	
33	104.7	79.3	77.8	73.7	74.2	67.4	96.2	75.9	79.6	77.3	69.3	70.5	71.4	135.6	117.4		76.6, 76.1, 76.1, 75.7	
34	105.6	79.2	77.5	73.7	74.0	68.0	96.0	75.8	79.5	77.0	69.1	70.5	69.5	29.9	29.0		76.6, 76.1, 76.1, 76.0, 73.0	
37	102.8	77.5	76.2	71.7	66.3	69.3	92.1	73.8	70.3	76.5	70.0	62.7	70.2	134.1	117.3	101.5	75.4, 74.9, 71.4	
38	104.0	78.5	75.4	72.8	67.7	70.3	94.2	74.9	78.2	77.0	70.1	67.9	71.4	135.7	117.3	102.3	76.3, 76.3, 73.0	
39	104.6	78.5	75.3	72.8	67.8	70.4	94.1	75.0	78.3	77.0	70.1	68.2	68.6	30.8	29.0	102.1	76.4, 76.3, 73.1	

[a] The solvents employed were $CDCl_3$ for **20**–**21**, **23**, **27**, **32** and **37**, and CD_3OD for the others.
[b] The formulas in the parentheses apply to **25**, **29** and **34** due to the substitution to cysteamine.

*4-Pentenyl 3,6-di-O-acetyl-2,4-di-O-benzyl-β-D-galactopyranoside (**6**).*—To a stirred solution of **4** (1.60 g, 2.93 mmol) in 20 mL of dry 1:1 CH_2Cl_2–Et_2O, $LiAlH_4$ (890 mg) was added portionwise and allowed to react for 5 min at 22 °C, followed by the dropwise addition of an $AlCl_3$ (2.93 g) suspension in dry Et_2O (7 mL) over 7 min. The mixture was boiled under reflux for 1 h.[25] After quenching with EtOAc and ice, the suspension was filtered through Celite and the residual solid rinsed several times with acetone. The residue was diluted with $CHCl_3$, washed with water, and chromatographed on a column of silica gel (5:1 toluene–EtOAc) to yield a mixture of 3,4-OH and 3,6-OH (**5**) derivatives (0.583 g, 47%, R_f 0.51 (1:4 toluene–EtOAc)). Acetylation of the resultant mixture with Ac_2O (3.5 mL) and dry Py (3.5 mL) overnight, permitted separation by column chromatography on silica gel (8:1 hexane–EtOAc), affording the title compound **6** (0.184 g, 26%, R_f 0.21 (4:1 toluene–EtOAc)) and 3,4-O-acetylated isomer (0.48 g, 69%, R_f 0.38 (4:1 toluene–EtOAc)). **6**; $[\alpha]_D^{21}$ +14.6° ($CHCl_3$). Anal. Calcd for $C_{29}H_{36}O_8$: C, 67.95; H, 7.08. Found: C, 67.82; H, 7.12.

*2-Propenyl 4,6-O-benzylidene-3-O-(4-methoxybenzyl)-β-D-galactopyranoside (**9**).*—A mixture of **7** (9.95 g, 45.2 mmol),[35] benzaldehyde (32 mL, 305 mmol) and formic acid (32 mL, 848 mmol) was stirred for 0.5 h at 22 °C. Addition of Et_2O gave crystalline **8**. The residual solution was neutralized with solid $NaHCO_3$ and Et_2O and petroleum ether were added so that more **8** could be obtained as an amorphous solid. The solid was recrystallized from MeOH and Et_2O to afford **8** (9.69g, 70%); mp 175–176 °C (Lit.: 178–179 °C[36]); R_f 0.23 (1:4 toluene–EtOAc). A suspension of **8** (4.13 g, 13.4 mmol) in toluene (40 mL) was azeotropically distilled in the presence of Bu_2SnO (3.66 g, 1.1 fold excess) in the same manner as for **3**. To the mixture, tetrabutylammonium iodide (6.43 g, 1.3 fold excess) and 4-methoxybenzyl chloride (2.41 mL, 1.3 fold excess) were added and the mixture was refluxed for 30 min. The solution was diluted with $CHCl_3$ and washed with water. The organic layer was dried with Na_2SO_4 and crystallized from EtOH to yield the title compound **9**. The mother liquor was chromatographed on silica gel (8:1 toluene–EtOAc) to afford additional **9**. The total yield was 4.63 g (81%); mp 165–165.5 °C; $[\alpha]_D^{27}$ +32.8° ($CHCl_3$); R_f 0.21 (2:1 toluene–EtOAc). Anal. Calcd for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 67.10; H, 6.49.

*2-Propenyl 2-O-benzyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)-β-D-galactopyranoside (**10**).*—To a stirred solution of **9** (5.15 g, 12.0 mmol) in 50 mL dry THF, NaH (60%, 2.88 g, 72 mmol) and benzyl bromide (8.29 mL, 69.7 mmol) were added sequentially and allowed to react overnight at 22 °C. The mixture was processed as for **4**, chromatographed on a column of silica gel and eluted with 2:1 toluene–$CHCl_3$. The residue was crystallized from EtOH to afford the title product **10** (5.46 g, 88%); mp 115–116 °C; $[\alpha]_D^{25}$ +38.7° ($CHCl_3$); R_f 0.39 (4:1 toluene–EtOAc). Anal. Calcd for $C_{31}H_{34}O_7$: C, 71.80; H, 6.61. Found: C, 71.77; H, 6.76.

*2-Propenyl 2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (**11**).*—A solution of **10** (4.91 g, 9.47 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.57 g, 11.3 mmol) in 50 mL 25:1 CH_2Cl_2–H_2O, was stirred for 1 h at 22 °C. The mixture was diluted with $CHCl_3$ and washed twice with $NaHCO_3$. The organic layer was dried with Na_2SO_4, concentrated and applied to a column of silica gel eluted by 8:1 toluene–EtOAc. The residue was evaporated in vacuo and crystallized from toluene at 4 °C to afford **11** (2.93 g, 78%); mp 73.5–74 °C; $[\alpha]_D^{27}$ +12.6° ($CHCl_3$); R_f 0.32 (2:1 toluene–EtOAc). Anal. Calcd for $C_{23}H_{26}O_6$: C, 66.33; H, 6.78. Found: C, 66.35; H, 6.72.

*2-Propenyl 3-O-(4-methoxybenzyl)-β-D-galactopyranoside (**12**).*—Compound **9** (3.95 g, 9.22 mmol) was treated with 80% aqueous AcOH (20 mL) for 50 min at 86 °C. The solution was evaporated in vacuo (toluene). The residue was made neutral with aqueous $NaHCO_3$, the solution was evaporated, and the residue extracted with acetone several times for chromatography on a silica gel column (1:4 toluene–EtOAc). The residue was crystallized from EtOH and toluene to afford the title compound **12** (1.85 g, 59%); mp 115–116 °C; $[\alpha]_D^{24}$ +16.6° (MeOH); R_f 0.52 (8:1

EtOAc–MeOH). Anal. Calcd for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.90; H, 7.10.

2-Propenyl 2,4-di-O-*benzyl-3*-O-*(4-methoxybenzyl)-β-D-galactopyranoside* (**14**).—A mixture of **12** (0.34 g, 1 mmol) and Ph_3CCl (0.56 g, 2 mmol) in dry Py (4 mL) was heated for 18 h at 37 °C. Ice was added, and the mixture concentrated in vacuo, and the residue chromatographed on silica gel (2:1 hexane–EtOAc) to yield **13** as a syrup (0.505 g, 86.7%, R_f 0.36 (2:1 toluene–EtOAc)). Compound **13** was 2,4-O-benzylated in dry DMF and processed in the same way as **4**, employing a 6-fold excess of NaH and a 5-fold excess of benzyl bromide. The resultant mixture was treated with 80% aqueous AcOH (4 mL) for 1 h at 90 °C, concentrated in vacuo and subjected to chromatography on silica gel, eluting with 3:1 hexane–EtOAc. The residue was crystallized from EtOH to afford the title compound **14** (0.279 g, 62%, as needles); mp 94.5–96.0 °C; $[\alpha]_D^{24}$ +18.5° ($CHCl_3$); R_f 0.29 (2:1 toluene–EtOAc). Anal. Calcd for $C_{31}H_{36}O_7$: C, 71.52; H, 6.97. Found: C, 71.25; H, 6.97.

2-Propenyl 6-O-*acetyl-2,4-di*-O-*benzyl-β-D-galactopyranoside* (**15**).—Compound **14** (0.399 g, 0.766 mmol) was acetylated conventionally with Ac_2O (4 mL) in dry Py (4 mL). The residue was dissolved in 20:1 CH_2Cl_2–H_2O (5.25 mL) and treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.191 g) as described for **11**. After washing with aqueous $NaHCO_3$ and concentrating, the mixture was chromatographed using 3:1 hexane–EtOAc to afford the title compound **15** (0.283 g, 96%); $[\alpha]_D^{27}$ –0.6° ($CHCl_3$); R_f 0.23 (4:1 toluene–EtOAc). Anal. Calcd for $C_{25}H_{30}O_7$: C, 67.86; H, 6.83. Found: C, 67.65; H, 6.75.

2-Propenyl 3-O-*acetyl-2,4,6-tri*-O-*benzyl-β-D-galactopyranoside* (**17**).—A stirred solution of **12** (0.749 g, 2.20 mmol) in dry DMF (8 mL), chilled on ice, was treated with NaH (60%, 0.555 g) and benzyl bromide (1.57 mL, 13.2 mmol) was added dropwise. After stirring overnight at 24 °C, the reaction was quenched with MeOH, the solution evaporated in vacuo and the residue processed as described for **4**. The product **16** (R_f 0.51, 4:1 toluene–EtOAc) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (550 mg) in 20:1 CH_2Cl_2–water (10.5 mL) and processed as for **11**. After column chromatography on silica gel (4:1 hexane–EtOAc), the residue was acetylated with Ac_2O and Py to afford the title compound **17** (1.04 g, 89%); $[\alpha]_D^{27}$ +40.7° ($CHCl_3$); R_f 0.44 (4:1 toluene–EtOAc). Anal. Calcd for $C_{32}H_{36}O_7$: C, 72.16; H, 6.81. Found: C, 71.84; H, 7.03.

3-O-*Acetyl-2,4,6-tri*-O-*benzyl-β-D-galactopyranosyl trichloroacetimidate* (**19**).—A mixture of **17** (1.04 g, 1.95 mmol) and $RhCl(PPh_3)_3$ (0.37 g) in 7:3:1 EtOH–toluene–water (22 mL) was refluxed for 20 h and treated with aqueous 2 N HCl (1 mL) under reflux for 1 h.[37] After being neutralized with 1 M $NaHCO_3$ (2 mL), the mixture was evaporated, diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$. The residue was chromatographed on a column of silica gel, eluted with 3:1 hexane–EtOAc, to yield an α/β mixture of **18** (0.773 g, 81%, R_f 0.33, 0.37, (2:1 toluene–EtOAc)). To a solution of **18** (0.773 g, 1.57 mmol) in dry CH_2Cl_2 (15 mL), trichloroacetonitrile (0.647 mL) and NaH (60%, 96 mg) were added sequentially, and the solution was stirred for 1 h at 22 °C.[38] Chromatography on silica gel, eluting with 15:1 hexane–EtOAc plus 0.1% Et_3N, gave the title compound **19** (0.748 g, 75%); R_f 0.49 (8:1 toluene–EtOAc). Anal. Calcd for $C_{31}H_{32}O_7Cl_3N{\cdot}H_2O$: C, 56.85; H, 5.23; N, 2.14. Found: C, 56.59; H, 4.84; N, 2.27.

2-Propenyl 2-O-*benzyl-4,6*-O-*benzylidene-3*-O-*[2*-O-*benzyl-4,6*-O-*benzylidene-3*-O-*(4-methoxybenzyl)-α-D-galactopyranosyl]-β-D-galactopyranoside* (**20**) *and 2-Propenyl 2*-O-*benzyl-4,6*-O-*benzylidene-3*-O-*[2*-O-*benzyl-4,6*-O-*benzylidene-3*-O-*(4-methoxybenzyl)-β-D-galactopyranosyl]-β-D-galactopyranoside* (**21**).—The donor compound **4** (0.599 g, 1.10 mmol) and acceptor compound **11** (0.398 g, 1.0 mmol) were dissolved in 10 mL of dry 1:4 CH_2Cl_2–Et_2O in the presence of ground 4 Å molecular sieves. Iodinium di-*sym*-collidine perchlorate (0.93 g) freshly prepared according to the established method[39] was added to the solution under stirring. After 20 h, the mixture was filtered through Celite, diluted with $CHCl_3$, and washed sequentially with aqueous H_2SO_4 and $NaHCO_3$. The residue was subjected to chromatography on silica gel,

eluting stepwise with 2:3, 1:2, and 1:4 hexane–$CHCl_3$ such that the α and β anomers (**20** and **21**, R_f 0.56 and 0.48 (2:1 toluene–EtOAc), respectively) were separated efficiently. Each anomer was crystallized from EtOH to yield **20** (0.412 g, 48%) and **21** (0.155 g, 18%). Compound **20** had mp 171.5–172.5 °C; $[\alpha]_D^{27}$ + 123° ($CHCl_3$). Anal. Calcd for $C_{51}H_{55}O_{13}$: C, 71.31 H, 6.34. Found: C, 71.03; H, 6.27. Compound **21** had mp 190–191.5 °C; $[\alpha]_D^{19}$ + 46.2° ($CHCl_3$). Anal. Calcd for $C_{51}H_{55}O_{13}$: C, 71.31 H, 6.34. Found: C, 71.17; H, 6.24.

*2-Propenyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**23**).*—To a stirred solution of **20** (0.389 g, 0.453 mmol) in 9 mL dry 1:1 CH_2Cl_2–Et_2O, was added $LiAlH_4$ (0.138 g) portionwise and allowed to react at 26 °C for 10 min, followed by dropwise addition of $AlCl_3$ (478 mg) suspended in dry Et_2O (3 mL). The mixture was refluxed for 30 min, quenched sequentially with EtOAc and MeOH, and the residue filtered off through Celite, washing the solid with MeOH several times. Chromatography on silica gel gave **22** (0.128 g, 33%, R_f 0.48 (1:4 toluene–EtOAc)), as well as de-3′-O-methoxybenzylated **23** (0.017 g, 5.1%, R_f 0.43 (1:4 toluene–EtOAc)), and the 6-*O*-benzyl derivative (0.052 g, 13%, R_f 0.69 (1:4 toluene–EtOAc)). Compound **22** (0.102 g, 0.119 mmol) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone as described for **11** to afford the title compound **23** (0.069 g, 78%); $[\alpha]_D^{24}$ + 86.5° ($CHCl_3$). Anal. Calcd for $C_{43}H_{50}O_{11}\cdot 0.5H_2O$: C, 68.69; H, 6.84. Found: C, 68.51; H, 7.00.

*2-Propenyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-sulfo-α-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside trisodium salt (**24**).*—A mixture of **23** (68.9 mg, 0.0928 mmol) and sulfur trioxide–Py (133 mg, 3 fold excess) in 1:1 dry Py–DMF (1 mL) was heated for 18 h at 56 °C. The reaction was quenched with 1.67 mL of 1 M $NaHCO_3$ and the solvent was evaporated in vacuo. The residue was dissolved in MeOH and filtered through Celite to remove some salt. Chromatography on a Sephadex LH-20 column (30 cm), eluted with MeOH, afforded the title compound **24** (74 mg, 76%); $[\alpha]_D^{15}$ + 69.7° (MeOH); R_f 0.42 (3:2:1 EtOAc–2-propanol–water). Anal. Calcd. For $C_{43}H_{47}O_{20}S_3Na_3\cdot 8H_2O$: C, 43.29; H, 5.32, Found: C, 43.30; H, 4.90.

*3-(2-Aminoethylthio)propyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-sulfo-α-D-galactopyranosyl)-β-D-galactopyranoside trisodium salt (**25**).*—Compound **24** (42.9 mg, 40.9 μmol) was dissolved in 1:1 MeOH–water (1 mL) and allowed to react with cysteamine HCl (48 mg) for 1.5 h at 24 °C, under UV irradiation (264 nm). Chromatography of the mixture on a column of Sephadex LH-20 (1 × 48 cm), eluted with MeOH supplemented with 0.5% Et_3N, afforded the tri(Et_3N) salt (49.0 mg, 88%). The counter-ion was changed into Na by the addition of 108 μL of 1 M $NaHCO_3$ and evaporation to give the title compound **25**; R_f 0.11 (6:2:1 EtOAc–AcOH–water). FAB-MS data: (+) 1127 (M + H), 1105 (M + 2H − Na); (−) 1103 (M − Na).

*3-(2-Aminoethylthio)propyl-6-*O*-sulfo-3-*O*-(3,6-di-*O*-sulfo-α-D-galactopyranosyl)-β-D-galactopyranoside (**26**).*—Compound **25** (30.7 mg, 27.3 μmol) was dried, placed on ice and ice-chilled trifluoroacetic acid (0.3 mL), bromotrimethylsilane (52.8 μL, 1 M final) and thioanisole (46.8 μL, 1 M final) were added. After 30 min on ice, the reaction was stopped with 3.84 mL 1 M $NaHCO_3$. The desired compound (20.5 mg, 98%) was isolated on a column of Sephadex LH-20 column (1 × 47 cm) eluted with 1:1 MeOH–water, 0.5% Et_3N; R_f 0.11 (3:2:1 EtOAc–AcOH–water). FAB-MS data: (+) 745 (M + 2H − Na).

*2-Propenyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**27**).*—Reductive cleavage of the benzylidene groups in **21** (0.327 g, 0.381 mmol) was performed as for **20**, employing $LiAlH_4$ (0.118 g, 8-fold excess) and $AlCl_3$ (0.411 g). In this step, the 3′-*O*-methoxybenzyl group was removed simultaneously, as determined by NMR and elemental analysis. After quenching the reaction with EtOAc and MeOH, the mixture was filtered through a layer of Celite, and washed with MeOH. The filtrate and washings were concentrated in vacuo and chromatographed on a column of silica gel (1:2 toluene–EtOAc) to yield **27** (100 mg, 35%) along with other positional isomers (81 mg,

29%). Compound **27**; $[\alpha]_D^{20}$ −4.9° ($CHCl_3$); R_f 0.26 (1:4 toluene–EtOAc). Anal. Calcd. for $C_{43}H_{50}O_{11}\cdot H_2O$: C, 67.88; H, 6.89. Found: C, 68.13; H, 6.78.

*2-Propenyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-sulfo-β-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside trisodium salt (**28**).*—Compound **27** (90.3 mg, 121.6 μmol) was O-sulfated by sulfur trioxide–Py (174 mg, 3-fold excess) in dry 1:1 Py–DMF (4 mL) for 16 h at 56 °C. After quenching with 2.19 mL of 1 M $NaHCO_3$, the residue was processed as for **24**. Chromatography on a column (1 × 19 cm) of Sephadex LH-20 with MeOH gave the title compound **28** (126.8 mg, 99%); $[\alpha]_D^{16}$ + 15.9° (MeOH); R_f 0.38 (3:2:1 EtOAc–isopropanol–water). Anal. Calcd. For $C_{43}H_{47}Na_3O_{20}S_3\cdot 6H_2O$: C, 44.64; H, 5.14, Found: C, 43.84; H, 4.77.

*3-(2-Aminoethylthio)propyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-sulfo-β-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside trisodium salt (**29**).*—Compound **28** (29.5 mg, 28.1 μmol) was dissolved in 1:1 MeOH–water (1 mL) and allowed to react with cysteamine HCl (33.8 mg) for 1 h at 24 °C, under UV irradiation (254 nm). The mixture was subjected to chromatography on a Sephadex LH-20 column (1 × 47 cm), eluted with MeOH supplemented with 0.5% Et_3N, to afford the tri(Et_3N) salt (30.5 mg, 94%). The ion form was converted as for **25** to afford the title compound **29**; R_f 0.12 (6:2:1 EtOAc–AcOH–water). FAB-MS data: (+) 1127 (M + H), 1105 (M + 2H − Na); (−) 1103 (M − Na).

*3-(2-Aminoethylthio)propyl 3-*O*-(3,6-di-*O*-sulfo-β-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside trisodium salt (**30**).*—Compound **29** (29.5 mg, 26.2 μmol) was deprotected as for **26**. The desired compound (16.8 mg, 84%) was isolated by using a column (1 × 47 cm) of Sephadex LH-20 eluted with 1:1 MeOH–water, 0.5% Et_3N; R_f 0.11 (3:2:1 EtOAc–AcOH–water). FAB-MS data: (+) 745 (M + 2H − Na).

*2-Propenyl 2,4-*O*-benzyl-3-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**32**).*—A mixture of **15** (262 mg, 0.592 mmol) and **19** (504 mg, 0.791 mmol) in CH_2Cl_2 (10 mL) was chilled at −80 °C, and a 10% solution of BF_3–Et_2O complex (1.07 mL) in CH_2Cl_2 was added dropwise in the presence of ground molecular sieves (4Å). After stirring at for 18 h 24 °C, the mixture was filtered through Celite and chromatographed on a column of silica gel (5:1 hexane–EtOAc) to afford **31** (342 mg, 52%, R_f 0.29 (4:1 toluene–EtOAc)). Compound **31** was deacetylated in 41 mM $NaOCH_3$ in dry MeOH (3 mL) to give the title compound **32** (275 mg, 89%); $[\alpha]_D^{25}$ + 49.5° ($CHCl_3$); R_f 0.51 (1:1 toluene–EtOAc). Anal. Calcd. for $C_{50}H_{56}O_{11}$: C, 72.10; H, 6.78. Found: C, 71.86; H, 6.80.

*2-Propenyl 2,4-di-*O*-benzyl-3-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-sulfo-α-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside disodium salt (**33**).*—Syrupy **32** (30.5 mg, 36.6 μmol) was dissolved in dry 1:1 Py–DMF (0.6 mL) and subjected to O-sulfation by sulfur trioxide–Py (35 mg, 3-fold excess) for 20 h at 56 °C. The resulting mixture was processed as for **24**, to afford the title compound **33** (31 mg, 82%); $[\alpha]_D^{15}$ + 58.2° (MeOH); R_f 0.38 (6:2:1 EtOAc–isopropanol–water). Anal. Calcd. for $C_{50}H_{54}Na_2O_{17}S_2\cdot 3H_2O$: C, 55.04; H, 5.54. Found: C, 55.00; H, 5.28.

*3-(2-Aminoethylthio)propyl 2,4-di-*O*-benzyl-3-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-sulfo-α-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside disodium salt (**34**).*—A solution of **33** (27.2 mg, 26.2 μmol) in 2:1 MeOH–water (1 mL) was allowed to react with cysteamine HCl (48 mg) for 2 h at 24 °C under UV irradiation (254 nm). The mixture was chromatographed and handled as described for **25** to afford the tri(Et_3N) salt (32.1 mg, 96%). The ion form was converted as for **25** to give the title compound **34**; R_f 0.41 (6:2:1 EtOAc–AcOH–water). FAB-MS data: (−) 1091 (M − Na).

*3-(2-Aminoethylthio)propyl 3-*O*-(3-*O*-sulfo-α-D-galactopyranosyl)-6-*O*-sulfo-β-D-galactopyranoside disodium salt (**35**).*—Debenzylation of **34** (27.8 mg, 25 μmol) was performed in the same way as for **26**. The yield was 13.6 mg (82%); R_f 0.15 (3:2:1 EtOAc–AcOH–water). FAB-MS data: (−) 641 (M − Na).

*2-Propenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,4-di-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**37**).*—Glycosidation between **6** (174 mg, 0.340 mmol) and **11** (113 mg, 0.283 mmol) was performed in dry 1:4 CH_2Cl_2–Et_2O (10 mL) using freshly prepared

iodinium di-*sym*-collidine perchlorate (0.20 g) as described for **20** and **21**. The resultant mixture was processed as for **20**, and chromatographed on a column of silica gel eluted with 1:1 toluene–EtOAc, to afford **36** (78 mg, 33%); R_f 0.40 (2:1 toluene–EtOAc), while leaving a significant amount of unreacted **11** (60 mg, 53%). Compound **36** was deacetylated by conventional transesterification to give **37** in quantitative yield; $[\alpha]_D^{15}$ +99.0° ($CHCl_3$); R_f 0.56 (1:4 toluene–EtOAc). Anal. Calcd. for $C_{43}H_{48}O_{11}\cdot H_2O$: C, 68.05; H, 6.64. Found: C, 68.07; H, 6.42.

2-Propenyl 2-O-*benzyl-4,6*-O-*benzylidene-3*-O-*(2,4-di*-O-*benzyl-3,6-di*-O-*sulfo-α-D-galactopyranosyl)-β-D-galactopyranoside disodium salt (**38**).*—Compound **37** (32.2 mg) was O-sulfated by sulfur trioxide–Py (42 mg) and processed as for **24**. Column chromatography gave the title compound **38** (36.8 mg, 90%); $[\alpha]_D^{15}$ +70.4° (MeOH); R_f 0.24 (6:2:1 EtOAc–isopropanol–water). Anal. Calcd. for $C_{43}H_{46}O_{17}S_2Na_2\cdot 2H_2O$: C, 52.65; H, 5.14. Found: C, 52.62; H, 5.31.

3-(2-Aminoethylthio)propyl 2-O-*benzyl-4,6*-O-*benzylidene-3*-O-*(2,4-di*-O-*benzyl-3,6-di*-O-*sulfo-α-D-galactopyranosyl)-β-D-galactopyranoside disodium salt (**39**).*—A solution of **38** (36.8 mg, 38.9 μmol) in 4:1 MeOH–water (1 mL) was reacted with cysteamine HCl (90 mg) for 2 h at 24 °C under UV irradiation (254 nm). The tri(Et_3N) salt (40.2 mg, 88%) was obtained as described for **25**. The ion form was converted as for **25** to afford the title compound **39**; R_f 0.28 (6:2:1 EtOAc–AcOH–water). FAB-MS data: (+) 1023 (M + H), 1000 (M + 2H − Na).

3-(2-Aminoethylthio)propyl 3-O-*(3,6-di*-O-*sulfo-α-D-galactopyranosyl)-β-D-galactopyranoside (**40**).*—Compound **39** (40.1 mg, 34 μmol) was proccessed as for **26**, to give **40** (21.8 mg, 97%); R_f 0.14 (3:2:1 EtOAc–AcOH–water). FAB-MS data: (−) 641 (M − Na), (+) 643 (M + 2H − Na).

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