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Synthesis and ^{1}H NMR characterization of the six isomeric mono-*O*-sulfates of 8-methoxycarbonyloct-1-yl *O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside

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Abstract

All six isomeric mono-*O*-sulfates of β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-O-$(CH_2)_8$COOMe (LacNAc-MCO) have been chemically synthesized and characterized by high resolution ^{1}H NMR spectroscopy. Sulfation causes characteristic substitution-site-specific downfield shifts of ^{1}H NMR signals. The $^4C_1$ chair conformation of both pyranose residues of LacNAc are unaffected by mono-*O*-sulfation, and, with the exception of the 3-*O*-sulfate derivative, glycosidic torsion angles are also unaffected.

Keywords: *N*-Acetyllactosamine; Sulfated oligosaccharides; ^{1}H NMR

1. Introduction

Sulfated oligosaccharides are widespread in nature and are thought to be involved in many important biological recognition processes [1–4] (see also citations in ref. [5]). There has been increasing interest in the role of sulfated oligosaccharides in cell adhesion processes, and it has been shown that *N*-acetyllactosamine (LacNAc)-based

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oligosaccharides containing sulfate groups, such as 3′-*O*-sulfated Lewis^a {β-D-(3-*O*-SO_3H)Gal*p*-(1 → 3)[α-L-Fuc*p*-(1 → 4)]-β-D-Glc*p*NAc-R} for instance, can act in vitro as ligands for the human endothelial adhesion molecule E-selectin [4]. In addition, a variety of sulfated structures, including 6′-*O*-sulfated sialyl Lewis^x {α-Neu-(2 → 3)-β-D-(6-*O*-SO_3H)Gal*p*-(1 → 4)[α-L-Fuc*p*-(1 → 3)]-β-D-GlcNAc*p*-R}, have been found on GlyCAM-1, a putative ligand for the lymphocyte adhesion molecule L-selectin [6].

The prospective key role of the sulfate group(s) in such processes has prompted efforts to determine the structure/site of sulfation of naturally occurring LacNAc-containing oligosaccharides. Such studies have typically relied on radiolabelling the oligosaccharides of interest, followed by extensive chromatographic analysis of the structures, and fragments thereof [6]. Recent studies have demonstrated the usefulness of FAB-mass spectrometry (FABMS) in characterizing sulfated human respiratory mucus oligosaccharides [7,8], and combined *endo*-glycosidase digestion/^{1}H NMR spectroscopy has successfully been used to fingerprint keratan sulfate structures from bovine articular cartilage [9].

A number of syntheses of sulfated LacNAc-based structures have been reported [4,10,11], and the regioselective sulfation [5,12,13] of such saccharides has also received attention of late. Synthetic sulfated LacNAc derivatives have found application in the differentiation of α-1,3-fucosyl transferase activities in mammalian tissue and sera [14–16], and in studies relating to the control of the biosynthesis of putative selectin ligands [17].

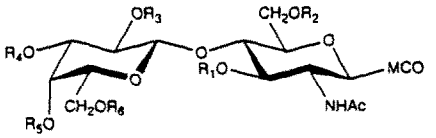
A panel of mono-*O*-sulfated LacNAc derivatives might prove useful as standards for the development of NMR and MS methods for localization of sulfate groups in LacNAc-based oligosaccharides. They might also serve as potential acceptor substrates for glycosyltransferases in systematic kinetic studies aimed at delineating the biosynthetic routes leading to sulfated oligosaccharides. Here we report the synthesis and high resolution ^{1}H NMR characterization of the systematically modified series of all six isomeric mono-*O*-sulfates of the 8-methoxycarbonyloctyl glycoside of *N*-acetyllactosamine (LacNAc-MCO).

2. Results and discussion

Chemical synthesis.—The six isomeric mono-*O*-sulfates of LacNAc-MCO, **1–6**, were prepared using established synthetic protocols. The approach adopted involved the synthesis of suitable partially blocked disaccharides, followed by sulfation and deprotection to give the target compounds.

The synthesis of all blocked disaccharides began from known [18] 8-methoxycarbonyloct-1-yl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside **7**, which was prepared using a modified literature procedure.

Glycosylation of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, followed by deacetylation of the crude product mixture gave disaccharide **8** in 84% yield (Scheme 1). Acetylation of **8** followed by hydrogenation to remove the two benzyl protecting groups produced diol **9** (96%). Selective mono-*O*-benzoylation of **9** gave **10** (87%), which on sulfation with trimethylamine–sulphur trioxide complex [19] and



1 $R_1 = SO_3^-Na^+$, R_2 - R_6 = H

2 R_1 = H, $R_2 = SO_3^-Na^+$, R_3 - R_6 = H

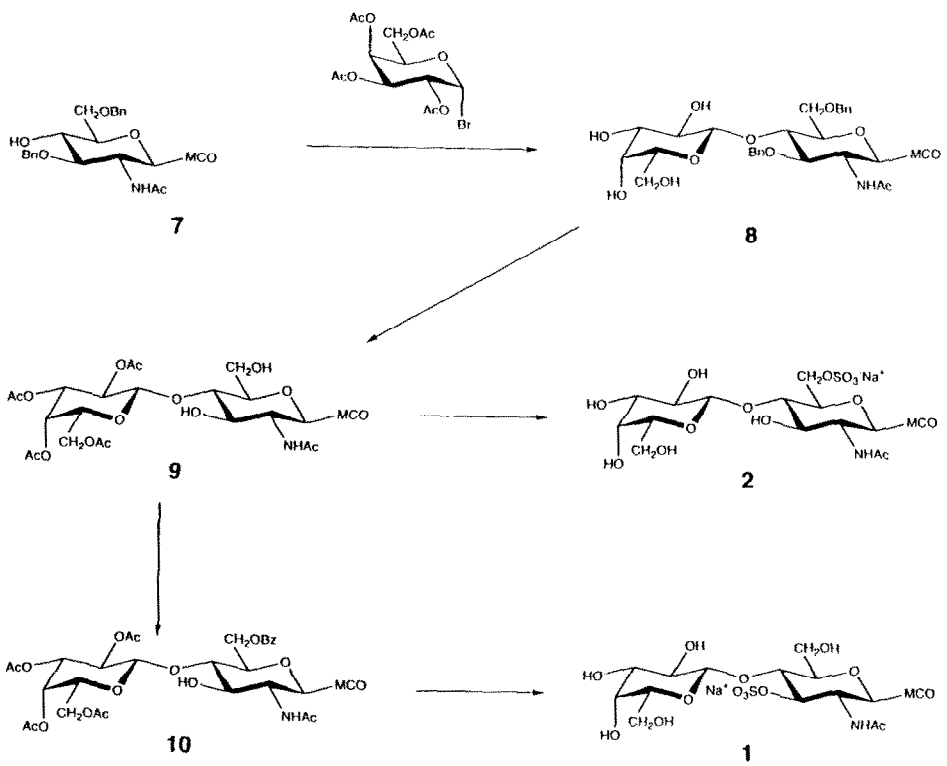
3 R_1 - R_2 = H, $R_3 = SO_3^-Na^+$, R_4 - R_6 = H

4 R_1 - R_3 = H, $R_4 = SO_3^-Na^+$, R_5 - R_6 = H

5 R_1 - R_4 = H, $R_5 = SO_3^-Na^+$, R_6 = H

6 R_1 - R_5 = H, $R_6 = SO_3^-Na^+$

MCO = $O(CH_2)_8COOMe$



Scheme 1.

deprotection afforded the 3-*O*-sulfate **1**. Alternatively, direct mono-*O*-sulfation of diol **9** gave the primary mono-*O*-sulfate as the principal product, which on deprotection gave the 6-*O*-sulfate **2**.

Glycosylation of **7** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl bromide [20] gave disaccharide **11** (70%), which produced the 2′-alcohol **12** (95%) on treatment with NaOMe (Scheme 2). Sulfation of **12**, followed by hydrogenation furnished the 2′-*O*-sulfate **3**.

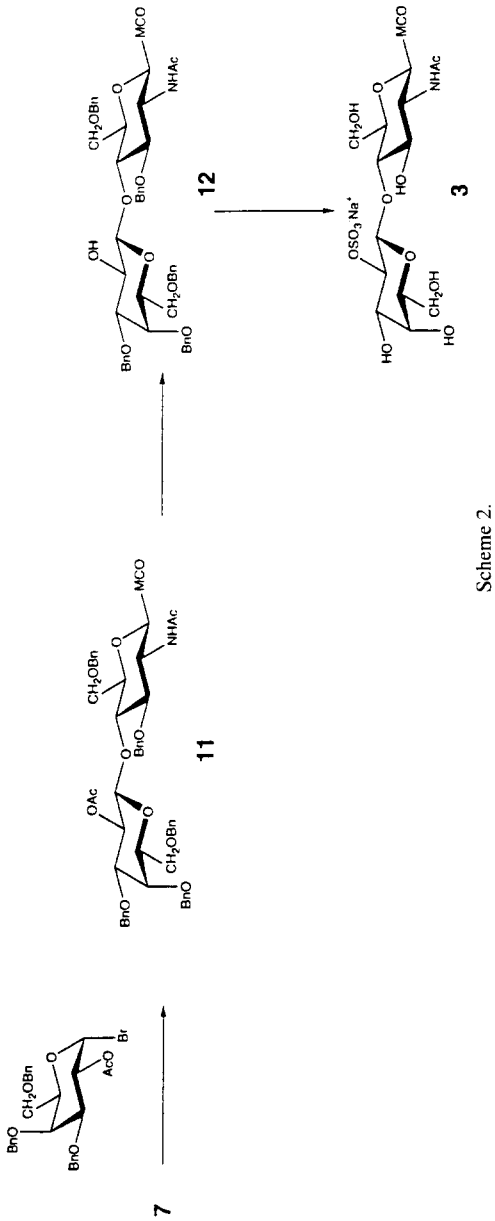
The sulfates **4–6** were prepared from disaccharide **8**. Treatment of **8** with acetone and *p*-toluenesulfonic acid for 16 h, followed by benzylation of the resulting product, gave the 3′,4′-acetal **13** (75%). In contrast, isopropylidenation of **8** under kinetic control, as described by Jacquinet and co-workers [21], followed by benzylation gave the 4′,6′-acetal **14** (70%) (Scheme 3). The structures of **13** and **14** were confirmed by comparison with literature data for related compounds [21].

De-isopropylidenation of **13** with aqueous acetic acid gave diol **15** (86%), which was converted to acetate **16** (95%) by treatment with trimethylorthoacetate and subsequent acid catalyzed rearrangement of the intermediate orthoesters [22]. Sulfation of **16**, followed by deprotection gave the 3′-*O*-sulfate **4**.

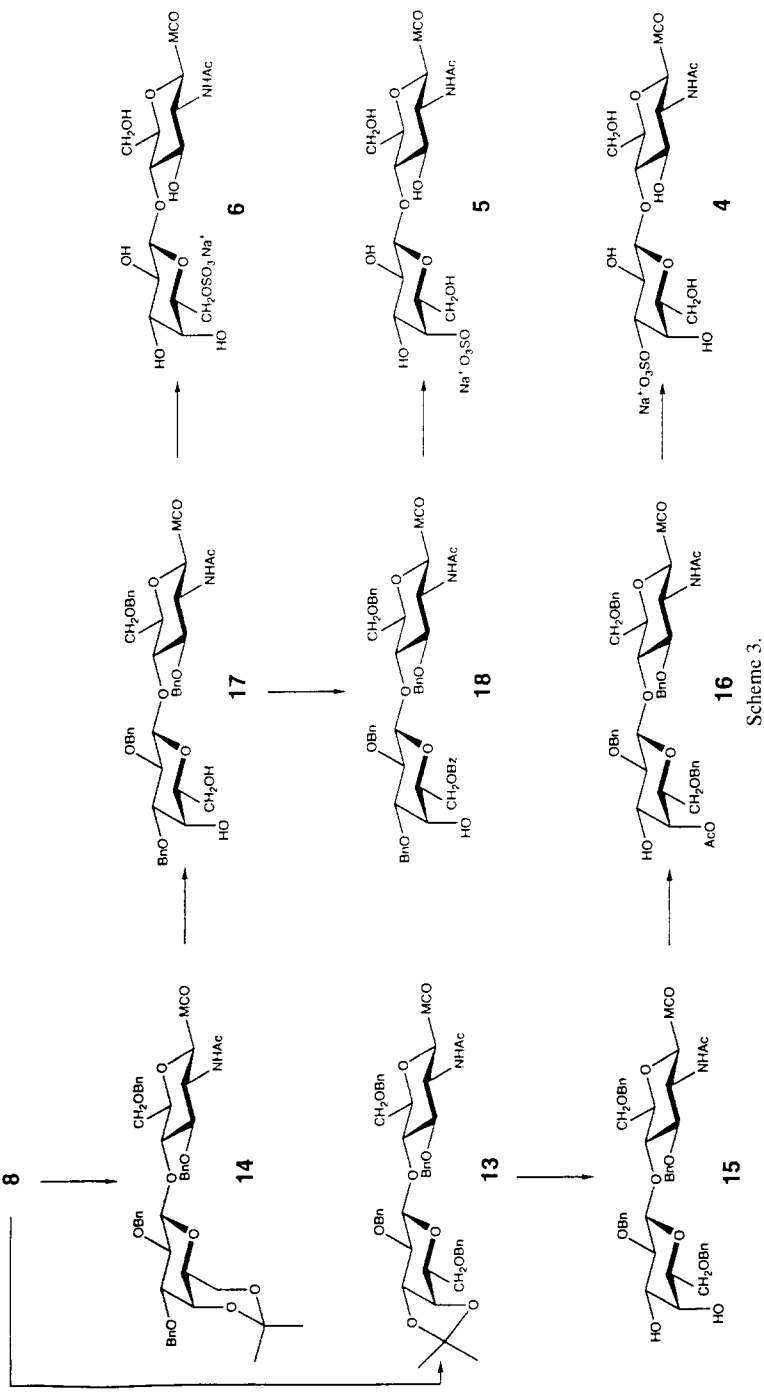
De-isopropylidenation of **14** gave the 4′,6′-diol **17** (85%), which was selectively mono-*O*-benzoylated to give **18** (73%). Sulfation of **18**, followed by deprotection gave 4′-*O*-sulfate **5**. Alternatively, direct mono-*O*-sulfation of diol **17**, followed by deprotection, gave the primary mono-*O*-sulfate as the principal product, which on deprotection gave the 6′-*O*-sulfate **6**.

Structural characterization.—Compounds **1–6** gave similar, but not identical, FABMS spectra. All gave a major ion at 678, corresponding to the sodium adduct of the sodium salt of the sulfated disaccharide. In addition, a major fragment derived from this ion was observed at 576 corresponding to a loss of SO_3Na and addition of a proton. The major differences in the spectra were the relative ratios of these peaks.

Proton chemical shifts and coupling constants of the six LacNAc-MCO mono-*O*-sulfates **1–6** and unsubstituted LacNAc-MCO are summarized in Tables 1 and 2. Characteristic ^{1}H NMR signal patterns of each isomeric mono-*O*-sulfate and unsubstituted LacNAc-MCO are shown in Fig. 1, and chemical shift changes induced by the sulfate-substitution are shown in Fig. 2. The effects are strongest at the substitution site and are typically of the order of +0.7 ppm (ca. +0.5 ppm each in case of substitution at the six position), as observed for the mono-*O*-sulfates of methyl α-D-galactopyranoside [23]. The deshielding effects decrease strongly with increasing distance from the substitution site [23] and, with one exception, hardly affect the unsubstituted pyranose ring or the aglycon. Consequently, the effects are very site-specific which is beneficial in identifying the substitution site in unknown, sulfate-containing carbohydrates [24]. The only slight exception to this rule is LacNAc-MCO 3-*O*-sulfate **1**. In this case, relatively weak but significant high- and low-field shifts were observed across both pyranose systems. This is indicative of a possible change in the overall orientation of the two pyranose rings relative to each other, likely due to steric effects caused by the adjacent substitution sites (i.e. H-3 and H-1′ → H-4). Sulfation adjacent to a glycosidic linkage has previously been shown to perturb the glycosidic torsion angles of chondroitin sulfate related disaccharides [25].



Scheme 2.



Scheme 3.

Table 1
^{1}H Chemical shifts [a] of sulfated LacNAc-MCO disaccharides [b,c]

H-1′	H-2′	H-3′	H-4′	H-5′	H-6a′	H-6b′	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	HNAc
LacNAc-MCO [d]														
4.47	3.54	3.67	3.93	3.72	3.76	3.74	4.52	3.71	3.73	3.70	3.57	3.98	3.83	2.03
LacNAc-MCO 3-*O*-sulfate (**1**)														
4.43	3.54	3.63	3.87	3.64	3.84	3.68	4.63	3.82	4.47	3.86	3.61	4.03	3.89	1.99
LacNAc-MCO 6-*O*-sulfate (**2**)														
4.53	3.53	3.67	3.93	3.72	3.76	3.74	4.54	3.73	[e]	[e]	3.79	4.40	4.31	2.03
LacNAc-MCO 2′-*O*-sulfate (**3**)														
4.63	4.27	3.85	3.99	3.74	3.78	3.76	4.51	3.72	[e]	[e]	3.57	4.01	3.90	2.03
LacNAc-MCO 3′-*O*-sulfate (**4**)														
4.59	3.68	4.34	4.29	3.78	3.78	3.76	4.52	3.71	3.73	3.72	3.58	3.99	3.84	2.03
LacNAc-MCO 4′-*O*-sulfate (**5**)														
4.53	3.56	3.81	4.67	3.85	3.82	3.78	4.52	3.71	3.71	3.71	3.58	3.99	3.84	2.03
LacNAc-MCO 6′-*O*-sulfate (**6**)														
4.51	3.54	3.69	3.99	3.97	4.20	4.18	4.52	3.71	3.74	3.69	3.61	3.98	3.83	2.03

[a] Chemical shifts are referenced to external 0.1% acetone at 2.225 ppm recorded under identical experimental conditions.
[b] All data were recorded on a Varian Unity spectrometer operating a 500 MHz. Concentrations were between 2 and 4 mM in D_2O. The temperature was 30.0 ± 0.1°C.
[c] Geminal protons are not assigned stereospecifically. The larger chemical shift was arbitrarily assigned to H6a and H6a′, respectively.
[d] The chemical shifts of the aglycone are: 3.89 (H-1), 3.59 (H-1′), 1.54 (H-2), 1.30 (H-3–H-6), 1.60 (H-7), 2.39 (H-8) and 3.69 ppm (OCH_3). Except for the aglyconic H-1′ in LacNAc-MCO 3-*O*-sulfate **1** (+0.02 ppm relative to unsulfated LacNAc-MCO), they are unchanged in all cases ($< \pm 0.01$ ppm).
[e] Due to extensive spectral overlap, precise chemical shifts cannot be deduced. However, it is very likely that shifts do not deviate from unsubstituted LacNAc-MCO by more than ± 0.01 ppm.

The analysis of the coupling constants for **1–6** suffers greatly from intense spectral overlap in general, and higher order effects present in the Glc*p*NAc moiety in particular. The chemical shifts of H-2, H-3 and H-4 are in many cases nearly identical. Together with the large coupling constants between them, this leads to higher order spectra in this range as well as in the H-1 signal (virtual coupling). As with chemical shifts, changes are most noticeable at the site of substitution, particularly for the 6- and 6′-*O*-sulfates. An increase (+2 Hz) in $J_{5'6a'}$ on sulfation of the 6′-position of LacNAc-MCO appears to suggest a conformational perturbation about the C-5′–C-6′ bond, which contrasts to the reported lack of significant change in C-5–C-6 bond rotamer populations on 6-*O*-sulfation of methyl α-D-galactopyranoside [23].

Taken together these data suggest that mono-*O*-sulfation does not exert a pronounced effect on the $^4C_1$ chair conformation of either residue in LacNAc derivatives and, with the exception of the 3-*O*-sulfate **1**, glycosidic torsion angles also appear to be unaffected.

3. Experimental

General methods.—All reagents and solvents were purified and dried according to standard procedures [26]. All solid reactants for glycosylation reactions were dried in

Table 2
Coupling constants [a] of sulfated LacNAc-MCO and changes relative to unsubstituted LacNAc-MCO [b,c]

$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{5'6a'}$	$J_{5'6b'}$	$J_{6a'b'}$	J_{12}	J_{23}	J_{34}	J_{45}	J_{56a}	J_{56b}	J_{6ab}
LacNAc-MCO													
7.8	9.9	3.4	<1	3.3	[d]	[d]	8.0	[d]	[d]	[d]	2.3	5.2	12.2
LacNAc-MCO 3-*O*-sulfate (**1**)													
7.8	10.0	3.5	<1	[d]	4.0	11.9	8.6	10.2	8.9	[d]	2.3	5.1	12.3
0.0	+0.1	+0.1	0.0	–	–	–	+0.6	–	–	–	0.0	−0.1	+0.1
LacNAc-MCO 6-*O*-sulfate (**2**)													
7.8	10.0	3.4	<1	[d]	[d]	[d]	8.0	[d]	[d]	[d]	1.4	4.1	11.0
0.0	+0.1	0.0	0.0	–	–	–	0.0	–	–	–	−0.9	−1.1	−1.2
LacNAc-MCO 2′-*O*-sulfate (**3**)													
7.8	9.7	3.4	<1	3.2	~5.6	11.1	8.2 [e]	[d]	[d]	[d]	2.3	4.9	12.3
0.0	−0.2	0.0	0.0	−0.1	–	–	+0.2	–	–	–	0.0	−0.3	+0.1
LacNAc-MCO 3′-*O*-sulfate (**4**)													
7.8	9.8	3.3	<1	[d]	[d]	[d]	8.1	[d]	[d]	[d]	2.2	5.2	12.2
0.0	−0.1	+0.1	0.0	–	–	–	+0.1	–	–	–	−0.1	0.0	0.0
LacNAc-MCO 4′-*O*-sulfate (**5**)													
7.8	10.1	3.4	<1	4.0	8.2	11.9	8.1 [e]	[d]	[d]	[d]	2.3	5.2	12.2
0.0	+0.2	0.0	0.0	+0.7	–	–	+0.1	–	–	–	0.0	0.0	0.0
LacNAc-MCO 6′-*O*-sulfate (**6**)													
7.8	10.0	3.4	<1	5.3	6.9	10.9	7.8	10.1	10.1	9.5	2.3	5.2	12.2
0.0	+0.1	0.0	0.0	+2.0	–	–	−0.2	–	–	–	0.0	0.0	0.0

[a] Coupling constants in Hz at 30.0 ± 0.01°C (magnitude values for $J_{6a,b}$).
[b,c] See footnotes b and c, Table 1.
[d] Due to extensive spectral overlap, coupling constants could not be determined with an accuracy necessary for a meaningful comparison.
[e] Result of a first order analysis. The signals clearly show higher order effects (virtual coupling).

vacuo over phosphorus pentoxide for at least 8 h prior to use. Crushed molecular sieves (4 Å, BDH) were heated at 180°C for at least 24 h prior to use. Unless otherwise stated, all reactions were carried out at ambient temperature. In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions, the organic extracts were dried (Na_2SO_4 or $MgSO_4$), and concentrated in vacuo at <40°C (bath). TLC was performed on Silica Gel 60-F_{254} (Merck) with detection by charring following immersion of the TLC plate in a solution of 10% H_2SO_4 in ethanol. Analytical TLC was typically performed using solutions containing varying proportions of toluene–EtOAc or dichloromethane–MeOH as eluants. Column chromatography was performed with Silica Gel 60 (230–400 mesh, Merck). C_{18} Sep-Pak sample preparation cartridges were from Waters Associates (Mississauga, ON). Optical rotations were measured at the sodium D-line with a Perkin–Elmer 241 polarimeter at 22 ± 2°C.

Routine ^{1}H NMR spectra were recorded at 360 MHz (Bruker WM-360) and were referenced with respect to internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD). Only partial NMR data are reported for protected compounds. Other spectral features were in accord with proposed structures.

FAB mass spectra were recorded on an AEI MS-9 spectrometer using Xe as the bombarding gas with 5:1 1,4-dithiothreitol–1,4-dithioerythritol as the matrix.

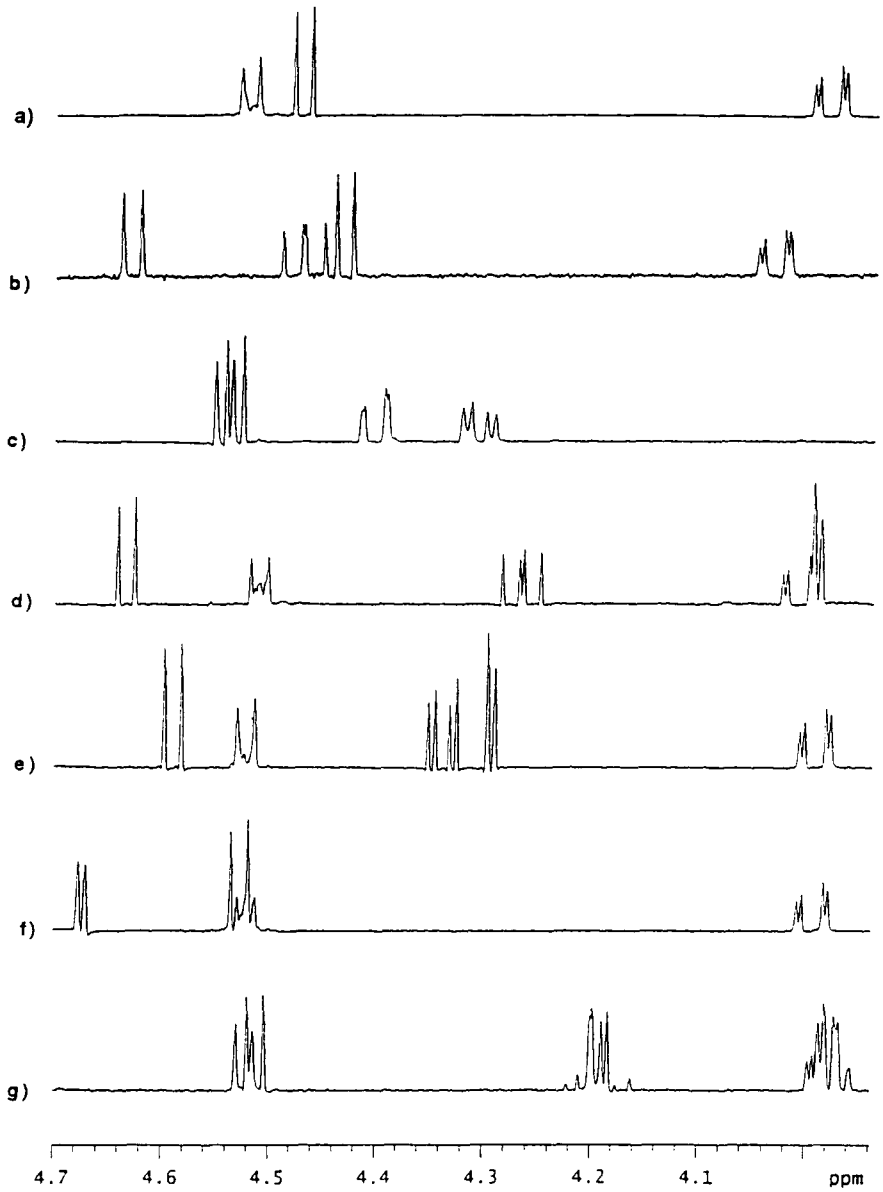


Fig. 1. Characteristic ^{1}H NMR patterns obtained for unsubstituted LacNAc-MCO and mono-*O*-sulfated LacNAc-MCO derivatives **1–6**: (a) unsubstituted LacNAc-MCO; (b) LacNAc-MCO 3-*O*-sulfate (**1**); (c) LacNAc-MCO 6-*O*-sulfate (**2**); (d) LacNAc-MCO 2′-*O*-sulfate (**3**); (e) LacNAc-MCO 3′-*O*-sulfate (**4**); (f) LacNAc-MCO 4′-*O*-sulfate (**5**); (g) LacNAc-MCO 6′-*O*-sulfate (**6**).

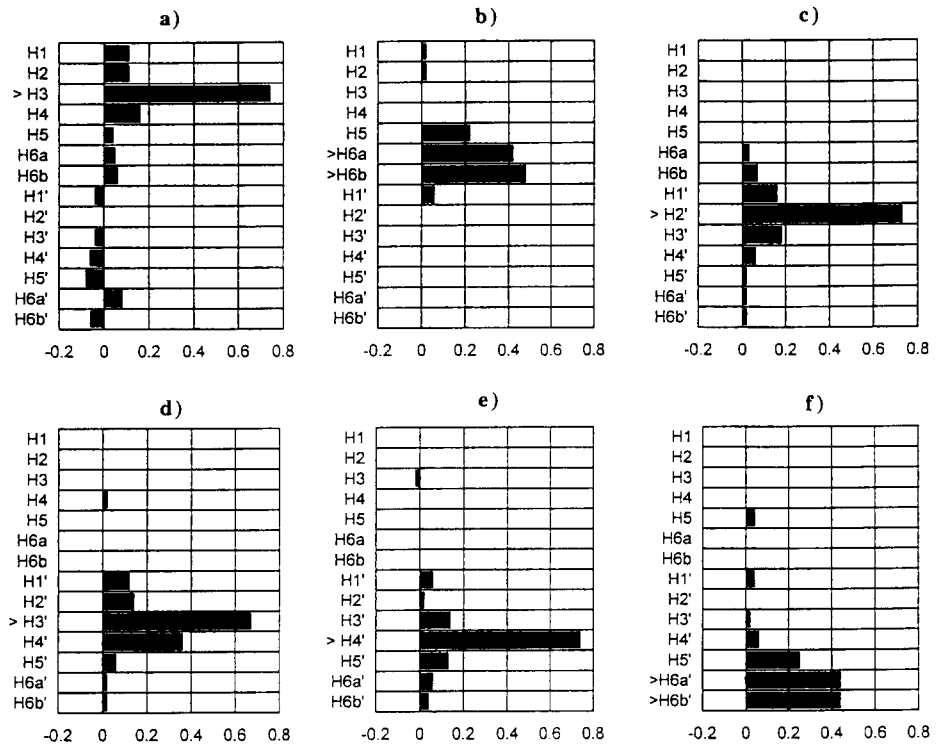


Fig. 2. Proton chemical shift changes induced by sulfate substitution in different positions of LacNAc-MCO. Chemical shift changes are quoted in ppm relative to unsubstituted LacNAc-MCO. Only changes larger than ± 0.01 ppm are shown. No chemical shift changes were observed for aglycone protons or NHAc except $+0.02$ ppm for H-1′ and -0.04 ppm for the acetamide methyl signal in LacNAc-MCO 3-*O*-sulfate (**1**), respectively.

*8-Methoxycarbonyloct-1-yl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**7**).—To a solution of 8-methoxycarbonyloct-1-yl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucoyranoside [18] (3 g, 6.26 mmol) in dry dimethylformamide (50 mL) was added benzyl bromide (12.5 mmol, 2.14 g, 1.49 mL). The mixture was cooled on an ice-bath and sodium hydride was added portionwise (0.25 mol equivalents at a time) until the reaction was judged complete by TLC. The reaction was quenched by the careful addition of iced water (200 mL), and allowed to stir for 10 min. The resulting precipitate was filtered off and dried overnight in vacuo over phosphorus pentoxide. The benzylated product was obtained in $> 90\%$ yield, and was of sufficient purity ($> 95\%$ by TLC) for use without further purification.

A mixture of the benzylated product (3.4 g, 6 mmol), sodium cyanoborohydride (3.77 g, 60 mmol), 3 Å molecular sieves (3 g) and a crystal of methyl orange in anhydrous tetrahydrofuran (100 mL) was stirred at ice-bath temperature under a nitrogen atmosphere. A saturated solution of HCl in ether was added dropwise until the pink colour of the indicator persisted. When TLC showed the reaction to be complete, the reaction

mixture was diluted with dichloromethane (200 mL) and solids were removed by filtration. The filtrate was washed with saturated $NaHCO_3$ solution and water, dried, and concentrated. The resulting oily residue was dissolved in dichloromethane–methanol (1:1, 10 mL) and slowly loaded onto a column of Amberlite MB-1 (50 mL). The resin was washed with dichloromethane–methanol (1:1, 500 mL), and the eluate was evaporated to provide a syrup which was further purified by silica gel chromatography (applied in dichloromethane, eluted with toluene–EtOAc, 2:1 → 1:2) to given compound **7** (2.39 g, 67% over two steps) as a white solid. Analytical data for **7** were in accordance with the literature [18].

*8-Methoxycarbonyloct-1-yl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**8**).—A solution of the protected saccharide **7** (1.2 g, 2.1 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyransoyl bromide (1.73 g, 4.2 mmol) in dichloroethane (20 mL) containing crushed 4 Å molecular sieves (3 g) was stirred under nitrogen for 3 h and cooled to −30°C. Silver trifluoromethanesulfonate (1.62 g, 6.3 mmol) was added and stirring was continued until TLC indicated the reaction was complete. Collidine (8 mmol, 1.06 mL) was added, the mixture was diluted with dichloromethane, and solids were removed by filtration through Celite. The filtrate was washed successively with 2 M HCl, water, and saturated $NaHCO_3$ solution, and dried. Concentration gave a white foamy residue which was dissloved in methanol (30 mL) containing NaOMe (50 mg). After standing for 16 h, the solution was desalted by passage through Dowex AG-50X8 (H^+) (5 mL), and concentrated to dryness. Silica gel chromatography (dichloromethane–methanol, 20:1 → 5:1) gave **8** (1.29 g, 84%) as a crunchy solid, $[\alpha]_D$ +19.7° (*c* 0.29, $CHCl_3$). ^{1}H NMR (CD_3OD): δ 4.39 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1/1′), 4.45 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1/1′), 4.56 (d, 2 H, J_{gem} 11 Hz, 2 × OCH_2Ar), 4.65 (d, 1 H, J_{gem} 11 Hz, OCH_2Ar), 5.07 (d, 1 H, J_{gem} 11 Hz, OCH_2Ar). Anal. Calcd for $C_{38}H_{55}NO_{13}$ (733.86): C, 62.20; H, 7.55; N, 1.91. Found: C, 61.86; H, 7.60; N, 1.91.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(tetra-*O*-acetyl-β-*D*-galactopyranosyl)-2-deoxy-β-*D*-glucopyranoside* (**9**).—Compound **8** (327 mg, 446 μmol) was dissolved in pyridine–dichloromethane 1:1 (10 mL), cooled on an ice bath, and acetic anhydride (2.5 mL) and 4-dimethylaminopyridine (10 mg) were added. The mixture was allowed to warm to room temperature over 4 h and concentrated to dryness. Silica gel chromatography (toluene–EtOAc, 1:1) gave 2-acetamido-4-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (400 mg, quantitative) as an oil. ^{1}H NMR ($CDCl_3$): δ 1.91, 1.97, 2.00, 2.02, 2.12 (5 × CH_3CO), 4.49 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1) 4.73 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.88 (dd, 1 H, $J_{2'3'}$ 10, $J_{3'4'}$ 3.5 Hz, H-3′), 5.10 (dd, 1 H, $J_{1',2'}$, $J_{2'3'}$, H-2′), 5.30 (br d, 1 H, $J_{3'4'}$, $J_{4'5'}$ 3.5 Hz, H-4′), 6.00 (d, 1 H, J_{NH} 9 Hz, NH).

A solution of the above oil (240 mg, 265 μmol) in methanol (15 mL) containing palladium (5% on charcoal; 100 mg) was subjected to hydrogenation until TLC showed the reaction to be complete (3 h). The suspension was filtered through Celite and concentrated to an oil. Silica gel chromatography (dichloromethane–MeOH, 40:1) then gave **9** (308 mg, 96%) as a white solid, $[\alpha]_D$ + 5.6° (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 6 H, 2 × CH_3CO), 2.01, 2.07, 2.12 (3 × CH_3CO), 4.84 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.69 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1/1′), 5.00 (dd, 1 H, $J_{2'3'}$ 9, $J_{3'4'}$ 3.5

Hz, H-3′), 5.03 (br d, 1 H, $J_{3'4'}$, $J_{4'5'}$ 3.5 Hz, H-4′), 5.15 (dd, 1 H, $J_{1'2'}$, $J_{2'3'}$, H-2′), 6.03 (d, 1 H, J_{NH} 9 Hz, NH). Anal. Calcd for $C_{32}H_{51}NO_{17}$ (721.76): C, 53.25; H, 7.12; N,1.94. Found: C, 52.94; H, 7.12; N, 1.97.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(tetra-*O*-acetyl-β-*D*-galactopyranosyl)-6-*O*-benzoyl-2-deoxy-β-*D*-glucopyranoside* (**10**).—Compound **9** (137 mg, 190 μmol) was dissolved in pyridine–dichloromethane 1:5 (6 mL), cooled on an ice bath, and benzoyl chloride (28 μl, 238 μmol) was added. The mixture was allowed to warm to room temperature, quenched with methanol (100 μL), and concentrated to dryness. Silica gel chromatography (EtOAc → EtOAc–MeOH, 10:1) gave **10** (137 mg, 87%) as a foam, which was converted to a crunchy solid on addition and evaporation of diethyl ether. $[\alpha]_D$ +9.1° (*c* 0.22, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.93, 1.97, 2.03, 2.04, 2.13 (5 × CH_3CO), 4.58 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.75 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1/1′), 4.92 (dd, 1 H, $J_{2'3'}$ 9, $J_{3'4'}$ 3.5 Hz, H-3′), 5.18 (dd, H 1, $J_{1',2'}$, $J_{2',3'}$, H-2′), 5.33 (br d, 1 H, $J_{3'4'}$, $J_{4',5'}$ 3.5 Hz, H-4′), 5.96 (br d, 1 H, J_{NH} 9 Hz, NH), 7.40–8.05 (m, 5 H, Ar). Anal. Calcd for $C_{39}H_{55}NO_{18}$ (825.87): C, 56.72; H, 6.71; N, 1.70. Found: C, 56.75; H, 6.49; N, 1.69.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**11**).—A solution of compound **7** (200 mg, 350 μmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl bromide [20] (275 mg, 494 μmol) in dichloroethane (10 mL) containing crushed 4 Å molecular sieves (1.5 g) was stirred under nitrogen for 3 h and cooled to −30°C. Silver trifluoromethanesulfonate (162 mg, 630 μmol) was added and stirring was continued until TLC indicated the reaction was complete. Collidine (1 mmol, 85 μL) was added, the mixture was diluted with dichloromethane, and solids were removed by filtration through Celite. The filtrate was washed successively with 2 M HCl, water, and saturated $NaHCO_3$ solution, and dried. Concentration gave an oil which was subjected to purification by silica gel chromatography (hexane–EtOAc, 4:1 → 1:1) to give **11** (260 mg, 70%) as a colourless oil, $[\alpha]_D$ −15.0° (*c* 0.12, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.01, 2.07 (2 × CH_3CO), 5.32 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2′), 6.22 (br d, 1 H, J_{NH} 9 Hz, NH). Anal. Calcd for $C_{61}H_{75}NO_{14}.H_2O$ (1064.29): C, 68.84; H, 7.29; N, 1.32. Found: C, 69.07; H, 7.21; N, 1.40. FABMS Calcd for $[C_{61}H_{75}NO_{14}]H^+$: 1046.5266. Measured M + H$^+$ 1046.5277.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**12**).—A solution of compound **11** (280 mg, 268 mmol) in methanol (10 mL) containing sodium methoxide (20 mg) was stirred at room temperature overnight and neutralized by passage through a column of Dowex AG-50X8 (H$^+$) (2 mL). The eluate was concentrated to dryness and the resulting residue was purified by silica gel chromatography (toluene–EtOAc, 3:1 → 2:1). Compound **12** (255 mg, 95%) was obtained as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, CH_3CO), 4.50 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1/1′), 4.73 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1/1′). The ^{1}H NMR spectrum of **12** was similar to that of **11** except for the H-2′ signal which had moved upfield by approx. 1 ppm.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**13**).—A solution of partially protected disaccharide **8** (344 mg, 469 μmol) in acetone (15 mL) containing

p-toluenesulfonic acid (15 mg) was stirred at room temperature overnight. The reaction was quenched by addition of saturated $NaHCO_3$ solution (30 mL) and stirred for 10 min. The mixture was diluted with dichloromethane (100 mL), and the organic phase was separated, washed with saturated NaCl solution, and dried. Concentration gave the crude isopropylidene acetal as a foam which was used directly in the next step.

The above foam was dissolved in dimethylformamide (20 mL) and benzyl bromide was added (2.35 mmol, 280 μL). The solution was stirred on an ice-bath whilst sodium hydride was added portionwise (0.25 mol equivalents at a time) until TLC indicated the reaction to be complete. The reaction was quenched by the careful addition of methanol, and solvent removed in vacuo. The resulting residue was partitioned between dichloromethane and dilute HCl, the organic phase was separated, washed with water and saturated $NaHCO_3$ solution, and dried. Concentration gave an oil which was purifed by silica gel chromatography (toluene–ether, 3:1 → 1:1) to give the 3′,4′-acetal **13** (336 mg, 75%) as an oil, R_f 0.8 (toluene–EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.37, 1.40 (2 × acetal CH_3), 1.86 (s, 1 H, CH_3CO), 4.44 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.91 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 5.95 (br d, 1 H, J_{NH} 9 Hz, NH).

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**14**).—To a stirred solution of partially protected disaccharide **8** (240 mg, 327 μmol) in acetonitrile (15 mL) containing 2,2-dimethoxypropane (655 μmol, 80 μL) was added *p*-toluenesulfonic acid (5 mg). The reaction was monitored by TLC, and when no starting material remained (approx. 15 min) triethylamine (100 μL) was added and the mixture was evaporated to give an oily residue. The residue was partitioned between dichloromethane and water, and the organic extract was dried, concentrated, and used directly in the next step.

The above oil was benzylated and processed as described in the preparation of the isomeric acetal **13.** Silica gel chromatography (toluene–EtOAc, 3:1 → 1:1) gave the 4′,6′-acetal **14** (220 mg, 70%) as a colourless oil, R_f 0.4 (toluene–EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.40, 1.45 (2 × acetal CH_3), 1.86 (s, 1 H, CH_3CO), 2.88 (br s, 1 H, H-5′), 4.48 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.90 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 5.06 (d, 1 H, J_{gem} 12 Hz, CH_2Ar), 5.88 (br d, 1 H, J_{NH} 9 Hz, NH).

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**15**).—A solution of 3′,4′-isopropylidene acetal **13** (180 mg, 189 μmol) in 80% aqueous acetic acid (10 mL) was heated at 80°C for 2 h, cooled to room temperature, and evaporated to dryness. Residual acetic acid was removed by azeotropic distillation with toluene. Silica gel chromatography (toluene–EtOAc, 1:1 → 1:2) gave a colourless oil which on addition and evaporation of ether gave the 3′,4′-diol **15** (148 mg, 86%) as a solid, $[\alpha]_D$ +16.0° (*c* 0.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, CH_3CO), 4.44 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.90 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 5.90 (br d, 1 H, J_{NH} 9 Hz, NH). Anal. Calcd for $C_{52}H_{67}NO_{13}$ (914.11): C, 68.33; H, 7.39; N, 1.53. Found: C, 68.31; H, 7.53; N, 1.60.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**16**).—A solution of 3′,4′-diol **15** (125 mg, 137 μmol) in toluene (20 mL) containing trimethylorthoacetate (300

μL, 2.36 mmol) and *p*-toluenesulfonic acid (10 mg) was stirred at room temperature for 1 h, and the reaction was quenched by the addition of triethylamine (200 μL). The solution was concentrated to dryness and the residue was partitioned between dichloromethane and water. The organic phase was separated, washed with water, dried, and concentrated to give the mixed 3′,4′-methyl orthoacetates as an oil which was used without further purification.

The above oil was dissolved in 80% aqueous acetic acid (10 mL) and stirred for 10 min at 40°C. The solution was then cooled to room temperature and evaporated to dryness. Residual acetic acid was removed by azeotropic distillation with toluene. Silica gel chromatography (toluene–EtOAc, 3:1 → 2:1) gave an oil which on addition and evaporation of ether gave the 4′-*O*-acetate **16** (130 mg, quantitative) as a solid, $[\alpha]_D$ −5.5° (*c* 0.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.90, 2.01 (2 × CH_3CO), 4.47 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.96 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 5.32 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 3.5 Hz, H-4′), 5.93 (br d, 1 H, J_{NH} 9 Hz, NH). Anal. Calcd for $C_{54}H_{69}NO_{14}$ (956.15): C, 67.84; H, 7.27; N, 1.47. Found: C, 67.69; H, 7.35; N, 1.53.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(2,3-di-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**17**).—A solution of 4′,6′-isopropylidene acetal **14** (200 mg, 210 μmol) in 80% aqueous acetic acid (5 mL) was heated at 80°C for 2 h, cooled to room temperature, and evaporated to dryness. Residual acetic acid was removed by azeotropic distillation with toluene. Silica gel chromatography (toluene–EtOAc, 1:1 → 1:2) gave a colourless oil which on addition and evaporation of ether gave 4′,6′-diol **15** (165 mg, 85%) as a solid, $[\alpha]_D$ +18.1° (*c* 0.21, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3 H, CH_3CO), 4.41 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.91 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 6.10 (br d, 1 H, J_{NH} 9 Hz, NH). Anal. Calcd for $C_{52}H_{67}NO_{13}$ (914.11): C, 68.33; H, 7.39; N, 1.53. Found: C, 68.07; H, 7.23; N, 1.54.

*8-Methoxycarbonyloct-1-yl 2-acetamido-4-*O*-(6-O-benzoyl-2,3-di-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**18**).—Compound **17** (96 mg, 105 μmol) was dissolved in pyridine–dichloromethane 1:5 (6 mL), cooled on an ice-bath, and benzoyl chloride (36 μL, 316 μmol) was added. The reaction was closely monitored by TLC as it warmed slowly to warm to room temperature. When the reaction was complete, methanol (100 μL) was added and the mixture was concentrated to dryness. Silica gel chromotagraphy (toluene–EtOAc, 4:1 → 2:1) gave an oil which on addition and evaporation of ether gave the 6′-*O*-benzoate **18** (78 mg, 73%) as a crunchy solid, $[\alpha]_D$ +16.2° (*c* 0.13, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 1.87 (s, 3 H, CH_3CO), 4.44 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 4.91 (d, 1 H, $J_{1,2}$ 8 Hz, H-1/1′), 5.72 (br d, 1 H, J_{NH} 9 Hz, NH), 7.50–8.10 (m, 5 H, benzoyl). Anal. Calcd for $C_{59}H_{71}NO_{14}$ (1018.22): C, 69.60; H, 7.03; N, 1.38. Found: C, 69.15; H, 6.98; N, 1.46.

General procedure for the sulfation of partially protected disaccharides.—Stirred solutions of the partially protected disaccharides (approx. 40 μmol) in anhydrous pyridine (4 mL) containing sulfur trioxide–trimethylamine complex [19] (approx. 150 μmol) were kept at room temperature overnight. Reactions were quenched by the addition of methanol (100 μL), and the mixtures were evaporated to dryness. The resulting residues were subjected to silica gel chromatography (dichloromethane–MeOH, 10:1 containing 0.2% v/v pyridine) to give the mono-*O*-sulfates as a white solid (80–95%).

In addition to the desired mono-*O*-sulfates, sulfation of diols **9** and **17** gave rise to small amounts (< 5%) of polar materials, presumably di-*O*-sulfates, which were not characterized.

General procedure for the deprotection of sulfated disaccharides.—Sulfated disaccharides were deprotected by treatment with NaOMe and/or by hydrogenation as necessary.

Deacylation. Sulfated disaccharides (approx. 40 μmol) in anhydrous methanol (2 mL) containing NaOMe (approx. 150 μmol) were kept at room temperature until TLC indicated deacylation to be complete (typically 8–12 h). The mixture was neutralized by passage through Dowex AG-50X8 (H^+) (5 mL), and concentrated to dryness.

Hydrogenation. Sulfated disaccharides (approx. 40 μmol) in anhydrous MeOH (10 mL) containing palladium (10% on charcoal; 100 mg) were stirrred under a positive pressure of hydrogen until hydrogenolysis was complete (2–4 days). The mixture was filtered through MeOH–washed Celite and concentrated to dryness.

The deprotected mono-sulfates were purified by anion-exchange (DEAE Sephadex A-50) and reverse-phase (Sep-Pak C_{18}) chromatography as described [27] for related sialylated sugars.

Deprotection and purification as described gave the LacNAc-MCO mono-*O*-sulfates **1**–**6** in 70–85% yield. All gave proton NMR (Tables 1 and 2) and FABMS data consistent with their proposed structures.

FABMS: % intensities are reported relative to the major ion = 100%. LacNAc-MCO 3-*O*-sulfate **1**: 678 (40%), 576 (67%); LacNAc-MCO 6-*O*-sulfate **2**: 678 (39%), 576 (43%); LacNAc-MCO 2′-*O*-sulfate **3**: 678 (83%), 576 (14%); LacNAc-MCO 3′-*O*-sulfate **4**: 678 (53%), 576 (67%); LacNAc-MCO 4′-*O*-sulfate **5**: 678 (56%), 576 (47%); LacNAc-MCO 6′-*O*-sulfate **6**: 678 (97%), 576 (100%).

*^{1}H NMR analysis of LacNAc-MCO mono-*O*-sulfates* **1**–**6**.—All NMR spectra were recorded on a Varian Unity 500 spectrometer with a 5 mm Varian *z*-gradient inverse-detection, triple-resonance probe. The sample concentrations were between 2 and 4 mM in D_2O. All spectra were recorded under temperature controlled conditions at 30.0 ± 0.01°C. For data acquisition and processing VNMR software version 4.3A was used. Gradient-enhanced two-dimensional experiments [28,29] were recorded non-spinning in absolute-value mode with a sweep width of 2500 Hz in both dimensions and 4K data points in F2 (no zero-filling) and 512 (zero-filled to 1K) data points in F1, resulting in digital resolutions of 1.2 and 4.8 Hz/Pt, respectively. All pulses were calibrated 90° pulses of 7.5 μs duration. Mixing times in the GTOCSY [30] experiments varied from 100 to 250 ms at a spin lock field strength of 5 kHz. One to five transients per t_1-increment were recorded in GCOSY [30] experiments (2–10 in GTOCSY) to account for differences in sample concentrations. All gradients were rectangular in shape, applied in the *z*-direction and of the following strength and duration: GCOSY: 3.0 G/cm for 2 ms, GTOCSY: 4.0 G/cm for 2 ms with gradient rise and fall times of 10 μs in all cases. Prior to Fourier transformation the FIDs were multiplied by unshifted sine-bell square functions of width $t_2/2$ and $t_1/2$, respectively.

The chemical shifts of H-2, H-3 and H-4 of the glucose-NAc moiety in unsubstituted LacNAc could only be determined in a two-dimensional HMQC-TOCSY [31] experiment recorded with a short 20 ms mixing time; 64 scans per t_1-increment with 4K

(zero-filled to 8K) data points in F2 and 256 (zero-filled to 512) in F1 were acquired in 17 h. Direct and relayed responses were recorded with a different phase [32,33] to facilitate data analysis.

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