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Synthesis and utility of sulfated chromogenic carbohydrate model substrates for measuring activities of mucin-desulfating enzymes

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This paper is dedicated to Professor Joachim Thiem, Institut für Organische Chemie, Universität Hamburg, to mark his 60th birthday

Abstract

A chromogenic substrate, 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 6-sodium sulfate was synthesized and used in combination with β-*N*-acetylhexosaminidase for detection of the sulfatase, MdsA, by release of 4-nitrophenol. MdsA was originally isolated from the bacterium *Prevotella* strain RS2 and is believed to be involved in desulfation of sulfomucins, major components of the mucus barrier protecting the human colon surface. The *exo* nature of the MdsA sulfatase was indicated by its inability to de-esterify the disaccharide 4-nitrophenyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside 6-sodium sulfate. This latter compound was prepared from monosaccharide precursors by two different methods, the shorter requiring just six steps from 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and giving an overall yield of 26.4%. The syntheses of 4-nitrophenyl β-D-galactopyranoside 3-triethylammonium sulfate and 6-triethylammonium sulfate and their use in combination with β-galactosidase as chromogenic substrates for detecting *Bacteroides fragilis* sulfatases with different specificities was also demonstrated.

Keywords: Chromogenic sugar sulfates; Disaccharide sulfate; Monosaccharide sulfate; *exo*-Sulfatase; Sulfomucin

1. Introduction

The gastrointestinal (GI) tract is covered by a mucus barrier at its mucosal surface, and the main structural components of the barrier are the glycoprotein 'mucins', the carbohydrate portions of which are oligosaccharides containing D-galactose, L-fucose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and *N*-acetylneuraminic acid. Importantly, some of these oligosaccharides may carry sulfate ester groups. Mucins containing higher levels of sulfate are generally referred to as sulfomucins and are secreted in specific regions of the GI tract—particularly in those regions heavily colonized by bacteria, such as the mouth and colon.[1] Desulfation by bacterial enzymes is thought to be one of the rate-limiting steps in the degradation of the sulfomucin barrier.[1–4]

There are different ways in which the mucin oligosaccharide chains can be sulfated, three of the major structural motifs being 2-acetamido-2-deoxy-D-glucopyranose 6-sulfate (GlcNAc 6-OSO_3H) (internal and terminal), D-galactopyranose 6-sulfate (Gal 6-OSO_3H) (terminal) and D-galactopyranose 3-sulfate (Gal 3-OSO_3H) (terminal).[5,6] Enzymes that desulfate model substrates containing these moieties include a *Prevotella* strain RS2 enzyme called MdsA. It can partially desulfate rat gastric mucin and is specific for GlcNAc 6-OSO_3H residues.[7,8] It is not known whether MdsA acts on external and/or internal GlcNAc 6-OSO_3H units of the mucin oligosaccharide chains, both of which are

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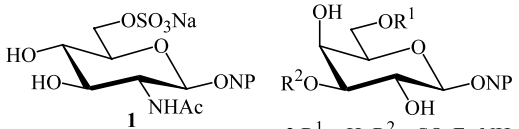
found.[9,10] Internal GlcNAc 6-OSO_3H entities generally have a β-D-galactopyranosyl unit attached at O-3 or O-4.[11] For sulfated galactose residues, the galactose-3-sulfatase(s) and galactose-6-sulfatase(s) from *Bacteroides fragilis* can de-esterify appropriate model substrates.[12]

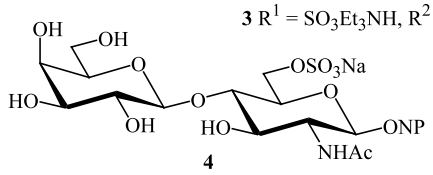
This paper describes (i) chemical syntheses of the 4-nitrophenyl β-D-glycopyranosides of GlcNAc 6-OSO_3Na and β-D-Gal-(1→4)-GlcNAc 6-OSO_3Na (**1** and **4**, respectively) and potential use of these substrates by the MdsA enzyme; and (ii) syntheses of the analogous β-D-glycopyranosides of Gal 3-OSO_3Et_3NH and Gal 6-OSO_3Et_3NH (**2** and **3**, respectively) and their cleavage by *B. fragilis* sulfatases.



1

2 R^1 = H, R^2 = SO_3Et_3NH

3 R^1 = SO_3Et_3NH, R^2 = H

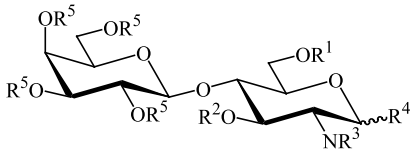


4

NP = 4-nitrophenyl

2. Results and discussion

Syntheses of substrates **1**–**4**.—Compound **1**, which is commercially available,[13] has been synthesized previously by direct sulfation of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (GlcNAc–ONP) followed by purification on ion exchange resin.[14] In the present work it was made by standard methods from the known 4-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside.[15]



	R^1	R^2	R^3	R^4	R^5
7	PMB	H	TCP	SPh (β)	Ac
8	PMB	Ac	TCP	SPh (β)	Ac
9	PMB	Ac	H,Ac	SPh (β)	Ac
10	H	Ac	H,Ac	SPh (β)	Ac
11	TBDMS	Ac	H,Ac	SPh (β)	Ac
12	TBDMS	Ac	H,Ac	Cl (α)	Ac
13	TBDMS	Ac	H,Ac	ONP (β)	Ac
14	H	Ac	H,Ac	ONP (β)	Ac
15	SO_3Na	Ac	H,Ac	ONP (β)	Ac
16	H	H	H,Ac	ONP (β)	H

7→8→9→10→11→12→13→14→15→4

↓

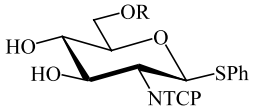
16

Scheme 1.

For the preparation of the D-galactose 3-sulfate-based substrate **2**, the known 4-nitrophenyl 4,6-*O*-isopropylidene-β-D-galactopyranoside,[16] made by the kinetically controlled acetonation of 4-nitrophenyl β-D-galactopyranoside (Gal-ONP), was used. The acetal was also converted into the thermodynamically preferred 3,4-isopropylidene isomer[17] for the preparation of the D-galactoside 6-sulfate **3**. In both cases the tin activation-sulfation technique[18] was used to introduce the sulfate esters, and in each instance the protecting acetal group was removed by selective, acid-catalysed hydrolysis. The yield of sulfates **2** and **3** were 39 and 41%, respectively, from the glycoside acetals. Alternatively, 6-sulfate **3** was obtained in 44% yield by direct sulfation of Gal-ONP. Compound **2** has also previously been prepared by direct stannylenation-sulfation of Gal-ONP (77%), and in the same work ester **3** was made from the glycoside in $>80\%$ yield by selective enzymatic de-*O*-sulfation of 4-nitrophenyl β-D-galactopyranoside 3,6-disulfate,[19,20] which is the major product of direct selective disulfation.

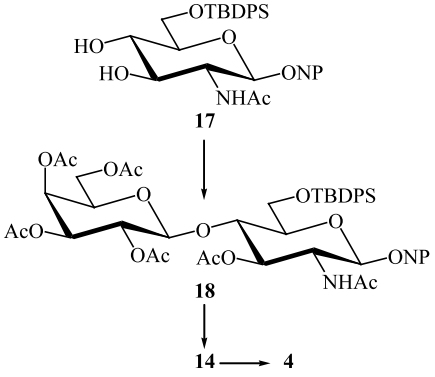
For reasons that will be alluded to below, a successful route to the lactosamine-based **4** appeared to lie in forming the aryl glycosidic bond at a late stage in the synthesis, and glycosyl chloride **12** was selected as a key precursor (Scheme 1)—particularly since 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[21] has been efficiently converted into its β-linked 4-nitrophenyl glycoside by treatment with Amberlyst A-26 (4-nitrophenoxide) resin.[22] Recently, Lay et al.[23] described the synthesis of thioglycoside **5** and found it to be subject to highly selective glycosylation at O-4. We therefore followed this guidance and prepared the corresponding 6-*O*-(4-methoxybenzyl) (PMB) ether **6** from phenyl 2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside.[23] In this work the latter compound was prepared by de-esterifying the corresponding 3,4,6-triacetate using Ellervik and Magnusson's method for deacetylating tetrachlorophthalimido derivatives.[24] Glycosylation of compound **6** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[25,26] in CH_2Cl_2 with boron trifluoride etherate ($BF_3{\cdot}OEt_2$) as catalyst gave the β-(1→4)-linked disaccharide **7** in 59% yield. No α-linked or O-3-bonded isomers were detected, but a slight amount of PMB ether bond cleavage occurred. Attempts to form a 4-nitrophenyl glycoside from the 3-acetate **8** of compound **7** by treatment with 4-nitrophenol in the presence of the thiophilic promoters *N*-iodosuccinimide (NIS)–triflic acid, NIS–trimethylsilyl triflate, NIS–silver triflate (AgOTf) or methylsulfenyl bromide[27]–AgOTf led mainly to a rapid loss of

the PMB ether group notwithstanding the finding that such ethers can survive glycosylation conditions involving the use of thiophilic activators.[28] When the *N*-tetrachlorophthaloyl group of compound **8** was replaced by *N*-acetyl upon treatment with 1,2-diaminoethane, then acetic anhydride and pyridine, and the product **9** was tested with bromine[29,30] or chlorine[31] in dichloromethane solution in order to form the glycosyl bromide or chloride, some loss of the PMB ether group was again observed. Removal of the ether protecting group of **9** by treatment with trifluoroacetic acid (TFA) in the presence of thiophenol gave crude alcohol **10**, which was silylated to give the fully protected **11** conversion of which on treatment with chlorine in dichloromethane proceeded rapidly and efficiently to give the required α-D-chloride **12** (^{1}H NMR, $CDCl_3$, δ 6.17, d, $J_{1,2}$ 3.7 Hz, H-1).

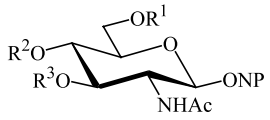


5 R = Bn
6 R = PMB

TCP = tetrachlorophthaloyl



Scheme 2.



	R^1	R^2	R^3
19	-4-MeO-C_6H_4CH-		TBDMS
20	PMB	H	TBDMS
21	-4-MeO-C_6H_4CH-		All
22	H	H	All
23	TBDMS	H	All
24	TBDMS	H	H
25	TBDMS	H	AllOCO
26	TBDMS	All	H
27	TBDMS	All	All

19→20; 21→22→23; 24→25→23+26+27

Scheme 3.

Glycosylation of 4-nitrophenol with crude chloride **12** under phase transfer-catalysed conditions afforded β-D-lactosaminide **13** (^{1}H NMR, $CDCl_3$, δ 5.21, d, $J_{1,2}$ 6.1 Hz, H-1) in 56% yield. Removal of the 6-*O*-silyl ether was accomplished in high yield with HF–pyridine to produce **14** which was sulfated to give **15** and de-*O*-acetylated under standard conditions to afford **4** (^{1}H NMR D_2O, δ 4.49, d, H-6_a; 4.35, dd, H-6_b; ^{13}C NMR D_2O, δ 67.5, C-6). Compound **14** was also de-*O*-acetylated to give the known **16**,[22,32] the desulfated analogue of **4**, which was used as a positive standard in the *Prevotella* sulfatase assays.

The preparation of sulfate **4** from phenyl 2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside by this route required 11 steps and proceeded in an overall yield of just 2.8%. The potentially shorter route to glycosylating agent **12** using the 6-*O*-TBDMS ether analogue of **6** as starting material was not viable; treatment of the *O*-unprotected thioglycoside under standard silylating conditions (TBDMSCl–imidazole–DMF, TBDMSCl–pyridine or TBDMSOTf–2,6-dimethylpyridine–CH_2Cl_2) gave none of the desired product—presumably because of the base sensitivity of the *N*-protecting group.

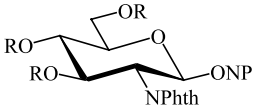
Taking advantage of the high selectivity of glycosylation at O-4 of 6-*O*-TBDPS-substituted-*N*-acetylglucosaminide derivatives, and in particular the specific access it affords to the *N*-acetyllactosamine framework in a new, simple and efficient way,[33] we developed an abbreviated route to compound **14** (Scheme 2). When 4-nitrophenyl glucosaminide **17**, made by selective substitution of GlcNAc–ONP,[34] was glycosylated with tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[25,26] with $BF_3{\cdot}OEt_2$ as promotor it gave, following acetylation, disaccharide derivative **18** (50% from the triol). Desilylation of **18** with HF–Py afforded the previously prepared **14**, the cleavage of the bulkier TBDPS ether however requiring substantially longer time than did that of the corresponding TBDMS ether **13**. By this abbreviated route, sulfate **4** was obtained in six steps from GlcNAc–ONP in 26.4% overall yield.

In the course of the development of the above routes to compound **4**, various others were explored briefly and several points of chemical significance were noted. Compound **20**, made by highly selective reductive ring opening of the acetal group of compound **19** (Scheme 3), was tested as a glycosyl acceptor, but it failed to react with tetra-*O*-benzoyl-α-D-galactopyranosyl bromide in the presence of AgOTf as promoter or with the corresponding acetylated trichoroacetimidate together with $BF_3{\cdot}OEt_2$. Likewise allyl ether **23**, made by the sequence 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[35] **→21→22→23** did not react under glycosylating conditions with tetra-*O*-acetyl-α-D-galactopyranosyl bromide or trichloroacetimidate. Attempts to make the allyl ether

23 from the corresponding 3-allyl carbonate **25** (which was made from the 3,4-diol **24**) by heating in THF solution with $Pd(PPh_3)_4$[36] were unsatisfactory, the required compound **23** being obtained in only 19% yield together with the 4-substituted allyl isomer **26** (24%) and the 2,3-diallyl ether **27** (14%).

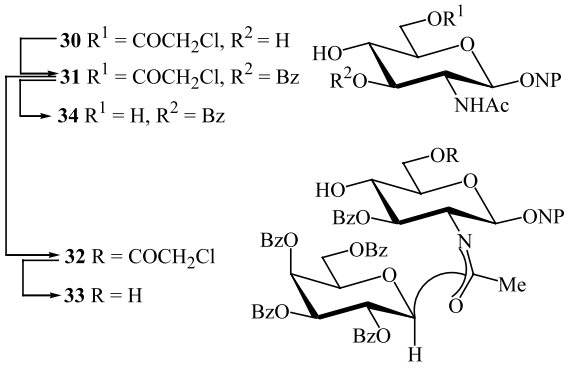
Although 2-acetamido-2-deoxy-D-glucopyranosides having ester groups at C-3 can be difficult to glycosylate at O-4, sometimes requiring specific adaptations for efficient reaction,[33,37] the occasional report of such direct substitution has appeared.[38] On the other hand, analogues with ether groups at C-3 can be efficiently glycosylated[33,39] and the lack of response of compounds **20** and **23** to glycosylation must presumably be ascribed to the combined bulks of the protecting groups on O-3 and O-6.

Since 6-*O*-substituted-2-deoxy-2-phthalimido-D-glucopyranosides can be glycosylated preferentially at O-4,[33,40–42] this approach was also considered for the preparation of compound **4**. 4-Nitrophenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**28**) was made from the corresponding glycosyl acetate,[43] but Zemplén de-esterification to give triol **29** was inefficient, suggesting that the base-catalysed transformation of the *N*-phthaloyl group to *N*-acetyl, which this approach to compound **4** would require, could prove problematical.



28 R = Ac
29 R = H

When the 3-benzoate **31**, made by stannylation–chloroacetylation of GlcNAc–ONP[34] to give the 6-ester **30** (Scheme 4) followed by 3-selective benzoylation, was glycosylated, reaction did not occur at the C-4 hydroxyl group as expected, but at the ambident acetamido group, apparently in like manner to that observed on glycosylation of benzyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide.[44] The product in this latter case was assigned the structure with the introduced glucosyl substituent bonded to the amide nitrogen atom on the grounds of the absence and presence of exchangeable NH and OH resonances, respectively, in the ^{1}H NMR spectrum. This evidence, however, does not preclude the possibility that glycosylation had occurred at the oxygen atom of the enolic form of the acetamido group. Similarly, in the present work it was concluded that the acetamido group of chloroacetate **31** had undergone glycosylation to give the incompletely characterized compound **32**. NMR evidence of its dechloroacetylated derivative **33** did not permit clear distinction between the O- and N-linked possibilities, but chemical evidence given below favours the former.



Scheme 4.

During work with the primary chloroacetates **30**–**32**, their chloroacetyl groups were cleaved when small samples were either left in methanol at ambient temperature overnight or heated under reflux briefly in this solvent. This may represent an advantageous way of removing this protecting group which is normally cleaved with the aid of acid or base catalysts or with such reagents as thiourea.[45] By the uncatalysed method, methanolysis of chloroacetate **31** gave the known[46] 4,6-diol **34**, and analogously **32** produced **33** (Scheme 4). In the latter case, however, monosaccharide derivative **31** was sometimes isolated instead of the disaccharide diol **33** indicating the reactive nature of the inter-sugar linkage in **32**. This reactivity was also reflected by failed attempts to de-esterify compound **33** under Zemplén conditions. In this case, TLC and ^{1}H NMR analysis of the crude products indicated the presence of GlcNAc–ONP and galactose. On the basis of the fragility of the inter-unit bond of compound **32** the acetimidate structure with glycosylation on the amido oxygen atom is favoured. Attempts to explore the nature of the inter-unit bond by crystallographic methods were frustrated by 'twinning' of the crystals of compound **33**.

Substrate specificity of recombinant Prevotella *sulfatase MdsA*[7,8].—The substrate specificity of MdsA was tested using D-glucose 6-sodium sulfate (Glc 6-OSO_3Na) and sulfates **1** and **4** together with and without added auxiliary glycosidases (*Aspergillus oryzae* extract, Sigma G 7138, 1993 catalogue, which has β-galactosidase and β-*N*-acetylhexosaminidase activities). It was expected that enzymic desulfation of **1** would render it a substrate for the β-*N*-acetylhexosaminidase with the consequent release of 4-nitrophenol. Similarly, enzymic desulfation of **4** and sequential galactosidase and β-*N*-acetylhexosaminidase action should release 4-nitrophenol. From the results given in Table 1 and those obtained in control experiments, the following conclusions were drawn about the sulfatase specificity: (i) there was a tenfold difference between the rates of

Table 1
Reactions of D-glucose 6-sodium sulfate (16 mM), 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 6-sodium sulfate (**1**) (1 mM) and 4-nitrophenyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside 6-sodium sulfate (**4**) (1 mM) catalysed by MdsA sulfatase

Substrate	Sulfatase reaction time (min)	Glycosidase added [a]	Product quantified	Products observed [b]	Specific activity [c] ± 20
Glc 6-SO_3Na	30	no	glucose	ND	2511
1	20	yes	nitrophenol	ND	267
1	20	no	nitrophenol	ND	0
1	60	yes	nitrophenol	nitrophenol, GlcNAc, GlcNAc–ONP (trace)	358
1	60	no	nitrophenol	GlcNAc–ONP	6
4	20	yes	nitrophenol	ND	0
4	20	no	nitrophenol	ND	0
4	60	yes	nitrophenol	Gal (trace)	0
4	60	no	nitrophenol	ND	0

[a] *Asperigillus oryzae* extract (Sigma G7138, 1993 catalogue) containing β-galactosidase and β-*N*-acetylhexosaminidase activities. For sulfate **1**, incubations were carried out in the presence of the sulfatase and glycosidases. For compound **4**, the substrate was incubated first with the sulfatase, the reaction was terminated, and the glycosidases were added prior to a second incubation.
[b] By paper chromatography after 60 min reaction; ND, not determined; GlcNAc–ONP, 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside; Gal, D-galactose.
[c] nmol product min^{-1} (mg sulfatase protein)$^{-1}$.

product formation from Glc 6-OSO_3Na and ester **1**, but as the concentrations of substrates and reaction conditions were different, we cannot speculate on the reason(s) for the variation; (ii) the 6-ester (**1**) was not a substrate for the auxiliary glycosidases, but following its desulfation by MdsA, it was cleaved by this enzyme preparation to give GlcNAc, 4-nitrophenol, and sulfate. The reaction catalysed by the sulfatase precedes glycosidic bond cleavage (although the relative rates of the two reactions were not determined), since the action of MdsA on ester **1** without the auxiliary glycosidases was shown to give GlcNAc–ONP (by paper chromatography), and only a trivial amount of free 4-nitrophenol, if any; and (iii) disaccharide sulfate **4** did not react in the presence of the sulfatase to give the unesterified compound **16** since, after attempted desulfation of **4**, the auxiliary glycosidases failed to liberate 4-nitrophenol. The auxiliary glycosidases by themselves did release 4-nitrophenol, Gal and GlcNAc from compound **16**. To confirm that compound **4** did not react in the presence of the sulfatase, the concentrations of the auxiliary glycosidases were increased up to tenfold without change to the initial observations (data not shown). These results, obtained largely by quantitative colorimetric methods, were confirmed by paper chromatographic examination of the reaction products.

The assay results using glycoside esters **1** and **4** (Table 1) indicate that the sulfatase MdsA desulfates the GlcNAc 6-OSO_3Na moiety when it is terminal, but not when it is substituted at the 4 position by β-D-Gal, i.e., when it is internal within an oligosaccharide chain. Thus, the enzyme appears to be an '*exo*-sulfatase'.

Substrate specificity of the B. fragilis *sulfatases.*— Two galactose-6-sulfatase and two galactose-3-sulfatase isozymes were partly-purified by DEAE chromatography from the *B. fragilis* cell free extract. Galactose-3-sulfatase and galactose-6-sulfatase activities (Table 2) were assayed by release of 4-nitrophenol from 4-nitrophenylgalactoside sulfates **2** or **3**, respectively, as substrates in combination with the same auxiliary glycosidase mixture as used in the *Prevotella* case above, although only the β-galactosidase activity was required in this case. Auxiliary glycosidases alone did not cleave 4-nitrophenol from the sulfated substrates. Gal-ONP was shown by paper chromatography to be the product formed from the action of each of the partially-purified sulfatases on their own, confirming that the *B. fragilis* enzymes were sulfatases rather than glycosidases. Each of the sulfatase enzymes was substrate specific. This is consistent with the observation by Salyers et al.[47] that the predominant Gram-negative bacteria from the colon often possess more than one isozyme catalysing a single step in polysaccharide degradation. The above data represent another example of this phenomenon, the purpose of which may be to circumvent metabolic disruption by mutation to a critical gene.

The *B. fragilis* cell-free extract alone was capable of releasing 4-nitrophenol from the sulfated substrates, presumably because it contains sulfatase and galactosidase activities. The natural substrates for these galactose-3-sulfatases and galactose-6-sulfatases are not known precisely, but since *B. fragilis* is able to use mucin for growth,[48] we speculate that they may be

involved in desulfating oligosaccharide chains that terminate in Gal 6-OSO_3H or Gal 3-OSO_3H, such groups being chain-terminating.[2] Possible confirmation will have to await the isolation of pure enzymes and their testing with appropriate mucins.

It is anticipated that the activity of mucin-desulfating enzymes in diverse bacteria present in the digestive tract should be measurable using the model substrates described above, and assays for bacterial sulfatases that de-esterify saccharide moieties containing GlcNAc 6-OSO_3H, Gal 3-OSO_3H, and Gal 6-OSO_3H in faeces of patients with colon diseases, in dental plaque of patients with oral diseases and in pulmonary sputum from patients with lung diseases, could lead to important new means of assessing specific bacterial involvement in these conditions.[3,49,50]

3. Experimental

General chemical methods.—Melting points were measured on a Reichert hot stage microscope and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter and are in units of 10^{-1} deg cm^2 g^{-1} (conventionally °). TLC was performed on glass or aluminium backed Silica Gel 60 F254 (E. Merck) with detection by UV absorption and/or by heating after dipping in $(NH_4)_6Mo_7O_{24}{\cdot}6\ H_2O$ (5 g) and $Ce(SO_4)_2$ (100 mg) in 5% aq H_2SO_4 (100 mL) solution. Chromatography (flash column) was performed on Scharlau or Merck Silica Gel 60 (40–60 μm). Chromatography solvents were distilled prior to use. Anhydrous solvents were those commercially available. Organic solutions were dried over $MgSO_4$ and evaporated under reduced pressure. All air-sensitive reactions were performed under argon. NMR spectra were recorded on a Bruker AC300E spectrometer at 300 MHz (^1H) for solutions in $CDCl_3$, $(CD_3)_2SO$ (internal Me_4Si, δ 0) or D_2O (internal acetone δ 2.23) or at 75.5 MHz (^{13}C) for solutions in $CDCl_3$ (centre line δ 77.0), $(CD_3)_2SO$ (centre line δ 39.7) or D_2O (internal acetone, δ 31.5). Assignments of ^1H and ^{13}C resonances were based on 2D (^1H–^1H DQF-COSY, ^1H–^{13}C HSQC) and DEPT experiments. The ^{13}C spectra gave unambiguous data on the numbers of protons bonded to each carbon atom; these are expressed as s, d, t, and q being the multiplicities expected in C,H undecoupled spectra. High-resolution +ve FABMS determinations were performed on a VG70-250S (VG Analytical) double focussing, magnetic sector mass spectrometer equipped with a standard liquid secondary caesium ion or sodium ion gun in a glycerol or nitrobenzyl alcohol matrix. IR data were recorded on a Perkin–Elmer 1600 series FTIR spectrometer.

*4-Nitrophenyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside 6-sodium sulfate* (**1**).—To a solution of 4-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside[15] (302 mg, 0.71 mmol), in dry DMF (12 mL) was added SO_3·pyridine (450 mg, 1.42 mmol), and the mixture was stirred at ambient temperature for 2 h. Methanol (0.2 mL) was added and the volatiles were removed to leave a colourless oil which was dissolved in MeOH (26 mL) and NaOMe–MeOH solution (2.0 mL, 1 M) was added. The mixture was stirred at ambient temperature for 1.5 h (during which time a precipitate formed), neutralized with Amberlite IRC 50 (H^+) resin, water was added to dissolve the precipitate, and the resin was filtered off and the volatiles were evaporated. Chromatography [5:4:1 CH_2Cl_2–MeOH–NH_4OH (d 0.88)] afforded the title compound **1** as the ammonium salt which was converted into its sodium salt by passage through a Dowex 50X8 100 (Na^+) resin column (10 × 2.5 cm) eluted with water. The fractions containing sodium salt **1** were evaporated and the resulting solid was suspended in MeOH (1.0 mL), centrifuged (3000 rpm, 5 min) and the MeOH decanted off. This process

Table 2
Reactions of the 3-sulfate (**2**) and the 6-sulfate (**3**) of 4-nitrophenyl β-D-galactopyranoside catalysed by *B. fragilis* sulfatases

Enzyme	Sample	Substrate	Specific activity [a]	Presence of endogenous galactosidase	Products formed with sulfatase [c]	
					Without glycosidase [b]	With glycosidase [b]
Galactose 3-sulfatase	Cell free extract	**2**	11.49	yes	ND	ND
Galactose 3-sulfatase	DEAE fraction a	**2**	223.7	no	Gal-ONP	Gal, nitrophenol
Galactose 3-sulfatase	DEAE fraction c	**2**	130.7	no	Gal-ONP	Gal, nitrophenol
Galactose 6-sulfatase	Cell free extract	**3**	7.37	yes	ND	ND
Galactose 6-sulfatase	DEAE fraction b	**3**	407.5	no	Gal-ONP	Gal, nitrophenol
Galactose 6-sulfatase	DEAE fraction d	**3**	111.9	trace	Gal-ONP	Gal, nitrophenol

[a] nmol 4-Nitrophenol released min^{-1} (mg enzyme protein)$^{-1}$.
[b] *Asperigillus oryzae* extract (Sigma G7138, 1993 catalogue) containing β-galactosidase and β-*N*-acetylhexosaminidase activities.
[c] By paper chromatography; ND, not determined; Gal-ONP, 4-nitrophenyl β-D-galactopyranoside; Gal, D-galactose.

was repeated twice and **1** was obtained as a colourless solid after drying (228 mg, 72%); $[\alpha]_D^{23}$ −31° (*c* 0.57, water); IR ($CHBr_3$ mull): *v* 3693–3170 (s), 1643 (s), 1585 (s), 1510 (s), 1349 (s), 1248 (s), 1071 (s) cm^{-1}; ^1H NMR (D_2O): δ 8.12 (d, 2 H, *J* 9.2 Hz, ArH), 7.17 (d, 2 H, *J* 9.2 Hz, ArH), 5.32 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.43 (dd, 1 H, $J_{6a,6b}$ 11.4, $J_{5,6a}$ 1.8 Hz, H-6a), 4.26 (dd, 1 H, $J_{5,6b}$, 5.8 Hz, H-6b), 4.07 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2) 3.95 (ddd, 1 H, H-5), 3.74 (t, 1 H, H-3), 3.64 (t, 1 H, H-4), 2.04 (s, 3 H, Ac); ^{13}C NMR (D_2O): δ 176.2 (s), 162.9 (s), 143.9 (s), 127.4 (d), 117.8 (d), 99.9 (d, C-1), 75.4 (d, C-5), 74.5 (d, C-3), 70.7 (d, C-4), 68.2 (t, C-6), 56.5 (d, C-2), 23.4 (q, Me). FABMS: *m*/*z* Calcd. for $C_{14}H_{18}N_2NaO_{11}S$ $[M+H]^+$ 445.0529. Found: 445.0548.

4-Nitrophenyl β-D-galactopyranoside 3-triethylammonium sulfate (**2**).—Dibutyltin(IV) oxide (610 mg, 2.45 mmol) was added to a solution of 4-nitrophenyl 4,6-*O*-isopropylidene-β-D-galactopyranoside[16] (830 mg, 2.43 mmol) in toluene (40 mL) and the mixture was heated under reflux in a Dean–Stark apparatus for 1 h. The toluene was evaporated and the residue dissolved in DMF (20 mL), cooled to 0 °C, SO_3·pyridine (406 mg, 2.55 mmol) added, the mixture was stirred at ambient temperature overnight and the volatiles were evaporated. Excess 9:1 EtOH–Et_3N (15 mL) was added and evaporated and the residue was chromatographed (60:20:1 toluene–EtOH–Et_3N) to give 4-nitrophenyl 4,6-*O*-isopropylidene-β-D-galactopyranoside 3-triethylammonium sulfate as a colourless solid (870 mg, 69%). TLC (20:20:1 toluene–EtOH–Et_3N) R_f 0.37. To a suspension of this product (800 mg, 1.53 mmol) in EtOH (30 mL) was added Amberlyst A15 (H^+) resin (1.00 g) and the mixture was stirred for 2 h at ambient temperature then filtered. Excess Et_3N (2 mL) was added and evaporated then the residue chromatographed twice (20:20:1 toluene–EtOH–Et_3N then 90:10:1 CH_2Cl_2–MeOH–Et_3N) to give **2** as a colourless gum (420 mg, 57%); $[\alpha]_D^{21}$ −34° (*c* 2.4, MeOH); TLC (20:20:1 toluene–EtOH–Et_3N): R_f 0.26, (90:10:1 CH_2Cl_2–MeOH–Et_3N): R_f 0.24; IR (neat): *v* 3669–3145 (s), 1590 (s), 1510 (s), 1488 (s), 1339 (s), 1248 (s), 1072 (s), 986 (s) cm^{-1}; ^1H NMR (CD_3OD): δ 8.21 (d, 2 H, *J* 9.2 Hz, ArH), 7.26 (d, 2 H, *J* 9.3 Hz, ArH), 5.15 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.39–4.33 (m, 2 H, H-4, H-3), 4.01 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 3.90–3.70 (m, 3 H, H-6a, H-6b, H-5), 3.22 (q, 6 H, *J* 7.3 Hz, CH_3CH_2N), 1.31 (t, 9 H, CH_3CH_2N); ^{13}C NMR (CD_3OD): δ 163.8 (s), 143.8 (s), 126.6 (d), 117.8 (d), 102.0 (d, C-1), 81.6 (d, C-3), 76.9 (d, C-5), 70.3 (d, C-2), 68.3 (d, C-4), 62.2 (t, C-6), 48.0 (t, CH_3CH_2N), 9.2 (q, CH_3CH_2N). FABMS: *m*/*z* Calcd. for $C_{18}H_{31}N_2O_{11}S$ $[M+H]^+$ 483.1649. Found: 483.1639.

4-Nitrophenyl β-D-galactopyranoside.—Bis[tributyltin(IV)] oxide (19.6 mL, 38.5 mmol) was added to a solution of 4-nitrophenol (10.7 g, 76.9 mmol) in benzene (100 mL) and the mixture heated under reflux in a Dean–Stark apparatus for 1 h. After cooling to ambient temperature, the volatiles were evaporated and the residue dissolved in CH_2Cl_2 (100 mL). β-D-Galactose pentaacetate (20.0 g, 51.2 mmol) was added followed by BF_3·OEt_2 (9.5 mL, 74.97 mmol) and the mixture stirred for 5.5 h at ambient temperature, left at 4 °C overnight, and stirred a further 6 h at ambient temperature. It was washed with aq NaOH (1 M), water, dried, and evaporated and dissolved in CH_3CN. The solution was washed with hexanes, evaporated, and the residue crystallized from EtOH to give 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (16.5 g, 69%), a portion (10.0 g, 21.3 mmol) of which was dissolved in CH_2Cl_2 (20 mL) and MeOH (150 mL). Aqueous sodium hydroxide solution (5 mL, 1 M) was added and the mixture stirred for 2 h at ambient temperature, neutralized with Dowex 50X8 100 (H^+) resin, filtered, the filtrate was evaporated and the residue crystallized from EtOH (5.19 g, 81%); mp 183–184 °C, lit. 181–182 °C (EtOH);[51] $[\alpha]_D^{20}$ −86° (*c* 1.12, water), lit. $[\alpha]_D^{20}$ −74.7° (*c* 9.0, water).[51]

4-Nitrophenyl β-D-galactopyranoside 6-triethylammonium sulfate (**3**).

Method A. To a solution of 4-nitrophenyl β-D-galactopyranoside (1.00 g, 3.32 mmol), in dry DMF (10 mL) at −10 → −15 °C, SO_3·pyridine (550 mg, 3.46 mmol) was added and the mixture was allowed to warm to ambient temperature overnight. The solvent was evaporated and the residue chromatographed (200:100:1 → 100:100:1 EtOAc–EtOH–Et_3N) to give sulfate **3** as a colourless foam (700 mg, 44%); $[\alpha]_D^{20}$ −54° (*c* 0.625, MeOH); TLC (100:100:1 toluene–EtOH–Et_3N): R_f 0.14; IR (neat): *v* 3658–3145 (br,s), 1590 (s), 1515 (s), 1494 (s), 1339 (s), 1248 (s), 1077 (s), 1034 (s), 997 (s) cm^{-1}; ^1H NMR (CD_3OD): δ 8.23 (d, 2 H, *J* 9.2 Hz, ArH), 7.28 (d, 2 H, *J* 9.3 Hz, ArH), 5.02 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.26–4.13 (m, 2 H, H-6a, H-6b), 4.07 (br.t, 1 H, $J_{5,6a} = J_{5,6b}$ 6.2 Hz, H-5), 3.96 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 3.83 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 3.62 (dd, 1 H, H-3), 3.20 (q, 6 H, *J* 7.3 Hz, CH_3CH_2N), 1.30 (t, 9 H, CH_3CH_2N); ^{13}C NMR (CD_3OD): δ 163.9 (s), 143.8 (s), 126.6 (d), 117.8 (d), 102.2 (d, C-1), 74.8 (d, C-5), 74.5 (d, C-3), 71.9 (d, C-2), 69.9 (d, C-4), 67.8 (t, C-6), 48.0 (t, CH_3CH_2N), 9.2 (q, CH_3CH_2N). FABMS: *m*/*z* Calcd. for $C_{18}H_{31}N_2O_{11}S$ $[M+H]^+$ 483.1649 Found: 483.1665.

Method B. Dibutyltin(IV) oxide (270 mg, 1.08 mmol) was added to a solution of 4-nitrophenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside[17] (330 mg, 0.97 mmol) in toluene (10 mL) and the mixture was heated under reflux in a Dean–Stark apparatus until clear (2 h). The toluene was evaporated and the residue suspended in DMF (4 mL), cooled to 0 °C then SO_3·pyridine (172 mg, 1.08 mmol) was added. After a few minutes most solids had dissolved. The mixture was

stirred overnight at ambient temperature, the volatiles were evaporated and the residue was chromatographed (60:20:1 toluene–EtOAc–Et_3N) to give 4-nitrophenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside 6-triethylammonium sulfate (340 mg, 67%). A sample (60 mg, 0.115 mmol) was dissolved in EtOH (10 mL), Amberlyst A15 (H^+) resin (1.00 g) was added and the mixture was stirred at ambient temperature overnight. After filtration and evaporation of the solvent the residue was chromatographed (100:100:1 toluene–EtOH–Et_3N) to give sulfate **3** as a colourless foam (34 mg, 61%), identical to that made by method A.

*Phenyl 2-deoxy-6-*O*-(4-methoxybenzyl)-2-tetrachlorophthalimido-1-thio-β-*D*-glucopyranoside* (**6**).—Sodium methoxide in MeOH (14.5 mL, 1 M)[24] was added to a solution of phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside[23] (7.60 g, 11.4 mmol) in MeOH (875 mL) and CH_2Cl_2 (15 mL) at 40–45 °C. The solution was stirred for 15 min, acidified to ~pH 4 with Dowex 50X8 100 (H^+) resin and filtered. Evaporation of the filtrate afforded phenyl 2-deoxy-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside[23] as a cream coloured solid (5.62 g, 91%), a portion (4.30 g, 7.98 mmol) of which was suspended in benzene (600 mL), $(Bu_3Sn)_2O$ (4.47 mL, 8.78 mmol) was added and the mixture was heated under reflux in a Dean–Stark apparatus for 16 h. After cooling, concentration to ~120 mL and the addition of Bu_4NI (3.54 g, 9.58 mmol) and 4-methoxybenzyl chloride (1.6 mL, 11.97 mmol), the mixture was heated to 95 °C for 2 h, further chloride (1 mL) was added and heating was continued for 2 h during which time all solids had dissolved. A third portion of the chloride (2.5 mL) and Bu_4NI (3.00g) were added and heating at 95 °C with stirring was continued for a further 16 h. The volatiles were evaporated and chromatography (9:1 toluene–acetone) of the residue gave the ether **6** as a yellow foam which on heating to 85 °C under vacuum for 30 min became a yellow glass (1.99 g, 38%); $[\alpha]_D^{20}$ +24° (*c* 1.00, $CHCl_3$); TLC (4:1 toluene–acetone): R_f 0.58; ^{1}H NMR ($CDCl_3$): δ 7.38–7.35 (m, 2 H, ArH), 7.25–7.20 (m, 5 H, ArH), 6.89 (d, 2 H, *J* 8.6 Hz, ArH), 5.55 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.55, 4.49 (ABq, 2 H, *J* 11.5 Hz, CH_2Ar), 4.37–4.30 (m, 1 H, H-3), 4.22 (t, 1 H, H-2), 3.86–3.77 (m, 4 H, H-6a, OMe), 3.73 (dd, 1 H, $J_{6a,6b}$ 10.3, $J_{5,6b}$ 4.3 Hz, H-6b), 3.67–3.55 (m, 2 H, H-5, H-4), 3.15 (br.s, 1 H, exchanged with D_2O, OH), 2.54 (br.s, 1 H, exchanged with D_2O, OH); ^{13}C NMR ($CDCl_3$): δ 163.5 (br. s), 162.9 (br.s), 159.4 (s), 140.3 (s), 132.3 (d), 131.9 (s), 129.6 (s), 129.4(d), 128.9 (d), 127.9 (d), 127.2 (br.s), 113.9 (d), 83.1 (d, C-1), 77.9 (d, C-4 or C-5), 73.5 (d, C-4 or C-5), 73.4, (t, CH_2Ar), 72.2 (d, C-3), 70.0 (t, C-6), 56.0 (d, C-2), 55.3 (q, OMe). FABMS: *m*/*z* Calcd. for $C_{28}H_{22}Cl_4NO_7S$ $(M-H)^+$: 655.9871. Found: 655.9840.

*Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-deoxy-6-*O*-(4-methoxybenzyl)-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside* (**7**).—A solution of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (170 μL, 1 M) was added to a solution of **6** (2.05 g, 3.10 mmol) and tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[25,26] (2.30 g, 4.67 mmol) in anhyd CH_2Cl_2 (8 mL) and hexanes (8 mL) at −30 °C. The mixture was stirred for 1.5 h during which time a colourless precipitate formed. Et_3N (1.0 mL) was added, the mixture was warmed to ambient temperature and the volatiles were evaporated. Chromatography (3:7→4:6 EtOAc–hexanes) afforded disaccharide derivative **8** as a yellow foam which on trituration with EtOH gave an amorphous solid (1.80 g, 59%); $[\alpha]_D^{21}$ +27° (*c* 1.32, $CHCl_3$); TLC (3:2 EtOAc–hexanes): R_f 0.41; ^{1}H NMR ($CDCl_3$): δ 7.42–7.39 (m, 2 H, ArH), 7.29–7.20 (m, 5 H, ArH), 6.92 (dd, 2 H, *J* 6.8, 2.0 Hz, ArH), 5.54 (d, 1 H, $J_{1,2}$ 10.4 Hz, H-1), 5.33 (d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4′), 5.16 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{1',2'}$ 8.0 Hz, H-2′), 4.95 (dd, 1 H, H-3′), 4.54 (d, 1 H, *J* 11.6 Hz, CH_aAr), 4.52–4.43 (m, 2 H, H-1′, CH_bAr), 4.35 (br. t, 1 H, after D_2O exchange became a dd, $J_{2,3}$ 10.3, $J_{3,4}$ 8.0 Hz, H-3), 4.23 (t, 1 H, H-2), 4.07–4.04 (m, 2 H, H-6a′, H-6b′), 4.03 (d, 1 H, $J_{OH,3}$ 0.9 Hz, exchanged with D_2O, OH), 3.91 (br.t, 1 H, $J_{5',6a'} \sim J_{5',6b'} \sim 6.2$ Hz, H-5′), 3.83 (s, 3 H, OMe), 3.76–3.63 (m, 4 H, H-6a, H-6b, H-5, H-4), 2.12, 2.00, 1.98, 1.97, (4s, 12 H, 4 Ac); ^{13}C NMR ($CDCl_3$): δ 170.5 (s), 170.0 (s), 169.9 (s), 169.0 (s), 163.4 (s), 162.5 (s), 159.4 (s), 140.3 (s), 132.6 (d), 131.7 (s), 130.1 (s), 129.8 (s), 129.5 (d), 128.9 (d), 128.0 (d), 127.3 (s), 114.0 (d), 101.5 (d), 82.9 (d), 81.5 (d), 78.3 (d), 73.4 (t), 71.3 (d), 70.7 (d), 70.4 (d), 68.7 (d), 67.7 (t), 66.8 (d), 61.4 (t), 55.9 (d), 55.3 (q), 20.7 (q), 20.53 (q), 20.45 (q). FABMS: *m*/*z* Calcd. for $C_{42}H_{41}Cl_4CsNO_{16}S$ $[M+Cs]^+$: 1119.9955. Found: 1119.9928.

*Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(4-methoxybenzyl)-2-tetrachlorophthalimido-1-thio-β-D-glucopyranoside* (**8**).—A solution of **7** (1.85 g, 1.87 mmol) in pyridine (10 mL) and Ac_2O (5 mL) was left to stand at ambient temperature for 16 h then poured into ice-water and extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with aq satd $NaHCO_3$, water, dried and evaporated to a yellow foam. Chromatography (2:3 EtOAc–hexanes) gave **8** as a colourless foam (1.62 g, 84%); $[\alpha]_D^{19}$ +31° (*c* 1.43, $CHCl_3$); TLC (1:1 EtOAc–hexanes): R_f 0.44; ^{1}H NMR ($CDCl_3$): δ 7.42–7.39 (m, 2 H, ArH), 7.31–7.22 (m, 5 H, ArH), 6.94 (dd, 2 H, *J* 6.7, 2.0 Hz, ArH), 5.65 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.61 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 8.9 Hz, H-3), 5.28 (d, 1 H, $J_{3',4'}$ 3.1, H-4′), 5.02 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{1',2'}$ 7.9 Hz, H-2′), 4.86 (dd 1 H, H-3′), 4.68, 4.46 (ABq, 2 H, *J* 11.6 Hz, CH_2Ar), 4.53 (d, 1 H, H-1′), 4.27 (t, 1 H, H-2), 4.04–3.98 (m, 3 H, H-6a′, H-6b′, H-4), 3.83 (s, 3 H, OMe), 3.76 (br.s, 2 H, H-6a, H-6b), 3.69–3.60 (m, 2 H,

H-5′, H-5), 2.11 2.04, 1.96, 1.95, 1.89, (5s, 15 H, 5 Ac); ^{13}C NMR ($CDCl_3$): δ 170.5 (s), 170.3 (s), 170.2 (s), 170.1 (s), 168.8 (s), 163.1 (s), 162.5 (s), 159.5 (s), 140.7 (s), 140.4 (s), 133.1 (d), 131.1 (s), 130.1 (s), 129.9 (s), 129.6 (d), 129.0 (d), 128.3 (d), 127.2 (s), 126.9 (s), 114.0 (d), 100.4 (d), 82.5 (d), 79.0 (d), 74.9 (d), 73.3 (t), 72.1 (d), 71.0 (d), 70.5 (d), 69.1 (d), 67.2 (t), 66.8 (d), 60.8 (t), 55.3 (q), 54.8 (d), 20.6 (q), 20.5 (q). FABMS: *m/z* Calcd. for $C_{44}H_{44}Cl_4NO_{17}S$ $[M+H]^+$: 1030.1084. Found: 1030.1030.

Phenyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3*-O-*acetyl-2-deoxy-1-thio-β*-D-*glucopyranoside* (**10**).—Dry 1,2-diaminoethane (0.37 mL, 5.5 mmol) was added to a solution of **8** (1.13 g, 1.10 mmol) in dry EtOH (30 mL, denatured with 5% Pr^{i}OH) and the mixture was stirred at 60 °C for 4 h. Coevaporation several times with toluene gave a residue which was dissolved in pyridine (30 mL) and Ac_2O (15 mL) and left at ambient temperature for 2 days. The solvent was evaporated and the residue dissolved in EtOAc (50 mL). The solution was washed with aq HCl (10%), aq satd $NaHCO_3$, water, and dried and the solvent was evaporated. Chromatography (4:1 → 7:3 toluene–acetone) gave crude **9** as a bright yellow foam (530 mg). ^{1}H NMR ($CDCl_3$) indicated it to be approximately 90% pure. Treatment of **7** (1.78 g, 1.80 mmol) with 1,2-diaminoethane (0.6 mL, 9.00 mmol) in a similar way to that used for compound **8** and then Ac_2O–pyridine followed by chromatography (50:50:1 → 50:50:2 toluene–EtOAc–MeOH) also gave acetamido compound **9** (941 mg) as a pale yellow foam of approximately 90–95% purity. To a solution of crude **9** (810 mg, ~1.01 mmol) in CH_2Cl_2 (30 mL) was added PhSH (15 mL) and TFA (10 mL) and the mixture was left to stand at ambient temperature for 15 min. The volatiles were evaporated and the residue filtered through a small plug of SiOH (eluted with 1:1 EtOAc–hexanes then 85:15 EtOAc–MeOH) to give crude alcohol **10** as a yellow gum after evaporation. Chromatography (50:50:1 → 50:50:2 toluene–EtOAc–MeOH) gave pure **10** as a colourless foam (603 mg, 87%); $[\alpha]_D^{20}$ −22° (*c* 1.11, $CHCl_3$); TLC (50:50:3 toluene–EtOAc–MeOH): R_f 0.36; ^{1}H NMR ($CDCl_3$): δ 7.48–7.38 (m, 2 H, ArH), 7.35–7.22 (m, 3 H, ArH), 5.85 (d, 1 H, $J_{NH,2}$ 9.6 Hz, exchanged with D_2O, NH), 5.34 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4′), 5.13–5.03 (m, 2 H, H-2′, H-3), 4.99 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-3′), 4.75 (d, 1 H, $J_{1,2}$ 10.4 Hz, H-1), 4.61 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.19–4.04 (m, 3 H, H-6a′, H-6b′, H-2), 3.96–3.73 (m, 3 H, H-5′, H-6a, H-4), 3.72 (dd, 1 H, $J_{6a,6b}$ 12.3, $J_{5,6b}$ 3.1 Hz, H-6b), 3.42 (br.dt, 1 H, H-5), 2.12, 2.06, 2.05, 2.04, 1.98, 1.96, (6s, 19 H, 6 Ac, OH); ^{13}C NMR ($CDCl_3$): δ 171.1 (s), 170.3 (s), 170.1 (s), 170.0 (s), 169.2 (s), 132.9 (s), 131.8 (d), 129.0 (d), 127.8 (d), 101.0 (d), 86.9 (d), 79.0 (d), 74.8 (d), 74.4 (d), 70.9 (d), 70.6 (d), 69.3 (d), 66.8 (d), 60.9 (t), 60.7 (t), 53.0 (d), 23.2 (q), 20.8 (q), 20.7 (q), 20.6 (q), 20.5 (q). FABMS: *m/z* Calcd. for $C_{30}H_{40}NO_{15}S$ $[M+H]^+$: 686.2119. Found: 686.2101.

Phenyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3*-O-*acetyl-6*-O-tert-*butyldimethylsilyl-2-deoxy-1-thio-β*-D-*glucopyranoside* (**11**).—2,6-Dimethylpyridine (0.19 mL, 1.64 mmol) was added to a solution of 6-ol **10** (560 mg, 0.82 mmol) in CH_2Cl_2 (6 mL) and the mixture was cooled to −20 °C. TBDMSOTf (0.28 mL, 1.23 mmol) was added dropwise, the mixture stirred for 30 min, warmed to ambient temperature and stirred for a further 15 min. The reaction mixture was diluted with EtOAc (50 mL) and washed with aq HCl (10%), aq satd $NaHCO_3$, dried and the solvent was evaporated to leave a gum. Chromatography (4:1 toluene–acetone) gave silyl ether **11** as a colourless foam (521 mg, 79%); $[\alpha]_D^{22}$ −26° (*c* 1.13, $CHCl_3$); TLC (7:3 toluene–acetone): R_f 0.32; ^{1}H NMR ($CDCl_3$): δ 7.50–7.45 (m, 2 H, ArH), 7.29–7.26 (m, 3 H, ArH), 5.54 (d, 1 H, $J_{NH,2}$ 9.5 Hz, exchanged with D_2O, NH), 5.35, (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′), 5.08 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{1',2'}$ 7.9 Hz, H-2′), 5.01 (t, 1 H $J_{3,4}=J_{2,3}$ 9.1 Hz, H-3), 4.93 (dd, 1 H, H-3′), 4.66 (d, 1 H, H-1′), 4.62 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1), 4.15–4.01 (m, 3 H, H-6a′, H-6b′, H-2), 3.94 (t, 1 H, H-4), 3.91–3.77 (m, 3 H, H-5′, H-6a, H-6b), 3.34 (dt, 1 H, $J_{5,6a}$ 1.9 Hz, H-5), 2.13, 2.00, 1.98, 1.96 (4s, 12 H, 4 Ac), 2.05 (s, 6 H, 2 Ac), 0.93 (s, 9 H, Si-Bu^t), 0.12, 0.10 (2s, 6 H, 2 Si-Me); ^{13}C NMR ($CDCl_3$): δ 171.1 (s), 170.3 (s), 170.14 (s), 170.05 (s), 169.9 (s), 169.0 (s), 133.2 (s), 132.4 (d), 128.9 (d), 127.8 (d), 100.2 (d), 87.0 (d), 79.6 (d), 74.2 (d), 73.8 (d), 71.1 (d), 70.7 (d), 69.3 (d), 66.9 (d), 61.2 (t), 61.1 (t), 52.9 (d), 25.9 (q), 23.3 (q), 20.8 (q), 20.7 (q), 20.6 (q), 20.5 (q), 18.3 (s), −5.0 (q), −5.3 (q). FABMS: *m/z* Calcd. for $C_{36}H_{54}NO_{15}SSi$ $[M+H]^+$: 800.2984. Found: 800.2961.

4-Nitrophenyl (2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3*-O-*acetyl-6*-O-tert-*butyldimethylsilyl-2-deoxy-β*-D-*glucopyranoside* (**13**).—A solution of Cl_2 in CH_2Cl_2 (0.81 mL, 0.42 mmol, from a stock solution of 37 mg mL^{-1} of Cl_2 in CH_2Cl_2) was added to a solution of **11** (280 mg, 0.35 mmol) in CH_2Cl_2 (3 mL) and the mixture was left at ambient temperature for 15 min. The solvent was evaporated to give crude glycosyl chloride **12** as a pale yellow gum. ^{1}H NMR ($CDCl_3$ δ 6.17, 1 H, $J_{1,2}$ 3.7 Hz, H-1). 4-Nitrophenol (97 mg, 0.71 mmol), aq K_2CO_3 (1 mL, 1 M) and Bu_4NHSO_4 (140 mg, 0.35 mmol) were added to a solution of this chloride in CH_2Cl_2 (2 mL) and the mixture was stirred vigorously for 16 h. The mixture was diluted with CH_2Cl_2 (20 mL) and the organic phase washed with water (3 × 5 mL), dried and the volatiles were evaporated. Chromatography (4:1 toluene–acetone) gave aryl glycoside **13** as a colourless foam (161 mg, 56%); $[\alpha]_D^{21}$ −42° (*c* 1.23, $CHCl_3$); TLC (7:3 toluene–acetone): R_f 0.32; ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 2 H, *J* 9.2 Hz, ArH), 7.05 (d, 2 H, *J* 9.2 Hz, ArH), 6.00

(d, 1 H, $J_{NH,2}$ 9.2 Hz, exchanged with D_2O, NH), 5.39 (d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4′), 5.21 (d, 1 H, $J_{1,2}$ 6.1 Hz H-1), 5.18–5.12 (m, 2 H, H-2′, H-3), 5.01 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-3′), 4.60 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.33 (br.q, 1 H, after D_2O exchange became a dd, $J_{2,3}$ 7.9 Hz, H-2), 4.20–4.07 (d, 2 H, *J* 7.0 Hz, H-6a′, H-6b′), 4.01 (t, 1 H, $J_{4,5} = J_{3,4}$ 6.7 Hz, H-4), 3.95 (dd, 1 H, $J_{6a,6b}$ 10.4, $J_{5,6a}$ 4.2 Hz, H-6a), 3.87 (t, 1 H, H-5′), 3.72–3.59 (m, 2 H, H-6b, H-5), 2.16, 2.11, 2.08, 2.06, 2.02, 1.99, (6s, 18 H, 6 Ac), 0.88 (s, 9 H, Si-Bu^t), 0.01 (s, 3 H, Si-Me), 0.00 (Si-Me + Si-Me$_4$); ^{13}C NMR ($CDCl_3$): δ 170.6 (s), 170.3 (s), 170.1 (s), 170.0 (s), 169.4 (s), 161.6 (s), 142.8 (s), 125.7 (d), 116.5 (d), 100.3 (d, C-1′), 98.0 (d, C-1), 75.9 (d, C-5), 73.0 (d, C-4), 71.3 (d, C-2′ or C-3), 71.0 (d, C-5′), 70.7 (d, C-3′), 69.3 (d, C-2′ or C-3), 66.8 (d, C-4′), 61.2 (t, C-6), 61.1 (t, C-6′), 51.7 (d, C-2), 25.8 (q), 23.2 (q), 20.9 (q), 20.8 (q), 20.6 (q), 20.5 (q), 20.4 (q), 18.2, (s, Si-Bu^t), −5.3 (q, Si-Me), −5.4 (q, Si-Me). FABMS: *m/z* Calcd. for $C_{36}H_{53}N_2O_{18}Si$ $[M+H]^+$: 829.3063. Found: 829.3057.

*4-Nitrophenyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**14**).—Hydrogen fluoride–pyridine (0.3 mL, 65–75%) was added to a solution of **13** (140 mg, 0.17 mmol) in THF (2 mL) contained in a plastic vial at 0 °C. The mixture was warmed to ambient temperature and left for 1.5 h, diluted with EtOAc (30 mL) and the solution was washed with aq satd $NaHCO_3$, dried and the volatiles were evaporated. Chromatography (3:2 toluene–acetone) afforded the 6-hydroxy compound **14** as a colourless amorphous solid (112 mg, 92%); $[\alpha]_D^{21}$ −25° (*c* 0.71, $CHCl_3$). TLC (7:3 toluene–acetone): R_f 0.16; ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 2 H, *J* 9.2 Hz, ArH), 7.07 (d, 2 H, *J* 9.2 Hz, ArH), 5.91 (d, 1 H, $J_{NH,2}$ 9.2 Hz, exchanged with D_2O, NH), 5.38 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4′), 5.25–5.19 (m, 2 H, H-3, H-1), 5.14 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{1',2'}$ 7.8 Hz, H-2′), 5.03 (dd, 1 H, H-3′), 4.64 (d, 1 H, H-1′), 4.33 (q, 1 H, after D_2O exchange became a dd, $J_{2,3}$ 9.7, $J_{1,2}$ 7.7 Hz, H-2), 4.18–4.09 (m, 2 H, H-6a′, H-6b′), 4.03 (t, 1 H, $J_{4,5} = J_{3,4}$ 8.6 Hz, H-4), 3.98–3.87 (m, 2 H, H-6a, H-5′), 3.83–3.75 (m, 1 H, after D_2O exchange became a dd, $J_{6a,6b}$ 12.3, $J_{5,6b}$ 3.3 Hz, H-6b), 3.63 (dt, 1 H, H-5), 2.11, 2.09, 2.08, 2.07, 2.00, 1.98 (6s, 18 H, 6 Ac), 1.87 (dd, 1 H, $J_{OH,6a}$ 8.5, $J_{OH,6b}$ 4.9 Hz, exchanged with D_2O, OH-6); ^{13}C NMR ($CDCl_3$): δ 171.1 (s), 170.4 (s), 170.0 (s), 169.9 (s), 169.6 (s), 161.5 (s), 142.9 (s), 125.8 (d), 116.3 (d), 100.9 (d), 98.2 (d), 75.7 (d), 74.35 (d), 72.40 (d), 70.7 (d), 69.5 (d), 66.7 (d), 60.8 (t), 60.5 (t), 52.9 (d), 23.2 (q), 20.9 (q), 20.7 (q), 20.6 (q), 20.5 (q), 20.3 (q). FABMS: *m/z* Calcd. for $C_{30}H_{39}N_2O_{18}$ $[M+H]^+$: 715.2198. Found: 715.2204. Similar treatment of the TBDPS ether (**18**) (720 mg, 0.76 mmol) with HF–pyridine (2.0 mL) for 48 h also gave compound **14** (460 mg, 85%).

*4-Nitrophenyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside 6-sodium sulfate* (**15**).—Sulfur trioxide·Me_3N (292 mg, 2.1 mmol) was added to a solution of **14** (250 mg, 0.35 mmol) in dry DMF (6 mL) and the mixture was heated to 40 °C for 3 h. MeOH (1 mL) was added and the volatiles were removed. The residue was dissolved in MeOH and passed through a column (10 × 2.5 cm) of Dowex 50X8 100 (Na^+) resin eluted with MeOH. The fractions containing **15** were evaporated and the residue was chromatographed (9:1 CH_2Cl_2–MeOH), to afford sulfate **15** as a colourless amorphous solid (225 mg, 79%); $[\alpha]_D^{20}$ −21° (*c* 1.24, MeOH); TLC (9:1 CH_2Cl_2–MeOH): R_f 0.1; ^{1}H NMR (CD_3OD): δ 8.22 (d, 2 H, *J* 9.2 Hz, ArH), 7.22 (d, 2 H, *J* 9.3 Hz, ArH), 5.38 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4′), 5.35 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.27–5.18 (m, 1 H, H-3), 5.13 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-3′), 5.03 (dd, 1 H, $J_{1',2'}$ 7.8 Hz, H-2′), 4.88 (d, 1 H, H-1′), 4.37 (d, 1 H, $J_{6a,6b}$ 10.8 Hz, H-6a), 4.28–4.07 (m, 5 H, H-6a′, H-6b′, H-6b, H-5′, H-2), 4.01–3.87 (m, 2 H, H-5, H-4), 2.13, 2.12, 2.07, 2.05, 1.92, 1.90 (6s, 18 H, 6 Ac); ^{13}C NMR ($CDCl_3$): δ 173.5 (s), 172.0 (s), 171.9 (s), 171.6 (s), 171.4 (s), 163.2 (s), 144.1 (s), 126.7 (d), 117.8 (d), 101.5 (d, C-1′), 99.1 (d, C-1), 76.3 (d, C-4 or C-5), 74.9, (d, C-4 or C-5), 74.0 (d, C-3), 72.6 (d, C-3′), 71.7 (d, C-5′), 70.5 (d, C-2′), 68.8 (d, C-4′), 66.5 (t, C-6), 62.3 (t, C-6′), 55.0 (d, C-2), 22.7 (q), 21.0 (q), 20.8 (q), 20.6 (q), 20.4 (q). FABMS: *m/z* Calcd. for $C_{30}H_{38}N_2NaO_{21}S$ $[M+H]^+$: 817.1586. Found: 817.1566.

*4-Nitrophenyl β-*D*-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**16**).—To a solution of **14** (20 mg, 0.028 mmol) in MeOH (2 mL) was added NaOMe in MeOH solution (0.1 mL, 1 M) and the mixture was left at ambient temperature for 2 h. Dilution with MeOH (5 mL), acidification to ~pH 4 with Dowex 50X8 100 (H^+) resin, filtration and evaporation gave a colourless crystalline solid (12 mg, 86%); $[\alpha]_D^{21}$ −6° (*c* 0.47, Me_2SO), lit. $[\alpha]_D$ −4° (*c* 0.50, Me_2SO).[22] ^{1}H NMR ($[CD_3]_2SO$) in agreement with literature data;[22] mp 207–209 °C (MeOH), lit. 213 °C (MeOH);[32] $[\alpha]_D^{21}$ −24° (*c* 0.74, water), lit. $[\alpha]_D^{25}$ −18° (*c* 1.0, water);[32] ^{1}H and ^{13}C NMR (D_2O) in agreement with literature data.[32]

*4-Nitrophenyl β-*D*-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside 6-sodium sulfate* (**4**).—To a solution of compound **15** (192 mg, 0.24 mmol) in MeOH (15 mL) was added NaOMe in MeOH (0.19 mL, 1 M) and the mixture was left at ambient temperature for 2 h. Acidification to ~ pH 4 with Dowex 50X8 100 (H^+) resin, filtration and evaporation gave a syrup that was dissolved in MeOH and passed through a Dowex 50X8 100 (Na^+) resin column (10 × 2.5 cm) eluted with MeOH. Fractions containing the title compound were evaporated. Chromatography (11.7:5:1 CH_2Cl_2–MeOH–water) followed by passage

through a plug of Biogel P-2 resin (water) gave sulfate salt **4** as a colourless solid (111 mg, 78%); $[\alpha]_D^{21}$ −37° (*c* 0.62, water); TLC (11.7:5:1 CH_2Cl_2–MeOH–water): R_f 0.28; IR ($CHBr_3$ mull): *v* 3659–3159 (br, s), 1653 (s), 1590 (s), 1515 (s), 1344 (s), 1248 (s), 1072 (s) cm^{-1}; ^1H NMR (D_2O): δ 8.25 (d, 2 H, *J* 9.2 Hz, ArH), 7.21 (d, 2 H, *J* 9.2 Hz, Ar), 5.36 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.57 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.49 (d, 1 H, $J_{6a,6b}$ 11.0 Hz, H-6a), 4.35 (dd, 1 H, $J_{5,6b}$ 4.8 Hz, H-6b), 4.16–4.04 (m, 2 H, H-5, H-2), 3.95 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.92–3.84 (m, 2 H, H-4, H-3), 3.83–3.72 (m, 3 H, H-6a′, H-6b′, H-5′), 3.70 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3′), 3.53 (dd, 1 H, H-2′), 2.03 (s, 3 H, Ac); ^{13}C NMR (D_2O): δ 176.1 (s), 162.8 (s), 143.8 (s), 127.3 (d), 117.7 (d), 103.9 (d, C-1′), 99.7 (d, C-1), 78.6 (d, C-4), 76.6 (d, C-5′), 74.2 (d, C-5), 73.7 (d, C-3′), 73.2 (d, C-3), 72.2 (d, C-2′), 69.9 (d, C-4′), 67.5 (t, C-6), 62.3 (t, C-6′), 56.1 (d, C-2), 23.4 (q, Me). FABMS: *m*/*z* Calcd. for $C_{20}H_{28}N_2NaO_{16}S$ $[M+H]^+$: 607.1057. Found: 607.1040.

*4-Nitrophenyl 2-acetamido-6-*O-tert-*butyldiphenylsilyl-2-deoxy-β-*D-*glucopyranoside* (**17**).—*tert*-Butylchlorodiphenylsilane (1.96 mL, 7.53 mmol) was added to 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[34] (2.00 g, 5.8 mmol) in pyridine (30 mL) and the solution was stirred at ambient temperature for 16 h. The solvent was evaporated and the residue dissolved in EtOAc then washed with aq HCl (10%), aq satd $NaHCO_3$, dried and the solvent evaporated. Chromatography (19:1 CH_2Cl_2–MeOH) gave the silyl ether **17** as a colourless foam (3.21 g, 95%); $[\alpha]_D^{20}$ −69° (*c* 1.01, $CHCl_3$); TLC (9:1 CH_2Cl_2–MeOH): R_f 0.43; ^1H NMR ($CDCl_3$): δ 8.07 (d, 2 H, *J* 9.2 Hz, ArH), 7.62 (d, 4 H, *J* 6.7 Hz, ArH), 7.42–7.25 (m, 6 H, ArH), 7.07 (d, 2 H, *J* 9.2 Hz, ArH), 5.86 (br.d, 1 H, $J_{NH,2}$ 6.0 Hz, exchanged with D_2O, NH), 5.30 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.29 (d, 1 H, *J* 3.1 Hz, exchanged with D_2O, OH), 4.03 (dd, 1 H, $J_{6a,6b}$ 11.0, $J_{5,6a}$ 2.8 Hz, H-6a), 3.96–3.82 (m, 2 H, H-6b, H-3), 3.81–3.71 (m, 1 H, after D_2O exchange became a dd, $J_{2,3}$ 10.1 Hz, H-2), 3.70–3.55 (m, 2 H, H-5, H-4), 2.97 (s, 1 H, exchanged with D_2O, OH), 2.07 (s, 3 H, Ac), 1.05 (s, 9 H, Si-Bu^t); ^{13}C NMR ($CDCl_3$): δ 172.6 (s), 161.7 (s), 142.6 (s), 135.6 (d), 135.5 (d), 133.0 (s), 132.8 (s), 129.8 (d), 127.7 (d), 125.7 (d), 116.4 (d), 97.9 (d), 76.7 (d), 75.1 (d), 71.5 (d), 63.8 (t), 56.8 (d), 26.8 (q), 23.5 (q), 19.2 (s). FABMS: *m*/*z* Calcd. for $C_{30}H_{37}N_2O_8Si$ $[M+H]^+$: 581.2319. Found: 581.2320.

*4-Nitrophenyl (2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1→4)-2-acetamido-3-*O-*acetyl-6-*O-tert-*butyldiphenylsilyl-2-deoxy-β-*D-*glucopyranoside* (**18**).—Powdered 4 Å molecular sieve (3.0 g) was added to a solution of diol **17** (636 mg, 1.10 mmol) and tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[25,26] (812 mg, 1.65 mmol) in dry CH_2Cl_2 (30 mL). The mixture was stirred at ambient temperature for 2 h, cooled to −30 °C and $BF_3{\cdot}OEt_2$ (0.135 mL, 1.10 mmol) was added dropwise over 10 min. After 1.5 h Et_3N (1.0 mL) was added and the mixture warmed to ambient temperature and the volatiles were evaporated. Chromatography (7:3 toluene–acetone) gave a monohydroxy product (556 mg) (R_f 0.22) which was dissolved in pyridine (10 mL) and Ac_2O (5 mL) and left at ambient temperature for 48 h. The volatiles were evaporated and the residue was dissolved in EtOAc, washed with aq HCl (10%), aq satd $NaHCO_3$, dried and the volatiles again evaporated. Chromatography (7:3 EtOAc–hexanes) gave **18** as a colourless foam (560 mg, 53%); $[\alpha]_D^{21}$ −31° (*c* 1.32, $CHCl_3$); TLC (4:1 EtOAc–hexanes): R_f 0.52; ^1H NMR ($CDCl_3$): δ 8.14 (d, 2 H, *J* 9.1 Hz, ArH), 7.64–7.57 (m, 4 H, ArH), 7.45–7.26 (m, 4 H, ArH), 7.12 (t, 2 H, *J* 7.6 Hz, ArH), 7.03 (d, 2 H, *J* 9.1 Hz, ArH), 5.98 (d, 1 H, $J_{NH,2}$ 9.1 Hz, exchanged with D_2O, NH), 5.37 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 5.22–5.15 (m 2 H, H-3, H-1), 5.11 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{1',2'}$ 7.9 Hz, H-2′), 4.99 (dd, 1 H, H-3′), 4.73 (d, 1 H, H-1′), 4.39 (q, 1 H, after D_2O exchange became a dd, $J_{2,3}$ 8.7, $J_{1,2}$ 6.9 Hz, H-2), 4.21 (t, 1 H, $J_{4,5}=J_{3,4}$ 7.7 Hz, H-4), 4.14 (d, 2 H, *J* 6.6 Hz, H-6a′, H-6b′), 3.99 (dd, 1 H, $J_{6a,6b}$ 11.3, $J_{5,6a}$ 3.6 Hz, H-6a), 3.85–3.73 (m, 2 H, H-5′, H-6b), 3.65–3.54 (m, 1 H, H-5), 2.11, 2.07, 2.06, 1.84 (4s, 12 H, 4 Ac), 2.02 (s, 6 H, 2 Ac), 1.05 (s, 9 H, Si-Bu^t); ^{13}C NMR ($CDCl_3$): δ 171.0 (s), 170.3 (s), 169.9 (s), 169.1 (s), 161.6 (s), 142.7 (s), 135.7 (d), 135.3 (d), 133.0 (s), 131.8 (s), 130.1 (d), 129.9 (d), 127.9 (d), 127.6 (d), 125.6 (d), 116.5 (d), 100.2 (d, C-1′), 98.0 (d, C-1), 75.5 (d, C-5), 73.2 (d, C-4), 71.7 (d, C-3), 70.9 (d, C-5′), 70.8 (d, C-3′), 69.4 (d, C-2′) 66.8 (d, C-4′), 61.3 (t, C-6), 61.1 (t, C-6′), 52.3 (d, C-2), 26.8 (q), 23.2 (q), 20.9 (q), 20.6 (q), 20.5 (q), 20.3 (q), 19.2 (s, Si-Bu^t). FABMS: *m*/*z* Calcd. for $C_{46}H_{57}N_2O_{18}Si$ $[M+H]^+$: 953.3376. Found: 953.3362.

*4-Nitrophenyl 2-acetamido-3-*O-tert-*butyldimethylsilyl-2-deoxy-4,6-*O-*(4-methoxybenzylidene)-β-*D-*glucopyranoside* (**19**).—Imidazole (2.19 g, 32.2 mmol) and TBDMSCl (2.42 g, 16.1 mmol) were added to a solution of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[35] (4.90 g, 10.7 mmol) in dry DMF (10 mL) and the mixture was left at ambient temperature for 16 h then heated at 40 °C for 3 h. The solvent was evaporated and the residue dissolved in EtOAc. The solution was washed with water, dried and the solvent evaporated. Chromatography (3:7→4:6 EtOAc–hexanes) gave silyl ether **19** as a colourless foam (3.1 g, 61%); mp (EtOAc–hexanes, needles) 138–140 °C; $[\alpha]_D^{21}$ −25° (*c* 0.79, $CHCl_3$); TLC (2:3 EtOAc–hexanes): R_f 0.56; ^1H NMR ($CDCl_3$): δ 8.19 (d, 2 H, *J* 9.2 Hz, ArH), 7.40 (d, 2 H, *J* 8.7 Hz, ArH), 7.06 (d, 2 H, *J* 9.2 Hz, ArH), 6.89 (d, 2 H, *J* 8.8 Hz, ArH), 5.83 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.78 (d, 1 H, $J_{NH,2}$ 8.6 Hz, exchanged with D_2O, NH), 5.47 (s, 1 H, CHAr), 4.48 (t, 1 H, $J_{3,4}=J_{2,3}$ 9.2 Hz, H-3), 4.42–4.23

(m, 1 H, H-6a), 3.81 (s, 3 H, OMe), 3.79–3.65 (m, 2 H, H-6b, H-5), 3.53–3.42 (m, 2 H, H-4, H-2), 1.98 (s, 3 H, Ac), 0.85 (s, 9 H, Si-Bu^t), 0.03, −0.02 (2s, 6 H, 2 Si-Me); ^{13}C NMR ($CDCl_3$): δ 170.6 (s), 161.8 (s), 160.2 (s), 142.9 (s), 129.5 (s), 127.6 (d), 125.8 (d), 116.6 (d), 113.6 (d), 101.9 (d, *C*HAr), 97.7 (d, C-1), 82.1 (d, C-4), 70.9 (d, C-3), 68.5 (t, C-6), 66.5 (d, C-5), 59.4 (d, C-2), 55.3 (q, OMe), 25.8 (q), 23.7 (q), 18.2 (s, Si-Bu^t), −4.1 (q, Si-Me), −5.0 (q, Si-Me). FABMS: *m*/*z* Calcd. for $C_{28}H_{39}N_2O_9Si$ $[M+H]^+$: 575.2425. Found: 575.2373.

*4-Nitrophenyl 2-acetamido-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**20**).—Powdered 3Å molecular sieve (15 g) was added to a solution of **19** (3.00 g, 5.2 mmol) and $NaCNBH_3$[52] (1.64 g, 26.1 mmol) in dry DMF (40 mL) and the mixture was cooled to 0 °C. A solution of TFA (3.99 mL, 51.7 mmol) in DMF (30 mL) was added dropwise over 30 min, and the mixture was stirred for 2 h then at ambient temperature for 4 h. TLC analysis indicated the presence mainly of starting material and a trace of the required title compound. An excess of $NaCNBH_3$ (4.2 g) and TFA (6 mL) in DMF (6 mL) was added in portions over a period of 2 days but TLC indicated little change in the ratio of the two components. The mixture was filtered into aq satd $NaHCO_3$ and the solution was extracted with $CHCl_3$ (5 × 50 mL). The combined organic extracts were washed with water (20 mL), dried and evaporated. Chromatography (2:3 → 1:1 EtOAc–hexanes) gave 6-ether **20** together with 15–20% of the 4-*O*-PMB regioisomer as an inseparable mixture (420 mg, 14%). Crystallization from EtOH–water gave the pure title compound **20** as a colourless solid (240 mg, 8%); mp 79–81 °C; $[\alpha]_D^{21}$ −35° (*c* 0.87, $CHCl_3$); TLC (1:1 EtOAc–hexanes): R_f 0.47; ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 2 H, *J* 9.2 Hz, ArH), 7.22 (d, 2 H, *J* 8.6 Hz, ArH), 7.05 (d, 2 H, *J* 9.2 Hz, ArH), 6.86 (d, 2 H, *J* 8.6 Hz, ArH), 5.67 (d, 1 H, $J_{NH,2}$ 7.8 Hz, exchanged with D_2O, NH), 5.66 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.52, 4.43 (ABq, 2 H, *J* 11.6 Hz, CH_2Ar), 4.17 (dd, 1 H, *J* 9.4, 8.1 Hz, H-3), 3.81 (s, 3 H, OMe), 3.77–3.66 (m, 3 H, H-6a, H-6b, H-5), 3.59–3.46 (m, 2 H, H-4, H-2), 2.42 (d, 1 H, $J_{OH,4}$ 3.2 Hz, exchanged with D_2O, OH-4), 1.97 (s, 3 H, Ac), 0.91 (s, 9 H, Si-Bu^t), 0.14, 0.09 (2s, 6 H, 2 Si-Me); ^{13}C NMR ($CDCl_3$): δ 170.4 (s), 162.0 (s), 159.4 (s), 142.6 (s), 129.7 (s), 129.4 (d), 125.7 (d), 116.5 (d), 113.9 (d), 97.2 (d), 74.5 (d), 73.9 (d), 73.3 (t), 73.0 (d), 69.7 (t), 57.8 (d), 55.3 (q), 25.8 (q), 23.7 (q), 18.2 (s), −4.2 (q), −4.7 (q). FABMS: *m*/*z* Calcd. for $C_{28}H_{40}CsN_2O_9Si$ $[M+Cs]^+$: 709.1557. Found: 709.1584.

*4-Nitrophenyl 2-acetamido-3-*O*-allyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-*D*-glucopyranoside* (**21**).—Barium hydroxide octahydrate (1.47 g, 4.66 mmol), BaO (4.38 g, 28.6 mmol), and allyl bromide (1.12 mL, 12.98 mmol) were added to a solution of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[35] (3.06 g, 6.65 mmol) in dry DMF (30 mL) and the mixture was stirred at ambient temperature for 2 h. Dilution with 9:1 CH_2Cl_2–MeOH (50 mL) and filtration through Celite followed by removal of the solvent gave a residue that was extracted with hot 9:1 CH_2Cl_2–MeOH (4 × 50 mL). The extracts were combined, the solvent was removed and chromatography of the further residue (99:1 → 19:1 CH_2Cl_2–MeOH) gave, after recrystallization from a large volume of acetone, allyl ether **21** as a colourless solid (1.21 g, 36%); mp 285–287 °C; $[\alpha]_D^{21}$ −3° (*c* 0.67, Me_2SO); TLC (19:1 CH_2Cl_2–MeOH): R_f 0.48; ^{1}H NMR ($[CD_3]_2SO$): δ 8.21 (d, 2 H, *J* 9.2 Hz, ArH), 8.09 (d, 1 H, $J_{NH,2}$ 8.9 Hz, NH), 7.37 (d, 2 H, *J* 8.7 Hz, ArH), 7.25 (d, 2 H, *J* 9.2 Hz, ArH), 6.95 (d, 2 H, *J* 8.7 Hz, ArH), 5.90–5.77 (m, 1 H, CH_2CH=CH_2), 5.64 (s, 1 H, CHAr), 5.45 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.22 (dd, 1 H, J_{trans} 17.3, J_{gem} 1.7 Hz, CH_2CH=C*H*H), 5.10 (dd, 1 H, J_{cis} 10.5 Hz, CH_2CH=CH*H*), 4.27–4.18 (m, 2 H, C*H*HCH=CH_2, H-6a), 4.09 (dd, 1 H, *J* 13.4, 5.4 Hz, CH*H*CH=CH_2), 4.00–3.86 (m, 1 H, H-2), 3.80–3.70 (m, 4 H, H-6b, H-5, H-4, H-3), 3.76 (s, 3 H, OMe), 1.82 (s, 3 H, Ac); ^{13}C NMR ($[CD_3]_2SO$): δ 170.5 (s), 162.8 (s), 160.6 (s), 143.2 (s), 136.5 (d), 131.0 (s), 128.4 (d), 126.9 (d), 117.7 (d), 117.0 (t), 114.5 (d), 101.3 (d), 99.3 (d), 81.5 (d), 78.9 (d), 73.3 (t), 68.6 (t), 66.9 (d), 56.2 (q), 23.9 (q). FABMS: *m*/*z* Calcd. for $C_{25}H_{29}N_2O_9$ $[M+H]^+$: 501.1873. Found: 501.1894.

*4-Nitrophenyl 2-acetamido-3-*O*-allyl-6-*O*-tert-butyldimethylsilyl-2-deoxy-β-*D*-glucopyranoside* (**23**).—Acetyl chloride (0.5 mL) was added to MeOH (100 mL) followed by compound **21** (1.12 g, 2.24 mmol) and the solution left 30 min at ambient temperature. The neutralized (Amberlyst A21 resin) solution on removal of the solvent gave 4-nitrophenyl 2-acetamido-3-*O*-allyl-2-deoxy-β-D-glucopyranoside (**22**) as a cream coloured solid that was washed with Et_2O and dried (600 mg, 70%); ^{1}H NMR ($[CD_3]_2SO$): δ 8.52 (d, 2 H, *J* 9.2 Hz, ArH), 7.95 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 7.18 (d, 2 H, *J* 9.2 Hz, ArH), 5.93–5.81 (m, 1 H, CH_2CH=CH_2), 5.33 (d, 1 H, $J_{OH,4}$ 5.7 Hz, OH-4), 5.24–5.18 (m, 2 H, *trans* CH_2CHCH*H*, H-1), 5.08 (dd, 1 H, J_{cis} 11.8, J_{gem} 1.4 Hz, CH_2CH=C*H*H), 4.62 (t, 1 H, $J_{OH,6}$ 5.5 Hz, OH-6), 4.28 (dd, 1 H, *J* 13.2, 5.3 Hz, C*H*HCH=CH_2), 4.10 (dd, 1 H, C*H*HCH=CH_2), 3.83–3.69 (m, 2 H, H-6a, H-2), 3.54–3.35 (m, 4 H, H-6b, H-5, H-4, H-3), 1.80 (s, 3 H, Ac). To a solution of **22** (699 mg, 1.83 mmol) in dry DMF (3 mL) was added imidazole (199 mg, 2.93 mmol) and TBDMSCl (331 mg, 2.20 mmol). The mixture was stirred for 45 min at ambient temperature and the solvent evaporated. Chromatography (7:3 EtOAc–hexanes) gave the title compound **23** as a colourless foam (650 mg, 72%); $[\alpha]_D^{21}$ −33° (*c* 1.17, $CHCl_3$); TLC (3:2 EtOAc–hexanes) R_f 0.30; ^{1}H NMR ($CDCl_3$): δ 8.17 (d, 2 H, *J* 9.2 Hz, ArH), 7.06 (2 H, *J* 9.2 Hz, ArH), 6.06–5.89 (m, 1 H, CH_2CH=CH_2), 5.75–5.70 (m, 2 H,

after D_2O exchange became a d, 1 H, $J_{1,2}$ 8.0 Hz, H-1, NH), 5.31 (dd, 1 H, J_{trans} 17.2, J_{gem} 1.5 Hz, CH_2-CH=C*H*H), 5.22 (br.d, 1 H, J_{cis} 10.4 Hz, CH_2CH=C*H*H), 4.33 (dd, 1 H, *J* 12.7, 5.8 Hz, C*H*HCH=CH_2), 4.22 (dd, 1 H, CH*H*CH=CH_2), 4.06 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 8.1 Hz, H-3), 3.94–3.80 (m, 2 H, H-6a, H-6b), 3.71–3.55 (m, 2 H, H-5, H-4), 3.47 (q, 1 H, after D_2O exchange became a dd, H-2), 2.97 (d, 1 H, $J_{OH,4}$ 2.0 Hz, exchanged with D_2O, OH-4), 1.99 (s, 3 H, Ac), 0.89 (s, 9 H, Si-Bu^t), 0.06 (s, 3 H, Si-Me), 0.05 (s, 3 H, Si-Me); ^{13}C NMR ($CDCl_3$): δ 170.7 (s), 161.9 (s), 142.7 (s), 134.8 (d), 125.7 (d), 117.5 (t), 116.6 (d), 97.2 (d), 79.9 (d), 75.2 (d), 73.5 (t), 72.9 (d), 64.1 (t), 56.7 (d), 25.8 (q), 23.6 (q), 18.2 (s), −5.5 (q). FABMS: *m*/*z* Calcd. for $C_{23}H_{37}N_2O_8Si$ $[M+H]^+$: 497.2319. Found: 497.2346.

*4-Nitrophenyl 2-acetamido-6-*O-tert-*butyldimethylsilyl-2-deoxy-β-*D-*glucopyranoside* (**24**).—Imidazole (630 mg, 9.25 mmol) and TBDMSCl (1.06 g, 7.01 mmol) were added to a solution of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[34] (2.00 g, 5.84 mmol) in dry DMF (9 mL) and the mixture was stirred at ambient temperature for 30 min, then the solvent was evaporated. Chromatography (93:7 CH_2Cl_2–MeOH) gave silyl ether **24** as a colourless amorphous solid (2.65 g, quant.); $[\alpha]_D^{20}$ −29° (*c* 0.56, MeOH); TLC (9:1 CH_2Cl_2–MeOH): R_f 0.37; ^{1}H NMR ($[CD_3]_2SO$): δ 8.17 (d, 2 H, *J* 9.2 Hz, ArH), 7.82 (d, 1 H, $J_{NH,2}$ 8.9 Hz, exchanged with D_2O, NH), 7.16 (d, 2 H, *J* 9.2 Hz, ArH), 5.22–5.11 (m, 3 H, after D_2O exchange became a d, 1 H, $J_{1,2}$ 8.4 Hz, H-1, OH-4, OH-3), 3.90 (d, 1 H, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.75–3.65 (m, 2 H, H-6b, H-2), 3.49–3.40 (m, 2 H, H-5, H-3), 3.24–3.16 (m, 1 H, after D_2O exchange became a t, $J_{4,5} = J_{3,4}$ 9.3 Hz, H-4), 1.82 (s, 3 H, Ac), 0.83 (s, 9 H, Si-Bu^t), −0.01 (s, 3 H, Si-Me), −0.05 (s, 3 H, Si-Me); ^{13}C NMR ($[CD_3]_2SO$): δ 169.6 (s), 162.4 (s), 142.0 (s), 125.7 (d), 116.9 (d), 98.5 (d), 77.3 (d), 74.0 (d), 70.0 (d), 62.6 (t), 55.4 (d), 25.9 (q), 23.2 (q), 18.1 (s), −5.1 (q), −5.2 (q). FABMS: *m*/*z* Calcd. for $C_{20}H_{33}N_2O_8Si$ $[M+H]^+$: 457.2006. Found: 457.2004.

*4-Nitrophenyl 2-acetamido-3-*O-*allyloxycarbonyl-6-*O-tert-*butyldimethylsilyl-2-deoxy-β-*D-*glucopyranoside* (**25**).—Allyl chloroformate (1.45 mL, 11.03 mmol) was added dropwise to a solution of diol **24** (2.40 g, 5.26 mmol) in pyridine (80 mL) and THF (40 mL) at −60 °C. The mixture was stirred for 2 h then allowed to warm to −10 °C and left for 16 h then warmed to ambient temperature and the volatiles were evaporated. Chromatography (19:1 → 4:1 toluene–acetone) gave the carbonate **25** as a colourless gum which solidified on standing overnight (675 mg, 24%); mp (EtOAc–hexanes) 97–98 °C; $[\alpha]_D^{19}$ −28° (*c* 0.84, $CHCl_3$); TLC (4:1 toluene–acetone): R_f 0.22; ^{1}H NMR ($CDCl_3$): δ 8.16 (d, 2 H, *J* 9.2 Hz, ArH), 7.08 (2 H, *J* 9.2 Hz, ArH), 6.03 (d, 1 H, $J_{NH,2}$ 8.5 Hz, exchanged with D_2O, NH), 6.00–5.86 (m, 1 H, CH_2CH=CH_2), 5.46 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.37 (dd, 1 H, J_{trans} 17.2, J_{gem} 1.0 Hz, CH_2-CH=C*H*H), 5.30 (dd, 1 H, J_{cis} 10.4, CH_2CH=C*H*H), 5.20 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.1 Hz, H-3), 4.72–4.59 (m, 2 H, CH_2CH=CH_2), 4.03 (q, after D_2O exchange became a dd, 1 H, H-2), 3.96–3.88 (m, 2 H, after D_2O exchange became a d, *J* 5.0 Hz H-6a, H-6b), 3.82 (dt, 1 H, $J_{4,5}$ 3.9 Hz, after D_2O exchange became a t, H-4), 3.75–3.66 (m, 1 H, H-5), 3.28 (d, 1 H, $J_{OH,4}$ 3.9 Hz, OH-4), 1.93 (s, 3 H, Ac), 0.83 (s, 9 H, Si-Bu^t), 0.05, 0.04 (2s, 6 H, 2 Si-Me); ^{13}C NMR ($CDCl_3$): δ 170.7 (s), 161.8 (s), 155.6 (s), 142.9 (s), 131.1 (d), 125.7 (d), 119.3 (t), 116.7 (d), 97.8 (d), 78.5 (d), 75.3 (d), 70.6 (d), 69.1 (t), 63.8 (t), 54.8 (d), 25.8 (q), 23.4 (q), 18.2 (s), −5.5 (q). FABMS: *m*/*z* Calcd. for $C_{24}H_{37}N_2O_{10}Si$ $[M+H]^+$: 541.2218. Found: 541.2210.

*4-Nitrophenyl 2-acetamido-3-*O-*allyl-6-*O-tert-*butyldimethylsilyl-2-deoxy-β-*D-*glucopyranoside* (**23**), *4-nitrophenyl 2-acetamido-4-*O-*allyl-6-*O-tert-*butyldimethylsilyl-2-deoxy-β-*D-*glucopyranoside* (**26**) *and 4-nitrophenyl 2-acetamido-2,3-di-*O-*allyl-6-*O-tert-*butyldimethylsilyl-2-deoxy-β-*D-*glucopyranoside* (**27**).—Freshly prepared $Pd(PPh_3)_4$ (10 mg) was added to a solution of carbonate **25** (625 mg 1.16 mmol) in THF (2 mL, freshly distilled from LAH), and the mixture was heated under reflux for 20 min then the solvent was evaporated. Chromatography (2:3 EtOAc–hexanes) gave the following products in order of elution:

Diallyl ether **27** as a colourless solid (90 mg, 14%); mp (EtOAc–hexanes, fine needles) 147–148 °C; $[\alpha]_D^{20}$ −38° (*c* 1.02, $CHCl_3$); TLC (3:2 EtOAc–hexanes): R_f 0.62; ^{1}H NMR ($CDCl_3$): δ 8.16 (d, 2 H, *J* 9.2 Hz, ArH), 7.07 (2 H, *J* 9.2 Hz, ArH), 6.02–5.85 (m, 3 H, after D_2O exchange became a m, 2 H, 2 × CH_2CH=CH_2, NH), 5.57 (d, 1 H, $J_{1,2}$ 6.9 Hz, H-1), 5.29 (d, 2 H, J_{trans} 17.2 Hz, 2 × CH_2CH=C*H*H), 5.20 (d, 2 H, J_{cis} 10.3 Hz, 2 × CH_2CH=C*H*H), 4.31–4.14 (m, 4 H, 2 × CH_2CH=CH_2), 4.01–3.91 (m, 2 H, H-6a, H-3), 3.74–3.59 (m, 3 H, H-6b, H-5, H-2), 3.50 (t, 1 H, $J_{3,4} = J_{4,5}$ 7.6 Hz, H-4), 1.99 (s, 3 H, Ac), 0.86 (s, 9 H, Si-Bu^t), −0.01, −0.04 (2s, 6 H, 2 Si-Me); ^{13}C NMR ($CDCl_3$): δ 170.4 (s), 162.0 (s), 142.6 (s), 134.7 (d), 134.5 (d), 125.7 (d), 117.2 (t), 116.6 (d), 97.0 (d), 78.6 (d), 76.7 (d), 76.5 (d), 73.3 (t), 73.0 (t), 62.2 (t), 55.2 (d), 25.8 (q), 23.6 (q), 18.2 (s), −5.3 (q), −5.4 (q). FABMS: *m*/*z* Calcd. for $C_{26}H_{41}N_2O_8Si$ $[M+H]^+$: 537.2632. Found: 537.2603.

The 3-allyl ether **23** (110 mg, 19%) gave identical ^{1}H and ^{13}C NMR data to those of the previously prepared sample.

The 4-allyl ether **26** as a colourless foam (140 mg, 24%); $[\alpha]_D^{20}$ −66° (*c* 1.6, $CHCl_3$); TLC (3:2 EtOAc–hexanes): R_f 0.13; ^{1}H NMR ($CDCl_3$): δ 8.14 (d, 2 H, *J* 9.2 Hz, ArH), 7.08 (2 H, *J* 9.2 Hz, ArH), 6.01–5.88 (m, 2 H, after D_2O exchange became a m, 1 H, CH_2-C*H*=CH_2, NH), 5.32–5.23 (m, 2 H, *trans* CH_2CH=C*H*H, H-1), 5.20 (d, 1 H, J_{cis} 10.4 Hz, CH_2CH=C*H*H), 4.35 (dd, 1 H, *J* 12.6, 5.7 Hz, C*H*HCH=CH_2), 4.22 (dd,

1 H, C*H*HCH=CH_2), 4.01 (dt, 1 H, $J_{2,3} = J_{3,4} \sim 8.4$, $J_{3,OH}$ 3.6 Hz, after D_2O exchange became a t, partially overlapped with H-6a, H-3), 3.94 (dd, 1 H, $J_{6a,6b}$ 11.3, $J_{5,6a}$ 2.1 Hz, H-6a), 3.86–3.75 (m, 2 H, H-6b, H-2), 3.72 (d, 1 H, exchanged with D_2O, OH-3), 3.57–3.51 (m, 1 H, H-5), 3.42 (t, 1 H, $J_{4,5}$ 8.4 Hz, H-4), 2.06 (s, 3 H, Ac), 0.87 (s, 9 H, Si-Bu^t), 0.01, –0.02 (2s, 6 H, 2 Si-Me); ^{13}C NMR ($CDCl_3$): δ 172.2 (s), 162.0 (s), 142.5 (s), 134.8 (d), 125.7 (d), 117.3 (t), 116.6 (d), 98.0 (d), 77.8 (d), 76.8 (d), 75.0 (d), 73.6 (t), 62.3 (t), 57.1 (d), 25.8 (q), 23.4 (q), 18.3 (s), −5.3 (q), −5.4 (q). FABMS: *m*/*z* Calcd. for $C_{23}H_{37}N_2O_8Si$ $[M+H]^+$: 497.2319. Found: 497.2318.

4-Nitrophenyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**28**).—Boron trifluoride etherate (0.18 mL, 1.45 mmol) was added to a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose[43] (1.4 g, 2.93 mmol) and 4-nitrophenol (816 mg, 5.87 mmol) in CH_2Cl_2 (25 mL) and the mixture was stirred at ambient temperature for 15 h. Further $BF_3{\cdot}OEt_2$ (0.54 mL) and 4-nitrophenol (408 mg) were added and stirring was continued for a further 21 h. Dichloromethane (50 mL) was added and the solution was washed with aq satd $NaHCO_3$, water, dried, and the solvent evaporated. Chromatography (3:7 → 2:3 EtOAc–hexane) gave glycoside **28** as a colourless gum which crystallized on addition of MeOH (704 mg, 44%, needles); mp 172–173 °C; $[\alpha]_D^{19}$ +77° (*c* 0.8, $CHCl_3$); TLC (1:1 EtOAc–hexanes): R_f 0.42; ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 2 H, *J* 9.3 Hz, ArH), 7.88–7.85 (m, 2 H, ArH), 7.78–7.74 (m, 2 H, ArH), 7.02 (d, 2 H, *J* 9.3 Hz, ArH), 6.14 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.88 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.3 Hz, H-3), 5.25 (t, 1 H, H-4), 4.65 (dd 1 H, H-2), 4.35 (dd, 1 H, $J_{6a,6b}$ 12.3, $J_{5,6a}$ 5.5 Hz, H-6a), 4.21 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 4.12–4.06 (m, 1 H, H-5), 2.11, 2.07, 1.90, (3s, 9 H, 3 Ac); ^{13}C NMR ($CDCl_3$): δ 170.4 (s), 170.0 (s), 169.4 (s), 167.6 (br.s), 160.9 (s), 143.3 (s), 134.6 (d), 131.2 (s), 125.7 (d), 123.8 (d), 116.8 (d), 95.4 (d), 72.5 (d), 70.4 (d), 68.6 (d), 61.9 (t), 54.3 (d), 20.7 (q), 20.6 (q), 20.3 (q). FABMS: *m*/*z* Calcd. for $C_{26}H_{24}CsN_2O_{12}$ $[M+Cs]^+$: 689.0384. Found: 689.0360.

4-Nitrophenyl 2-deoxy-2-phthalimido-β-D-*glucopyranoside* (**29**).—Sodium methoxide in MeOH (2 mL, 1 M) was added to a solution of triacetate **28** (690 mg 1.24 mmol) in a mixture of THF (15 mL) and MeOH (15 mL) and after 2 h at ambient temperature the mixture was acidified to ~pH 6 with Dowex 50X8 100 (H^+) resin, filtered and the solvent was evaporated. Chromatography (19:1 CH_2Cl_2–MeOH) gave triol **29** as a colourless solid (190 mg, 37%); mp (MeOH) 271–272 °C; $[\alpha]_D^{19}$ −51° (*c* 0.64, MeOH); TLC (19:1 CH_2Cl_2–MeOH): R_f 0.2; ^{1}H NMR ($[CD_3]_2SO$): δ 8.14 (d, 2 H, *J* 9.2 Hz, ArH), 8.00–7.77 (m, 4 H, ArH), 7.17 (d, 2 H, *J* 9.2 Hz, ArH), 5.93 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.61 (d, 1 H, $J_{OH,3}$ 4.7 Hz, OH-3), 5.36 (d, 1 H, $J_{OH,4}$ 5.7 Hz, OH-4), 4.71 (t, 1 H, $J_{OH,6}$ 5.9 Hz, OH-6), 4.22–4.03 (m, 2 H, H-3, H-2), 3.82–3.77 (m, 1 H, H-6a), 3.67–3.57 (m, 2 H, H-6b, H-5), 3.44–3.34 (m, 1 H, H-4); ^{13}C NMR ($[CD_3]_2SO$): δ 168.2 (br.s), 167.8 (br.s), 161.5 (s), 142.3 (s), 134.9 (d), 131.2 (s), 125.9 (d), 123.5 (br.s), 116.7 (d), 95.3 (d), 77.9 (d), 70.7 (d), 70.2 (d), 60.4 (t), 56.9 (d). FABMS: *m*/*z* Calcd. for $C_{20}H_{18}N_2NaO_9$ $]M+Na]^+$: 453.0910. Found: 453.0892.

4-Nitrophenyl 2-acetamido-6-O-*chloroacetyl-2-deoxy-β*-D-*glucopyranoside* (**30**).—Dibutyltin(IV) oxide (218 mg, 0.88 mmol) was added to a solution of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[34] (300 mg, 0.88 mmol) in dry MeOH (15 mL) and the mixture was heated under reflux until a clear solution was obtained (2 h). The solvent was evaporated and the residue suspended in dioxane (10 mL) and DMF (5 mL). Chloroacetyl chloride (0.21 mL, 2.64 mmol) was added, the mixture became clear and after 15 min, MeOH (3.0 mL) was added and the volatiles were evaporated. Chromatography (19:1 → 9:1 CH_2Cl_2–MeOH) gave the 6-ester **30** as a colourless solid which was washed with CH_2Cl_2, hexanes and dried (243 mg, 66%); mp (acetone–Et_2O) 216–217 °C; $[\alpha]_D^{20}$ −34° (*c* 0.72, Me_2SO); TLC (4:1 CH_2Cl_2–MeOH): R_f 0.63; ^{1}H NMR (4:1 $CDCl_3$–$[CD_3]_2SO$): δ 8.18 (d, 2 H, *J* 9.2 Hz, ArH), 7.65 (m, after D_2O exchange became a s, NH, $CHCl_3$), 7.11 (d, 2 H, *J* 9.2 Hz, ArH), 5.29 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.13 (d, 1 H, $J_{OH,4}$ 4.5 Hz, exchanged with D_2O, OH-4), 4.90 (d, 1 H $J_{OH,3}$, 4.4 Hz, exchanged with D_2O, OH-3), 4.59 (dd, 1 H, $J_{6a,6b}$ 11.8, $J_{5,6a}$ 1.8 Hz, H-6a), 4.32 (dd, 1 H $J_{5,6b}$ 6.9 Hz, H-6b), 4.15 (s, 2 H, CH_2Cl), 3.88 (q, 1 H, after D_2O exchange became a dd $J_{2,3}$ 10.2 Hz, H-2), 3.77–3.66 (m, 2 H, H-5, H-3), 3.45 (dt, 1 H, after D_2O exchange became a dd, *J* 9.6, 8.7 Hz, H-4), 1.96 (s, 3 H, Ac); ^{13}C NMR ($[CD_3]_2SO$): δ 169.6 (s), 167.3 (s), 162.1 (s), 142.1 (s), 125.9 (d), 116.7 (d), 98.3 (d), 74.1 (d), 73.6 (d), 70.2 (d), 64.7 (t), 55.3 (d), 41.3 (t), 23.2 (q). FABMS: *m*/*z* Calcd. for $C_{16}H_{20}ClN_2O_9$ $[M+H]^+$: 419.0857. Found: 419.0865.

4-Nitrophenyl 2-acetamido-3-O-*benzoyl-6*-O-*chloroacetyl-2-deoxy-β*-D-*glucopyranoside* (**31**).—Benzoyl chloride (0.52 mL, 4.5 mmol) was added dropwise over 10 min to a solution of chloroacetate **30** (900 mg, 2.15 mmol) in pyridine (20 mL) and THF (5 mL) at −60 °C. The mixture was stirred for 1.5 h, water (3.0 mL) was added and the solution was allowed to warm to ambient temperature. The solvent was evaporated, the residue dissolved in EtOAc and the solution was washed with 10% aq HCl, aq satd $NaHCO_3$, water and dried. The solvent was evaporated to low volume and the concentrate filtered through a plug of SiOH (EtOAc) and evaporated to a gum which was crystallized from EtOAc–hexanes to afford diester **31** as a colourless solid (696 mg). The mother liquors were evaporated and chromatographed (1:1 → 7:3 EtOAc–hexanes) to give further product (total 844 mg, 79%); mp 195–196 °C; $[\alpha]_D^{21}$ +34° (*c* 0.6, Me_2SO); TLC (7:3

EtOAc–hexanes): R_f 0.55; ^{1}H NMR ([CD$_3$]$_2$SO): δ 8.23 (d, 2 H, J 9.1 Hz, ArH), 8.08 (d, 1 H, $J_{NH,2}$ 9.2 Hz, NH), 7.96 (d, 2 H, J 7.3 Hz, ArH), 7.67 (t, 1 H, J 7.5 Hz, ArH), 7.54 (t, 2 H, J 7.7 Hz, ArH), 7.24 (d, 2 H, J 9.1 Hz, ArH), 5.80 (d, 1 H, $J_{OH,4}$ 6.1 Hz, OH-4), 5.50 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.28 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.7 Hz, H-3), 4.49 (d, 1 H, partially overlapped with ABq at 4.45, $J_{6a,6b} \sim 12$ Hz, H-6a), 4.45 (ABq, partially overlapped with d at 4.49, 2 H, J 15.5 Hz, CH_2Cl), 4.31 (dd, 1 H, $J_{5,6b}$ 6.0 Hz, H-6b), 4.17 (q, 1 H, H-2), 4.00–3.96 (m, 1 H, H-5), 3.70 (dt, 1 H, $J_{4,5}$ 6.1 Hz, H-4), 1.66 (s, 3 H, Ac); ^{13}C NMR ([CD$_3$]$_2$SO): δ 169.5 (s), 167.3 (s), 165.6 (s), 161.8 (s), 142.3 (s), 133.4 (d), 130.0 (s), 129.5 (d), 128.8 (d), 126.0 (d), 116.9 (d), 97.5 (d), 76.0 (d), 73.9 (d), 67.9 (d), 64.3 (t), 53.2 (d), 41.3 (t), 22.8 (q). FABMS: m/z Calcd. for $C_{23}H_{24}ClN_2O_{10}$ $[M+H]^+$: 523.1120. Found: 523.1106.

Glycosylation of alcohol **31** *and dechloroacetylation of the product* **32** (**33**).—Tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[53] (2.00 g, 3.03 mmol) and powdered 4 Å molecular sieve (3.0 g) were added to a solution of the alcohol **31** (794 mg, 1.52 mmol) in dry CH_2Cl_2 (20 mL) and dry toluene (4 mL). The mixture was stirred for 30 min then cooled to −20 °C. A solution of AgOTf (1.17 g, 4.55 mmol) and 2,4,6-collidine (0.38 mL, 2.89 mmol) in CH_2Cl_2 (20 mL) and toluene (4 mL) was added dropwise over 15 min. Stirring was carried out for 1.5 h, 10% aq $Na_2S_2O_3$ (20 mL) was added and the mixture warmed to ambient temperature and filtered through Celite. The filtrate was diluted with CH_2Cl_2 (20 mL) and washed with water, dried and the volatiles were evaporated. Chromatography (49:1 CH_2Cl_2–MeOH) gave crude chloroacetate **32** as a foam (1.01 g). TLC (24:1 CH_2Cl_2–MeOH) R_f 0.55. Crude **32** was dissolved in MeOH (15 mL) and heated under reflux for 45 min, cooled and the solvent was evaporated. Chromatography (4:1 toluene–acetone) gave the 4,6-diol **33** as a colourless amorphous solid (785 mg). Recrystallization from EtOH then from EtOAc–hexanes gave colourless fine needles; mp 140–142 °C; $[\alpha]_D^{18}$ +91° (*c* 1.4, $CHCl_3$); TLC (19:1 CH_2Cl_2–MeOH): R_f 0.53; IR ($CHBr_3$ mull): ν 3690–3145 (br, s), 1723 (s), 1595 (m), 1515 (m), 1493 (m), 1451 (m), 1339 (m), 1317 (m), 1269 (br, s), 1178 (m), 1071 (br, s), 1028 m) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.07–7.94 (m, 8 H, ArH), 7.76 (d, 2 H, J 7.5 Hz, ArH), 7.71 (d, 2 H, J 7.4 Hz, ArH), 7.62–7.50 (m, 3 H, ArH), 7.49–7.34 (m, 8 H, ArH), 7.19 (t, 4 H, J 7.4 Hz, ArH), 6.83 (d, 2 H, J 9.2 Hz, ArH), 6.22 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1′), 5.96 (d, 1 H, $J_{3'4'}$ 3.1 Hz, H-4′), 5.79 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2′), 5.61 (dd, 1 H, H-3′), 5.46 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.4 Hz, H-3), 5.19 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.37 (dd, 1 H, $J_{6a',6b'}$ 10.5, $J_{5,6a'}$ 5.0 Hz, H-6a′), 4.30–4.25 (m, 1 H, H-5′), 4.10–3.89 (m, 4 H, H-6b′, H-6a, H-6b, H-4), 3.85 (dd, 1 H, H-2), 3.79–3.74 (m, 1 H, H-5), 2.95 (d, 1 H, $J_{OH,4}$ 4.7 Hz, exchanged with D_2O, OH-4), 2.10 (s, 3 H, Ac), 2.00 (t, 1 H $J_{OH,6}$ 6.4 Hz, exchanged with D_2O, OH-6); ^{13}C NMR ($CDCl_3$): δ 166.8 (s), 165.8 (s), 165.4 (s), 165.0 (s), 163.0 (s), 161.5 (s), 142.9 (s), 133.6 (d), 133.4 (d), 133.2 (d), 130.0 (d), 129.8 (d), 129.7 (d), 129.4 (s), 129.1 (d), 129.0 (d), 128.7 (d), 128.5 (d), 128.4 (d), 128.3 (d), 125.8 (d), 116.1 (d), 100.2 (d, C-1), 92.3 (d, C-1′), 77.8 (d, C-3), 76.7 (d, C-5), 71.7 (d, C-3′), 71.3 (d, C-5′), 69.1 (d, C-4), 69.0 (d, C-2′), 67.7 (d, C-4′), 62.8 (d, C-2), 62.2 (t, C-6), 60.7 (t, C-6′), 15.7 (q, Me). FABMS: m/z Calcd. for $C_{55}H_{49}N_2O_{18}$ $[M+H]^+$: 1025.2980. Found: 1025.3024. Anal. Calcd for $C_{55}H_{48}N_2O_{18}$: C, 64.45; H, 4.72; N, 2.73. Found: C, 64.18; H, 4.81; N, 2.71.

*4-Nitrophenyl 2-acetamido-3-*O*-benzoyl-2-deoxy-β-*D*-glucopyranoside* (**34**).—Diester **31** (50 mg, 0.096 mmol) was heated in refluxing MeOH (6 mL) for 6 h. The solvent was evaporated and the residue chromatographed (7:3 EtOAc–hexanes) to give **34** as a colourless solid (40 mg, 93%); mp 160–161 °C (EtOAc–Et_2O–hexanes), lit. 163–165 °C (EtOAc–Et_2O);[46] $[\alpha]_D^{20}$ −4° (*c* 0.81, pyridine) lit. $[\alpha]_D^{20}$ −5° (*c* 1.12, pyridine);[46] ^{1}H NMR ([CD$_3$]$_2$SO): δ 8.22 (d, 2 H, J 9.2 Hz, ArH), 8.04 (d, 1 H, $J_{NH,2}$ 9.3 Hz, exchanged with D_2O, NH), 7.96 (d, 2 H, J 7.2 Hz, ArH), 7.66 (t, 1 H, J 7.4 Hz, ArH), 7.53 (t, 2 H, J 7.7 Hz, ArH), 7.24 (d, 2 H, J 9.3 Hz, ArH), 5.54 (br.s, 1 H, exchanged with D_2O, OH-4), 5.46 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.25 (br.t, 1 H, $J_{2,3} \sim J_{3,4} \sim 10.2$ Hz, H-3), 4.73 (br.t, 1 H, $J \sim 5.8$ Hz, exchanged with D_2O, OH-6), 4.12 (q, 1 H, H-2), 3.75 (br.dd, 1 H, $J_{6a,6b} \sim 10.8$, $J_{5,6a} \sim 5.0$ Hz, H-6a), 3.67–3.53 (m, 3 H, H-6b, H-5, H-4), 1.65 (s, 3 H, Ac); ^{13}C NMR ([CD$_3$]$_2$SO): δ 169.5 (s), 165.7 (s), 162.1 (s), 142.2 (s), 133.4 (d), 130.2 (s), 129.5 (d), 128.8 (d), 126.0 (d), 116.9 (d), 97.8 (d), 77.2 (d), 76.6 (d), 67.7 (d), 60.3 (t), 53.4 (d), 22.8 (q).

Prevotella *sulfatase assays*.—MdsA sulfatase was expressed from a recombinant plasmid system in *Bacteroides thetaiotaomicron*. The expression system was similar to that previously described,[8] except that a DNA sequence encoding a hexahistidine motif was added at the C-terminus of the *mdsA* gene, so that the expressed MdsA terminated in $(his)_6$. This enabled a one-step purification from periplasm by batch adsorption and elution from a Ni-NTA agarose resin using standard methods.[54] The MdsA specific activity [nmol glucose formed min^{-1} (mg enzyme)$^{-1}$] was determined using Glc 6-OSO_3Na desulfation with glucose being assayed as previously described.[7,8] A unit of MdsA sulfatase has been defined as the amount of enzyme required to release 1 μmol of glucose min^{-1} under standard assay conditions.[7] Desulfation rates of 4-nitrophenyl glycoside substrates containing GlcNAc 6-OSO_3Na were measured by determining the 4-nitrophenol released by added auxiliary glycosidases (*A. oryzae* extract, Sigma G7138, 1993 catalogue, which has β-galactosidase and β-*N*-acetylhexosaminidase activities), as described below.

Compound **1** (1 mM) was incubated for 0, 20, and 60 min with Tris–Cl buffer (37.5 mM, pH 7.4), β-mercaptoethanol (3mM), MdsA sulfatase (0.0061 units), and auxiliary glycosidases (7.3 units of β-galactosidase and 0.6 units of β-*N*-acetylhexosaminidase), in a final reaction volume of 0.08 mL. After incubation at 37 °C, reactions were terminated by addition of 0.5 M glycine buffer, pH 9.6 (0.92 mL) to raise the pH, and released 4-nitrophenol was measured at 410 nm. Controls without auxiliary glycosidases or without sulfatase were also carried out.

Compound **4** (1 mM) was incubated for 0, 20, and 60 min with Tris–Cl buffer, 37.5 (mM, pH 7.4), β-mercaptoethanol (3 mM) and MdsA sulfatase (0.0061 units) in a total volume of 0.07 mL. The sulfatase was then inactivated by heating for 2 min in a boiling water bath. After cooling, auxiliary glycosidases (0.01 mL containing 7.3 units of β-galactosidase and 0.6 units of β-*N*-acetylhexosaminidase) were added, and a second incubation continued for 30 min at 37 °C. It was terminated by addition of 0.5 M glycine buffer, pH 9.6, (0.92 mL) to raise the pH. The free 4-nitrophenol was measured at 410 nm. Controls without glycosidases were carried out.

Control experiments were conducted to ensure that the activities of the auxiliary glycosidases used were adequate. 4-Nitrophenyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**16**, 1 mM) was incubated with the auxiliary glycosidase mixture (7.3 units of β-galactosidase plus 0.6 units β-*N*-acetylhexosaminidase) and 37.5 mM Tris–Cl buffer, final volume 0.05 mL, for 110 min at 37 °C. Paper chromatography was used to detect products. Compound **4** (1 mM) was incubated with the auxiliary glycosidase extract (7.3 units of β-galactosidase plus 0.6 units β-*N*-acetylhexosaminidase) in 37.5 mM Tris Cl buffer in a final volume 0.05 mL, for 110 min at 37 °C. Ten times higher levels of auxiliary glycosidases and extended times of incubation were also used in other experiments with compound **4** to ensure they were not a limiting factor in product formation.

The β-glycosidase activities present in the auxiliary glycosidase extract were determined using 4-nitrophenyl-β-D-galactopyranoside or 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (1 mM), Tris–Cl buffer, (37.5 mM, pH 7.4) and 4-nitrophenol production determined after pH adjustment to 9.6, as above.

B. fragilis *sulfatase assays.—B. fragilis* ATCC 25285 was cultured and harvested as previously described.[12] Cell free extracts were made by 5 × 10 s of sonication (18 microns amplitude) with 30 s cooling on ice between sonications, with diisopropyl fluorophosphate (0.002%, w/v) added to minimize proteinase activity.[12] Cell debris was removed by centrifugation, the proteins precipitating between 40 and 90% ammonium sulfate saturation were selected, dissolved in Tris chloride buffer (20 mM, pH 7.4) desalted by dialysis against buffer, and the preparations were chromatographed on DEAE Sepharose. Fractions containing two separate galactose-3-sulfatase isozymes (fractions a and c, Table 2) and two separate galactose-6-sulfatase isozymes (fractions b and d, Table 2) were identified by use of the chromogenic substrates **2** and **3**. Desulfation was detected by incubating the substrate (1 mM) in Tris chloride buffer (20 mM, pH 7.4) containing auxiliary glycosidase mixture (7.3 units of β-galactosidase plus 0.6 units of β-*N*-acetylhexosaminidase) and 0.025 mL of a column fraction in a final volume 0.08 mL at 37 °C for 20 min, terminating the reaction by adding 0.5 M glycine buffer (0.92 mL) to pH 9.6 and measuring the absorption of the released 4-nitrophenol at 410 nm.

Ascending paper chromatography was carried out on the products formed from esters **2** and **3** after incubation with sulfatase-containing fractions from *B. fragilis*. The incubations were performed as above, except that they were carried out for 120 min with **2** or 180 min with **3**, and the auxiliary glycosidase mixture was omitted unless indicated. The chromatography solvent was 2:3:1 Bu^nOH–AcOH–NH_4OH (1 M).[55] Free 4-nitrophenol and its glycosides were visualized under ultra violet light, and reducing sugars were detected by the silver nitrate dip protocol.[56]

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