PERKIN 1

Esterase-catalysed regioselective 6-deacylation of hexopyranose per-acetates, acid-catalysed rearrangement to the 4-deprotected products and conversions of these into hexose 4- and 6-sulfates

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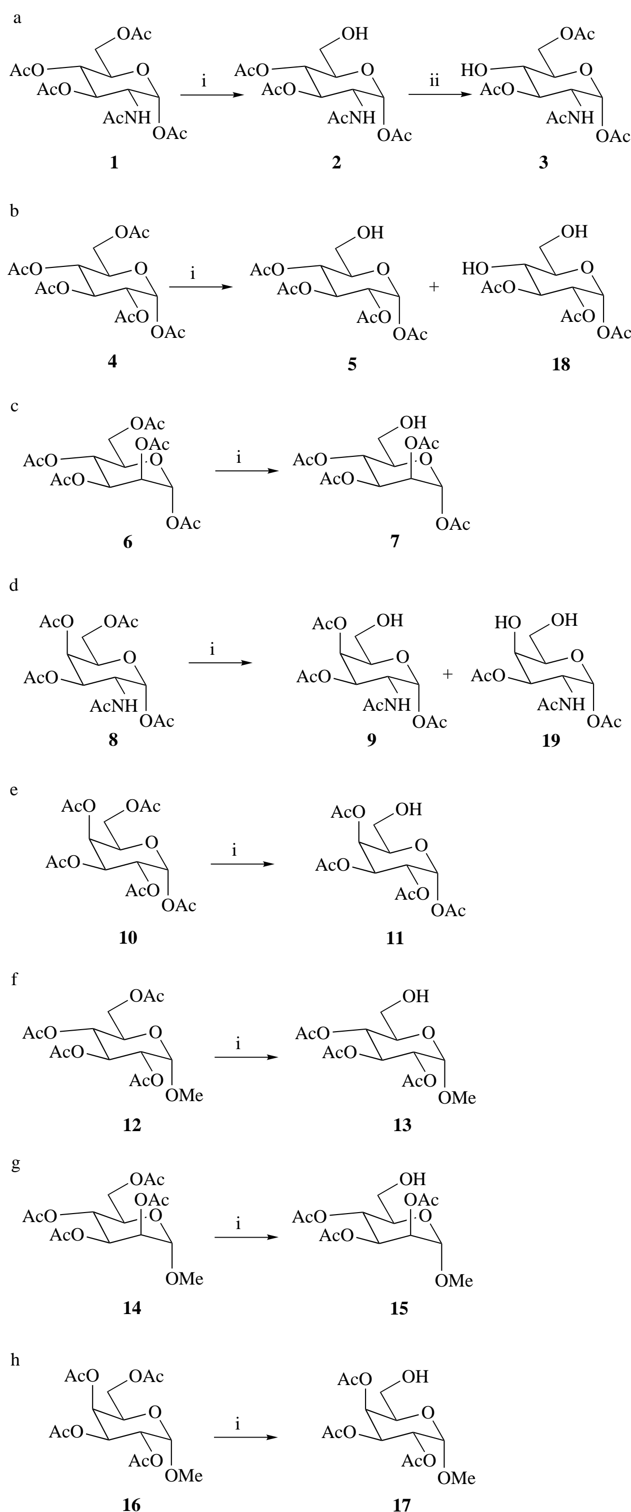
The esterase from *Rhodosporidium toruloides* has been used to catalyse the hydrolysis of a series of per-acetylated α-D-hexopyranoses and α-D-hexopyranosides. Per-acetylated glucose 4, mannose 6, *N*-acetylgalactosamine 8, galactose 10, methyl α-D-glucoside 12, methyl α-D-mannoside 14 and methyl α-D-galactoside 16 have been selectively cleaved at the C-6 position by the esterase to give the 6-OH derivatives 5, 7, 9, 11, 13, 15 and 17. Acid-catalysed rearrangement of acetates 5, 7, 13, 15, 11, 17 and 9 with 4→6 acetyl migration gives the corresponding 4-deprotected derivatives 22–28, respectively. Hydrolyses of β-D-glucose pentaacetate 20 and α-D-lactose octaacetate 21 have been attempted, but no hydrolyses have been observed. 1,2,3,6-Tetraacylated α-D-hexopyranoses 3 and 22, derivatives of *N*-acetylglucosamine and glucose respectively, and 2,3,6-triacetylated α-D-hexopyranosides 24 and 25, derivatives of glucose and mannose, respectively, have been hydrolysed by the esterase to the corresponding 4,6-dihydroxy acetates 29, 18, 30 and 31. Acylation of methyl α-D-glucopyranoside 32 catalysed by the esterase provides the C-6 monoacetate 33 and the C-3 monoacetate 34 in 4 and 5% yield, respectively. The sodium salts of *N*-acetylglucosamine, glucose, *N*-acetylgalactosamine, galactose and mannose 6-sulfates 38–42, respectively, are prepared in two steps from the 6-deacetylated hexopyranoses 2, 5, 9, 11 and 7, respectively. The sodium salts of *N*-acetylglucosamine, glucose and mannose 4-sulfates 43–45, respectively, are prepared in two steps from the 4-deacetylated precursors 3, 22 and 26 which are obtained *via* acid catalysed 4→6 acyl migration of compounds 2, 5 and 7.

Introduction

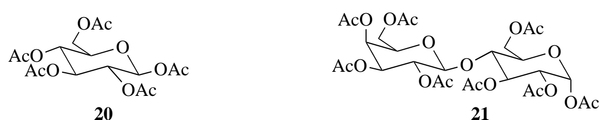
A major problem that faces the carbohydrate chemist in the construction of oligosaccharides is regioselectivity. A regioselective synthesis of an oligosaccharide ultimately necessitates the initial preparation of carbohydrate units with the required degree of protection. Strategies for the synthesis of partially protected mono- and oligo-saccharides using traditional chemical techniques have been developed. However, this approach is hindered by the requirement for multiple protection/deprotection steps. Alternatives have relied upon methods such as the partial deacylation of peracylated sugars. However, this approach is restricted by the position of deacylation and problems of poor selectivity. In contrast, enzymic techniques have been used successfully in the selective deacylation of peracylated sugars and hence have provided an effective method of manipulating protecting-group strategies in carbohydrate synthesis. Research involving enzyme-catalysed hydrolysis of peracylated sugars has provided an effective method of removing one or more acyl groups.[1] Most of this research has resulted in the selective cleavage of the anomeric ester in preference to the cleavage of other primary and secondary esters. However, when a sugar is derivatized at the anomeric centre as, for example, with methyl glycosides, cleavage of the primary ester group was commonly observed. Previously we reported the regioselective deacetylation of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose **1** to give the 6-OH derivative **2** using the crude esterase from *Rhodosporidium toruloides*.[2] The 6-OH derivative **2** was subsequently converted to the corresponding 4-OH derivative **3** *via* a 4→6 acetyl migration. It was envisaged that the combined use of chemical and enzymic techniques would provide a simple general route to the preparation of 6-OH and 4-OH derivatives of acetylated monosaccharides, which could be further used in the field of carbohydrate chemistry. Accordingly we have extended this study to glucose, galactose, *N*-acetylgalactosamine and mannose. In this paper is reported the use of the chemoenzymic method in the preparation of a series of 4-OH, 6-OH and 4,6-$(OH)_2$ derivatives of acetylated α-D-hexopyranoses using the crude esterase from *R. toruloides*, and acetylation of methyl α-D-glucopyranoside catalysed by the enzyme. Also reported is the conversion of the 4- and 6-deprotected species into the corresponding hexose monosulfates.

Results and discussion

Eight α-D-peracetylated monosaccharides and derivatives were subjected to the esterase-catalysed hydrolysis (Scheme 1). The hydrolyses were conducted by suspending the substrate and the esterase in citrate buffer (pH 5.0). The mixture was stirred at 30 °C and the reaction was monitored by thin-layer chromatography (TLC). Once the starting material was consumed, the enzyme was removed by filtration. The filtrate was then purified by conventional solvent/solvent extraction followed by column chromatography or crystallization. The results are summarized in Scheme 1b–h and Table 1. Per-acetylated α-D-glucose **4**, α-D-mannose **6**, *N*-acetyl α-D-galactosamine **8** and α-D-galactose **10**, gave the corresponding 6-hydroxy derivatives **5**, **7**, **9** and **11**, respectively, in good to excellent yield. The α-D-glycoside acetates **12**, **14** and **16** were converted similarly into the 6-hydroxy derivatives **13**, **15** and **17**, respectively. The site of the deacetylation was established by comparing the ^{1}H NMR spectra of the C-6 deacetylated products with those of the starting materials. In the C-6 deprotected sugars, C-6 protons in the ^{1}H NMR spectra are shifted upfield owing to the loss of the deshielding effect of the acetate groups. Hydrolyses of per-acetylated glucose **4** and *N*-acetylgalactosamine **8** gave the C-6 deprotected products **5** and **9** and small amounts of the dihydroxy derivatives **18** and **19** respectively. It was assumed that the dihydroxy derivatives were formed by a 4→6 acyl migration, followed by hydrolysis. The hydrolysis of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose **20** was also investigated. However, no hydrolysis was observed. Extending the incubation period of the reaction



Scheme 1 *Reagents*: i, Esterase from *R. toruloides*; ii, HOAc



did not result in hydrolysis of the substrate and upon work-up only starting material was isolated. The result suggested that the β-isomer was not a suitable substrate for the enzyme. The enzyme also failed to catalyse hydrolysis of α-D-lactose octaacetate **21**. One potential problem in this experiment was the insolubility of α-lactose octaacetate in the citrate phosphate buffer used. To overcome this problem, the solvent system described by Khan *et al.* was used.[3] The substrate, dissolved

Table 1 *Rhodosporidium toruloides* esterase-catalysed hydrolysis of (1),(2),3,4,6-(tetra)penta-*O*-acetyl-α-D-hexopyranoses

Per-acetylated sugar (mmol)	Product (% yield)
4 (85.38)	**5** (54)[a]
6 (7.69)	**7** (88)
8 (0.95)	**9** (73)[b]
10 (5.49)	**11** (67)
12 (22.60)	**13** (77)
14 (4.12)	**15** (70)
16 (5.66)	**17** (85)

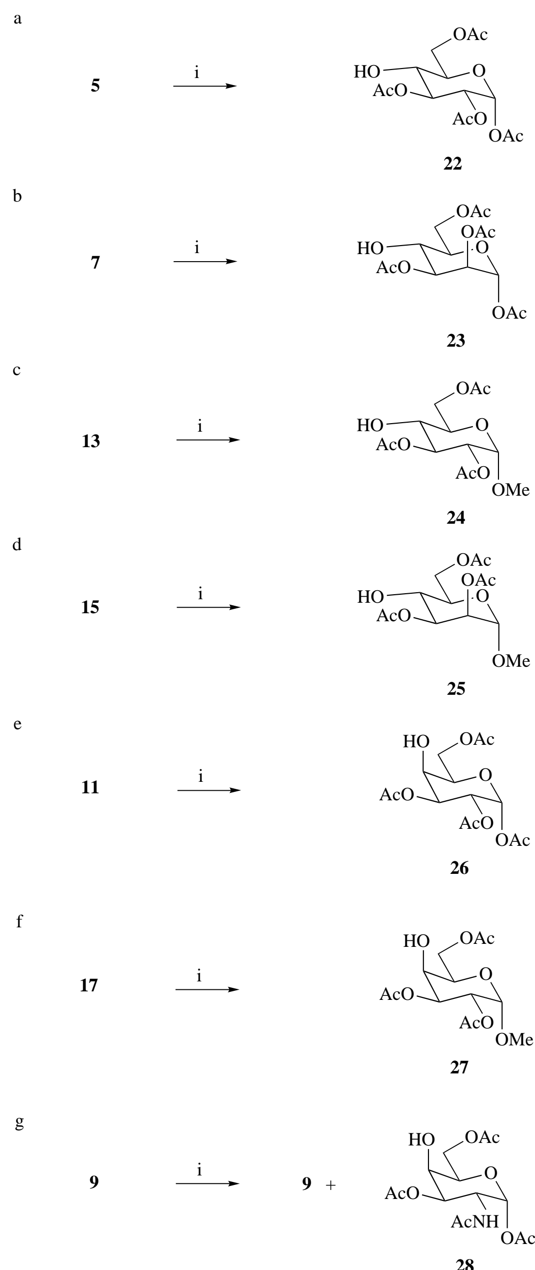
[a] 4,6-$(OH)_2$ product **18** was isolated in 1% yield. [b] 4,6-$(OH)_2$ product **19** was isolated in 6% yield.

Table 2 Conversion of the C-6 deprotected monosaccharides into the C-4 deprotected monosaccharides *via* acid-catalysed acyl migration

Substrate (mmol)	Time (*t*/h)	Product	Yield (%)
5 (11.90)	16	**22**	60
7 (3.59)	44	**23**	92
9 (39.20)	16	**28** and **9** (1 : 1)	
11 (1.45)	16	**26**	
13 (0.99)	16	**24**	63
15 (0.96)	16	**25**	49
17 (2.55)	16	**27**	

in tetrahydrofuran (THF)–acetone (1 : 1, v/v), was added to the buffer containing the enzyme. The reaction mixture was maintained at 30 °C and was monitored by TLC. However, the reaction was still unsuccessful. Upon work-up only starting material was isolated.

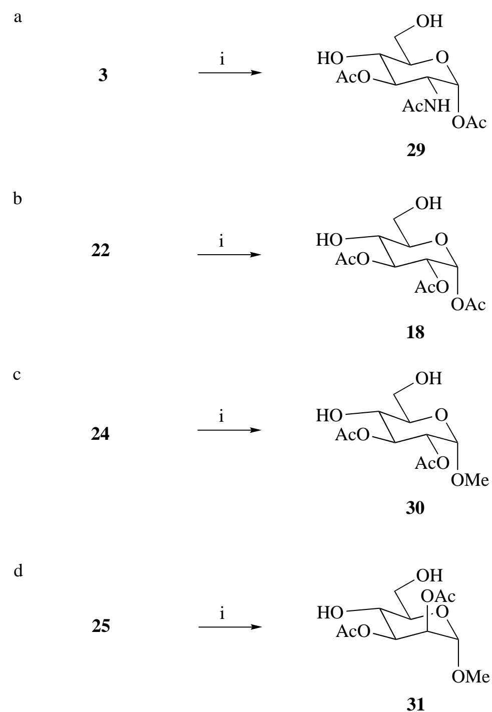
Within the field of carbohydrate chemistry there is considerable interest in the development of simple approaches for the preparation of C-4 unprotected monosaccharides which could be useful building blocks for the preparation of higher oligosaccharides containing the 1→4 linkage. It is well documented that acyl groups have the propensity to migrate under both acidic and basic conditions.[4] Albert *et al.* previously reported that the use of an O-4-to-O-6 acetyl migration in partially acetylated hexopyranosides had provided a generally applicable method for the regiospecific deprotection of hydroxy groups at C-4.[5] Having prepared a series of C-6 deprotected monosaccharides, we investigated the possibility of using a 4→6 acyl migration to convert the C-6 deprotected monosaccharides into their corresponding C-4 deprotected monosaccharides. The migration was carried out as follows: the C-6 deprotected sugar was dissolved in toluene, acetic acid (1%, v/v) was added and the solution was heated to 80 °C for various periods depending on the substrate. The products were purified by crystallization or by flash chromatography. The results are illustrated in Scheme 2 and Table 2. Acetates **5**, **7**, **13** and **15** (derivatives, respectively, of α-D-glucose, α-D-mannose, methyl α-D-glucoside and methyl α-D-mannoside) were successfully converted into the respective 4-OH derivatives **22**, **23**, **24** and **25**. The products were characterized by ^1H and ^{13}C NMR spectroscopy. In the above examples the ^1H NMR spectra of the products showed an upfield shift of the H-4 signal accompanied by a corresponding downfield shift of the H-6 signal. The remaining ^1H signals were virtually unaffected. This indicated that acyl migration from O-4 to O-6 had occurred. Conversion of the galactosides **11** and **17** into the corresponding 4-OH derivatives **26** and **27** was achieved. However, the products could not be purified because further acyl migration occurred giving complex mixtures of products. Conversion of acetate **9** into the corresponding 4-OH isomer **28** also was problematical. Acetate **9** was found to be insoluble in toluene and hence no reaction took place. Dimethylformamide (DMF) was used as a co-solvent to improve the solubility. Thus, compound **9** was initially dissolved in DMF, toluene and acetic acid were added, and the reaction mixture was heated to 80 °C. After 16 h the



Scheme 2 *Reagents and conditions:* i, HOAc, toluene, 80 °C

^{1}H NMR spectrum showed that there was a ~1 : 1 mixture of isomers **9** and **28**. The mixture was heated at 80 °C for 3 days. The ^{1}H NMR spectrum still showed a 1 : 1 mixture of the 4-OH and 6-OH products. Isolation of the required 4-OH species **28** was not achieved.

Partially protected monosaccharides can be manipulated in a variety of ways with a view to producing useful building blocks for the syntheses of higher oligosaccharides. One potential modification which was of interest was the possibility of employing the *Rhodosporidium* esterase for the subsequent removal of further acyl moieties. Thus, esterase-catalysed hydrolyses of 4-OH acetates **3**, **22**, **24** and **25** (derivatives respectively of *N*-acetylglucosamine, glucose, glucose and mannose) were examined. The results are shown in Scheme 3 and in Table 3. In all cases, the esterase hydrolysed the C-6 acetoxy group of the 4-hydroxy compound to give the corresponding 4,6-dihydroxy derivatives **29**, **18**, **30** and **31**. The position of cleavage was determined by comparison of the ^{1}H NMR spectra of the products with those of their respective starting materials. The ^{1}H NMR spectrum of each product showed a distinctive upfield shift of the H-6 signals, confirming that in all cases cleavage of the primary acetate had been achieved.

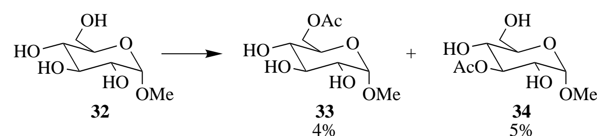


Scheme 3 *Reagents:* i, Esterase from *R. toruloides*

Table 3 *Rhodosporidium toruloides* esterase-catalysed hydrolysis of (1),(2),3,6-(tri)tetra-*O*-acetyl-α-D-hexopyranoses

Substrate (mmol)	Product	Yield (%)
3 (1.44)	**29**	82
22 (1.21)	**18**	72
24 (1.51)	**30**	35
25 (3.19)	**31**	60

The preferential acylation of primary over secondary hydroxy groups rarely can be achieved efficiently with free sugars. Recently, enzymic transformations have been used to effect specific modification of carbohydrates. In contrast to chemical techniques the regioselective acylation of sugars by enzyme-catalysed transesterification using activated esters has provided an efficient method of preparing monoacylated sugars.[6] Having successfully used the *R. toruloides* esterase for the regioselective deacylation of per-acylated monosaccharides, the possibility was investigated of using this enzyme for the selective acylation of methyl α-D-glucopyranoside **32**. The esterase-catalysed acylation was carried out with vinyl acetate in a THF–triethylamine solvent system.[6i] As shown in Scheme 4, the reaction yielded two monoacylated glucosides **33**

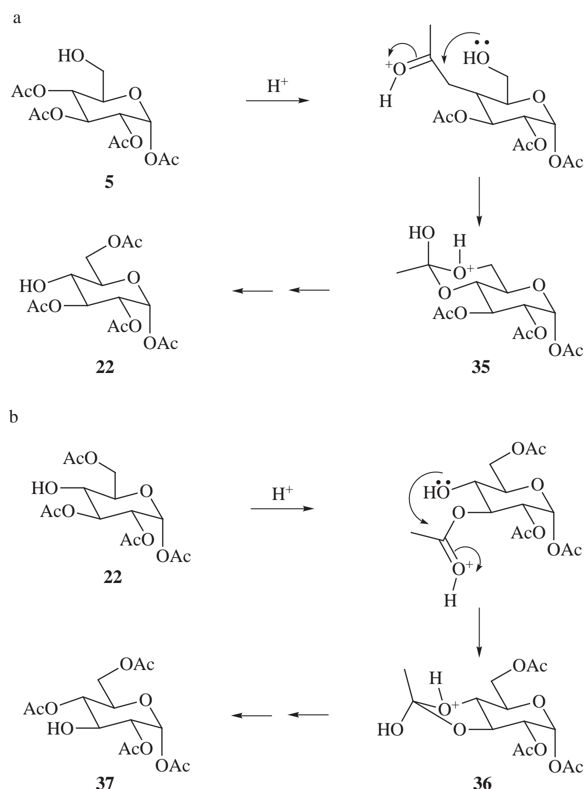


Scheme 4 *Reagents and conditions: R. toruloides*, vinyl acetate, THF, Et_3N, 25 °C

and **34** in 4 and 5% yield, respectively. Increasing the incubation period of the reaction did not improve yields. The chemical preparation of monoacylated glucosides **33** and **34**, as well as requiring multistep procedures, is complicated by the formation of mixtures of products, requiring complex purification techniques, ultimately lowering the yields of the required products.[7] In contrast, the esterase-catalysed acylation of glucosides **33** and **34** was achieved in one step, in low yields, but without optimization.

The ratios of the products of 4→6 acyl migration under the conditions reported in this study are under thermodynamic control. The fact that product ratios of 4- and 6-deprotected species are <100 : 1 indicated that the free energies of the isomers differ by <3 kcal mol^{-1}.† Energy minimization of the isomers using the programme PCMODEL indicated that they differed by <2 kcal mol^{-1}. This is the best result that could be expected at the MMX level and indicates that although it is not possible to explain the relative stabilities of the isomers in a qualitative way, the calculations give results that within the accuracy of the method are consistent with the experimental observations.

It was surprising that 4→6 acyl migration was not accompanied by some 3→4 acyl migration. No evidence of 3-deprotected species in the product mixture was found. Minimization of the 3-deprotected species in the glucose series showed that it had a free energy within 2 kcal mol^{-1} of the energies of the 4- and 6-deprotected species. However, the minimization energies of the oxonium ion intermediates generated during acyl migration (Scheme 5a,b) are very different.



Scheme 5

The presumed oxonium ion intermediate in 4→6 acyl migration (**35**, Scheme 5a) has a six-membered ring which can adopt a chair conformation. Energy minimization at the MMX level showed that the epimer shown, with the methyl group attached to the oxonium carbon atom in the equatorial position, had the lowest energy. This is shown in the Chem 3D rendering of the structure minimised using PCMODEL (Fig. 1a). This structure displays a hydrogen bond between the atoms indicated by arrows. The minimum-energy conformation found for the corresponding intermediate (**36**, Scheme 5b) for 3→4 acyl migration contains a more highly strained five-membered ring and also has an intramolecular hydrogen bond (arrows in Fig. 1b). The calculated energy difference between the 6-OH and 4-OH derivatives **5** and **22**, was 1.9 kcal mol^{-1}, the 6-OH compound **5** being the more stable. Similarly the calculated energy for intermediate **35** was 4.3 kcal mol^{-1} lower than that calculated for

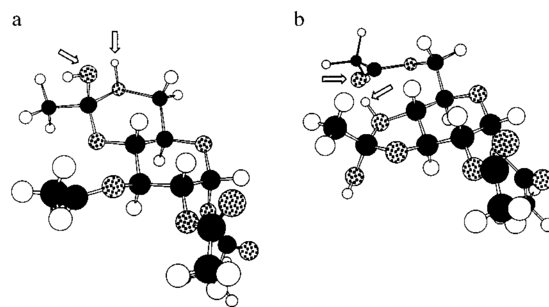


Fig. 1 Energy-minimized structures of the oxonium ion intermediates **35** and **36** (Scheme 5) in the acid-catalysed rearrangement of 6-deprotected acetates **5** and **22**, respectively

intermediate **36**. Accordingly the calculated difference in the differences in potential energy between substrates **5** and **22** and corresponding intermediates **35** and **36** for 4→6 and 3→4 acyl migration, respectively, was 6.2 kcal mol^{-1}. If the oxonium ion species **35** and **36** are assumed to be reasonable approximations to the transition states for the rate-determining steps in the corresponding acyl migrations (Hammond postulate), the value of 6.2 kcal mol^{-1} can also be taken as a reasonable approximation to the difference in activation energies for the two rearrangements. (The free energy of activation is assumed to be dominated by the enthalpy term; the entropic contribution is assumed to be approximately the same for the two reactions, since in both cases the motions of one hydroxy group and one acetoxy group are frozen in the transition state.) From the relationship $k_1/k_2 = \exp(\Delta\Delta G^{\ddagger}/RT)$, where k_1 and k_2 are the rate constants for 1→6 and 1→4 acyl migrations, respectively, and $\Delta\Delta G^{\ddagger}$ is the difference in activation energies for the two migrations, it follows that at 80 °C, the temperature at which the acyl migrations were carried out, $k_1/k_2 = 2.7 \times 10^4$. Although this figure can only be regarded as approximate, it does indicate that 3→4 acyl migration should be considerably slower than 4→6 migration. It is probable, therefore, that under the conditions of the rearrangement used in this work, 3→4 acyl migration was under kinetic control and was too slow to give observable amounts of 3-deprotected species in the product mixture.

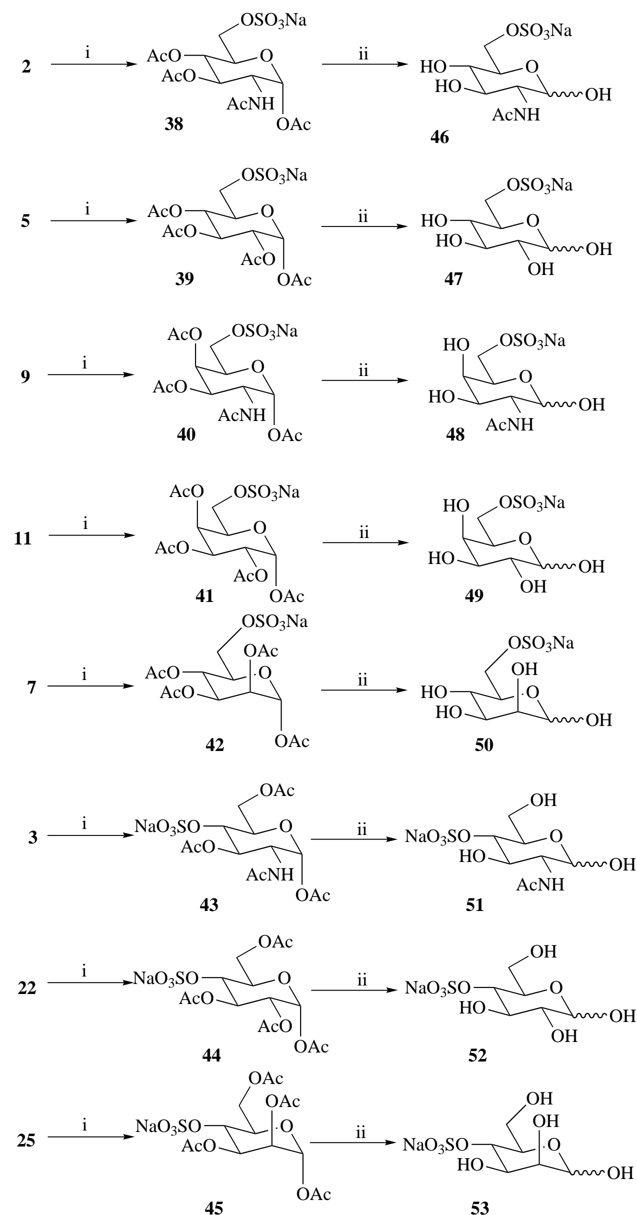
With the 4- and 6-deprotected acetates in hand, attention was turned to the conversions of these into the corresponding sulfates, since this would provide an efficient route to the latter class of compound. Sulfated carbohydrates are widely distributed in Nature. They occur in glycoprotein proteins bearing sulfated asparagine-linked oligosaccharides,[8] glycoprotein hormones,[9] brain and nerve tissue,[10] glycoproteins of several enveloped viruses,[11] lymphocyte proteoglycans and glycosaminoglycans,[12] tumour cells[13] and selectin-binding counter receptors such as SLex and ICAM-1.[14] They are also known to possess anti-viral activity,[15] inhibit natural cell-mediated cytotoxicity against K-562 target cells[16] and may be involved in the inhibition of cell migration during cancer metastasis.[17]

Synthesis of sulfated monosaccharides have attracted the interest of chemists over the last few decades.[18] They have usually been prepared by reaction of free sugars with sulfating reagents such as chlorosulfonic acid[19] or complexes formed by sulfur trioxide with pyridine[20] or DMF.[21] However, these reactions are not regiospecific and present the usual difficulties in the preparation of single isomers. They usually yield a mixture of products although these are dominated by the 6-sulfate. Separation techniques such as column chromatography using cellulose powder,[22] ion-exchange chromatography,[23] electrophoresis[24] and recrystallization of the brucine salt[25] have been used for resolving the isomers obtained by direct sulfation. However, these techniques are rather laborious and time consuming. For the synthesis of a single isomer with the sulfate group at a predetermined position, selective protection is required to mask hydroxy functions at all positions except the one to be sulfated. Chemical synthesis of hexoses with the

† 1 cal = 4.184 J.

required degree of protection is an ardous task. However, preparation of the C-6 and C-4 deprotected monosaccharides prompted us to investigate their conversion into corresponding hexose 6- and 4-sulfates.

The sulfur trioxide–pyridine complex was used as the sulfating reagent. Sulfation of **22** using sulfur trioxide–pyridine complex as the sulfating reagent and DMF as the solvent was attempted. Surprisingly, no reaction took place after heating at 90 °C for two days. When the same reaction was conducted in pyridine, the reaction was completed in a few hours at room temp. Accordingly, the sulfations were carried out by treating sulfur trioxide–pyridine complex with the hexose derivatives **2**, **5**, **9**, **11**, **7**, **3**, **22** and **25** (Scheme 6) in anhydrous pyridine. The



Scheme 6 *Reagents:* i, SO_3, pyridine; ii, MeONa

sugar sulfates produced were converted into sodium salts **38**–**45** (Scheme 6) by anion exchange using an ion-exchange resin. The reaction scales, times and yields are summarized in Table 4. The positions of the sulfate groups in 6-sulfates **38**–**42** were confirmed by comparing their ^{13}C NMR spectra with those of the corresponding 6-OH derivatives **2**, **5**, **9**, **11** and **7**. They all showed a downfield shift of the C-6 signal attributed to the deshielding effect of the attached sulfate group. ^{1}H NMR spectroscopy was used to confirm the structures of the 4-sulfates **43**–**45**. The ^{1}H NMR spectra of the sugar sulfates **43**–**45** all exhibited a downfield shift of the H-4 signals.

Table 4 Sulfation of (1),(2),3,(4)-(tri)tetra-*O*-acetyl-α-D-hexopyranoses

Starting material (mmol)	Reaction time (h)	Product (% yield)
2 (0.91)	5	**38** (63)
5 (1.17)	1.5	**39** (74)
9 (0.29)	2	**40** (93)
11 (0.58)	2	**41** (94)
7 (2.44)	2	**42** (89)
3 (1.12)	2	**43** (95)
22 (0.37)	2	**44** (98)
23 (0.23)	7	**45** (93)

Table 5 Deacetylation of the protected hexopyranose sulfates

Starting material (mmol)	Reaction time (h)	Product (% yield)
38 (0.36)	6	**46** (78)
39 (1.7)	3	**47** (92)
40 (0.17)	3	**48** (61)
41 (0.38)	2.5	**49** (83)
42 (0.67)	2	**50** (94)
43 (0.60)	8	**51** (82)
44 (0.36)	5	**52** (80)
45 (0.90)	4	**53** (88)

Deacetylation of acetate sulfates **38**–**45** was carried out at room temp. using 2.5 mol equiv. of sodium methoxide. The products **46**–**53** (Scheme 6) were purified by anion-exchange chromatography and gel filtration. Reaction scales and results are given in Table 5.

Conclusions

Hydrolysis of a series of α-D-per-acetylated monosaccharides catalysed by *R. toruloides* esterase results in cleavage of the primary acetoxy group with good selectivity. The work described in this paper provides a useful method for the regiospecific deacetylation at C-6 in various per-acetylated α-D-hexopyranoses and α-D-hexopyranosides. The advantage of this procedure is that C-6 deprotected monosaccharides can be isolated in one step. In contrast, the use of traditional chemical techniques requires the use of complex protection/deprotection strategies in order to obtain the required degree of protection. Utilisation of the propensity of acyl groups to migrate under acidic conditions has ultimately made possible the conversion of the C-6 partially acylated monosaccharides into the corresponding C-4 deprotected species. This procedure provides a simple method for regiospecific deprotection of the C-4 position of hexopyranosides. In addition, the present results demonstrate that the combined use of chemical and enzymic methods offers a convenient and expeditious method for the preparation of hexose 4- and 6-sulfates.

Experimental

The esterase from *R. toruloides* esterase was a gift from Glaxo Group Research (Glaxo-Wellcome). ^{1}H NMR spectra were recorded at 250 MHz using a Bruker ACF 250 spectrometer, or at 400 MHz using a Bruker ACP 400 spectrometer. ^{13}C NMR spectra were recorded at 63 MHz using a Bruker ACF 250 spectrometer or at 100 MHz using a Bruker ACP 400 spectrometer. *J*-Values are quoted in Hz. Mps were determined using a Stuart Scientific SMP 1 melting point apparatus and are uncorrected. Optical rotations were recorded on an Optical Activity Ltd model AA-1000 polarimeter at 589 nm (Na D-line) with a path length of 2 dm. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. Low-resolution mass spectra were recorded on Kratos MS 80 and VG Analytical Quattro 2 spectrometers. Fast-atom bombardment (FAB) mass spectra were recorded using *m*-nitrobenzyl alcohol (NOBA) or a mixture of glycerol–thioglycerol (1 : 1) as matrix. High-resolution mass spectra were

recorded on Kratos MS 80, VG Analytical ZAB-E or Bruker BioApex 9.4 T FTICR instruments. Software used was PCMODEL (Serena Software, Bloomington, Indiana) and Chem 3D (CambridgeSoft Corporation, Cambridge, Mass.). Potential energies are quoted in kcal mol^{-1} where 1 kcal = 4.184 kJ.

General procedure of esterase-catalysed hydrolyses of acetylated monosaccharides

Per-acetylated hexopyranose was suspended in citrate phosphate buffer (pH 5.0, 50 mmol/100 mmol). After addition of the *R. toruloides* esterase, the reaction mixture was stirred at 30 °C, and was followed by TLC. When complete hydrolysis of the per-acetylated sugar had occurred, the enzyme was filtered off and the filtrate was extracted with dichloromethane. The organic fraction was dried ($MgSO_4$), and concentrated by reduced-pressure evaporation. The residue was then purified by flash chromatography or crystallization.

General procedure for the conversion of C-6 deprotected monosaccharides into the C-4 deprotected monosaccharides

C-6 Deprotected monosaccharide was added to toluene and the mixture was heated to 80 °C. Acetic acid was added and the mixture was heated at 80 °C for 16 h. The solvent was removed under reduced pressure to give the C-4 deprotected product which was purified either by column chromatography or by crystallization.

1,2,3,4-Tetra-*O*-acetyl-α-D-glucopyranose 5

Glucose pentaacetate **4** (33.30 g, 85.38 mmol) was suspended in buffer (350 cm^3) at 30 °C. Esterase (340 mg) was added and the mixture was stirred for 16 h. TLC analysis (dichloromethane–methanol, 15:1, v/v) showed the formation of a major product (R_f 0.61) and a minor product (R_f 0.19). The solution was evaporated under reduced pressure to give a residue, which was extracted with ethyl acetate (2 × 100 cm^3) and ethanol (2 × 100 cm^3). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to yield a syrup. Purification by flash chromatography (dichloromethane–methanol, 150:1, v/v) yielded a syrup. Crystallization from diethyl ether yielded tetraacetate **5** (16.05 g, 54%). A minor product (R_f 0.19; 407 mg, 1.5%) was isolated, and identified by ^1H NMR as 1,2,3-tri-*O*-acetyl-α-D-glucopyranose **18**; *compound* **5**, mp 98–100 °C (lit.,[26] 99–101 °C) (Found: C, 48.32; H, 5.75. $C_{14}H_{20}O_{10}$ requires C, 48.26; H, 5.79%); $[\alpha]_D^{28}$ +113 (*c* 1.26, $CHCl_3$) {lit.,[26] $[\alpha]_D$ +119 (*c* 2.00, $CHCl_3$)}; ν_{max}(Nujol)/cm^{-1} 3500 (OH) and 1750 (C=O); δ_H(400 MHz; $CDCl_3$) 1.97 (3 H, s, CH_3CO), 1.99 (3 H, s, CH_3CO), 2.03 (3 H, s, CH_3CO), 2.13 (3 H, s, CH_3CO), 2.37 (1 H, m, OH), 3.55 (1 H, m, H^b-6), 3.68 (1 H, m, H^a-6), 3.89 (1 H, ddd, *J* 2.29, 4.19 and 10, H-5), 5.04 (1 H, dd, *J* 3.7 and 10, H-2), 5.07 (1 H, dd, *J* 9.8 and 10, H-4), 5.48 (1 H, app t, *J* 10 and 10, H-3) and 6.3 (1 H, d, *J* 3.7, H-1); δ_C(63 MHz; $CDCl_3$) 20.48 (CH_3CO), 20.58 (CH_3CO), 55.28 (OCH_3), 60.85 (C-6), 68.75 (C-4), 69.14 (C-2), 69.95 (C-3), 70.86 (C-5), 96.62 (C-1) and 169.96, 170.09 and 170.45 (C=O); *m/z* (relative abundance) (CI, NH_3) 366 [$(M + NH_4)^+$, 22%], 331 (6), 306 (14), 289 (72), 229 (34), 187 (39), 169 (26), 127 (10), 115 (49), 98 (28), 83 (43), 60 (34) and 43 (100); compound **18**, δ_H(250 MHz; D_2O), 2.00 (3 H, s, CH_3CO), 2.09 (3 H s, CH_3CO), 2.15 (3 H, s, CH_3CO), 3.83 (4 H, m, H-4, H-5 and H_2-6), 5.07 (1 H, dd, *J* 3.77 and 10.47, H-2), 5.36 (1 H, m, H-3) and 6.26 (1 H, d, *J* 3.77, H-1); δ_C(63 MHz; D_2O) 20.41 (CH_3CO), 20.77 (CH_3CO), 20.80 (CH_3CO), 61.21, 68.39, 69.28, 72.42, 73.84, 89.28 (C-1), 169.30 (C=O), 169.99 (C=O) and 171.38 (C=O).

1,2,3,4-Tetra-*O*-acetyl-α-D-mannopyranose 7

Mannose pentaacetate **6** (3.0 g, 7.69 mmol) was suspended in buffer (40 cm^3). Esterase (90 mg) was added and the mixture was stirred at 30 °C. After 20 h, the reaction mixture was filtered and the filtrate was freeze dried. The residue was suspended in ethyl acetate (10 cm^3) and subjected to flash chromatography (dichloromethane–methanol, 20:1, v/v) to yield compound **7** (2.35 g, 88%) as a syrup. The syrup was redissolved in water (15 cm^3), then freeze dried to give compound **7** as a hygroscopic powder [Found: $(M + Na)^+$, (FAB, Na) 371.0986. $C_{14}H_{20}NaO_{10}$ requires *m/z*, 371.095 41]; $[\alpha]_D^{27}$ +61.6 (*c* 0.34, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3475 (OH), 1725 (C=O) and 1250 (C–O–C); δ_H(400 MHz; $CDCl_3$) 1.97 (3 H, s, CH_3CO), 2.04 (3 H, s, CH_3CO), 2.12 (3 H, s, CH_3CO), 2.12 (3 H, s, CH_3CO), 2.46 (1 H, dd, *J* 5.97 and 8.06, OH), 3.59 (1 H, m, H^b-6), 3.68 (1 H, m, H^a-6), 3.81 (1 H, ddd, *J* 2.32, 4.39 and 10, H-5), 5.23 (1 H, dd, *J* 1.9 and 3.4, H-2), 5.26 (1 H, app t, *J* 10 and 10, H-4), 5.35 (1 H, dd, *J* 3.4 and 10, H-3) and 6.04 (1 H, d, *J* 1.90, H-1); δ_C(63 MHz; $CDCl_3$) 20.49 (CH_3CO), 20.53 (CH_3CO), 20.59 (CH_3CO), 20.71 (CH_3CO), 60.93 (C-6), 65.62 (C-4), 68.23 (C-2), 68.44 (C-3), 72.74 (C-5), 90.49 (C-1), 168.15 (C=O), 169.66 (C=O), 169.86 (C=O) and 170.24 (C=O); *m/z* (rel. ab.) FAB (NOBA) 371 [$(M + Na)^+$, 30%], 331 (15), 290 (26), 281 (22), 229 (37), 169 (40), 147 (53), 127 (100) and 109 (94).

2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-galactopyranose 9

Acetate **8** (200 mg, 5.14 × 10^{-4} mol) was suspended in buffer (9 cm^3). Esterase (80 mg) was added and the reaction mixture was stirred at 30 °C for 16 h before being filtered, and the filtrate was freeze dried. Ethyl acetate (5 cm^3) and methanol (2 cm^3) were added to the residue. The mixture was subjected to flash chromatography (dichloromethane–methanol, 15:1, v/v) to give title compound **9** (0.13 g, 73%) as a solid and diol **19** (9.8 mg, 6%) as a syrup; compound **9**, mp 189–181 °C [Found: $(M + Na)^+$, (FAB, Na) 371.1079. $C_{14}H_{21}NNaO_9$ requires *m/z*, 371.111 39]; $[\alpha]_D^{23}$ +150 (*c* 0.07, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3600–3300 (OH), 1750 (C=O), 1675 (C=O) and 1250 (C–O–C); δ_H(400 MHz; $CDCl_3$) 1.92 (3 H, s, CH_3CO), 2.02 (3 H, s, CH_3CO), 2.14 (3 H, s, CH_3CO), 2.17 (3 H, s, CH_3CO), 2.58 (1 H, m, OH), 3.45 (1 H, dd, *J* 6.4 and 11.63, H^b-6), 3.64 (1 H, dd, *J* 6.4 and 1.63, H^a-6), 4.04 (1 H, app t, *J* 6.4 and 6.4, H-5), 4.71 (1 H, ddd, *J* 3.7, 9.2 and 11.6, H-2), 5.22 (1 H, dd, *J* 3.2 and 11.6, H-3), 5.37 (1 H, d, *J* 3, H-4), 5.66 (1 H, d, *J* 9.2, NH) and 6.17 (1 H, d, *J* 3.7, H-1); δ_C(63 MHz; $CDCl_3$) 20.58 (CH_3CO), 20.63 (CH_3CO), 20.82 (CH_3CO), 22.98 (CH_3CO), 47.12 (C-2), 60.43 (C-6), 67.51 (C-4), 67.74 (C-3), 71.28 (C-5), 91.15 (C-1), 169.01 (C=O), 170.14 (C=O), 171.05 (C=O) and 171.12 (C=O); *m/z* (rel. ab.) FAB (GlythioNa) 370 [$(M + Na)^+$, 28%], 310 (12), 237 (15), 200 (10), 153 (11), 137 (28), 115 (86), 61 (59) and 44 (100); compound **19**, δ_H(250 MHz; $CDCl_3$) 1.90 (3 H, s, CH_3CO), 2.05 (3 H, s, CH_3CO), 2.13 (3 H, s, CH_3CO), 3.62–3.72 (2 H, m, H_2-6), 4.03 (1 H, app t, *J* 6.1 and 6.1, H-5), 4.14 (1 H, d, *J* 2.6, H-4), 4.52 (1 H, dd, *J* 3.78 and 11.34, H-2), 5.14 (1 H, dd, *J* 2.6 and 11.34, H-3) and 6.07 (1 H, d, *J* 3.78, H-1); δ_C(63 MHz; $CDCl_3$) 21.00 (CH_3CO), 21.07 (CH_3CO), 22.51 (CH_3CO), 47.22 (C-6), 61.61 (C-4), 66.87 (C-3), 71.17 (C-5), 73.65, 92.11 (C-1), 173.46 (C=O), 173.83 (C=O) and 175.49 (C=O).

1,2,3,4-Tetra-*O*-acetyl-α-D-galactopyranose 11

Galactose pentaacetate **10** (2.5 g, 6.41 mmol) was suspended in buffer (50 cm^3). Esterase (500 mg) was added and the mixture was stirred at 30 °C for 16 h. TLC (dichloromethane–methanol, 15:1, v/v) showed that the reaction was not complete. Further esterase (300 mg) and buffer (30 cm^3) were added. The mixture was stirred at 30 °C for another 7 h. The enzyme was removed by filtration and the filtrate was freeze dried. The residue was suspended in dichloromethane (25 cm^3). Water (2 cm^3) was added and the resulting mixture was subjected to flash chromatography (dichloromethane–methanol, 40:1, v/v) to give compound **11** as a syrup (1.5 g, 67%) (the syrup could be converted into a powder by dissolution in water followed by freeze drying) [Found: $(M + Na)^+$, (FAB, Na) 371.0986. $C_{14}H_{20}NaO_{10}$ requires *m/z*, 371.095 41]; $[\alpha]_D^{27}$ +67.1 (*c* 0.81, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3500 (OH), 1750 (C=O) and 1225 (C–O–C); δ_H(250 MHz; $CDCl_3$) 2.00 (3 H, s, CH_3CO), 2.01 (3 H, s, CH_3CO), 2.14 (3 H, s, CH_3CO), 2.16 (3 H, s, CH_3CO), 3.46

(1 H, dd, J 6.6 and 11.9, H^b-6), 3.66 (1 H, dd, J 6.6 and 11.9, H^a-6), 4.05 (1 H, app t, J 6.6 and 6.6, H-5), 5.35 (2 H, m, H-2, -3), 5.48 (1 H, d, J 1.19, H-4) and 6.36 (1 H, d, J 1.69, H-1); δ_C(63 MHz; $CDCl_3$) 20.42 (CH$_3$CO), 20.52 (CH$_3$CO), 20.77 (CH$_3$CO), 60.62 (C-6), 66.56, 67.33, 68.19 (C-4), 71.41 (C-5), 89.56 (C-1), 168.98, 169.80, 169.90 and 170.82 (C=O); m/z (rel. ab.) FAB (Glythio Na) 371 [(M + Na)$^+$, 100%], 311 (12), 289 (24), 229 (18), 169 (39), 127 (47) and 109 (71).

Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside 13

Glucopyranoside **12** (8.16 g, 22.6 mmol) was suspended in buffer (80 cm^3) at 30 °C. Esterase (240 mg) was added and the reaction mixture was stirred for 16 h, filtered, and extracted with dichloromethane (3 × 50 cm^3) and ethyl acetate (2 × 50 cm^3). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to yield a syrup. Crystallization from diethyl ether yielded *compound* **13** (5.54 g, 77%); mp 103–104 °C (lit.,[27] 109.5–110 °C; lit.,[28] 110 °C) (Found: C, 48.54; H, 6.24. $C_{13}H_{20}O_9$ requires C, 48.73; H, 6.29%); $[\alpha]_D^{28}$ +125 (c 0.72, $CHCl_3$) {lit.,[27] $[\alpha]_D$ +137 ($CHCl_3$); lit.,[28] 145.5}; ν_{max}(Nujol)/cm^{-1} 3500 (OH) and 1740 (C=O); δ_H(400 MHz; $CDCl_3$) 1.98 (3 H, s, CH_3CO), 2.02 (3 H, s, CH_3CO), 2.03 (3 H, s, CH_3CO), 2.39 (1 H, m, OH), 3.37 (3 H, s, OCH_3), 3.55 (1 H, m, H^b-6), 3.68 (1 H, m, H^a-6), 3.75 (1 H, ddd, J 2.3, 4.3 and 10, H-5), 4.88 (1 H, dd, J 3.6 and 10, H-2), 4.93 (1 H, d, J 3.6, H-1), 4.99 (1 H, dd, J 10 and 9.7, H-4) and 5.49 (1 H, dd, J 9.7 and 10, H-3); δ_C(63 MHz; $CDCl_3$) 20.48 (CH$_3$CO), 20.58 (CH$_3$CO), (CH$_3$CO), 55.28 (OCH_3), 60.85 (C-6), 68.75 (C-4), 69.14 (C-3), 69.95 (C-5), 70.86 (C-2), 96.62 (C-1), 169.96 (C=O), 170.09 (C=O) and 170.45 (C=O); m/z (rel. ab.) FAB (NOBA) 343 [(M + Na)$^+$, 48%], 331 (7), 321 [(M + H)$^+$, 7], 290 (9), 229 (34), 169 (100), 141 (28), 127 (85) and 109 (82).

Methyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside 15

Mannopyranoside **14** (1.49 g, 4.12 mmol) was suspended in buffer (15 cm^3) at 30 °C. Esterase (45 mg) was added and the mixture was stirred for 16 h before being filtered, and extracted with dichloromethane (2 × 30 cm^3). The organic extracts were dried ($MgSO_4$), filtered, and evaporated under reduced pressure to yield a syrup. Crystallization from diethyl ether yielded compound **15** as crystals (0.92 g, 70%); mp 95–96 °C (lit.,[29] 97–98 °C) (Found: C, 48.75; H, 6.29. Calc. for $C_{13}H_{20}O_9$: C, 48.73; H, 6.29%); $[\alpha]_D^{28}$ +54.5 (c 0.3, $CHCl_3$) {lit.,[29] $[\alpha]_D^{25}$ +54.9 (c 1.1, $CHCl_3$)}; ν_{max}(Nujol)/cm^{-1} 3500 (OH) and 1740 (C=O); δ_H(400 MHz; $CDCl_3$) 1.97 (3 H, s, CH_3CO), 2.04 (3 H, s, CH_3CO), 2.12 (3 H, s, CH_3CO), 2.43 (1 H, s, OH), 3.38 (3 H, s, OCH_3), 3.60 (1 H, dd, J 12.62 and 4.25, H^b-6), 3.72 (2 H, m, H-5, H^a-6), 4.69 (1 H, d, J 1.66, H-1), 5.22 (2 H, m, H-2, -4) and 5.35 (1 H, dd, J 3.44 and 10.16, H-3); δ_C(63 MHz; $CDCl_3$) 20.54 (CH$_3$CO), 20.58 (CH$_3$CO), 20.72 (CH$_3$CO), 55.12 (OCH_3), 61.11 (C-6), 66.29 (C-4), 68.69 (C-3), 69.39 (C-2), 70.33 (C-5), 98.47 (C-1) and 169.75, 169.95 and 170.69 (C=O); m/z (rel. ab.) FAB (NOBA) 343 [(M + Na)$^+$, 21%], 331 (14), 321 [(M + H)$^+$, 11], 319 (17), 290 (19), 229 (50), 169 (71), 141 (37), 127 (100) and 109 (93).

Methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranoside 17

Galactopyranoside **16** (2.0 g, 5.52 mmol) was suspended in buffer (40 cm^3). Esterase (70 mg) was added and the reaction mixture was stirred at 30 °C for 20 h. TLC analysis (dichloromethane–methanol; 9:1, v/v) indicated the reaction was not complete. Further esterase (100 mg) and buffer (40 cm^3) were added. The reaction mixture was stirred at 30 °C for another 5 h before being filtered and the filtrate was freeze dried. The residue was suspended in dichloromethane (10 cm^3) and purified by flash chromatography (diochloromethane–methanol, 40:1, v/v) to yield compound **17** (1.5 g, 85%) as a syrup (the syrup was converted into a hygroscopic powder by dissolution in water followed by freeze drying) [Found: (M + Na)$^+$, (FAB, Na) 343.1030. $C_{13}H_{20}NaO_9$ requires m/z, 343.1005]; $[\alpha]_D^{26}$ +149.2 (c 0.12, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3525 (OH), 1750 (C=O) and 1250 (C–O–C); δ_H(250 MHz; $CDCl_3$) 1.99 (3 H, s, CH_3CO), 2.08 (3 H, S, CH_3CO), 2.15 (3 H, s, CH_3CO), 3.40 (3 H, s, OCH_3), 3.49 (1 H, dd, J 6.11 and 11.63, H^b-6), 3.66 (1 H, dd, J 9.98 and 11.63, H^a-6), 4.04 (1 H, app t, J 6.6 and 6.6, H-5), 4.98 (1 H, d, J 3.48, H-1), 5.16 (1 H, dd, J 3.48 and 10.75, H-2), 5.36 (1 H, dd, J 3.49 and 10.75, H-3) and 5.42 (1 H, dd, J 3.49 and 10.75, H-4); δ_C(63 MHz; $CHCl_3$) 20.13 (CH$_3$CO), 20.28 (CH$_3$CO), 54.88 (OCH_3), 60.33 (C-6), 67.29 (C-3), 67.93 (C-2), 68.31 (C-4), 68.44 (C-5), 96.60 (C-1) and 169.56, 170.03 and 170.35 (C=O); m/z (rel. ab.) FAB (Glythio Na) 343 [(M + Na)$^+$, 100%], 289 (22), 229 (10), 169 (25), 127 (39) and 109 (45).

1,2,3,6-Tetra-*O*-acetyl-α-D-glucopyranose 22

Glucopyranose derivative **5** (4.08 g, 11.9 mmol) was added to toluene (76 cm^3) and the mixture was heated to 80 °C. Acetic acid (0.76 cm^3) was added and the reaction mixture was stirred at 80 °C for 16 h. The solvent was removed under reduced pressure to give a syrup. Crystallization from diethyl ether yielded *tetraacetate* **22** (2.44 g, 60%); mp 99–99.5 °C (Found: C, 48.38; H, 5.77. $C_{14}H_{20}O_{10}$ requires C, 48.26; H, 5.79%); $[\alpha]_D^{28}$ +67.5 (c 0.64, $CHCl_3$) {lit.,[26] $[\alpha]_D^{24}$ +56.8 (c 0.88, $CHCl_3$)}; ν_{max}(Nujol)/cm^{-1} 3500 (OH) and 1750 (C=O); δ_H(400 MHz; $CDCl_3$) 1.98 (3 H, s, CH_3CO), 2.07 (3 H, s, CH_3CO), 2.09 (3 H, s, CH_3CO), 2.13 (3 H, s, CH_3CO), 3.37 (1 H, br s, OH), 3.58 (1 H, app t, J 10 and 10, H-4), 3.92 (1 H, ddd, J 2.3, 3.9 and 10, H-5), 4.22 (1 H, dd, J 2.3 and 12.4, H^b-6), 4.44 (1 H, dd, J 3.9 and 12.4, H^a-6), 4.99 (1 H, dd, J 3.71 and 10, H-2), 5.29 (1 H, dd, J 10.1 and 10, H-3) and 6.25 (1 H, d, J 3.71, H-1); δ_C(63 MHz; $CDCl_3$) 20.35 (CH$_3$CO), 20.67 (CH$_3$CO), 20.73 (CH$_3$CO), 21.29 (CH$_3$CO), 62.4 (C-6), 68.26 (C-4), 69.14 (C-2), 71.92 (C-3), 72.14 (C-5), 89.18 (C-1), 168.97 (C=O), 169.87 (C=O), 171.09 (C=O) and 171.57 (C=O); m/z (rel. ab.) FAB (NOBA) 371 [(M + Na)$^+$, 29%], 349 [(M + H)$^+$, 5], 331 (9), 311 (5), 290 (10), 229 (100), 187 (24), 169 (21) and 127 (62).

1,2,3,6-Tetra-*O*-acetyl-α-D-mannopyranose 23

Mannopyranoside derivative **7** (1.25 g, 3.59 mmol) was dissolved in toluene (25 cm^3). Acetic acid (1 cm^3) was added and the mixture was heated at 80 °C. After 44 h, the solvent was removed by reduced pressure evaporation. The residue was purified by column chromatography (dichloromethane–methanol, 40:1, v/v) to yield a syrup. The syrup was dissolved in water and then freeze dried to give tetraacetate **23** (1.15 g, 92%) as a powder [Found: (M + Na)$^+$, (FAB, Na) 371.0957. $C_{14}H_{20}NaO_{10}$ requires m/z, 371.095 41]; $[\alpha]_D^{27}$ +27.3 (c 0.2, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3500 (OH), 1750 (C=O) and 1250 (C–O–C); δ_H(400 MHz; $CDCl_3$) 2.01 (3 H, s, CH_3CO), 2.07 (3 H, s, CH_3CO), 2.09 (3 H, s, CH_3CO), 2.10 (3 H, s, CH_3CO), 3.15 (1 H, d, J 4.49, OH), 3.88–3.79 (2 H, m, H-5, -4), 4.23 (1 H, dd, J 1.69 and 12.2, H^b-6), 4.43 (1 H, dd, J 4.02 and 12.2, H^a-6), 5.18–5.13 (2 H, m, H-2, -3) and 5.99 (1 H, d, J 1.72, H-1); δ_C(63 MHz; $CDCl_3$) 20.45 (CH$_3$CO), 20.53 (CH$_3$CO), 20.56 (CH$_3$CO), 20.63 (CH$_3$CO), 62.78 (C-6), 64.70 (C-4), 68.26 (C-2), 70.72 (C-3), 72.69 (C-5), 90.61 (C-1), 168.12 (C=O), 169.47 (C=O), 170.39 (C=O) and 171.49 (C=O); m/z (rel. ab.) FAB (NOBA) 371 [(M + Na)$^+$, 67%], 349 [(M + H)$^+$, 3], 331 (7), 290 (47), 287 (8), 229 (100), 187 (25) and 127 (76).

2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranose 28

Galactopyranose derivative **9** (45 mg, 39.2 mmol) was added to toluene (0.79 cm^3) and the mixture was heated to 80 °C. Acetic acid (0.01 cm^3) was added and the mixture was heated at 80 °C for 16 h. The solvent was removed under reduced pressure to give a solid. The ^{1}H NMR spectrum showed that the product was compound **28**; no acyl migration had taken place. This was probably due to the insolubility of product **28** in toluene. To improve the solubility of compound **28**, DMF was used as a

co-solvent. Glycoside **28** (45 mg, 3.92×10^{-2} mol) was initially dissolved in DMF (0.1 cm^3), toluene (0.79 cm^3) was added, and the reaction mixture was heated to 80 °C. Acetic acid (0.01 cm^3) was added and the mixture was heated at 80 °C. After 16 h the ^1H NMR spectrum showed that there was a ~1 : 1 mixture of compounds **28** and **9**. The mixture was heated at 80 °C for 3 days. The solvent was removed under reduced pressure to give a syrup. The ^1H NMR spectrum showed that there was still a ~1 : 1 mixture of compounds **28** and **9**. The addition of a more polar co-solvent had altered the position of the equilibrium. However, isolation of the required 4-deprotected product was not achieved effectively.

1,2,3,6-Tetra-*O*-acetyl-α-D-galactopyranose 26

Galactopyranose derivative **11** (503 mg, 1.45 mmol) was added to toluene (8.90 cm^3) and the mixture was heated to 80 °C. Acetic acid (0.09 cm^3) was added and the solution was heated at 80 °C for 16 h. The solvent was removed under reduced pressure to give a syrup in quantitative yield. The ^1H NMR spectrum showed that compound **26** had been formed as the major product but minor amounts of starting material **11** (12.5% as determined from the ^1H NMR integrals) were still present. Purification by crystallization or by column chromatography proved ineffective; compound **26** [Found: $(M + Na)^+$, (FAB, Na) 371.0938. $C_{14}H_{20}NaO_{10}$ requires *m/z*, 371.095 41]; $[\alpha]_D^{27}$ +136.3 (*c* 0.16, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3500 (OH), 1730 (C=O) and 1250 (C–O–C); δ_H(400 MHz; $CDCl_3$) 1.87 (3 H, s, CH_3CO), 1.93 (3 H, s, CH_3CO), 1.97 (3 H, s, CH_3CO), 2.01 (3 H, s, CH_3CO), 3.58 (1 H, br s, OH), 4.06 (3 H, m, H-4, -5, H^b-6), 4.14 (1 H, m, H^a-6), 5.08 (1 H, dd, *J* 2.80 and 10.8, H-3), 5.28 (1 H, dd, *J* 3.6 and 10.8, H-2) and 6.18 (1 H, d, *J* 3.6, H-1); δ_C(63 MHz; $CDCl_3$) 20.07 (CH_3CO), 20.29 (CH_3CO), 20.36 (CH_3CO), 20.40 (CH_3CO), 62.33 (C-6), 66.09 (C-2), 66.62, 69.47 (C-3), 69.90, 89.45 (C-1), 168.87 (C=O), 169.79 (C=O), 170.08 (C=O) and 170.69 (C=O); *m/z* (rel. ab.) FAB (GlythioNa) 371 [$(M + Na)^+$, 100%], 311 (12), 289 (19), 229 (10), 169 (12), 127 (28) and 109 (20).

Methyl 2,3,6-tri-*O*-acetyl-α-D-glucopyranoside 24

Glucopyranoside derivative **13** (317 mg, 9.91×10^{-4} mol) was added to toluene (5.6 cm^3) and the mixture was heated to 80 °C. Acetic acid (0.056 cm^3) was added to the solution, which was heated at 80 °C for 16 h. The solvent was removed under reduced pressure to give a syrup. Purification by flash chromatography (toluene–ethyl acetate, 5 : 1, v/v) yielded compound **24** (R_f 0.53; 199 mg, 63%) as a syrup [Found: $(M + Na)^+$, (FAB, Na) 343.0999. $C_{13}H_{20}NaO_9$ requires *m/z*, 343.1005]; $[\alpha]_D^{28}$ +120 (*c* 0.27, $CHCl_3$) (lit.,[30] $[\alpha]_D^{20}$ +100.8); ν_{max}(Nujol)/cm^{-1} 3550 (OH), 1750 (C=O) and 1250 (C–O–C); δ_H(400 MHz; $CDCl_3$) 2.06 (3 H, s, CH_3CO), 2.07 (3 H, s, CH_3CO), 2.10 (3 H, s, CH_3CO), 3.05 (1 H, d, *J* 5.4, OH), 3.38 (3 H, s, OCH_3), 3.54 (1 H, m, H-4), 3.80 (1 H, ddd, *J* 2.29, 4.4 and 10, H-5), 4.28 (1 H, dd, *J* 2.29 and 12.21, H^b-6), 4.44 (1 H, dd, *J* 4.4 and 12.21, H^a-6), 4.83 (1 H, dd, *J* 3.68 and 9.8, H-2), 4.88 (1 H, d, *J* 3.68, H-1) and 5.28 (1 H, app t, *J* 9.8 and 9.8, H-3); δ_C(63 MHz; $CDCl_3$) 20.62 (CH_3CO), 20.68 (CH_3CO), 20.73 (CH_3CO), 55.15 (OCH_3), 62.72 (C-6), 69.17 (C-4), 69.55 (C-5), 70.47 (C-2), 72.74 (C-3), 96.73 (C-1), 170.17 (C=O), 171.36 (C=O) and 171.46 (C=O); *m/z* (rel. ab.) FAB (NOBA) 343 [$(M + Na)^+$, 43%], 331 (9), 321 [$(M + H)^+$, 100], 303 (6), 290 (32), 229 (77), 169 (51), 127 (65), 115 (31) and 109 (51).

Methyl 2,3,6-tri-*O*-acetyl-α-D-mannopyranoside 25

Mannopyranoside derivative **15** (336 mg, 9.64×10^{-4} mol) was added to toluene (5.9 cm^3) and the mixture was heated to 80 °C. Acetic acid (0.06 cm^3) was added and the reaction mixture was stirred at 80 °C for 16 h. The solvent was removed under reduced pressure to give a syrup. Purification by chromatography (dichloromethane–methanol, 75 : 1, v/v) yielded compound **25** (164 mg, 49%) as a syrup [Found: $(M + Na)^+$, (FAB, Na) 343.1017. $C_{13}H_{20}NaO_9$ requires *m/z*, 343.1005]; $[\alpha]_D^{27}$ +30 (*c* 0.14, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3525 (OH), 1750 (C=O) and 1250 (C–O–C); δ_H(400 MHz; $CDCl_3$) 2.01 (3 H, s, CH_3CO), 2.07 (3 H, s, CH_3CO), 2.08 (3 H, s, CH_3CO), 2.98 (1 H, d, *J* 4.09, OH), 3.35 (3 H, s, OCH_3), 3.81–3.74 (2 H, m, H-4, -5), 4.29 (1 H, dd, *J* 1.16 and 12, H^b-6), 4.42 (1 H, dd, *J* 4.49 and 12, H^a-6), 4.63 (1 H, dd, *J* 1.57, H-1) and 5.17–5.09 (2 H, m, H-2, -3); δ_C(63 MHz; $CDCl_3$) 20.61 ($3 \times CH_3CO$), 54.91 (OCH_3), 63.19 (C-6), 65.42, 69.49 (C-2), 70.47, 71.34 (C-3), 98.43 (C-1), 169.83 (C=O), 170.59 (C=O) and 171.33 (C=O); *m/z* (rel. ab.) FAB (NOBA) 343 [$(M + Na)^+$, 41%], 321 [$(M + H)^+$, 100], 290 (19) and 229 (14).

Methyl 2,3,6-tri-*O*-acetyl-α-D-galactopyranoside 27

Galactopyranoside **17** (708 mg, 2.55 mmol) was added to toluene (12.5 cm^3) and the mixture was heated to 80 °C. Acetic acid (0.13 cm^3) was added to the solution, which was heated at 80 °C for 16 h. The solvent was removed under reduced pressure to give a syrup ion quantitative yield. Purification by crystallization or by column chromatography proved ineffective. The ^1H NMR spectrum showed that compound **27** had been formed as the major product but that minor amounts of starting material **17** (20% as determined from the ^1H NMR integrals) were still present. Purification by crystallization or by column chromatography proved ineffective; compound **27** [Found: $(M + Na)^+$, (FAB, Na) 343.1029. $C_{13}H_{20}NaO_9$ requires *m/z*, 343.1005); $[\alpha]_D^{27}$ +69.5 (*c* 0.79, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3550 (OH), 1750 (C=O) and 1225 (C–O–C); δ_H(400 MHz; $CDCl_3$) 1.94 (6 H, s, $2 \times CH_3CO$), 1.96 (3 H, s, CH_3CO), 2.81 (1 H, br s, OH), 3.26 (3 H, s, OCH_3), 3.91 (1 H, m, H-5), 3.99 (1 H, m, H-4), 4.14 (2 H, m, H_2-6), 4.82 (1 H, d, *J* 2.97, H-1) and 5.09 (2 H, m, H-2, -3); δ_C(63 MHz; $CDCl_3$) 2.304 (CH_3CO), 20.37 (CH_3CO), 20.41 (CH_3CO), 54.78 (OCH_3), 62.77 (C-6), 67.15, 67.18, 67.72 (C-2), 69.76 (C-3), 96.69 (C-1), 169.92 (C=O), 170.18 (C=O) and 170.62 (C=O); *m/z* (rel. ab.) FAB (GlythioNa) 343 [$(M + Na)^+$, 100%], 289 (52), 229 (18), 169 (40), 141 (20), 127 (56) and 103 (67).

2-Acetamido-1,3-di-*O*-acetyl-2-deoxy-α-D-glucopyranose 29

Glucopyranoside **3** (500 mg, 1.44 mmol) was suspended in buffer (6.25 cm^3) at 30 °C. Esterase (18 mg) was added and the reaction mixture was stirred at 30 °C for 16 h. The mixture was concentrated on the freeze drier, extracted with ethanol (3 × 5 cm^3), filtered and evaporated under reduced pressure to yield a solid. Recrystallization from acetone yielded compound **29** (0.36 g, 82%) as crystals; mp 166–167 °C (decomp.) [(Found: $(M + Na)^+$, (FAB Na) 328.0978. $C_{12}H_{19}NNaO_8$ requires *m/z*, 328.100 83]; $[\alpha]_D^{27}$ +62.5 (*c* 0.08, MeOH); ν_{max}(Nujol)/cm^{-1} 3700–3200 (OH), 1735 (C=O) and 1650 (C=O, NHAc); δ_H(400 MHz; D_2O) 1.93 (3 H, s, CH_3CO), 2.09 (3 H, s, CH_3CO), 2.19 (3 H, s, CH_3CO), 3.79 (4 H, m, H-4, -5, H_2-6), 4.28 (1 H, dd, *J* 3.68 and 10.89, H-2), 5.20 (1 H, dd, *J* 8.92 and 10.89, H-3) and 6.05 (1 H, d, *J* 3.68, H-1); δ_C(63 MHz; D_2O) 20.86 (CH_3CO), 20.92 (CH_3CO), 22.29 (CH_3CO), 51.19 (C-2), 60.62 (C-6), 67.85 (C-4), 73.61 (C-3), 74.45 (C-5), 91.74 (C-1), 173.20 (C=O), 174.21 (C=O) and 175.18 (C=O); *m/z* (rel. ab.) FAB (NOBA) 328 [$(M + Na)^+$, 55%], 306 [$(M + H)^+$, 15], 246 (100), 186 (18), 156 (9) and 144 (14).

1,2,3-Tri-*O*-acetyl-α-D-glucopyranoside 18

Glucopyranoside **22** (420 mg, 1.21 mmol) was suspended in buffer (5 cm^3) at 30 °C. Esterase (15 mg) was added and the reaction mixture was stirred at 30 °C. After 16 h, the reaction mixture was filtered, and then extracted with dichloromethane (2 × 50 cm^3) and ethyl acetate (2 × 10 cm^3). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to yield compound **18** (266 mg, 72%) as a syrup [Found: $(M + Na)^+$, (FAB, Na) 329.0849. $C_{12}H_{18}NaO_9$ requires *m/z*, 329.084 85]; $[\alpha]_D^{27}$ +89.3 (*c* 0.14, MeOH); ν_{max}(Nujol)/cm^{-1} 3450 (OH), 1725 (C=O) and 1250 (C–O–C); δ_H(400 MHz; D_2O) 2.07 (3 H, s, CH_3CO), 2.15 (3 H, s, CH_3CO),

2.24 (3 H, s, CH_3CO), 3.86 (4 H, m, H-4, -5, H_2-6), 5.07 (1 H, dd, *J* 3.68 and 10, H-2), 5.36 (1 H, app t, *J* 10 and 10, H-3) and 6.28 (1 H, d, *J* 3.68, H-1); δ_C(63 MHz; D_2O), 20.69 (CH_3CO), 20.97 (CH_3CO), 21.01 (CH_3CO), 60.59 (C-6), 67.49 (C-4), 70.54 (C-2), 73.29 (C-3), 74.63 (C-5), 90.33 (C-1), 173.03 (C–O), 173.29 (C=O) and 174.24 (C=O); *m*/*z* (rel. ab.) FAB (NOBA) 329 [(M + Na)$^+$, 45%], 307 [(M + H)$^+$, 15], 289 (17), 247 (100) and 187 (23).

Methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside 30

Glucopyranoside **24** (484 mg, 1.51 mmol) was suspended in buffer (6.25 cm^3) at 30 °C. Esterase (15 mg) was added and the reaction mixture was stirred at 30 °C. After 16 h, the reaction mixture was filtered, and concentrated on the freeze drier. The residue was extracted with ethyl acetate (2 × 10 cm^3), and the combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to give compound **30** (146 mg, 35%) as a syrup [(Found: (M + Na)$^+$, (FAB, Na) 301.0928. $C_{11}H_{18}NaO_8$ requires *m*/*z*, 301.089 93]; $[\alpha]_D^{27}$ +114.5 (*c* 0.1, MeOH); ν_{max}(Nujol)/cm^{-1} 3500 (OH), 1750 (C=O) and 1250 (C–O–C); δ_H(400 MHz; D_2O) 2.07 (3 H, s, CH_3CO), 2.10 (3 H, s, CH_3CO), 3.40 (3 H, s, OCH_3), 3.79–3.70 (3 H, m, H-4, -5, H^b-6), 3.86 (1 H, m, H^a-6), 4.92 (1 H, dd, *J* 3.7 and 10.13, H-2), 4.97 (1 H, d, *J* 3.7, H-1) and 5.24 (1 H, dd, *J* 8.96 and 10.13, H-3); δ_C(63 MHz; D_2O) 20.76 (CH_3CO), 20.97 (CH_3CO), 55.52 (OCH_3), 60.85 (C-6), 68.17 (C-4), 71.59 (C-2), 72.02 (C-5), 73.67 (C-3), 97.15 (C-1), 173.51 (C=O) and 174.26 (C=O); *m*/*z* (rel. ab.) FAB (NOBA) 579 [2(M + Na)$^+$, 5%], 557 [2(M + H)$^+$, 5], 301 [(M + Na)$^+$, 32], 279 [(M + H)$^+$, 66], 247 (100), 217 (7), 187 (28), 145 (8) and 127 (22).

Methyl 2,3-di-*O*-acetyl-α-D-mannopyranoside 31

Mannopyranoside **25** (1.02 mg, 3.19 mmol) was suspended in buffer (12.5 cm^3) at 30 °C. Esterase (36 mg) was added and the reaction mixture was stirred at 30 °C for 16 h before being filtered, and concentrated on the freeze drier. The residue was extracted with ethyl acetate (2 × 30 cm^3), and the combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to yield a solid. Recrystallization from acetone yielded compound **31** (531 mg, 60%), mp 136–137 °C [Found: (M + Na)$^+$, (FAB, Na) 301.0882. $C_{11}H_{18}NaO_8$ requires *m*/*z*, 301.089 93]; $[\alpha]_D^{27}$ +45.7 (*c* 0.7, MeOH); ν_{max}(Nujol)/cm^{-1} 3475 (OH), 1750 (C=O) and (C–O–C); δ_H(400 MHz; D_2O) 2.08 (3 H, s, CH_3CO), 2.17 (3 H, s, CH_3CO), 3.44 (3 H, s, OCH_3), 3.83–3.75 (2 H, m, H-5, H^b-6), 3.94–3.88 (2 H, m, H-4, H^a-6), 4.82 (1 H, d, *J* 1.7, H-1), 5.08 (1 H, dd, *J* 3.39 and 9.85, H-3) and 5.25 (1 H, dd, *J* 1.7 and 3.39, H-2); δ_C(63 MHz; D_2O) 20.85 (CH_3CO), 20.95 (CH_3CO), 55.66 (OCH_3), 61.19 (C-6), 65.04 (C-4), 70.22 (C-2), 72.76 (C-3), 73.19 (C-5), 98.86 (C-1), 173.63 (C=O) and 173.93 (C=O); *m*/*z* (rel. ab.) FAB (NOBA) 579 [2(M + Na)$^+$, 4%], 557 [2(M + H)$^+$, 8], 301 [(M + Na)$^+$, 30], 279 [(M + H)$^+$, 100], 278 (6), 277 (6), 259 (7), 247 (94) and 127 (25).

Methyl 6-*O*-acetyl-α-D-glucopyranoside 33 and methyl 3-*O*-acetyl-α-D-glucopyranoside 34

Triethylamine (2.00 cm^3, 7.17 × 10^{-3} mol), vinyl acetate (13.00 cm^3, 7.05 × 10^{-2} mol) and *R. toruloides* esterase (10 g) were added to a solution of glucopyranoside **32** (3.25 g, 1.68 × 10^{-2} mol) in THF (50 cm^3). After stirring of the mixture at 25 °C for 1.5 h, TLC analysis showed the formation of two products (R_f 0.42, 0.3; ethyl acetate–ethanol, 9 : 1, v/v). The suspension was filtered through Celite, the Celite was washed with ethyl acetate (3 × 30 cm^3), and the filtrate was evaporated under reduced pressure to give an orange syrup. Purification by flash chromatography (ethyl acetate–ethanol, 20 : 1, v/v) yielded products **33** (150 mg, 4%) and **34** as syrups. Crystallization from acetone yielded 3-acetate **34** (199 mg, 5%); 6-acetate **33** [Found: (M + Na)$^+$, (FAB, Na) 259.080 00. $C_9H_{16}NaO_7$ requires *m*/*z*, 259.079 37]; $[\alpha]_D^{22}$ +32.6 (*c* 0.46, MeOH); ν_{max}(Nujol)/cm^{-1} 3600–3100 (OH) and 1750 (C=O); δ_H(400 MHz; D_2O) 2.10 (3 H, s, CH_3CO), 3.39 (3 H, s, OCH_3), 3.42 (1 H, dd, *J* 9.2 and 10, H-4), 3.54 (1 H, dd, *J* 3.7 and 9.6, H-2), 3.64 (1 H, dd, *J* 9.6 and 9.2, H-3), 3.82 (1 H, ddd, *J* 2.3, 5.2 and 10, H-5), 4.24 (1 H, dd, *J* 5.2 and 12.2, H^b-6), 4.37 (1 H, dd, *J* 2.30 and 12.20, H^a-6) and 4.77 (1 H, d, *J* 3.77, H-1); δ_C(63 MHz; D_2O) 20.83 (CH_3CO), 55.79 (OCH_3), 64.04 (C-6), 69.89 (C-5), 70.19 (C-4), 71.79 (C-2), 73.59 (C-3), 100.01 (C-1) and 174.79 (C=O); *m*/*z* (FAB, ThioglyNa) 259 [(M + Na)$^+$, 100%]; compound **34**; mp 133–134 °C [Found: (M + Na)$^+$, (FAB, Na) 259.0798. $C_9H_{16}NaO_7$ requires *m*/*z*, 259.079 37]; $[\alpha]_D^{20}$ +162.5 (*c* 0.12, MeOH); ν_{max}(Nujol)/cm^{-1} 3600–3100 (OH) and 1740 (C=O); δ_H(250 MHz; D_2O) 2.14 (3 H, s, CH_3CO), 3.42 (3 H, s, OCH_3), 3.56 (1 H, dd, *J* 9.5 and 9.75, H-4), 3.77–3.68 (3 H, m, H-2, -5, H^b-6), 3.85 (1 H, dd, *J* 2.13 and 12.13, H^a-6), 4.83 (1 H, d, *J* 3.77, H-1) and 5.10 (1 H, app t, *J* 9.5 and 9.5, H-3); δ_C(63 MHz; D_2O) 21.19 (CH_3CO), 55.79 (OCH_3), 60.97 (C-6), 68.36 (C-4), 70.15 (C-5), 72.04 (C-2), 76.18 (C-3), 99.79 (C-1) and 174.64 (C=O); *m*/*z* (FAB, ThioglyNa) 259 [(M + Na)$^+$, 10%], 237 [(M + H)$^+$, 19], 205 (14), 145 (18), 75 (35) and 56 (50).

General procedure for the preparation of hexose acetate sulfates 38–45

Sulfur trioxide–pyridine complex was added to the 4- or 6-deprotected α-hexopyranose acetate in anhydrous pyridine. The mixture was stirred at room temp. and the reaction was followed by TLC (dichloromethane–methanol, 10 : 1, v/v). On completion of the reaction, water was added to destroy the excess of sulfur trioxide. Pyridine was removed by co-evaporation with water under reduced pressure (<35 °C). The residue was redissolved in water and passed through an anion-exchange column (Dowex 1X2-400, Cl^- form). The column was washed with water and eluted with aq. NaCl. Compound **45** was eluted with 0.2 M NaCl, compounds **39**, **43** and **44** were eluted with 0.5 M NaCl, compounds **38**, **40** and **42** were eluted with 1.0 M NaCl and compound **41** was eluted with 1.5 M NaCl. The eluate was concentrated and subjected to gel filtration with water as eluent (Sephadex G25, column dimensions 2.5 cm × 90 cm) to remove NaCl. Fractions containing the sulfated sugar were combined, concentrated and freeze dried to give the product.

General procedure for the deacetylation

Acetate and sodium methoxide (2.5 mol equiv.) were suspended in anhydrous methanol. The mixture was stirred at room temp. and the reaction was followed by TLC (propan-1-ol–nitromethane–water, 10 : 9 : 2, v/v). When deacetylation was complete, the reaction was quenched by the addition of water. The solution was neutralized by passage through a cation-exchange column (Dowex, 50W-X8, H^+ form). The neutralized solution was then passed through an anion-exchange column (Dowex 1X2-400, Cl^- form). The column was washed with water and eluted with aq. NaCl. Compounds **47**, **49**, **50**, **51** and **53** were eluted with 0.2 M NaCl, compounds **46** and **52** were eluted with 0.5 M NaCl and compound **48** was eluted with 1.0 M NaCl. The eluate was evaporated under reduced pressure (<35 °C) and the residue was subjected to gel filtration with water as eluent (Sephadex G25; column dimensions 2.5 cm × 90 cm). Carbohydrate-containing fractions were combined, concentrated and freeze dried to give the sulfated hexose.

Sodium 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose 6-sulfate 38

A mixture of the *N*-acetylglucosamine derivative **2**[2] (317 mg, 0.91 mmol), anhydrous pyridine (10 cm^3) and sulfur trioxide–pyridine complex (360 mg, 2.26 mmol) was stirred at room temp. for 5 h. Water (30 cm^3) was added and the product was purified as described in the general procedure to give sulfate **38** (260 mg, 63%) as a powder (decomp. 186 °C) [Found: (M + Na)$^+$, 472.0519. $C_{14}H_{20}NNa_2O_{12}S$ requires *m*/*z*, 472.0502];

$[\alpha]_D^{20}$ +62.7 (*c* 0.5, water); δ_H(400 MHz; D_2O) 1.96 (3 H, s, CH_3CO), 2.06 (3 H, s, CH_3CO), 2.10 (3 H, s, CH_3CO), 2.23 (3 H, s, CH_3CO), 4.11 (1 H, dd, *J* 2.3 and 11.6, H^a-6), 4.16 (1 H, dd, *J* 4 and 11.6, H^b-6), 4.34 (1 H, ddd, *J* 2.3, 4 and 10, H-5), 4.49 (1 H, dd, *J* 3.65 and 11, H-2), 5.17 (1 H, app t, *J* 10 and 10, H-4), 5.37 (1 H, dd, *J* 10 and 11, H-3) and 6.12 (1 H, d, *J* 3.65, H-1); δ_C(100 MHz; D_2O) 20.73 (CH_3CO), 20.84 (CH_3CO), 20.92 (CH_3CO), 22.36 (CH_3CO), 50.84 (C-2), 66.48 (C-6), 69.02, 70.09, 71.64, 91.26 (C-1), 172.87 (C=O), 173.22 (C=O), 173.90 (C=O) and 175.15 (C=O); *m*/*z* (rel. ab.) 472 [(M + Na)$^+$, 60%], 368 (35), 310 (35), 119 (40), 87 (60) and 82 (100).

Sodium 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose 6-sulfate 39
The glucose derivative **5** (406.2 mg, 1.17 mmol) was dissolved in anhydrous pyridine (10 cm^3). Sulfur trioxide–pyridine complex (470 mg, 2.95 mmol) was added. The mixture was stirred at room temp. for 1.5 h. Water (30 cm^3) was added and the product was purified as described in the general procedure to give compound **39** (390 mg, 74%) as a powder (decomp. 190 °C) [Found: (M + Na)$^+$ 473.0329. $C_{14}H_{19}Na_2O_{13}S$ requires *m*/*z*, 473.0342]; $[\alpha]_D^{23}$ +61.6 (*c* 0.25, water); δ_H(400 MHz; D_2O) 2.05 (3 H, s, CH_3CO), 2.06 (3 H, s, CH_3CO), 2.09 (3 H, s, CH_3CO), 2.22 (3 H, s, CH_3CO), 4.11 (1 H, dd, *J* 2.5 and 11.8, H^a-6), 4.15 (1 H, dd, *J* 3.8 and 11.8, H^b-6), 4.38 (1 H, ddd, *J* 2.5, 3.8 and 10.3, H-5), 5.21 (1 H, dd, *J* 9.8 and 10.3, H-4), 5.23 (1 H, dd, *J* 3.7 and 9.8, H-2), 5.50 (1 H, app t, *J* 9.8 and 9.8, H-3) and 6.30 (1 H, d, *J* 3.7, H-1); δ_C(100 MHz; D_2O) 20.63 (CH_3CO), 20.83 (2 × CH_3CO), 20.91 (CH_3CO), 66.36, 68.41, 69.88, 70.15, 71.34, 89.89 (C-1), 172.78 (C=O), 173.15 (2 × C=O) and 173.98 (C=O); *m*/*z* (rel. ab.) 473 [(M + Na)$^+$, 80%], 371 (40) and 149 (100).

Sodium 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-galactopyranose 6-sulfate 40
The *N*-acetylgalactosamine derivative **9** (100 mg, 0.29 mmol) was dissolved in anhydrous pyridine (5 cm^3). Sulfur trioxide–pyridine complex (120 mg, 0.75 mmol) was added. The mixture was stirred at room temp. for 2 h. Water (10 cm^3) was added and the mixture was freeze dried. The residue was purified as described in the general procedure to give sulfate **40** (125 mg, 93%) as a powder (decomp. 177 °C) [Found: (M + Na)$^+$ 472.0502. $C_{14}H_{20}NNa_2O_{12}S$ requires *m*/*z*, 472.0502]; $[\alpha]_D^{22}$ +70 (*c* 0.25, water); δ_H(400 MHz; D_2O) 1.93 (3 H, s, CH_3CO), 1.99 (3 H, s, CH_3CO), 2.17 (3 H, s, CH_3CO), 2.18 (3 H, s, CH_3CO), 4.00–4.08 (2 H, m, H_2-6), 4.51 (1 H, t, *J* 6, H-5), 4.58 (1 H, dd, *J* 3.65 and 11.6, H-2), 5.28 (1 H, dd, *J* 3 and 11.6, H-3), 5.52 (1 H, d, *J* 3, H-4) and 6.15 (1 H, d, *J* 3.65, H-1); δ_C(100 MHz; D_2O) 20.64 (2 × CH_3CO), 20.84 (CH_3CO), 22.32 (CH_3CO), 47.07 (C-2), 66.31 (C-6), 68.05, 68.80, 69.26, 91.67 (C-1), 172.98 (C=O), 173.46 (C=O), 173.95 (C=O) and 175.31 (C=O); *m*/*z* (rel. ab.) 472 [(M + Na)$^+$, 100%], 368 (40) and 310 (60).

Sodium 1,2,3,4-tetra-*O*-acetyl-α-D-galactopyranose 6-sulfate 41
A mixture of the galactose derivative **11** (200 mg, 0.58 mmol), anhydrous pyridine (10 cm^3) and sulfur trioxide–pyridine complex (250 mg, 1.57 mmol) was stirred at room temp. for 2 h. Water (30 cm^3) was added and the mixture was freeze dried. The residue was purified as described in the general procedure to give sulfate **41** (245 mg, 94%) as a powder [Found: (M + Na)$^+$, 473.0337. $C_{14}H_{19}Na_2O_{13}S$ requires *m*/*z*, 473.0342]; $[\alpha]_D^{22}$ +82.4 (*c* 0.25, water); δ_H(400 MHz; D_2O) 2.03 (3 H, s, CH_3CO), 2.07 (3 H, s, CH_3CO), 2.209 (3 H, s, CH_3CO), 2.211 (3 H, CH_3CO), 4.07 (1 H, dd, *J* 6.30 and 10.95, H^a-6), 4.10 (1 H, dd, *J* 6 and 10.95, H^b-6), 4.59 (1 H, app t, *J* 6 and 6.3, H-5), 5.39 (1 H, dd, *J* 3.7 and 10.6, H-2), 5.47 (1 H, dd, *J* 3 and 10.6, H-3), 5.60 (1 H, d, *J* 3, H-4) and 6.36 (1 H, d, *J* 3.7, H-1); δ_C(100 MHz; D_2O) 20.70 (2 × CH_3CO), 20.76 (CH_3CO), 20.89 (CH_3CO), 66.24, 67.40, 68.77, 68.86, 69.41, 90.38 (C-1), 172.93 (C=O), 173.29 (C=O), 173.46 (C=O) and 173.86 (C=O); *m*/*z* (rel. ab.) 473 [(M + Na)$^+$, 100%], 451 (50), 446 (50), 369 (60) and 311 (80).

Sodium 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose 6-sulfate 42
A mixture of mannopyranoside **7** (850 mg, 2.44 mmol), anhydrous pyridine (15 cm^3) and sulfur trioxide–pyridine complex (1.0 g, 6.28 mmol) was stirred at room temp. for 2 h. Water (30 cm^3) was added and the mixture was purified as described in the general procedure to give sulfate **42** (980 mg, 89%) as a powder (decomp. 166 °C) [Found: (M + Na)$^+$, 473.0333. $C_{14}H_{19}Na_2O_{13}S$ requires *m*/*z*, 473.0342]; $[\alpha]_D^{21}$ +58.2 (*c* 0.5, water); δ_H(400 MHz; D_2O) 2.04 (3 H, s, CH_3CO), 2.12 (3 H, s, CH_3CO), 2.21 (3 H, s, CH_3CO), 2.23 (3 H, s, CH_3CO), 4.13 (1 H, dd, *J* 2.3 and 11.6, H^a-6), 4.20 (1 H, dd, *J* 3.65 and 11.6, H^b-6), 4.34–4.38 (1 H, m, H-5), 5.37–5.45 (3 H, m, H-2, -3, -4) and 6.09 (1 H, d, *J* 2, H-1); δ_C(100 MHz; D_2O) 20.81 (2 × CH_3CO), 20.87 (2 × CH_3CO), 66.04 (C-6), 66.45, 69.21, 70.34, 70.86, 91.32 (C-1), 172.08 (C=O), 173.35 (C=O), 173.39 (C=O) and 173.51 (C=O); *m*/*z* (rel. ab.) 473 [(M + Na)$^+$, 100%], 451 (40), 311 (55) and 301 (30).

Sodium 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose 4-sulfate 43
The *N*-acetylglucosamine derivative **3** (390 mg, 1.12 mmol) was dissolved in anhydrous pyridine (13 cm^3). Sulfur trioxide–pyridine complex (340 mg, 2.50 mmol) was added. The mixture was stirred at room temp. for 2 h. Water (10 cm^3) was added and the mixture was then freeze dried. The residue was purified as described in the general procedure to give sulfate **43** (483 mg, 95%) as a powder (decomp. 177 °C) [Found: (M + Na)$^+$, 472.0510. $C_{14}H_{20}NNa_2O_{12}S$ requires *m*/*z*, 472.0502]; $[\alpha]_D^{19}$ +46.8 (*c* 0.25, water); δ_H(250 MHz; D_2O) 1.91 (3 H, s, CH_3CO), 2.04 (3 H, s, CH_3CO), 2.06 (3 H, s, CH_3CO), 2.16 (3 H, s, CH_3CO), 4.13–4.19 (1 H, m, H-5), 4.23 (1 H, dd, *J* 2.6 and 12.5, H^a-6), 4.31 (1 H, dd, *J* 3.8 and 12.5, H^b-6), 4.40 (1 H, dd, *J* 3.8 and 11, H-2), 4.51 (1 H, app t, *J* 9 and 9, H-4), 5.34 (1 H, dd, *J* 9 and 11, H-3) and 6.04 (1 H, d, *J* 3.78, H-1); δ_C(100 MHz; D_2O) 20.81 (2 × CH_3CO), 21.00 (CH_3CO), 22.23 (CH_3CO), 50.81 (C-2), 63.07 (C-6), 70.33, 71.08, 74.24, 91.10 (C-1), 172.87 (C=O), 173.92 (C=O), 174.57 (C=O) and 175.21 (C=O); *m*/*z* (rel. ab.) 472 [(M + Na)$^+$, 100%], 370 (50) and 149 (60).

Sodium 1,2,3,6-tetra-*O*-acetyl-α-D-glucopyranose 4-sulfate 44
A mixture of the glucose derivative **22** (130 mg, 0.37 mmol), anhydrous pyridine (10 cm^3) and sulfur trioxide–pyridine complex (140 mg, 0.88 mmol) was stirred at room temp. After 2 h, water (15 cm^3) was added and the mixture was freeze dried. The residue was purified as described in the general procedure to give sulfate **44** (165 mg, 98%) as a powder [Found: (M + Na)$^+$, 473.0342. $C_{14}H_{19}Na_2O_{13}S$ requires *m*/*z*, 473.0342]; $[\alpha]_D^{20}$ +56.6 (*c* 0.5, water); δ_H(250 MHz; D_2O) 1.88 (3 H, s, CH_3CO), 2.05 (3 H, s, CH_3CO), 2.08 (3 H, s, CH_3CO), 2.20 (3 H, s, CH_3CO), 4.19–4.32 (3 H, m, H-5, H_2-6), 4.58 (1 H, app t, *J* 9.3 and 9.3, H-4), 5.17 (1 H, dd, *J* 3.78 and 10.46, H-2), 5.51 (1 H, dd, *J* 9.3 and 10.46, H-3) and 6.25 (1 H, d, *J* 3.78, H-1); δ_C(63 MHz; D_2O) 20.79 (CH_3CO), 21.02 (2 × CH_3CO), 21.30 (CH_3CO), 63.14 (C-6), 69.98, 70.62, 71.02, 73.97, 89.88 (C-1), 172.97 (C=O), 173.35 (C=O), 174.29 (C=O) and 174.72 (C=O); *m*/*z* (rel. ab.) 473 [(M + Na)$^+$, 100%] and 371 (60).

Sodium 1,2,3,6-tetra-*O*-acetyl-α-D-mannopyranose 4-sulfate 45
A mixture of the mannose derivative **23** (80 mg, 0.23 mmol), anhydrous pyridine (5 cm^3) and sulfur trioxide–pyridine complex (90 mg, 0.57 mmol) was stirred at room temp. (15 °C). After 7 h, water (15 cm^3) was added and the mixture was purified as described in the general procedure to give sulfate **45** (97 mg, 93%) as a powder (decomp. 149 °C) [Found: (M + Na)$^+$, 473.0334. $C_{14}O_{19}Na_2O_{13}S$ requires *m*/*z*, 473.0342]; $[\alpha]_D^{22}$ +51.2

(c 0.25, water); δ_H(250 MHz; D_2O) 2.03 (3 H, s, CH_3CO), 2.08 (3 H, s, CH_3CO), 2.14 (3 H, s, CH_3CO), 2.17 (3 H, s, CH_3CO), 4.17–4.26 (2 H, m, H-5, H^a-6), 4.36 (1 H, dd, J 3.77 and 12.49, H^b-6), 4.70 (1 H, app t, J 9.9 and 9.9, H-4), 5.29 (1 H, dd, J 2 and 3.5, H-2), 5.44 (1 H, dd, J 3.5 and 9.9, H-3) and 6.00 (1 H, d, J 2, H-1); δ_C(100 MHz; D_2O) 20.87 (2 × CH$_3$CO), 21.05 (2 × CH$_3$CO), 63.14 (C-6), 69.60, 69.68, 71.10, 71.83, 91.08 (C-1), 172.08 (C=O), 173.28 (C=O), 173.60 (C=O) and 174.57 (C=O); m/z (rel. ab.) 473 [$(M + Na)^+$, 100%], 371 (90) and 289 (40).

Sodium 2-acetamido-2-deoxy-D-glucopyranose 6-sulfate 46
The sulfate **38** (160 mg, 0.36 mmol) and sodium methoxide (50 mg, 0.93 mmol) were suspended in anhydrous methanol (25 cm^3). The mixture was stirred at room temp. for 6 h. Water (25 cm^3) was added and the mixture was purified as described in the general procedure to give the sulfate **46** (90 mg, 78%) as a powder (decomp. 164 °C) [Found: $(M + Na)^+$, 346.0182. $C_8H_{14}NNa_2O_9S$ requires m/z, 346.0179]; $[\alpha]_D^{21}$ +24.2 (c 0.5, water); δ_H(400 MHz; D_2O) 1.95 (3 H, s, CH_3CO), 3.42–3.98 (4 H, m, 4 × HC–O), 4.11–4.26 (2 H, m, H_2-6), 4.64 (0.4 H, d, J 8.63, H-1β) and 5.11 (0.6 H, d, J 3.32, H-1α); δ_C(100 MHz; D_2O) 22.60 (CH$_3$CO), 22.87 (CH$_3$CO), 54.60, 57.21, 67.82, 70.17, 70.28, 70.41, 71.29, 74.37, 91.59 (C-1α), 95.68 (C-1β), 175.19 and 175.47; m/z (rel. ab.) 346 [$(M + Na)^+$, 25%], 244 (15) and 79 (100).

Sodium D-glucopyranose 6-sulfate 47
The sulfate **39** (766 mg, 1.70 mmol) and sodium methoxide (280 mg, 5.18 mmol) were suspended in anhydrous methanol (50 cm^3). The mixture was stirred at room temp. for 3 h. The reaction was quenched by addition of water (50 cm^3). The mixture was purified as described in the general procedure to give sulfate **47** (440 mg, 92%) as a powder (decomp. 170 °C) [Found: $(M + Na)^+$, 304.9915. $C_6H_{11}NNa_2O_9S$ requires m/z, 304.9914]; $[\alpha]_D^{14}$ +23.3 (c 0.5, water); δ_H(250 MHz; D_2O) 3.16–3.24 (0.6 H, m, HC-O), 3.37–3.70 (3 H, m, 3 × HC-O), 3.93–4.00 (0.4 H, m, HC-O), 4.10–4.29 (2 H, m, H_2-6), 4.61 (0.6 H, d, J 7.85, H-1β) and 5.17 (0.4 H, d, J 5.17, H-1α); δ_C(63 MHz; D_2O) 67.94, 70.05, 70.09, 70.30, 72.16, 73.44, 74.58, 74.79, 76.37, 92.99 (C-1α) and 96.81 (C-1β); m/z (rel. ab.) 305 [$(M + Na)^+$, 100%] and 99 (75).

Sodium 2-acetamido-2-deoxy-D-galactopyranose 6-sulfate 48
The sulfate **46** (78 mg, 0.17 mmol) and sodium methoxide (28 mg, 0.52 mmol) were suspended in anhydrous methanol (10 cm^3). The mixture was stirred at room temp. After 3 h, the reaction was quenched by addition of water (10 cm^3) and the mixture was purified as described in the general procedure to give sulfate **48** (34 mg, 61%) as a hygroscopic powder [Found: $(M + Na)^+$, 346.0176. $C_8H_{14}NNa_2O_9S$ requires m/z, 346.0179]; $[\alpha]_D^{22}$ +38.6 (c 0.5, water); δ_H(400 MHz; D_2O) 2.002 (3 H, s, CH_3CO), 2.004 (3 H, s, CH_3CO), 3.69 (0.4 H, dd, J 3.32 and 10.62, HC-O), 3.82–4.30 (6.6 H, m, HC-O), 4.62 (0.4 H, d, J 8.29, H-1β) and 5.19 (0.6 H, d, J 3.65, H-1α); δ_C(63 MHz; D_2O) 22.78 (CH$_3$CO), 23.03 (CH$_3$CO), 50.95, 54.30, 67.96, 68.09, 68.39, 68.48, 69.12, 69.20, 71.72, 73.57, 175.52 (C-1α) and 175.80 (C-1β); m/z (rel. ab.) 300 [$(M - Na)^-$, 100%], 199 (20) and 45 (55).

Sodium D-galactopyranose 6-sulfate 49
The sulfate **41** (173 mg, 0.38 mmol) and sodium methoxide (60 mg, 1.11 mmol) were suspended in anhydrous methanol (10 cm^3). The mixture was stirred at room temp. for 2.5 h. The reaction was quenched by the addition of water (10 cm^3) and the mixture was purified as described in the general procedure to give sulfate **49** (90 mg, 83%) as a powder (decomp. 150 °C) [Found: $(M + Na)^+$, 304.9913. $C_6H_{11}NNa_2O_9S$ requires m/z, 304.9914]; $[\alpha]_D^{14}$ +46.8 (c 0.5, water) {lit.,[31] $[\alpha]_D^{14}$ +47}; δ_H(250 MHz; D_2O) 3.45 (0.6 H, dd, J 7.85 and 9.88, HC-O), 3.63 (0.6 H, dd, J 3.48 and 9.88, HC-O), 3.76 (0.4 H, dd, J 3.48 and 10.46, H^a-6), 3.84 (1 H, dd, J 3.20 and 10.46, H^b-6), 3.88–4.29 (4 H, m, 4 × HC-O), 4.57 (0.6 H, d, J 7.85, H-1β) and 5.23 (0.4 H, d, J 3.78, H-1α); δ_C(63 MHz; D_2O) 68.15, 68.53, 69.06, 69.17, 69.36, 69.76, 69.94, 72.57, 73.39, 73.57, 93.21 and 87.28; m/z (rel. ab.) 259 [$(M - Na)^-$, 100%] and 97 (30).

Sodium D-mannopyranose 6-sulfate 50
The sulfate **42** (303 mg, 0.67 mmol) and sodium methoxide (110 mg, 2.04 mmol) were suspended in anhydrous methanol (20 cm^3). The mixture was stirred at room temp. for 2 h. Water (20 cm^3) was added and the mixture was purified as described in the general procedure to give sulfate **50** (180 mg, 94%) as a syrup [Found: $(M + Na)^+$, 304.9916. $C_6H_{11}NNa_2O_9S$ requires m/z, 304.9914]; $[\alpha]_D^{23}$ +17.8 (c 0.5, water); δ_H(250 MHz; D_2O) 3.55–3.72 (2 H, m, 2 × HC-O), 3.83 (0.57 H, dd, J 3.19 and 9.59, HC-O), 3.90–3.93 (1 H, m, HC-O), 3.95–4.02 (0.57 H, m, HC-O), 4.14–4.33 (2 H, m, H_2-6), 4.90 (0.43 H, d, J 0.87, H-1β) and 5.15 (0.57 H, d, J 1.45, H-1α); δ_C(63 MHz; D_2O) 67.04, 67.29, 68.26, 70.95, 71.23, 71.40, 73.01, 74.80, 94.63 and 95.00; m/z (rel. ab.) 305 [$(M + Na)^+$, 100%].

Sodium 2-acetamido-2-deoxy-D-glucopyranose 4-sulfate 51
The sulfate **43** (270 mg, 0.60 mmol) and sodium methoxide (70 mg, 0.13 mmol) were suspended in anhydrous methanol (45 cm^3). The mixture was stirred at room temp. for 8 h. The reaction was quenched by addition of water (40 cm^3) and the mixture was purified as described in the general procedure to give sulfate **51** (160 mg, 82%) as a powder (decomp. 157 °C) [Found: $(M + Na)^+$, 346.0179. $C_8H_{14}NNa_2O_9S$ requires m/z, 346.0179]; $[\alpha]_D^{22}$ +19.0 (c 0.5, water); δ_H(250 MHz; D_2O) 2.00 (3 H, s, CH_3CO), 3.52–4.23 (6 H, m, 6 × HC-O), 4.69 (0.4 H, d, J 8.48, H-1β) and 5.17 (0.6 H, d, J 2.62, H-1α); δ_C(63 MHz; D_2O) 22.76 (CH$_3$CO), 23.04 (CH$_3$CO), 54.74, 57.28, 61.20, 61.37, 70.06, 70.66, 73.19, 75.19, 77.77, 78.14, 91.28, 95.68, 175.40 (C=O) and 175.68 (C=O); m/z (rel. ab.) 346 [$(M + Na)^+$, 12%] and 79 (100).

Sodium D-glucopyranose 4-sulfate 52
The sulfate **44** (160 mg, 0.36 mmol) and sodium methoxide (60 mg, 1.11 mmol) were suspended in anhydrous methanol (25 cm^3). The mixture was stirred at room temp. After 5 h, the reaction was quenched by addition of water (25 cm^3). The mixture was purified as described in the general procedure to give sulfate **52** (80 mg, 80%) as a hygroscopic powder [Found: $(M + Na)^+$, 304.9916. $C_6H_{11}NNa_2O_9S$ requires m/z, 304.9914]; $[\alpha]_D^{14}$ +34.8 (c 0.5, water); δ_H(400 MHz; D_2O) 3.30 (0.6 H, dd, J 8 and 9.5, β anomer), 3.55–3.94 (5 H, m, 5 × HC-O), 4.12 (1 H, app t, J 9.4, H-4β), 4.13 (1 H, app t, J 9.6, H-4α), 4.64 (0.6 H, d, J 7.96, H-1β) and 5.21 (0.4 H, d, J 3.65, H-1α); δ_C(100 MHz; D_2O) 61.22, 61.39, 70.52, 72.04, 72.13, 74.74, 75.09, 75.18, 77.59, 77.82, 92.51 and 96.61; m/z (rel. ab.) 305 [$(M + Na)^+$, 100%], 203 (30) and 99 (15).

Sodium D-mannopyranose 4-sulfate 53
The sulfate **45** (400 mg, 0.90 mmol) and sodium methoxide (170 mg, 3.15 mmol) were suspended in anhydrous methanol (40 cm^3). The mixture was stirred at room temp. After 4 h, the reaction was quenched by addition of water (30 cm^3). The mixture was purified as described in the general procedure to give sulfate **53** (220 mg, 88%) as a powder (decomp. 171 °C) [Found: $(M + Na)^+$, 304.9911. $C_6H_{11}NNa_2O_9S$ requires m/z, 304.9914]; $[\alpha]_D^{14}$ +13.6 (c 0.5, water); δ_H(250 MHz; D_2O) 3.46 (0.33 H, ddd, J 2.33, 6.10 and 9.60, H-5α), 3.65–4.00 (4.67 H, m, 4.67 × HC-O), 4.25 (0.33 H, app t, J 9.6, H-4β), 4.32 (0.67 H, app t, J 9.6, H-4α), 4.86 (0.33 H, d, J 1.16, H-1β) and 5.12 (0.37 H, d, J 2.04, H-1α); δ_C(63 MHz; D_2O) 61.50, 61.56, 69.89, 71.56, 72.00, 72.60, 75.27, 75.85, 76.17, 94.40 and 94.45; m/z (rel. ab.) 259 [$(M - Na)^-$, 100%] and 97 (35).

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