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Carbohydrate Research 305 (1998) 351–361

CARBOHYDRATE RESEARCH

Diastereoselective resolution of 6-substituted glycosides via enzymatic hydrolysis

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Received 20 May 1997; accepted 29 July 1997

Abstract

The diastereoselectivity of the enzymatic hydrolyses of 4-nitrophenyl 6-deoxy-6-methyl-(*R*)- and (*S*)-sulfinyl-β-D-galactopyranoside (**1a,b**), 4-nitrophenyl 7-deoxy-D- and L-*glycero*-β-D-*galacto*-heptopyranoside (**2a,b**) and 4-nitrophenyl 6,7-anhydro-D- and L-*glycero*-β-D-*galacto*-heptopyranoside (**3a,b**) was investigated using a range of crude glycosidase preparations. It was shown that the enzymes display a high degree of discrimination between diastereomers thereby demonstrating the utility of glycosidases for the diastereomeric resolution of unnatural 6-substituted monosaccharide derivatives. © 1998 Elsevier Science Ltd.

Keywords: Enzymes; Glycosidase; Diastereoselectivity; Resolution; Transglycosylation; Unnatural glycosides

1. Introduction

During a recent investigation directed towards the incorporation of modified galactosides into biologically active oligosaccharides, a range of diastereomeric 6-substituted derivatives were obtained which required separation. Although this could be achieved by chromatography, it was evident that the development of a facile, enzyme-based 'resolution' method would be of considerable interest. Glycosidases are well known to exhibit diastereoselectivity in transglycosylation reactions involving racemic and *meso*-alcohols as acceptors [1–8]. However, there have been only a few examples describing stereoselectivity in the enzymatic hydrolysis of glycosides arising from a stereogenic centre incorporated in the aglycon [9,10]. In this communication, we describe, for the first time, methodology for discriminating between diastereomers in which asymmetry at C-6 provides the basis for selectivity.

2. Results and discussion

In order to investigate the feasibility of enzymatic resolution of 6-substituted glycosides, two sulfoxides

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PII S0008-6215(97)00246-2

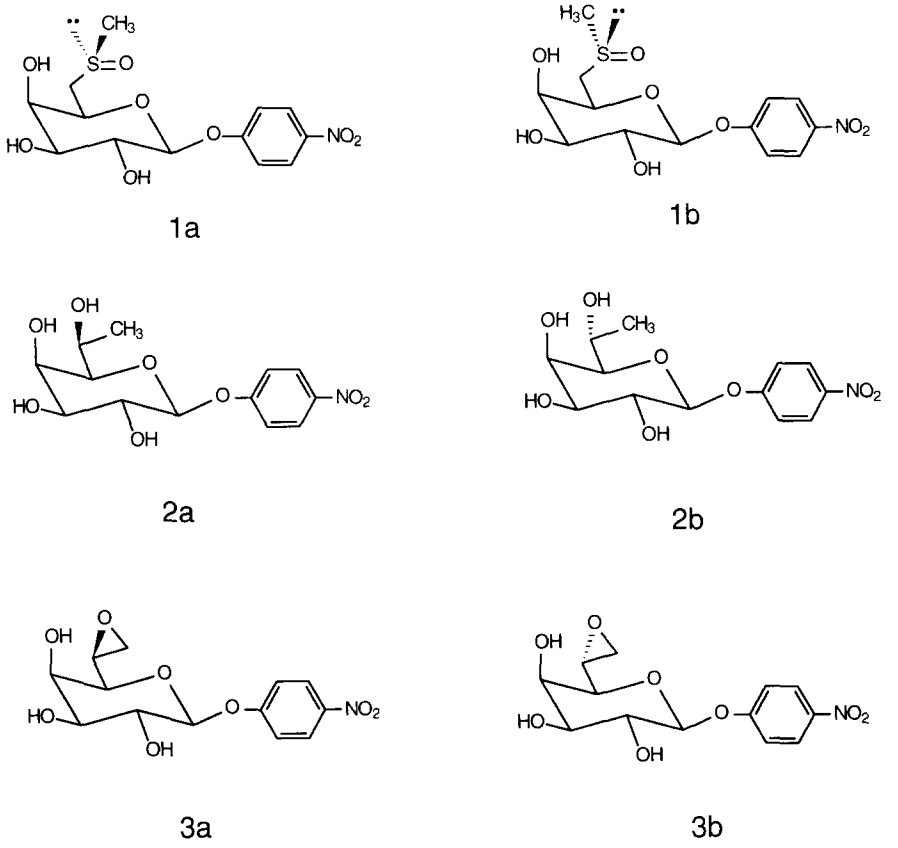
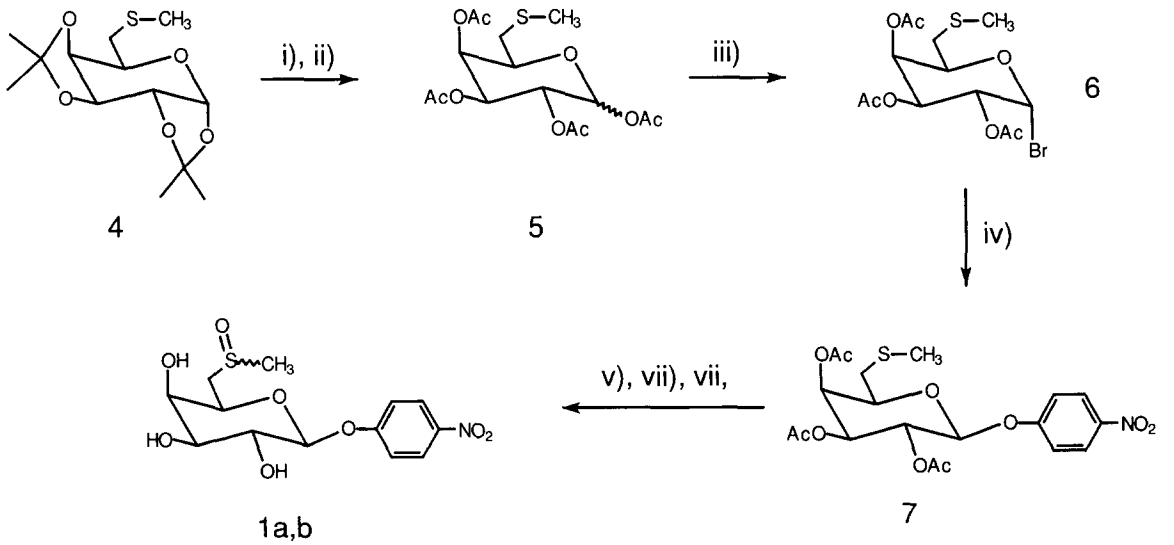


Fig. 1. Structures of diastereomers **1a**, **1b**, **2a**, **2b**, **3a**, and **3b**.

4-nitrophenyl 6-deoxy-6-methyl-(*R*)-sulfinyl-β-D-galactopyranoside (**1a**) and 4-nitrophenyl 6-deoxy-6-methyl-(*S*)-sulfinyl-β-D-galactopyranoside (**1b**) were selected for initial study (Fig. 1). These sulfoxides were prepared as shown in Scheme 1. Deacetalisation of 1,2:3,4-di-*O*-isopropylidene-6-*S*-methyl-6-sulfinyl-α-D-galactopyranose (**4**) [11] followed by acetylation afforded the fully protected derivative (**5**) as a 1:1.5



Scheme 1.

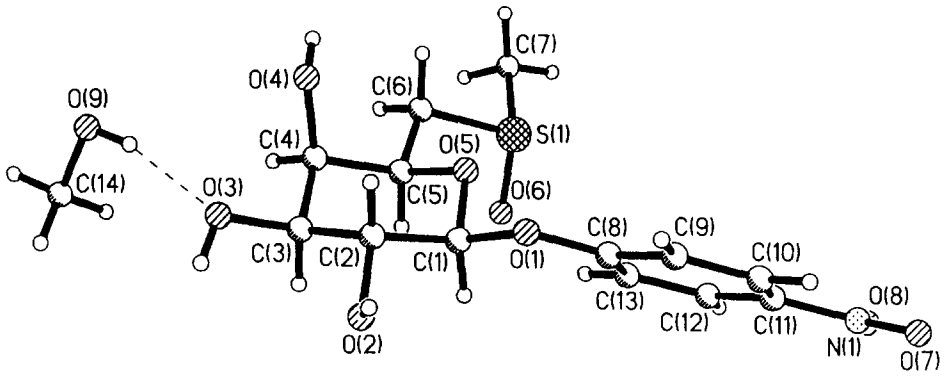


Fig. 2. Structure of sulfoxide **1b** showing the presence of one molecule of methanol in the asymmetric unit.

mixture of α:β anomers. After conversion to the α-bromide (**6**), the 4-nitrophenyl group was introduced under phase transfer conditions [12,13]. Controlled peracetic acid oxidation [14] of **7** then yielded diastereomeric sulfoxides which were separated by column chromatography and finally deprotected to **1a** and **1b**. The absolute stereochemistry of the more polar diastereomer (**1b**) was unequivocally established by single crystal X-ray analysis and shown to be *S* at the sulfoxide stereocentre (Fig. 2). Hence, the stereochemistry assigned to the less polar diastereomer (**1a**) was *R*.

A mixture of **1a** and **1b** was then incubated with a number of crude, commercially available enzyme preparations. Of the nine crude enzymes studied, the extract from *Helix aspersa* and limpet (sold as 'β-glucuronidase') and the extract from almond (sold as 'β-glucosidase') were found to hydrolyse **1b** significantly faster than **1a**, with the enzyme preparation of *H. aspersa* showing the greatest selectivity. As shown in Figs. 3 and 4a, this preparation selectively cleaved **1b** with practically no concomitant cleavage of the diastereomer **1a** after 48 h. It should be noted that the preparation is a crude extract containing a variety of glycosidic activities and, in particular, β-galactosidase activity. As a sufficiently high rate of hydrolysis was observed in these experiments, no attempt was made to isolate the enzyme responsible for the resolution.

In order to establish the generality of such diastereomeric resolutions, two further pairs of 6-substituted diastereomers (**2a,b** and **3a,b**) were investigated. Thus, 4-nitrophenyl 7-deoxy-D-*glycero*-β-D-*galacto*-heptopyranoside (**2a**) and 4-nitrophenyl 7-deoxy-L-*glycero*-β-D-*galacto*-heptopyranoside (**2b**) were prepared from a 2.5:1 (*L:D*) mixture (**9**) of 7-deoxy-D-*glycero*-α-D-*galacto*-1,2:3,4-di-*O*-isopropylidene-heptopyranose and 7-deoxy-L-*glycero*-α-D-*galacto*-1,2:3,4-di-*O*-isopropylidene-heptopyranose [15,16] (Scheme 2). Subsequent deacetalisation and acetylation afforded derivatives **10a,b** which were separated by column chromatography. Conversion of the individual diastereomers to their α-bromides (**11a**, **11b**) followed by treatment with 4-nitrophenol [12,13] and deprotection yielded the target β-glycosides **2a** and **2b**. Assignment of the configurations at the C-6 centres is based on the original work of Lemieux et al. [15] and Hoppe and Schöllkopf [16].

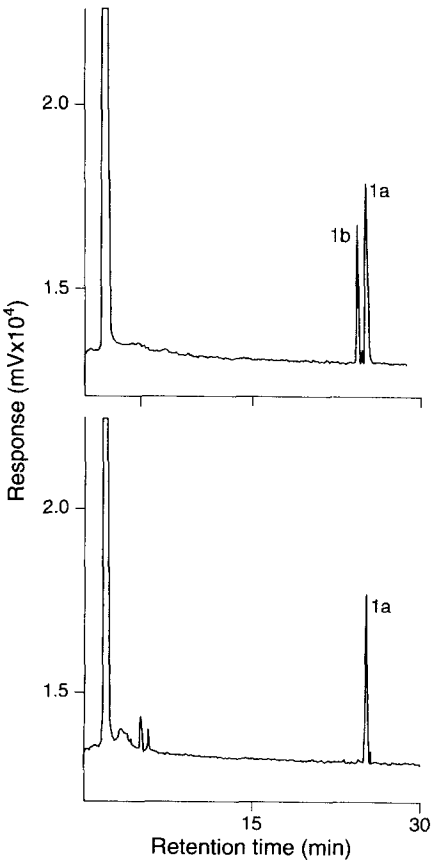
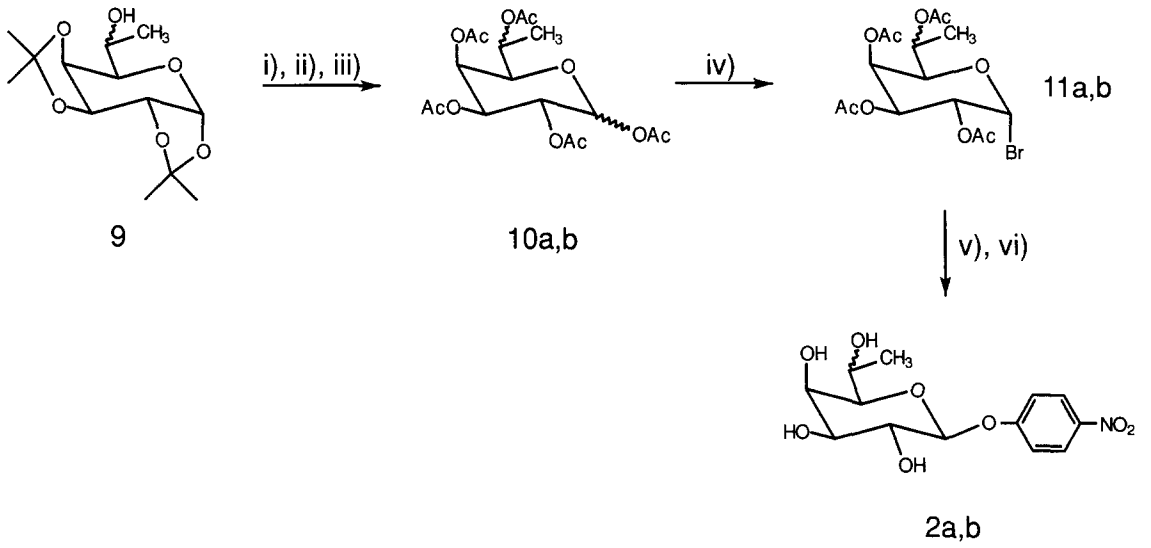


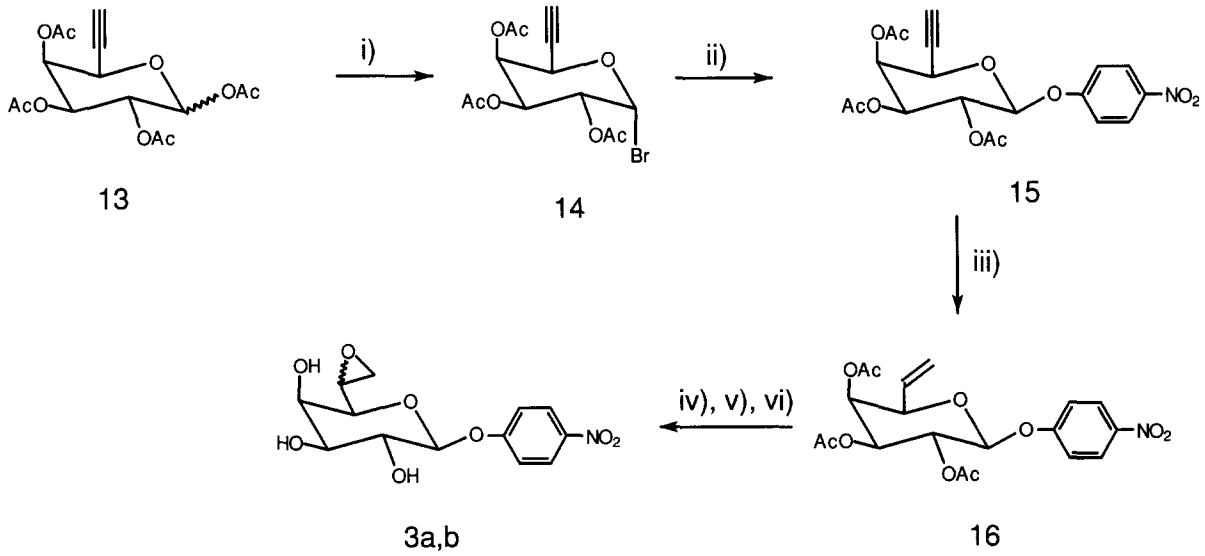
Fig. 3. HPLC resolution of diastereomers **1a** and **1b** before (top) and after (bottom) incubation with β-glucuronidase (*H. aspersa*) for 48 h.



Scheme 2.

4-Nitrophenyl 6,7-anhydro-L-*glycero*-β-D-*galacto*-heptopyranoside (**3a**) and 4-nitrophenyl 6,7-anhydro-D-*glycero*-β-D-*galacto*-heptopyranoside (**3b**) were prepared from 1,2,3,4-tetra-*O*-acetyl-6,7-dideoxy-D-*galacto*-hept-6-ynopyranose (**13**) [17] (Scheme 3). Introduction of the 4-nitrophenyl moiety in the usual way [12,13] was then followed by partial catalytic hydrogenation using Lindlar's Catalyst. The alkene (**15**) was then treated with *m*-chloroperbenzoic acid (MCPBA) to afford the diastereomeric epoxides which were separated by column chromatography and deprotected. Assignment of the D-*glycero* configuration to the more polar diastereomer (**3a**) and the L-*glycero* configuration to the less polar diastereomer (**3b**) is tentatively based on previous assignments [17].

Once obtained, **2a,b** and **3a,b** were subjected to enzymatic hydrolysis which proceeded with a high degree of specificity though, interestingly, with different enzymes. Thus, a β-galactosidase present in the crude extract from barley (sold as 'β-amylase') displayed exceptionally high activity towards **2a** as compared to **2b** which was hydrolysed very slowly (Fig. 4b). β-Galactosidase from *Escherichia coli* also



Scheme 3.

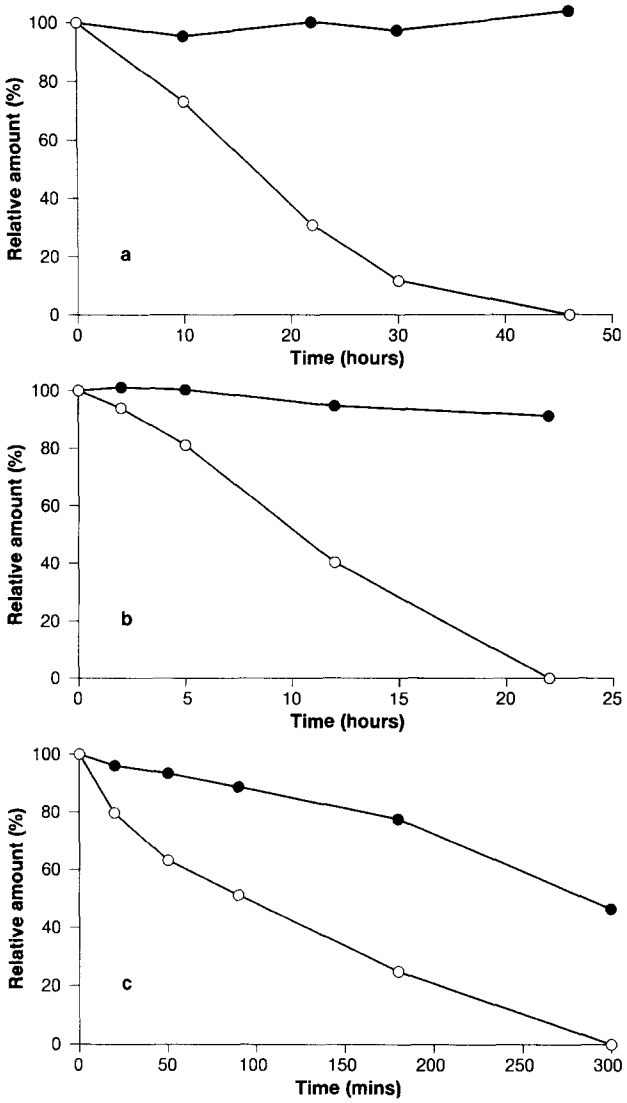


Fig. 4. The kinetics of enzymatic cleavage of **1a** (closed circles) and **1b** (open circles) (a, β-glucuronidase (*H. aspersa*)), **2a** (open circles) and **2b** (closed circles) (b, β-amylase) and **3a** (closed circles) and **3b** (open circles) (c. β-galactosidase (*Aspergillus oryzae*)).

showed a significantly enhanced selectivity towards **2a**. A more interesting pattern was observed in the hydrolysis of epoxides **3a** and **3b**. With this mixture, enzymes were found with opposite diastereoselectivities. Thus, snail acetone powder, almond meal and the β-glucuronidases from *H. aspersa* and limpet exhibited a preference for the hydrolysis of **3a**, whereas β-amylase from barley and the β-galactosidase from *A. oryzae* showed some preference for the diastereomer **3b**. Of these enzymes, the greatest discrimination was displayed by the β-galactosidase from *A. oryzae* (Fig. 4c). Once again, the nature of the enzymes responsible for the observed specificity was not investigated further, but in view of the relatively small number of crude enzyme preparations used in this study, it is anticipated that a wide range of glycosidases are capable of hydrolysing 6-substituted glycosides diastereoselectively. Further, with the ability of numerous enzymes to discriminate between 6-substituted diastereomeric glycosides, it may be possible to apply enzyme-catalysed transglycosylation and combine the resolution of diastereomers and the synthesis of unnatural disaccharides in a single step.

In conclusion, it has been shown that glycosidases display a marked diastereoselectivity in hydrolysis of substrates where the asymmetric centre resides at C-6 rather than in the aglycon. As several non-homologous substrates were selectively cleaved by a number of enzymes, it seems that the proposed methodology would be of general applicability to diastereomeric resolutions of unnatural 6-substituted glycosides either via enzymatic hydrolysis or transglycosylation.

3. Experimental

General methods.—Chemicals were obtained from Aldrich Chemical (Gillingham, Dorset, UK). All glycosyl bromides were used immediately although storage in a freezer for several days was possible. However, on prolonged storage decomposition was observed.

Snail acetone powder, β-glucuronidases from *H. aspersa*, limpet and scallop, β-galactosidases from *E. coli* and *A. oryzae*, β-amylase (barley), almond meal and β-glucosidase (almonds) were obtained from Sigma Chemical (Poole, Dorset, UK). Melting points were recorded on a Reichert Kofler hot stage and are uncorrected. NMR spectra were recorded with a Jeol GX270, Lambda 300 or GX400 spectrometers using Me_4Si or DSS as internal standard. Mass spectra (EI, CI and FAB) were obtained using a Fisons/VG Analytical Autospec system by the University of Bristol Mass Spectroscopy Service. Electrospray mass spectra were recorded on a VG Autospec X spectrometer at Glaxo Research, Stevenage, Hertfordshire. Elemental analyses were carried out by University of Bristol Microanalysis Service. TLC was performed on Silica Gel 60 (Merck) precoated on aluminium sheets. Pet. ether refers to low boiling 40–60 petroleum ether. Products were visualised using orcinol–ferric chloride spray reagent (Sigma). Preparative chromatography was performed by gradient elution from columns of Silica Gel (230–400

mesh, Merck 9385). HPLC analysis was carried out using a Gilson 305/306 pump system equipped with a Gilson 234 autoinjector and a Sedex 55 evaporative light scattering detector. The samples were analysed with a Hypersil Hypercarb column (5 μ, 100 mm × 4.6 mm) using linear gradients of acetonitrile–water at a flow rate of 0.75 ml min^{-1}: from 0 to 35% acetonitrile in 20 min followed by 35–60% in 5 min for **1** and **3** and from 0 to 60% over 20 min for **2**.

Enzymatic hydrolysis: Each diastereomeric pair of substrates **1–3** (0.5 mg) was dissolved in 0.05 M citrate buffer (0.5 mL, pH 5.2) and 0.1 mL of enzyme solution (6 mg/mL prepared in the same buffer) was added. The samples were incubated at 37 °C and aliquots withdrawn at appropriate time intervals for HPLC analysis.

*1,2,3,4-Tetra-*O*-acetyl-6-*S*-methyl-6-sulfinyl-*D*-galactoside* (**5**).—1,2:3,4-Di-*O*-isopropylidene-6-*S*-methyl-6-sulfinyl-α-D-galactoside (**4**) (12.50 g, 0.043 mol), prepared by the method of Madson et al. [11], was dissolved in glacial acetic acid (60 mL) and heated on a water bath. Water (55 mL) was added over 1 h in 5 mL portions and the reaction mixture heated for an additional 1.5 h, when TLC indicated disappearance of starting material [TLC R_f 0.22 Pet. Ether:EtOAc (1:1)]. The reaction mixture was concentrated in vacuo at a temperature not exceeding 40 °C. Ethanol (2 × 50 mL) was added to the thin syrup and concentration in vacuo gave a colourless oil which was not characterised but acetylated directly.

The crude material was dissolved in dry pyridine (90 mL) and cooled to 0 °C. Acetic anhydride (60 mL) was added dropwise over 2 min. After 18 h at RT, the reaction mixture was poured into water (50 mL) and on standing for several hours at 0 °C, crystalline (**5**) (12.21 g, 75%) precipitated as a 1:1.5 mixture of α:β anomers [TLC R_f 0.45 Pet. Ether:EtOAc (1:1)]; ν_{max} (neat film, NaCl)/cm^{-1} 1752 (s, C=O ester), 1370 (m), 1221 (s) and 1061 (m); δ_H (400 MHz; $CDCl_3$) 1.99 (s, 3 H, CH_3-β), 2.00 (s, 3 H, CH_3-α), 2.03 (s, 3 H, CH_3-α), 2.05 (s, 3 H, CH_3-β), 2.12 (br, s, 9 H, 2 × CH_3-β and CH_3-α), 2.17 (s, 3 H, CH_3-α), 2.17 (s, 3 H, CH_3-α), 2.18 (s, 3 H, CH_3-β), 2.52 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 7, H-6Aα), 2.56 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 7, H-6Aβ), 2.66 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 7, H-6Bα), 2.72 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 7, H-6Bβ), 3.92 (td, 1 H, $J_{5,6A}$ 7, $J_{5,6B}$ 7 and $J_{5,4}$ 1, H-5β), 4.22 (td, 1 H, $J_{5,6A}$ 7, $J_{5,6B}$ 7 and $J_{5,4}$ 1, H-5α), 5.10 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3β), 5.33 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 8, H-2β), 5.34 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 3, H-2α), 5.37 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3α), 5.54 (dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4β), 5.58 (br, dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4α), 5.69 (d, 1 H, $J_{1,2}$ 8, H-1β), and 6.37 (d, 1 H, $J_{1,2}$ 3, H-1α).

*2,3,4-Tri-*O*-acetyl-6-*S*-methyl-6-sulfinyl-α-D-galactosyl bromide* (**6**).—1,2,3,4-Tetra-*O*-acetyl-6-*S*-methyl-6-sulfinyl-D-galactoside (**5**) (12.21 g, 0.032 mol) was cooled to 0 °C and to it was added a 30% hydrogen bromide solution in glacial acetic acid (30 mL). The mixture was slowly allowed to warm up to RT and after 1.5 h TLC indicated bromination to be complete. $CHCl_3$ (100 mL) and ice water (50 mL) were added to the reaction mixture, the $CHCl_3$ layer separated and the aqueous layer extracted with $CHCl_3$ (50 mL). The combined organic extracts were washed with ice water (5 × 50 mL) until the washings were neutral, dried (Na_2SO_4) and the solvent removed in vacuo to give (**6**) (12.86 g, 100%) as a pale yellow foam [TLC R_f 0.36 Pet. Ether:EtOAc (1:1)]; δ_H (270 MHz; $CDCl_3$) 2.00 (s, 3 H, CH_3), 2.05 (br, s, 6 H, 2 × CH_3), 2.07 (s, 3 H, CH_3), 2.60 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 7, H-6A), 2.68 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 7, H-6B), 4.38 (td, 1 H, $J_{5,6A}$ 7, $J_{5,6B}$ 7 and $J_{5,4}$ 1, H-5), 5.02 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3), 5.42 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 3, H-2), 5.60 (br, dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4) and 6.72 (d, 1 H, $J_{1,2}$ 3, H-1).

*4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6-*S*-methyl-6-sulfinyl-β-D-galactopyranoside* (**7**).—A solution of 2,3,4-tri-*O*-acetyl-6-*S*-methyl-6-sulfinyl-α-D-galactosyl bromide (**6**) (12.86 g, 0.032 mol) in $CHCl_3$ (130 mL) was stirred vigorously with a solution of 4-nitrophenol (8.90 g, 0.064 mol) and benzyltriethylammonium chloride (6.07 g, 0.027 mol) in aqueous NaOH solution (66 mL, 1.25 M, 0.083 mol) and the reaction mixture heated under reflux. After 2 h, the reaction mixture was cooled to RT and diluted with water (100 mL). The two layers were then separated and the organic layer washed with aqueous NaOH solution (2 × 50 mL, 1.25 M), water (50 mL), dried (Na_2SO_4) and the solvent removed in vacuo to give a yellow oil which was purified by column chromatography [Pet. Ether:EtOAc (4:1)] to give (**7**) (9.60 g, 65%) as a foam [TLC R_f 0.33 Pet. Ether:EtOAc (1:1)]; ν_{max} (neat film, NaCl)/cm^{-1} 1749 (s, C=O ester), 1594 (w), 1522 (m), 1372 (m), 1344 (m), 1222 (s) and 1064 (s); δ_H (270 MHz; $CDCl_3$) 2.00 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 2.15 (s, 3 H, CH_3), 2.20 (s, 3 H, CH_3), 2.60 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 5, H-6A), 2.75 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 9, H-6B), 4.00 (ddd, 1 H, $J_{5,6B}$ 9, $J_{5,6A}$ 5 and $J_{5,4}$ 1, H-5), 5.15 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3), 5.20 (d, 1 H, $J_{1,2}$

7, H-1), 5.45 (br, dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4), 5.46 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 7, H-2), 7.18 (AA'BB', 2 H, J 8, 2 × H-2') and 8.24 (AA'BB', 2 H, J 8, 2 × H-3').

4-Nitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-6-methyl-(R)-sulfinyl-β-D-galactopyranoside (**8a**) and 4-nitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-6-methyl-(S)-sulfinyl-β-D-*galactopyranoside* (**8b**).—An equimolar quantity of a solution of H_2O_2 (1.08 mL, 27.5%, 8.75 mmol) in glacial acetic acid (20 mL) was added over a period of 4 h to a solution of 4-nitrophenyl 2,3,4-tri-*O*-acetyl-6-*S*-methyl-6-sulfinyl-β-D-galactopyranoside (**7**) (4.00 g, 8.75 mmol) in glacial acetic acid (20 mL). When all the peroxide had been consumed (negative reaction with starch-iodide), the solution was concentrated in vacuo to give a colourless oil which was purified by column chromatography [Pet. Ether:EtOAc (1:1 to 3:7)] to give (**8a**) (0.95 g, 23%) as a foam [TLC R_f 0.28 Pet. Ether:EtOAc (1:1)]; ν_{max} (neat film, NaCl)/cm^{-1} 1750 (s, C=O ester), 1522 (w), 1414 (m), 1263 (s), 1277 (s) and 1071 (s, S=O); δ_H (270 MHz; $CDCl_3$) 2.02 (s, 3 H, $OCOCH_3$), 2.10 (s, 3 H, $OCOCH_3$), 2.20 (s, 3 H, $OCOCH_3$), 2.59 (s, 3 H, $SOCH_3$), 2.84 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 9, H-6A), 2.90 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 4, H-6B), 4.70 (ddd, 1 H, $J_{5,6A}$ 9, $J_{5,6B}$ 4 and $J_{5,4}$ 1, H-5), 5.27 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3), 5.38 (d, 1 H, $J_{1,2}$ 7, H-1), 5.50 (dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4), 5.57 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 7, H-2), 7.25 (AA'BB', 2 H, J 8, 2 × H-2') and 8.23 (AA'BB', 2 H, J 8, 2 × H-3').

Further elution gave (**8b**) (2.24 g, 54%) as a foam [TLC R_f 0.20 Pet. Ether:EtOAc (1:1)]; ν_{max} (neat film, NaCl)/cm^{-1} 1756 (s, C=O ester), 1346 (w), 1240 (s) and 1064 (s, S=O); δ_H (270 MHz; $CDCl_3$) 2.10 (br, s, 6 H, 2 × $OCOCH_3$), 2.21 (s, 3 H, $OCOCH_3$), 2.68 (s, 3 H, $SOCH_3$), 2.85 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 3, H-6A), 2.90 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 11, H-6B), 4.56 (ddd, 1 H, $J_{5,6B}$ 11, $J_{5,6A}$ 3 and $J_{5,4}$ 1, H-5), 5.22 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3), 5.35 (d, 1 H, $J_{1,2}$ 7, H-1), 5.47 (dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4), 5.55 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 7, H-2), 7.18 (AA'BB', 2 H, J 8, 2 × H-2') and 8.24 (AA'BB', 2 H, J 8, 2 × H-3').

4-Nitrophenyl 6-deoxy-6-methyl-(R)-sulfinyl-β-D-galactopyranoside (**1a**).—To a solution of 4-nitrophenyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-methyl-(*R*)-sulfinyl-β-D-galactopyranoside (0.95 g, 2.01 mmol) in dry CH_3OH (10 mL) at 0 °C, was added a $NaOCH_3$ solution in CH_3OH (15 mL, 0.87 mmol) via cannula. The progress of the reaction was monitored by TLC and after 1.5 h at RT deacetylation was complete. Dowex H^+ resin was added in portions until pH 6, the resin filtered off and the filtrate concentrated in vacuo. Recrystallisation twice from CH_3OH gave (**1a**) (0.55 g, 78%) as spars, mp 201 °C [TLC R_f 0.19 EtOAc:MeOH (9:1)]; δ_H (300 MHz; CD_3OD) 2.67 (s, 3 H, $SOCH_3$), 3.14 (dd, 1 H, $J_{6A,6B}$ 14 and $J_{6A,5}$ 8, H-6A), 3.24 (dd, 1 H, $J_{6B,6A}$ 14 and $J_{6B,5}$ 4.5, H-6B), 3.67 (dd, 1 H, $J_{3,2}$ 9.5 and $J_{3,4}$ 3.5, H-3), 3.84 (dd, 1 H, $J_{2,3}$ 9.5 and $J_{2,1}$ 7.5, H-2), 3.86 (br, d, 1 H, $J_{4,3}$ 3.5, H-4), 4.28 (ddd, 1 H, $J_{5,6A}$ 8, $J_{5,6B}$ 4.5 and $J_{5,4}$ 1, H-5), 5.10 (d, 1 H, $J_{1,2}$ 7.5, H-1), 7.27 (AA'BB', 2 H, J 8, 2 × H-2') and 8.21 (AA'BB', 2 H, J 8, 2 × H-3'); δ_C (75.5 MHz; CD_3OD) 38.7 (CH_3, $SOCH_3$), 55.3 (CH_2, CH_2-6), 70.8 (CH, C-5), 71.5 (CH, C-2), 71.9 (CH, C-4), 74.4 (CH, C-3), 101.7 (CH, C-1), 117.9 (CH, C-2'), 126.7 (CH, C-3'), 143.0 (C, C-1') and 163.6 (C, C-4'). Anal. Calcd. for $C_{13}H_{17}NO_8S \cdot H_2O$ (365.078): C, 42.73; H, 5.25; N, 3.84; S, 8.76. Found: C, 42.82; H, 5.17; N, 4.14; S, 8.47.

4-Nitrophenyl 6-deoxy-6-methyl-(S)-sulfinyl-β-D-galactopyranoside (**1b**).—4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-methyl-(*S*)-sulfinyl-β-D-galactopyranoside (2.24 g, 4.73 mmol) was deacetylated as described above and recrystallised to give (**1b**) (1.08 g, 66%) as needles, mp 225 °C (CH_3OH:H_2O) [TLC R_f 0.16 EtOAc:CH_3OH (9:1)]; δ_H (300 MHz; Me_2SO-d_6) 2.84 (dd, 1 H, $J_{6A,6B}$ 13.5 and $J_{6A,5}$ 2.5, H-6A), 3.07 (dd, 1 H, $J_{6B,6A}$ 13.5 and $J_{6B,5}$ 11, H-6B), 3.55 (dd, 1 H, $J_{3,2}$ 9.5 and $J_{3,4}$ 3.5, H-3), 3.61 (dd, 1 H, $J_{2,3}$ 9.5 and $J_{2,1}$ 7.5, H-2), 3.64 (br, d, 1 H, $J_{4,3}$ 3, H-4), 3.73 (s, 3 H, $SOCH_3$), 4.07 (br, dd, 1 H, $J_{5,6B}$ 11, $J_{5,6A}$ 2.5 and $J_{5,4}$ 1, H-5), 5.04 (d, 1 H, $J_{1,2}$ 7.5, H-1), 7.20 (AA'BB', 2 H, J 8, 2 × H-2) and 8.18 (AA'BB', 2 H, J 8, 2 × H-3'); δ_C (75.5 MHz; Me_2SO-d_6) 38.7 (CH_3, $SOCH_3$), 55.7 (CH_2, CH_2-6), 69.3 (CH, C-5), 69.9. (CH, C-2), 70.3 (CH, C-4), 73.0 (CH, C-3), 100.3 (CH, C-1), 116.8 (CH, C-2'), 126.1 (CH, C-3'), 142.1 (C, C-1') and 162.5 (C, C-4'). Anal. Calcd. for $C_{13}H_{17}NO_8S \cdot CH_3OH$ (379.094): C, 44.32; H, 5.58; N, 3.69; S, 8.45. Found: C, 43.99; H, 5.12; N, 4.00; S, 8.20.

1,2,3,4,6-Penta-O-acetyl-7-deoxy-D-glycero-D-galacto-heptopyranoside (**10a**) and 1,2,3,4,6-penta-O-acetyl-7-deoxy-L-glycero-D-galacto-*heptopyranoside* (**10b**).—A mixture of 1,2:3,4-di-*O*-isopropylidene-7-deoxy-D-*glycero*-D-*galacto*-heptopyranoside and 1,2:3,4-di-*O*-isopropylidene-7-deoxy-L-*glycero*-D-*galacto*-heptopyranoside (**9**) (0.87 g, 3.16 mmol) in a ratio of 2.5:1 (L:D), prepared by known methodology [15,16], was dissolved in aqueous acetic acid (5 mL, 60%) and the mixture heated under reflux for 2.5 h,

when TLC indicated disappearance of starting material [TLC R_f 0.31 EtOAc]. The reaction mixture was concentrated in vacuo at a temperature not exceeding 40 °C. Ethanol (2 × 10 mL) was added to the thin syrup and concentration in vacuo gave a colourless oil which was not characterised but used directly for acetylation.

The crude material was dissolved in dry pyridine (15 mL) and cooled to 0 °C. Acetic anhydride (10 mL) was added dropwise over 2 min and after 18 h at RT, the reaction mixture was poured into water (10 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic extracts were washed with aqueous HCl (2 × 10 mL, 1 M), water (10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), dried (Na_2SO_4) and the solvent removed in vacuo to give an oil which was purified by column chromatography [Pet. Ether:EtOAc (3:1)] to give (**10a**) (0.21 g, 16%) as a foam in a 1:1.2 mixture of α:β anomers [TLC R_f 0.30 Pet. Ether:EtOAc (1:1)]; δ_H (270 MHz; $CDCl_3$) 1.24 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7α), 1.27 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7β), 1.98 (s, 3 H, $OCOCH_3\alpha$), 1.98 (s, 3 H, $OCOCH_3\beta$), 1.99 (s, 3 H, $OCOCH_3\beta$), 2.00 (s, 3 H, $OCOCH_3\alpha$), 2.03 (s, 3 H, $OCOCH_3\alpha$), 2.05 (s, 3 H, $OCOCH_3\beta$) 2.11 (s, 3 H, $OCOCH_3\alpha$), 2.12 (s, 3 H, $OCOCH_3\beta$), 2.13 (s, 3 H, $OCOCH_3\beta$), 2.17 (s, 3 H, $OCOCH_3\alpha$), 3.67 (dd, 1 H, $J_{5,6}$ 9 and $J_{5,4}$ 1, H-5β), 3.95 (br, dd, 1 H, $J_{5,6}$ 9 and $J_{5,4}$ 1, H-5α), 4.97 (2 × dq, 2 H, $J_{6,5}$ 9 and J_{6,CH_3-7} 6, H-6α and H-6β), 5.08 (dd, 1 H, $J_{3,2}$ 10.5 and $J_{3,4}$ 3.5, H-3β), 5.33 (m, 3 H, H-2α, H-2β and H-3α), 5.46 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4β), 5.54 (br, dd, 1 H, $J_{4,3}$ 2.5 and $J_{4,5}$ 1, H-4α), 5.67 (d, 1 H, $J_{1,2}$ 8, H-1β), and 6.38 (d, 1 H, $J_{1,2}$ 3, H-1α).

Further elution gave (**10b**) (0.69 g, 54%) as a foam in a 1:1.1 mixture of α:β anomers [TLC R_f 0.24 Pet. Ether:EtOAc (1:1)]; δ_H (270 MHz; $CDCl_3$) 1.16 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7α), 1.18 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7β), 2.00 (s, 3 H, $OCOCH_3\alpha$), 2.01 (s, 3 H, $OCOCH_3\alpha$), 2.04 (br, s, 9 H, 2 × $OCOCH_3\beta$ and $OCOCH_3\alpha$), 2.06 (s, 3 H, $OCOCH_3\beta$), 2.12 (s, 3 H, $OCOCH_3\beta$), 2.18 (s, 3 H, $OCOCH_3\beta$) 2.20 (s, 3 H, $OCOCH_3\alpha$), 2.12 (s, 3 H, $OCOCH_3\alpha$), 3.77 (br, d, 1 H, $J_{5,6}$ 9, H-5β), 4.04 (br, d, 1 H, $J_{5,6}$ 9, H-5α), 5.10 (m, 3 H, H-3β, H-6α and H-6β), 5.31 (m, 3 H, H-2α, H-2β and H-3α), 5.47 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4β), 5.50 (br, dd, 1 H, $J_{4,3}$ 2.5 and $J_{4,5}$ 1, H-4α), 5.62 (d, 1 H, $J_{1,2}$ 8, H-1β) and 6.35 (d, 1 H, $J_{1,2}$ 3, H-1α).

2,3,4,6-Tetra-O-*acetyl*-7-*deoxy*-D-glycero-α-D-galacto-*heptopyranosyl bromide* (**11a**).—1,2,3,4,6-Penta-*O*-acetyl-7-deoxy-D-*glycero*-α-D-*galacto*-heptopyranoside (**10a**) (164 mg, 0.40 mmol) was cooled to 0 °C and to it was added a 30% hydrogen bromide solution in glacial acetic acid (2 mL). The reaction mixture was slowly allowed to warm up to RT and bromination was complete (TLC) after 1 h. $CHCl_3$ (10 mL) and ice water (5 mL) were added to the reaction mixture, the $CHCl_3$ layer separated and the aqueous layer extracted with $CHCl_3$ (5 mL). The combined organic extracts were washed with ice water (5 × 5 mL) until the washings were neutral, dried (Na_2SO_4) and the solvent removed in vacuo to give (**11a**) (162 mg, 95%) as a foam [TLC R_f 0.43 Pet. Ether:EtOAc (1:1)].

2,3,4,6-Tetra-O-*acetyl*-7-*deoxy*-L-glycero-α-D-galacto-*heptopyranosyl bromide* (**11b**).—1,2,3,4,6-Penta-*O*-acetyl-7-deoxy-L-*glycero*-α-D-*galacto*-heptopyranoside (**10b**) (485 mg, 1.20 mmol) was brominated as described above to give (**11b**) (420 mg, 82%) as a foam [TLC R_f 0.69 EtOAc].

4-Nitrophenyl 2,3,4,6-tetra-O-*acetyl*-7-*deoxy*-D-glycero-β-D-galacto-*heptopyranoside* (**12a**).—A solution of 2,3,4,6-tetra-*O*-acetyl-7-deoxy-D-*glycero*-α-D-*galacto*-heptopyranosyl bromide (**11a**) (162 mg, 0.38 mmol) in $CHCl_3$ (5 mL) was stirred vigorously with a solution of 4-nitrophenol (0.11 g, 0.80 mmol) and benzyltriethylammonium chloride (0.08 g, 0.033 mmol) in aqueous NaOH solution (0.58 mL, 1.25 M, 0.70 mmol) and the reaction mixture heated under reflux.

After 3 h, the reaction mixture was cooled to RT and diluted with water (20 mL). The organic layer was washed with aqueous NaOH solution (2 × 10 mL, 1.25 M), water (10 mL), dried (Na_2SO_4) and the solvent removed in vacuo to give an oil which was purified by column chromatography [Pet. Ether:EtOAc (2:1)] to give (**12a**) (187 mg, 98%) as a foam [TLC R_f 0.12 Pet. Ether:EtOAc (1:1)]; δ_H (270 MHz; $CDCl_3$) 1.33 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7), 2.01 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.08 (s, 3 H, $OCOCH_3$), 2.14 (s, 3 H, $OCOCH_3$), 3.75 (br, d, 1 H, $J_{5,6}$ 9, H-5), 5.05 (dq, 1 H, $J_{6,5}$ 9 and J_{6,CH_3-7} 6, H-6), 5.15 (dd, 1 H, $J_{3,2}$ 10.5 and $J_{3,4}$ 3.5, H-3), 5.20 (d, 1 H, $J_{1,2}$ 8, H-1), 5.51 (dd, 1 H, $J_{2,3}$ 10.5 and $J_{2,1}$ 8, H-2), 5.53 (br, d, 1 H, $J_{4,3}$ 3.5, H-4), 7.05 (AA′BB′, 2 H, J 9, 2 × H-2′) and 8.23 (AA′BB′, 2 H, J 9, 2 × H-3′).

4-Nitrophenyl 2,3,4,6-tetra-O-*acetyl*-7-*deoxy*-L-glycero-β-D-galacto-*heptopyranoside* (**12b**).—A solution of 2,3,4,6-tetra-*O*-acetyl-7-deoxy-L-*glycero*-α-D-*galacto*-heptopyranosyl bromide (**11b**) (420 mg, 0.99 mmol) was converted to its 4-nitrophenyl derivative as described above. Column chromatography

[Pet. Ether:EtOAc (1.5:1)] gave (**12b**) (253 mg, 53%) as a foam [TLC R_f 0.21 Pet. Ether:EtOAc (1:1)]; δ_H (400 MHz; $CDCl_3$) 1.21 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7), 2.01 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.10 (s, 3 H, $OCOCH_3$), 2.21 (s, 3 H, $OCOCH_3$), 3.81 (br, d, 1 H, $J_{5,6}$ 9, H-5), 5.10 (m, 3 H, H-1, H-3 and H-6), 5.50 (m, 2 H, H-2 and H-4), 7.10 (AA'BB', 2 H, *J* 9, 2 × H-2') and 8.20 (AA'BB', 2 H, *J* 9, 2 × H-3').

4-Nitrophenyl 7-deoxy-D-glycero-*β*-D-galacto-*heptopyranoside* (**2a**).—Deacetylation of 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-7-deoxy-D-*glycero*-*α*-D-*galacto*-heptopyranoside (**12a**) (187 mg, 0.39 mmol) using the method described above gave (**2a**) (93 mg, 71%) as spars, mp 236 °C (CH_3OH) [Found: MNH_4^+, 333.1293. $C_{13}H_{21}N_2O_8$ requires *M*, 333.1298 (1.5 ppm)] [TLC R_f 0.14 EtOAc:CH_3OH (10:1)]; δ_H (400 MHz; D_2O) 1.21 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7), 3.44 (d, 1 H, $J_{5,6}$ 8, H-5), 3.69 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3), 3.79 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 8, H-2), 3.89 (dq, 1 H, $J_{6,5}$ 8 and J_{6,CH_3-7} 6, H-6), 4.12 (br, d, 1 H, $J_{4,3}$ 3, H-4), 5.10 (d, 1 H, $J_{1,2}$ 8, H-1), 7.14 (AA'BB', 2 H, *J* 8, 2 × H-2') and 8.16 (AA'BB', 2 H, *J* 8, 2 × H-2'). Anal. Calcd. for $C_{13}H_{17}NO_8$ (315.095): C, 49.51; H, 5.44; N, 4.44. Found: C, 49.88; H, 5.56; N, 4.05.

4-Nitrophenyl 7-deoxy-L-glycero-*β*-D-galacto-*heptopyranoside* (**2b**).—Deacetylation of 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-7-deoxy-L-*glycero*-*α*-D-*galacto*-heptopyranoside (**12b**) (253 mg, 0.52 mmol) as described above gave, after column chromatography [EtOAc:CH_3OH (15:1)], (**2b**) (0.14 g, 86%) as spars, mp 214 °C (EtOAc) [TLC R_f 0.21 EtOAc:CH_3OH (15:1)]; δ_H (270 MHz; CD_3OD) 1.28 (d, 3 H, $J_{CH_3-7,6}$ 6, CH_3-7), 3.47 (dd, 1 H, $J_{5,6}$ 7 and $J_{5,4}$ 1, H-5), 3.63 (dd, 1 H, $J_{3,2}$ 9 and $J_{3,4}$ 3, H-3), 3.89 (dd, 1 H, $J_{2,3}$ 9 and $J_{2,1}$ 7.5, H-2), 3.97 (dd, 1 H, $J_{4,3}$ 3 and $J_{4,5}$ 1, H-4), 4.08 (br, dq, 1 H, $J_{6,5}$ 7 and J_{6,CH_3-7} 6, H-6), 5.02 (d, 1 H, $J_{1,2}$ 7.5, H-1), 7.30 (AA'BB', 2 H, *J* 9, 2 × H-2') and 8.25 (AA'BB', 2 H, *J* 9, 2 × H-2'); δ_C (75.5 MHz; CD_3OD) 19.2 (CH_3, CH_3-7), 68.2 (CH, C-6), 70.8 (CH, C-4), 71.8 (CH, C-2), 74.6 (CH, C-3), 80.8 (CH, C-5), 102.3 (CH, C-1), 117.8 (CH, C-2'), 126.6 (CH, C-3'), 143.8 (C, C-1') and 164.1 (C, C-4'). Anal. Calcd. for $C_{13}H_{17}NO_8$ (315.095): C, 49.51; H, 5.44; N, 4.44. Found: C, 49.21; H, 5.37; N, 3.98.

2,3,4-Tri-O-*acetyl-6,7-dideoxy-α*-D-galacto-*hept-6-ynopyranosyl bromide* (**14**).—1,2,3,4-Tetra-*O*-acetyl-6,7-dideoxy-D-*galacto*-hept-6-ynopyranose (**13**) (4.58 g, 13.39 mmol), prepared by literature methodology [17], was cooled to 0 °C and to it was added a 30% hydrogen bromide solution in glacial acetic acid (20 mL). The reaction mixture was maintained at 0 °C and monitored by TLC which, after 2.5 h indicated disappearance of starting material [TLC R_f 0.45 Pet. Ether:EtOAc (1:1)]. $CHCl_3$ (100 mL) and ice water (50 mL) were added to the reaction mixture, the $CHCl_3$ layer separated and the aqueous layer extracted with $CHCl_3$ (50 mL). The combined organic extracts were washed with ice water (5 × 20 mL) until the washings were neutral, dried (Na_2SO_4) and the solvent removed in vacuo to give (**14**) (3.83 g, 79%) as a yellow foam [TLC R_f 0.58 Pet. Ether:EtOAc (1:1)]; δ_H (400 MHz; $CDCl_3$) 2.00 (s, 3 H, $OCOCH_3$), 2.05 (s, 3 H, $OCOCH_3$), 2.10 (s, 3 H, $OCOCH_3$), 2.50 (d, 1 H, $J_{7,5}$ 2, H-7), 5.02 (m, 1 H, H-5), 5.07 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3, H-3), 5.40 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 3, H-2), 5.60 (br, d, 1 H, $J_{4,3}$ 3, H-4) and 6.64 (d, 1 H, $J_{1,2}$ 3, H-1).

4-Nitrophenyl 2,3,4-tri-O-*acetyl-6,7-dideoxy-β*-D-galacto-*hept-6-ynopyranoside* (**15**).—2,3,4-Tri-*O*-acetyl-6,7-dideoxy-*α*-D-*galacto*-hept-6-ynopyranosyl bromide (**14**) (3.83 g, 10.55 mmol) in $CHCl_3$ (60 mL) was converted to its 4-nitrophenyl derivative (**15**) as described above. Extraction after 6 h gave an oil which was purified by column chromatography [Pet. Ether:EtOAc (2:1)] to give (**15**) (2.87 g, 65%) as spars, mp 150 °C (Pet. Ether:EtOAc), [TLC R_f 0.21 Pet. Ether:EtOAc (2:1)]; ν_{max} ($CHCl_3$)/cm^{-1} 3275 (m, C–H stretch), 3031 (s), 1765 (s, C=O ester), 1240 (s) and 1050 (s); δ_H (400 MHz; $CDCl_3$) 2.04 (s, 3 H, $OCOCH_3$), 2.07 (s, 3 H, $OCOCH_3$), 2.24 (s, 3 H, $OCOCH_3$), 2.53 (d, 1 H, $J_{7,5}$ 2, H-7), 4.66 (dd, 1 H, $J_{5,7}$ 2 and $J_{5,4}$ 1.5, H-5), 5.15 (dd, 1 H, $J_{3,2}$ 10.5 and $J_{3,4}$ 3.5, H-3), 5.18 (d, 1 H, $J_{1,2}$ 8, H-1), 5.52 (dd, 1 H, $J_{2,3}$ 10.5 and $J_{2,1}$ 8, H-2), 5.57 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1.5, H-4), 7.11 (AA'BB', 2 H, *J* 9, 2 × H-2') and 8.22 (AA'BB', 2 H, *J* 9, 2 × H-3'); δ_C (75.5 MHz; $CDCl_3$) 20.6 (CH_3, $OCOCH_3$), 20.6 (CH_3, $OCOCH_3$), 20.7 (CH_3, $OCOCH_3$), 65.1 (CH, C-5), 68.0 (CH, C-2), 68.6 (CH, C-4), 69.9 (CH, C-3), 75.7 (CH, C-7), 98.2 (CH, C-1), 116.7 (CH, C-2'), 125.9 (CH, C-3'), 143.3 (C, C-1'), 161.1 (C, C-4'), 169.2 (C, $OCOCH_3$), 170.0 (C, $OCOCH_3$) and 170.1 (C, $OCOCH_3$). Signal arising from the acetylenic carbon (C-6) was not observed.

4-Nitrophenyl 2,3,4-tri-O-*acetyl-6,7-dideoxy-β*-D-galacto-*hept-6-enopyranoside* (**16**).—4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6,7-dideoxy-*β*-D-*galacto*-hept-6-ynopyranoside (**15**) (2.00 g, 4.75 mmol) in EtOAc (200 mL) was cooled to 0 °C and hydrogenated in the presence of Lindlar's Catalyst (5% palladium on calcium carbonate poisoned with lead (200 mg)). The

reaction was monitored by TLC and after 3 h at RT. the mixture was filtered through Celite. The filtrate was concentrated in vacuo to give (**16**) (2.00 g, 100%) as spars, mp 164 °C (EtOAc) [TLC R_f 0.24 Pet. Ether:EtOAc (2:1)]; ν_{max} ($CHCl_3$)/cm^{-1} 3010 (w, olefinic C–H), 1754 (s, C=O ester), 1615 (w, C=C), 1595 (s), 1523 (s), 1497 (w), 1348 (s), 1220 (br, s) and 1059 (br, s); δ_H (400 MHz; $CDCl_3$) 2.03 (s, 3 H, $OCOCH_3$), 2.08 (s, 3 H, $OCOCH_3$), 2.16 (s, 3 H, $OCOCH_3$), 4.40 (br, dd, 1 H, $J_{5,6}$ 3.5 and $J_{5,4}$ 1.5, H-5), 5.18 (dd, 1 H, $J_{3,2}$ 10.5 and $J_{3,4}$ 3.5, H-3), 5.21 (d, 1 H, $J_{1,2}$ 8, H-1), 5.31 (d, 1 H, $J_{7cis,6}$ 10.5, H-7cis), 5.41 (d, 1 H, $J_{7trans,6}$ 17.5, H-7trans), 5.47 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4), 5.55 (dd, 1 H, $J_{2,3}$ 10.5 and $J_{2,1}$ 8, H-2), 5.75 (ddd, 1 H, $J_{6,7trans}$ 17.5, $J_{6,7cis}$ 10.5 and $J_{6,5}$ 3.5, H-6), 7.10 (AA′BB′, 2 H, *J* 9, 2 × H-2′) and 8.22 (AA′BB′, 2 H, *J* 9, 2 × H-3′). Anal. Calcd. for $C_{19}H_{21}NO_{10}$ (423.117): C, 53.90; H, 5.00; N, 3.31. Found: C, 53.70; H, 4.88; N, 3.51.

*4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6,7-anhydro-*L-glycero-*β*-D-galacto-heptopyranoside* (**17a**) and 4-nitrophenyl 2,3,4-tri-O-acetyl-6,7-anhydro-D-glycero-*β*-D-galacto-*heptopyranoside* (**17b**).—4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6,7-dideoxy-*β*-D-*galacto*-hept-6-enopyranoside (**16**) (300 mg, 0.71 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C and *m*-chloroperbenzoic acid (0.61 g, 3.54 mmol) added. The reaction was allowed to warm up to RT and maintained at ambient temperature for 80 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed successively with aqueous $Na_2S_2O_3$ (100 mL), saturated aqueous $NaHCO_3$ (30 mL) and water (2 × 60 mL). The organic extracts were dried (Na_2SO_4) and concentrated in vacuo to a give an oil which was purified by column chromatography [Pet. Ether:EtOAc (100:0 to 70:30)] to give unreacted starting material (60 mg).

Further elution gave (**17a**) (166 mg, 66%) as a foam [TLC R_f 0.18 Pet. Ether:EtOAc (2:1)]; δ_H (270 MHz; $CDCl_3$) 2.04 (s, 3 H, $OCOCH_3$), 2.08 (s, 3 H, $OCOCH_3$), 2.20 (s, 3 H, $OCOCH_3$), 2.82 (d, 2 H, $J_{CH_2-7,6}$ 4, CH_2-7), 3.12 (dt, 1 H, J_{6,CH_2-7} 4 and $J_{6,5}$ 3.5, H-6), 3.81 (dd, 1 H, $J_{5,6}$ 3.5 and $J_{5,4}$ 1, H-5), 5.14 (d, 1 H, $J_{1,2}$ 8, H-1), 5.15 (dd, 1 H, $J_{3,2}$ 10.5 and $J_{3,4}$ 3.5, H-3), 5.53 (dd, 1 H, $J_{2,3}$ 10.5 and $J_{2,1}$ 8, H-2), 5.60 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4), 7.06 (AA′BB′, 2 H, *J* 9, 2 × H-2′) and 8.22 (AA′BB′, 2 H, *J* 9, 2 × H-3′); δ_C (75.5 MHz; $CDCl_3$) 20.6 (CH_3, $OCOCH_3$), 20.7 (CH_3, $OCOCH_3$), 20.7 (CH_3, $OCOCH_3$), 45.1 (CH_2, CH_2-7), 49.5 (CH, C-6), 67.6 (CH, C-4), 68.4 (CH, C-2), 70.5 (CH, C-3), 73.1 (CH, C-5), 98.7 (CH, C-1), 116.6 (CH, C-2′), 125.9 (CH, C-3′), 143.2 (C, C-1′), 161.3 (C, C-4′), 169.3 (C, $OCOCH_3$), 170.0 (C, $OCOCH_3$) and 170.1 (C, $OCOCH_3$).

Further elution gave (**17b**) (59 mg, 23%) as a foam [TLC R_f 0.14 Pet. Ether:EtOAc (2:1)]; δ_H (270 MHz; $CDCl_3$) 2.02 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.06 (s, 3 H, $OCOCH_3$), 2.67 (dd, 1 H, $J_{7A,7B}$ 4.5 and $J_{7A,6}$ 2.5, H-7A), 2.81 (dd, 1 H, $J_{7B,7A}$ 4.5 and $J_{7B,6}$ 4, H-7B), 3.14 (ddd, 1 H, $J_{6,5}$ 6, $J_{6,7B}$ 4 and $J_{6,7A}$ 2.5, H-6), 3.53 (dd, 1 H, $J_{5,6}$ 6 and $J_{5,4}$ 1, H-5), 5.10 (dd, 1 H, $J_{3,2}$ 10.5 and $J_{3,4}$ 3.5, H-3), 5.17 (d, 1 H, $J_{1,2}$ 8, H-1), 5.53 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4), 5.55 (dd, 1 H, $J_{2,3}$ 10.5 and $J_{2,1}$ 8, H-2), 7.09 (AA′BB′, 2 H, *J* 9, 2 × H-2′) and 8.20 (AA′BB′, 2 H, *J* 9, 2 × H-3′); δ_C (75.5 MHz; $CDCl_3$) 20.6 (CH_3, $OCOCH_3$), 20.7 (CH_3, $OCOCH_3$), 20.7 (CH_3, $OCOCH_3$), 43.5 (CH_2, CH_2-7), 50.5 (CH, C-6), 68.2 (CH, C-2 or C-4), 68.3 (CH, C-2 or C-4), 70.4 (CH, C-3), 75.7 (CH, C-5), 98.6 (CH, C-1), 116.6 (CH, C-2′), 125.9 (CH, C-3′), 143.2 (C, C-1′), 161.3 (C, C-4′), 169.3 (C, $OCOCH_3$), 170.0 (C, $OCOCH_3$) and 170.3 (C, $OCOCH_3$).

*4-Nitrophenyl 6,7-anhydro-*L-glycero-*β*-D-galacto-*heptopyranoside* (**3a**).—4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6,7-anhydro-L-*glycero*-*β*-D-*galacto*-heptopyranoside (**17a**) (166 mg, 0.38 mmol) was deacetylated as described above to give a solid which, after column chromatography [EtOAc:CH_3OH (15:1)], gave (**3a**) (104 mg, 88%) as spars, mp 281 °C (EtOAc:CH_3OH) [Found: MNH_4^+, 331.1135. $C_{13}H_{19}N_2O_8$ requires *M*, 333.1141 (1.9 ppm)] [TLC R_f 0.07 Pet. Ether:EtOAc (2:1)]; δ_H (400 MHz; CD_3OD) 2.87 (d, 2 H, $J_{CH_2-7,6}$ 3, CH_2-7), 3.30 (dt, 1 H, $J_{6,5}$ 5.5 and J_{6,CH_2-7} 3, H-6), 3.58 (dd, 1 H, $J_{5,6}$ 5.5 and $J_{5,4}$ 1, H-5), 3.65 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3.5, H-3), 3.89 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 8, H-2), 4.04 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4), 5.05 (d, 1 H, $J_{1,2}$ 8, H-1), 7.24 (AA′BB′, 2 H, *J* 9, 2 × H-2′) and 8.26 (AA′BB′, 2 H, *J* 9, 2 × H-3′).

*4-Nitrophenyl 6,7-anhydro-*D-glycero-*β*-D-galacto-*heptopyranoside* (**3b**).—4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6,7-anhydro-D-*glycero*-*β*-D-*galacto*-heptopyranoside (**17b**) (59 mg, 0.13 mmol) was deacetylated as described above. Column chromatography [EtOAc:CH_3OH (15:1)] of the resultant solid gave (**3b**) (41 mg, 100%) as spars, mp 118 °C (EtOAc:CH_3OH) [TLC R_f 0.12 EtOAc:CH_3OH (15:1)]; δ_H (400 MHz; CD_3OD) 2.76 (dd, 1 H, $J_{7A,7B}$ 5 and $J_{7A,6}$ 2.5, H-7A), 2.88 (dd, 1 H, $J_{7B,7A}$ 5 and $J_{7B,6}$ 4.5, H-7B), 3.37 (m, 1 H, H-6), 3.39 (dd, 1 H, $J_{5,6}$ 5 and $J_{5,4}$ 1, H-5), 3.63 (dd, 1 H, $J_{3,2}$ 10 and $J_{3,4}$ 3.5, H-3), 3.91 (dd, 1 H, $J_{2,3}$ 10 and $J_{2,1}$ 8, H-2), 3.99 (dd, 1 H, $J_{4,3}$ 3.5 and $J_{4,5}$ 1, H-4), 5.05 (d, 1 H,

$J_{1,2}$ 8, H-1), 7.28 (AA′BB′, 2 H, J 9, 2 × H-2′) and 8.27 (AA′BB′, 2 H, J 9, 2 × H-3′).

Acknowledgements

The financial support of GlaxoWellcome and the BBSRC under the DTI-sponsored LINK Scheme Initiative is gratefully acknowledged.

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