Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 3, pp. 709-720, 1996

0957-4166/96 $15.00 + 0.00

S0957-4166(96)00066-3

Chemoenzymatic Synthesis of Ethyl 1-thio-(β-D-galactopyranosyl)-*O*-β-D-glycopyranosyl Disaccharides using the β-Galactosidase from *Bacillus circulans*.

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Abstract: Different ethyl 1-thio-β-D-disaccharides have been synthesised by transgalactosylation using the β-galactosidase from *Bacillus circulans* as biocatalyst. This β-galactosidase shows mainly a β-1-4 specificity in the galactosyl transfer. Gal-β-(1-4)-*O*-β-D-GlcSEt **15** was obtained in 36% yield, Gal-β-(1-4)-*O*-α-D-GlcSEt **19** in 30% yield, Gal-β-(1-4)-*O*-β-D-GalSEt **17** in 60% yield, Gal-β-(1-4)-*O*-β-D-GalNAcSEt **20** in 49% yield, Gal-β-(1-4)-*O*-β-D-Gal-β-(1-4)-*O*-β-D-GalNAcSEt **21** in 9% yield, Gal-β-(1-6)-*O*-β-D-GlcSEt **16** in 3% yield and Gal-β-(1-3)-*O*-β-D-XylSEt **18** in 25% yield. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The oligosaccharide components in glycoconjugates are involved in many biological processes such as cell-cell recognition and communication, growth regulation and antibody interactions.[1] Nowadays there is an increasing need to obtain these compounds for biological studies but their chemical synthesis, although well-developed over the last decade,[2] is still a time-consuming approach often using expensive and toxic catalysts.

Alternatively, straightforward enzymatic procedures have been successfully applied for the synthesis of oligosaccharides[3]. In this type of enzymatic synthesis, glycosyl transferases are widely used to perform regiospecific galactosylation and sialylation in a preparative scale[4] but these enzymes belonging generally to the Leloir pathway are still difficult to obtain and to handle. Moreover they need expensive cofactors as glycosyl donors. On the other hand, glycosyl hydrolases (glycosidases) can be also used to synthesise di- and trisaccharides in a kinetically controlled reaction where a glycosyl donor is used to transfer its glycosyl residue to a sugar acceptor presents in the reaction medium.[5] In spite of the increasing work carried out with glycosyl hydrolases their main drawback is the lack of regioselectivity which limits their use for synthetic purposes. The few exceptions are the β-(1-6) specificity of the β-galactosidase from *E. coli*[6a] , the partial β-(1-3) specificity of the β-galactosidase from bovine testes[6b] and the β-(1-4) specificity of the β-*N*-acetylhexosaminidase from *Aspergillus oryzae*.[5b-d]

In an important contribution to glycosidase-catalysed oligosaccharide synthesis, T. Usui et al.[7a,b] reported recently the use of a β-galactosidase from *Bacillus circulans* to synthesise some β-D-(1-4) galactosyl disaccharides bearing a GlcNAc or a GalNAc residue at the reducing end. Some β-(1-6) linkages were produced as well but to the best of our knowledge it was the first time that a preparative scale β-D-galactosyl transfer has been shown to occur preferentially at the *O*-4 position using a galactosyl hydrolase. Previous

enzymatic syntheses of oligosaccharides having a β-D-Gal-(1-4) linkage such as *N*-acetyllactosamine[8] or larger oligosaccharides[9] were carried out by using bovine β-D-galactosyl transferase (E.C. 2.4.1.22) which catalyses the transfer of a β-D-galactopyranosyl unit from UDP-galactose to the hydroxyl group at 4-position of GlcNAc or related glycosides.[10] Accordingly, there is a real synthetic interest in the use of galactosidase to perform the same type of galactosyl transfer. In the present study we examined the potential of the β-galactosidase from *Bacillus circulans* to synthesise different ethyl 1-thio-(β-D-galactosyl)-*O*-β-D-glycopyranosides. Such compounds with an activated thioethyl group at the anomeric centre can be used for the construction of larger oligosaccharides using the block synthesis approach[11] or they can be coupled with a suitable spacer for covalent binding to solid supports.[12]

RESULTS AND DISCUSSION

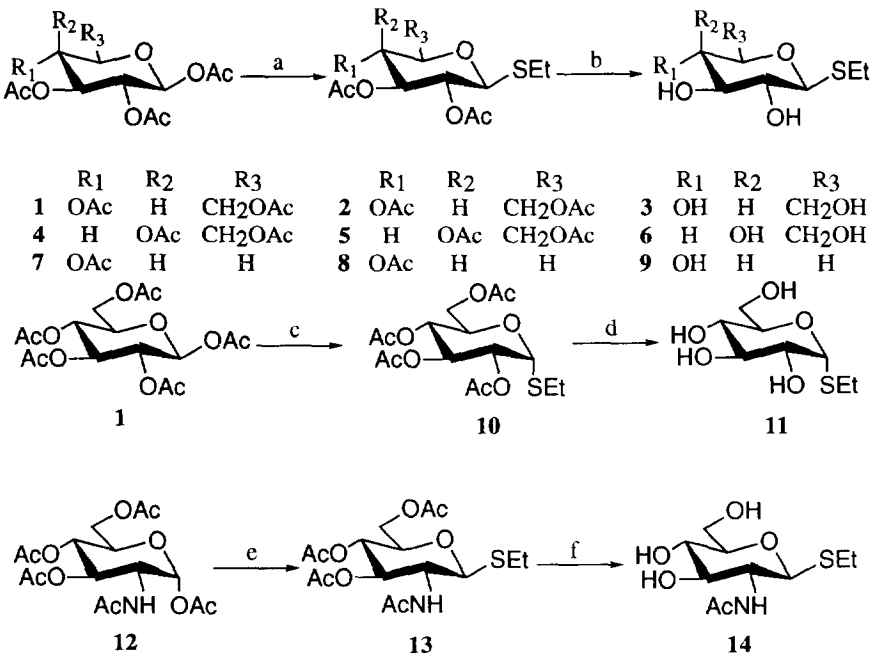
Synthesis of the glycosyl acceptors.

The first step was to synthesise several ethyl 1-thio-β-D-glycopyranosides which could be used as glycosyl acceptors during galactosyl transfer. In order to shorten the chemical synthesis of such compounds the per-acetates were used as starting materials. The β-acetates **1**,**4** and **7** were reacted with ethanethiol in the presence of tin tetrachloride[13] at -30° C to give compounds **2**, **5** and **8** in 75 to 79% yield (Scheme 1). To obtain compound **10** with the thioethyl group in the α-position, use of the classical benzyl non-participating group at C-2 could not be made owing to the possibility that the sulfur might poison the hydrogenation catalyst needed to deprotect the per-benzylated compound at the end of the synthesis. Fortunately, it is known that isomerisation of acetylated alkyl 1-thio-β-D-glucopyranosides into the corresponding α-anomers occurs in the presence of Lewis acids[14]. Accordingly, it was decided to carry out the reaction with the β-acetate **1** in dichloromethane at room temperature for a longer reaction time (48 h) and to compare the effectiveness of tin tetrachloride ($SnCl_4$) and ferric trichloride ($FeCl_3$) as Lewis acids for the isomerisation *in situ* of the synthesised ethyl 2,3,4,6 tetra-*O*-acetyl-1-thio-β-glucopyranoside **2** into the corresponding α-anomer. The results (Table 1) show that the overall yield in the synthesis is better with $FeCl_3$ (63%) than with $SnCl_4$ (57%) but $SnCl_4$ gave a better yield for ethyl 2,3,4,6 tetra-O-acetyl-1-thio-α-D-glucopyranoside **10** (36%) than $FeCl_3$ (28%). To perform the large scale synthesis of compound **10** $SnCl_4$ was used. The synthesis of ethyl 2-acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **13** was performed by reacting the corresponding α-acetate with ethylthiotrimethylsilane in the presence of zinc iodide[15] to give the required compound in 70% yield.

Table 1. Effect of the Lewis acid on the synthesis of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α and β-D-glucopyranosides **2** and **10**

	% yield of **2**	% yield of **10**	% yield of **2+10**
$SnCl_4$[a]	21	36	57
$FeCl_3$[a]	35	28	63

a: Reagents and conditions: 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranoside (**1**), EtSH, CH_2Cl_2, r.t., 43 h.



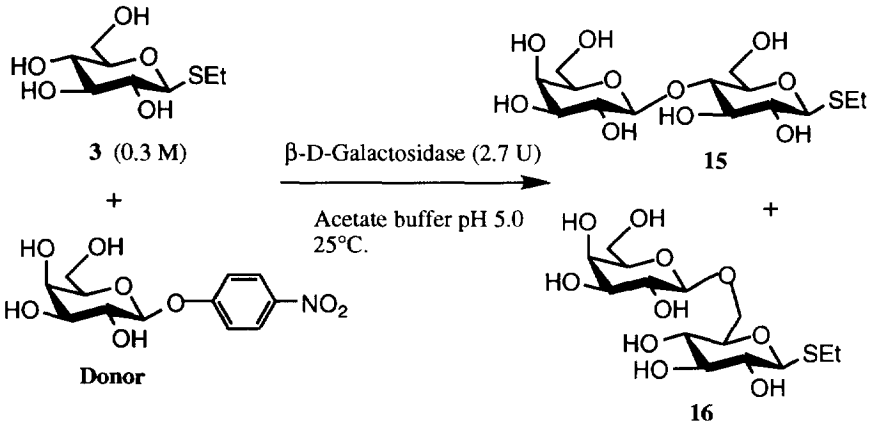
Scheme 1. Synthesis of ethyl 1-thioglycopyranoside acceptors. Reagents and conditions: *(a)* EtSH, $SnCl_4$, $ClCH_2CH_2Cl$, -35°C to -17°C, 65 min.; *(b)* MeONa, MeOH, r.t., 20 h; *(c)* EtSH, $SnCl_4$, CH_2Cl_2, r.t., 43 h; *(d)* MeONa, MeOH, r.t., 20 h; *(e)* Et_3SSiMe_3, ZnI_2, $ClCH_2CH_2Cl$, 50°C, 8 h; *(f)* MeONa, MeOH, r.t., 3 h.

Finally, compounds **2**, **5**, **8**, **10**, **13** were deacetylated using sodium methoxide in methanol to give the corresponding ethyl 1-thio-D-glycopyranosides **3**, **6**, **9**, **11** and **14** in quantitative yields.

Enzymatic galactosyl transfer.

The enzymatic galactosyl transfers were carried out with the different ethyl 1-thio acceptors under improved conditions (0.3 M of acceptor and 0.05 M of donor) to obtain optimum yields and to minimise the formation of side-products resulting from the transfer of the glycosyl donor to itself. The effect of donor concentration is shown in Scheme 2. With the acceptor concentration fixed at 0.3 M, the decrease of donor concentration from 0.1 M to 0.05 M decreased the reaction time to 50%, increased the overall yield by 33% and favoured the synthesis of the β-D-(1-4) linked disaccharide **15** relative to the β-D-(1-6) linked disaccharide **16.**

Using these defined conditions, the enzymatic synthesis of different disaccharides was investigated. As shown in Scheme 3, when ethyl 1-thio-β-D-glucopyranoside **3** was used as acceptor, β-D-Gal-(1-4)-*O*-β-D-GlcSEt **15** was obtained in 36% yield together with a small amount (3%) of β-D-Gal-(1-6)-*O*-β-D-GlcSEt **16**. The two regioisomers were easily purified using a carbon-Celite column. The thioethyl group at the reducing end apparently allowed, through specific interaction with the carbon-Celite mixture, a good separation between the two isomers. This is not the case when the reducing end is free[6] or blocked by an *O*-methyl

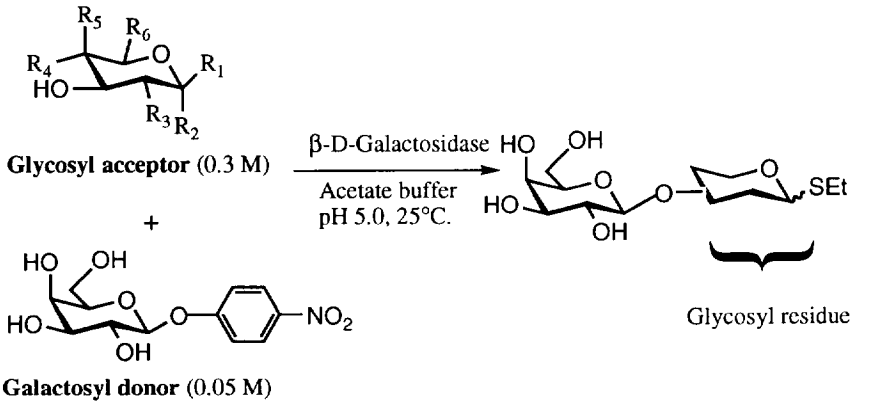


Donor concentration (M)	Reaction time (h)	Yield **15**+**16** (%)	Ratio **15** : **16**
0.1	17	30	5 : 1
0.05	8	40	13 : 1

Scheme 2. Effect of donor concentration on the β-galactosidase-catalysed synthesis of ethyl 1-thio-(β-D-galactopyranosyl)-(1-4)-*O*-β-D-glucopyranoside **15** and ethyl 1-thio-(β-D-galactopyranosyl)-(1-6)-*O*-β-D-glucopyranoside **16**.

group.[5b-d] Both disaccharides were eluted with a 75 : 25 water-ethanol mixture but the β-(1-6) disaccharide **16** was eluted in the first fractions. When ethyl 1-thio-α-D-glucopyranoside **11** was used as acceptor, β-D-Gal-(1-4)-*O*-α-D-GlcSEt **19** was formed as the sole product in 30% yield. By contrast with the results obtained by Nilsson[5a] with the β-galactosidase from *E. coli*, and as found by us with the β-N-acetylhexosaminidase[5b-d] from *Aspergillus oryzae*, the anomeric configuration of the acceptor did not markedly influence the regioselectivity of transfer with the β-galactosidase from *Bacillus circulans* . When ethyl 1-thio-α-D-glucopyranoside **11** was used as acceptor, none of the β-(1-6) linked disaccharide was formed but the β-(1-4) isomer was formed as the major product. When ethyl 1-thio-β-D-galactopyranoside **6** was used as acceptor, β-D-Gal-(1-4)-*O*-β-D-GalSEt **17** was obtained as a single compound in 60% yield. This disaccharide has been isolated from partial hydrolysates of several polysaccharides[16] with biological roles. With ethyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside **14** as acceptor, β-D-Gal-(1-4)-*O*-β-D-GlcNAcSEt **20** was obtained in 49% yield and a trisaccharide β-D-Gal-(1-4)-*O*-β-D-Gal-(1-4)-*O*-β-D-GlcNAcSEt **21** was obtained in 9% yield. Both compounds were separated by carbon-Celite chromatography. Compound **20** was eluted with an

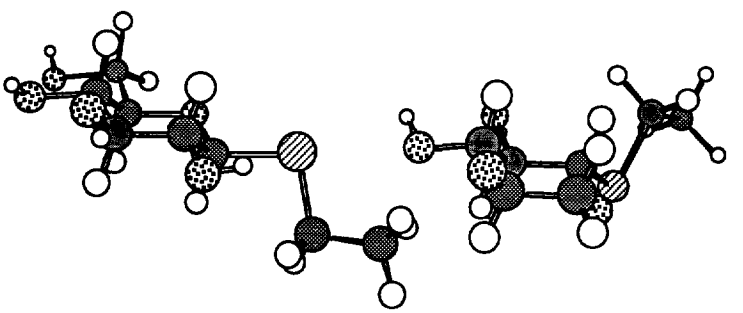
85 : 15 water-ethanol mixture and trisaccharide **21** with a 75 : 25 water-ethanol mixture. Compound **20** could be a useful intermediate in the enzymatic synthesis of Lewis x glycosides of primary alcohols as an alternative to the azide method.[17] Synthesis of the trisaccharide **21** suggests that the β-galactosidase from *Bacillus circulans* can be used for the synthesis of larger oligosaccharides while still retaining its β-(1-4) selectivity. Finally when ethyl 1-thio-β-D-xylopyranoside **9** was used as acceptor, β-D-Gal-(1-3)-*O*-β-D-XylSEt **18** was obtained as the sole product in 25% yield. β-D-Galactosyl-D-xylopyranosides are components of biologically important proteoglycans and xyloglucans.[18] It was noteworthy that the transfer was to C-3 of xylose, in contrast to the predominant C-4 transfer with the hexopyranose substrates. The linkage position was defined



Glycosyl acceptors							Disaccharides			
Cpd.	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**Cpd.**	**Glycosyl Residue**	**Yield (%)**	**Rel. Rate (%)**
3	SEt	H	OH	OH	H	CH_2OH	**15**	(1-4)-GlcβSEt	36	77
3	SEt	H	OH	OH	H	CH_2OH	**16**	(1-6)-GlcβSEt	3	n.d.
6	SEt	H	OH	H	OH	CH_2OH	**17**	(1-4)-GalβSEt	60	5.5
9	SEt	H	OH	OH	H	H	**18**	(1-3)-XylβSEt	25	9.5
11	H	SEt	OH	OH	H	CH_2OH	**19**	(1-4)-GlcαSEt	30	100
14	SEt	H	NHAc	OH	H	CH_2OH	**20**	(1-4)-GlcNAcβSEt	49	85

Scheme 3. β-Galactosidase-catalysed transgalactosylation on ethyl 1-thio-D-glycopyranosides as acceptors using *p*-nitrophenyl-β-D-galactopyranoside as donor.

by NMR studies. Thus the resonances of H-3′, H-4′, H-3 and H-5 had very similar chemical shifts, but were nevertheless readily distinguishable in the 2D ^{1}H-^{1}H shift correlation spectrum, and just distinguishable in the ^{13}C-^{1}H shift correlation. In this spectrum, only C-1′ and C-3 showed glycosylation shifts, each of *ca* +8 ppm. An NOE-difference spectrum with pre-irradiation at H-1' was also consistent with the simultaneous proximity of H-3′, H-3 and H-5. Conversely, no NOE could be seen, either way, between H-1′ and either H-5 proton, although one would be expected if the glycosidic link were 1→4. This result shows that the presence of the OH group in position 6 on the pyranoside ring is essential to galactosyl transfer to the OH group in position 4. This may be due to the combined effect of a stabilising interaction between the lone electron pair of the 6-OH group and an electrophilic centre in the enzyme as well as a possible steric interaction from the hydroxymethyl group. The structures of ethyl 1-thio-β-D-glucopyranoside **3** and ethyl 1-thio-β-D-xylopyranoside **9** were minimised using PCMODEL, and the $^{4}C_1$ conformation of glycoside **3** was compared with the $^{3}C_O$ conformation of glycoside **9** using Chem 3D to represent the minimised structures (Figure). It can be seen that the arrangement of hydroxyl groups at C-3 and C-4 of the D-glucopyranoside **3** are very similar to the arrangement of the hydroxyl groups at C-2 and C-3 in the D-xylopyranoside **9**. It can be inferred that with the loss of the important contribution of the hydroxymethyl group to the bound conformation of the D-glucopyranoside **3**, the hydroxyl groups at C-2 and C-3 become major determinants of the conformation of the D-xylopyranoside bound at the active site.



3 **9**

Figure. Minimised structures of ethyl 1-thio-β-D-glucopyranoside **3** and ethyl 1-thio-β-D-xylopyranoside **9**.

The enzymatic synthesis of some β-D-Gal-β-D-Xyl derivatives has been attempted by López et al.[19] using the β-galactosidase from *E. coli* but the best result gave a mixture of β-(1-3) and β-(1-4) linked disaccharides with an overall yield of 30%. The present results show that the enzyme from *B. circulans* is a very useful catalyst for the construction of Gal (β1→4) linkages using unprotected donor and acceptor sugars.

EXPERIMENTAL

Materials. Commercially available β-D-galactosidase (E.C. 3.2.1.23; from *Bacillus circulans* "Biolacta") was a gift from Daiwa Kasei Co., Ltd., Osaka, Japan. All other chemicals were obtained from commercial sources.

Methods. NMR spectra were recorded on Bruker AC-400 or 250 MHz spectrometers. The structure of the enzymatically synthesised disaccharides were assigned by proton-proton shift correlation, carbon-proton shift correlation and DEPT-experiments. (1-3), (1-4) and (1-6)-Linkages were identified by the marked down-field shift of the C-3, C-4 and C-6 resonances. The FABMS spectra were recorded with a Kratos MS80 spectrometer. Optical rotations were determined with an Optical Activity Ltd. AA. 1000 polarimeter. Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. HPLC was performed with a graphitised carbon column (Hypercarb S, 4.6 mm x 100 mm) on a Gilson liquid chromatograph equipped with a light scattering detector (Sedex 55). Elution was effected with a gradient of H_2O-CH_3CN at a flow rate of 0.75 ml/min. All non-enzymatic reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents. TLC was performed on 25 mm E. Merck silica gel plates (60F-254) with detection by spraying the plates with 10% aq. H_2SO_4 in methanol and heating. Column chromatography was performed on Merck silica gel (60, particle size 0.040-0.063 mm). All ethyl 1-thioglycosides were obtained as oils after deacetylation. As they were homogeneous by NMR, no further crystallisation was attempted and the products were used directly. The carbon-Celite column for the separation of the enzymatically synthesised disaccharides was prepared by mixing equal parts by weight of activated carbon (Darco G-60, Aldrich) and Celite (Celite 535, Fluka) in water. The mixture was packed into a glass column under pressure. Molecular modelling was carried out using PCMODEL (Serena Software, Bloomington, Indiana) and presented using Chem 3D (Cambridge Scientific Computing, Cambridge, Mass.).

Enzyme assay. β-D-galactosidase activity was assayed spectrophotometrically as follows. In a thermostated spectrophotometer cuvette at 33° C were added 2 ml of a 2.1 mM *p*-nitrophenyl β-D-galactopyranoside solution in sodium acetate buffer (50 mM, pH 5.0) and 50 µl of an enzyme solution (4 mg/ml). The amount of *p*-nitrophenol liberated was measured continuously at 380 nm. The activity that will hydrolyses 1 µmole of substrate per minute under the above conditions is defined as 1 unit (U).

General methods for the enzymatic galactosylation and disaccharide purification. To a solution of ethyl 1-thio-D-acceptor (≈2 mmol) and *p*-nitrophenyl β-D-galactopyranoside (≈0.3 mmol) in 6.8 ml of sodium acetate buffer (50 mM, pH 5.0) was added the β-D-galactosidase from *Bacillus circulans* (1.39 mg, 2.7 U). The progress of the reaction was monitored by HPLC as described above. After all of the *p*-nitrophenyl β-D-galactopyranoside had been consumed, the reaction was quenched by heating for 5 min at 100° C. The reaction mixture was then directly loaded on to the carbon-Celite column. The column was first eluted with water (200 ml) and then with a linear gradient of 0 to 35% (v:v) of ethanol. Under these conditions compounds **17** and **20** were eluted with 15% (v : v) of ethanol. Compounds **15**, **16**, **19** and **21** were eluted with 25% (v : v) of ethanol and compound **18** was eluted with 35% (v : v) of ethanol.

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside 2. 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranoside **1** (6.16g, 15.78 mmol) was dissolved in $ClCH_2CH_2Cl$ (200ml) containing 4Å molecular sieves (5g) and ethanethiol (2.31 ml, 31.25 mmol). After stirring at -30°C for 1 h, $SnCl_4$ (1.83 ml, 16.61 mmol) was added and the reaction medium was allowed to warm to -17°C over 1 h. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (150 ml). The mixture was stirred for 1 h, then filtered through a pad of Celite. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (150 ml). The organic layers were combined, washed with H_2O (200 ml), dried ($MgSO_4$) and evaporated. Crystallisation (Et_2O : light

petroleum) afforded the glycoside **2** as white crystals (4.82 g, 79%). M.p. 83-84°C; $[\alpha]_D^{22}$ -29.2 (c 1.00, $CHCl_3$), [lit.[13b] m.p. 84-85°C, $[\alpha]_D^{22}$ = -27.9 (c 1.9, $CHCl_3$)]; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.16 (t, 1 H, *J* 9.2 Hz, H-4), 5.06-4.93 (m, 2 H, H-2, H-3), 4.43 (d, 1 H, *J* 9.8 Hz, H-1), 4.21 (dd, 1 H, *J* 12.3, 4.8 Hz, H-6a), 4.06 (dd, 1 H, *J* 12.3, 2.4 Hz, H-6b), 3.68-3.61 (m, 1 H, H-5), 2.72-2.54 (m, 2 H, S$\underline{CH_2}$CH3), 2.01 (s, 3 H, CO$\underline{CH_3}$), 1.99 (s, 3 H, CO$\underline{CH_3}$), 1.96 (s, 3 H, CO$\underline{CH_3}$), 1.94 (s, 3 H, CO$\underline{CH_3}$), 1.20 (t, 3 H, *J* 7.4 Hz, $CH_2\underline{CH_3}$); ^{13}C-NMR (250 MHz, $CDCl_3$): δ 170.5 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 169.3 (2 x $\underline{C}OCH_3$), 83.3 (C-1), 75.7, 73.7, 69.6, 68.1, 62.0 (C-6), 24.0 (S$\underline{CH_2}$CH3), 20.6, 20.4, 14.7 (SCH2$\underline{CH_3}$); Found: C, 48.86; H, 6.16. $C_{16}H_{24}O_9S$ requires: C, 49.00; H, 6.12%.

Ethyl 1-thio-β-D-glucopyranoside 3. To a solution of **1** (4.03 g, 10.28 mmol) in MeOH (100 ml) a catalytic amount of NaOMe (55.5 mg, 1.02 mmol) was added. After 20 h at room temperature the mixture was neutralised with Amberlyst 15, filtered and concentrated to yield **3** quantitatively as a syrup. $[\alpha]_D^{24}$ = -53.5 (c 0.74, MeOH) [lit.[20] -55.1 ((H_2O))]; ^{1}H-NMR (250 MHz, D_2O): δ 4.49 (d, 1 H, *J* 9.8 Hz, H-1), 3.85 (dd, 1 H, *J* 12.4, 1.9 Hz, H-6b), 3.65 (dd, 1 H, *J* 12.4, 5.3 Hz, H-6a), 3.48-3.23 (m, 4 H, H-2,3,4,5), 2.82-2.60 (m, 2 H, S$\underline{CH_2}$CH3), 1.23 (t, 3 H, *J* 7.4 Hz, SCH2$\underline{CH_3}$); ^{13}C-NMR (250 MHz, D_2O): δ 85.9 (C-1), 80.6, 78.0, 73.0, 70.3, 61.7 (C-6), 25.0 (S$\underline{CH_2}$CH3), 15.3 (SCH2$\underline{CH_3}$); m/z (FAB): 247 (MNa^+).

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside 10. (a) Catalysis by $SnCl_4$. 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranoside **1** (5.03g, 12.88 mmol) was dissolved in CH_2Cl_2 (30ml) containing 4Å molecular sieves (5g) and ethanethiol (1.90 ml, 25.28 mmol). The mixture was stirred at room temperature for 1 h, $SnCl_4$ (1.80 ml, 15.31 mmol) was added and the reaction medium was stirred at r. t. After 43 h the mixture was diluted with CH_2Cl_2 (30 ml), quenched by addition of saturated aq. $NaHCO_3$ (40 ml) and stirred for 1 h. After worked-up as described for glycoside **2**, the crude product was purified by chromatography (silica, 65% Et_2O in petroleum ether) to afford 1.81g of **10** (36%) and 1.05g of **2** (21%) as oils, which could both be crystallised (diethyl ether:light petroleum). Data for **10**: M.p. 98-99°C; $[\alpha]_D^{23}$ = +211.6 (c 1.0, $CHCl_3$), [lit.[14] m.p. 96-97 °C, $[\alpha]_D^{22}$ = +204 (c 1, $CHCl_3$)]; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.68 (d, 1 H, *J* 5.7 Hz, H-1), 5.31 (dd, 1 H, *J10.1, 9.5* Hz, H-4), 5.08-4.98 (m, 2 H, H-2,3), 3.47-4.40 (m, 1 H, H-5), 4.30 (dd, 1 H, *J* 12.3, 4.6 Hz, H-6a), 4.07 (dd, 1 H, *J* 12.3, 2.2 Hz, H-6b), 2.63-2.48 (m, 2 H, S$\underline{CH_2}$CH3), 2.08 (s, 3 H, CO$\underline{CH_3}$), 2.06 (s, 3 H, CO$\underline{CH_3}$), 2.03 (s, 3 H, CO$\underline{CH_3}$), 2.01 (s, 3 H, CO$\underline{CH_3}$), 1.26 (t, 3 H, *J* 7.4 Hz, $CH_2\underline{CH_3}$); ^{13}C-NMR (250 MHz, $CDCl_3$): δ 170.5 ($\underline{C}OCH_3$), 169.8 (2 x $\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 81.5 (C-1), 70.5, 70.4, 68.4, 67.3, 61.8 (C-6), 24.1 (S$\underline{CH_2}$CH3), 20.6, 20.6, 20.5, 14.5 (SCH2$\underline{CH_3}$); Found: C, 48.76; H, 6.15. $C_{16}H_{24}O_9S$ requires: C, 49.00; H, 6.12. **(b) Catalysis by $FeCl_3$.** 1,2,3,4-Penta-*O*-acetyl-β-D-glucopyranoside 1 (4.99 g, 12.79 mmol) was heated with $FeCl_3$ (2.49 g, 15.35 mmol) as described above for the reaction with $SnCl_4$ to give **10** (1.38 g, 28 %) and 2 (1.72 g, 35 %).

Ethyl 1-thio-α-D-glucopyranoside 11. As described for **3**, a solution of **10** (2.71 g, 6.90 mmol) in MeOH (60 ml) was treated with a catalytic amount of NaOMe (37.2 mg, 0.69 mmol) to give quantitatively **11** as a syrup.$[\alpha]_D^{23}$ = +303.8 (c 0.62, MeOH) [lit.[20] +269 (H_2O)]; ^{1}H-NMR (400 MHz, D_2O): δ 5.40 (d, 1 H, *J* 5.5 Hz, H-1), 4.03-3.98 (m, 1 H, H-5), 3.84-3.72 (m, 3 H, H-2,6a,6b), 3.54 (dd, 1 H, *J* 9.7, 9.2 Hz, H-3), 3.36 (dd, 1 H, *J* 9.8, 9.4 Hz, H-4), 2.67-2.54 (m, 2 H, S$\underline{CH_2}$CH3), 1.23 (t, 3 H, *J* 7.4 Hz, SCH2$\underline{CH_3}$); ^{13}C-NMR (400 MHz, D_2O): δ 85.6 (C-1), 74.3, 72.8, 71.6, 70.4, 61.2 (C-6), 24.7 (S$\underline{CH_2}$CH3), 15.8 (SCH2$\underline{CH_3}$); m/z (FAB): 247 $(M+Na)^+$.

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 5. As described for **2**, 1,2,3,4,6-penta-*O*-acetyl-β-D-galacopyranoside **4** (6.14 g, 15.74 mmol) was treated with $SnCl_4$ (2.00 ml, 17.02 mmol) and ethanethiol (2.31 ml, 31.25 mmol). Crystallisation (Et_2O : light petroleum) afforded **5** as white crystals (4.09g, 79%). M.p. 77-78°C; $[\alpha]_D^{28}$ = -8.1 (c 0.98, $CHCl_3$) [lit.[21] m.p. 74.5-75 °C, $[\alpha]_D^{21}$ = -8.5 (c 2.3, $CHCl_3$)]; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.36 (dd, 1 H, *J* 3.3, 1.0 Hz, H-4), 5.17 (dd, 1 H, *J* 9.9, 9.8 Hz), 4.98 (dd, 1 H, *J* 10.0, 3.3 Hz, H-6a), 4.43 (d, 1 H, *J* 9.8 Hz, H-1), 4.14-4.00 (m, 2 H), 3.90-3.84 (m, 1 H), 2.74-2.56 (m, 2 H, S$\underline{CH_2}$CH3), 2.09 (s, 3 H, CO$\underline{CH_3}$), 2.00 (s, 3 H, CO$\underline{CH_3}$), 1.98 (s, 3 H, CO$\underline{CH_3}$), 1.92 (s, 3 H, CO$\underline{CH_3}$), 1.22 (t, 3 H, *J* 7.4 Hz); ^{13}C-NMR (250 MHz, $CDCl_3$): δ 170.3 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 83.9 (C-1), 74.2, 71.7, 67.1, 67.0, 61.3 (C-6), 24.3 (S$\underline{CH_2}$CH3), 20.7, 20.6, 20.6, 20.5, 14.7 (SCH2$\underline{CH_3}$); Found: C, 48.94; H, 6.17. $C_{16}H_{24}O_9S$ requires: C, 49.00; H, 6.12%.

Ethyl 1-thio-β-D-galactopyranoside 6. As described for **3**, a solution of **5** (4.03 g, 10.28 mmol) in MeOH (120 ml) was treated with a catalytic amount of NaOMe (55.5 mg, 1.02 mmol) to give quantitatively **6** as a syrup. $[\alpha]_D^{24}$ =-23.2 (c 0.59, MeOH) [lit.[20] -23.5 (H_2O)]; ^{1}H-NMR (250 MHz, D_2O): δ 4.24 (d, 1 H, *J* 9.5 Hz, H-1), 3.73 (apparent d, 1 H, *J* 3.1 Hz, H-4), 3.57-3.27 (m, 5 H), 2.60-2.44 (m, 2 H, S$\underline{CH_2}$CH3), 1.03 (t, 3 H, *J* 7.4 Hz, SCH2$\underline{CH_3}$); ^{13}C-NMR (250 MHz, D_2O): δ 86.4 (C-1), 79.7, 74.7 70.4, 69.6, 61.9 (C-6), 25.0 (S$\underline{CH_2}$CH3), 15.3 (SCH2$\underline{CH_3}$); m/z (FAB): 247 ($M+Na^+$).

Ethyl 2-Acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside 13. 1,3,4,6-Tetra-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranoside **12** (4.03 g 10.75 mmol) was dissolved in $ClCH_2CH_2Cl$ (80 ml). After heating at 50°C, ZnI_2 (23.63 g, 74.03 mmol) and phenylthiotrimethylsilane (7 ml, 43.25 mmol) were added and the reaction mixture was stirred for 6 h at 50°C. The reaction medium was filtered through a pad of Celite, washed with saturated aq. $NaHCO_3$ (100 ml) and H_2O (100 ml). The organic layer was dried ($MgSO_4$) and evaporated. Crystallisation (EtOH:light petroleum) afforded **13** as white crystals (2.98 g, 70%). M.p. 194-195°C; $[\alpha]_D^{21}$ = -43.4 (c 0.52, MeOH), [lit.[15] m.p. 194-196 °C, $[\alpha]_D$ = -42 (c 1.0, $CHCl_3$)]; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.74 (d, 1 H, J 9.3 Hz, NH), 5.21-4.98 (m, 2 H, H-3,4), 4.59 (d, 1 H, J 10.1 Hz, H-1), 4.25-4.01 (m, 3 H, H-2,6a,6b), 3.72-3.75 (m, 1 H, H-5), 2.77-2.59 (m, 2 H, S$\underline{CH_2}$CH3), 2.05 (s, 3 H, CO$\underline{CH_3}$), 2.01 (s, 3 H, CO$\underline{CH_3}$), 2.00 (s, 3 H, CO$\underline{CH_3}$), 1.94 (s, 3 H, CO$\underline{CH_3}$), 1.24 (t, 3 H, *J* 7.4 Hz); ^{13}C-NMR (250 MHz, $CDCl_3$: δ 173.1 ($\underline{C}OCH_3$), 172.3 ($\underline{C}OCH_3$), 171.8 ($\underline{C}OCH_3$), 171.2 ($\underline{C}OCH_3$), 85.0 (C-1), 76.8, 75.2, 70.1 (C-5), 63.4 (C-6), 54.2 (C-2), 25.0 (S$\underline{CH_2}$CH3), 22.7, 20.6, 20.5, 20.5, 15.4 (SCH2$\underline{CH_3}$); Found: C, 48.91; H, 6.31. $C_{16}H_{25}O_8NS$ requires: C, 49.09; H, 6.43.

Ethyl 2-Acetamido-2-deoxy-1-thio-β-D-glucopyranoside 14. As described for **3**, a solution of **13** (1.03 g, 2.65 mmol) in MeOH (15 ml) was treated with a catalytic amount of NaOMe (14.3 mg, 0.26 mmol) and stirred for 3 h at r.t. to give quantitatively **14** as a solid. $[\alpha]_D^{21}$ = -43.7 (c 0.53, MeOH); ^{1}H-NMR (250 MHz, CD_3OD): δ 4.52 (d, 1 H, *J* 10.4 Hz, H-1), 3.90 (dd, 1 H, *J* 12.0, 1.8 Hz, H-6b), 3.81-3.66 (m, 2 H, H-2,6a), 3.51-3.27 (m, 3 H, H-3,4,5), 2.83-2.63 (m, 2 H, S$\underline{CH_2}$CH3), 2.01 (s, 3 H, NHCO$\underline{CH_3}$), 1.27 (t, 3 H, J 7.4 Hz, SCH2$\underline{CH_3}$); ^{13}C-NMR (250 MHz, CD_3OD): δ 172.3 (NH$\underline{C}OCH_3$), 85.5 (C-1), 82.1, 77.4, 71.9, 62.9 (C-6), 56.2 (C-2), 24.8 (S$\underline{CH_2}$CH3), 22.9 (NHCO$\underline{CH_3}$), 15.4 (SCH2$\underline{CH_3}$); m/z (FAB): 288 $(M+Na)^+$.

Ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside 8. As described for **2**, 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside **7** (4.49g, 14.12 mmol) was treated with $SnCl_4$ (1.90 ml, 16.17 mmol) and ethanethiol (2.10 ml, 28.39 mmol). Crystallisation (Et_2O : light petroleum) afforded **8** as white crystals (3.39 g, 75%). M.p. 100-101°C; $[\alpha]_D^{23}$ = -88.2 (c 0.67, $CHCl_3$), [lit.[13a] m.p. 99.5°C, $[\alpha]_D^{20}$ = -82.2 (c 1.0, $CHCl_3$)]; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.15 (dd, 1 H, *J* 8.4, 8.4 Hz, H-3), 4.97-4.88 (m, 2 H, H-2,4), 4.50 (d, 1 H, *J* 8.4 Hz, H-

1), 4.19 (dd, 1 H, *J* 11.6, 4.9 Hz, H-5a), 3.35 (dd, 1 H, *J* 11.6, 9.0 Hz, H-5b), 2.76-2.58 (m, 2 H, SCH$_2$CH$_3$), 1.23 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH$_3$); ^{13}C-NMR (250 MHz, CDCl$_3$): δ 169.8 (COCH$_3$), 169.6 (COCH$_3$), 169.3 (COCH$_3$), 83.4 (C-1), 72.1, 69.6, 68.5, 65.4, 24.0 (SCH$_2$CH$_3$), 20.6, 20.6, 20.6, 14.6 (SCH$_2$CH$_3$); Found: C, 49.17; H, 6.30. $C_{13}H_{20}O_7S$ requires: C, 48.73; H, 6.29.

Ethyl 1-thio-β-D-xylopyranoside 9. As described for **3**, a solution of **8** (1.01 g, 3.15 mmol) in MeOH (20 ml) was treated with a catalytic amount of NaOMe (17.0 mg, 0.31 mmol) to give quantitatively **9** as a solid; $[\alpha]_D^{31}$ = -83.1 (c 0.89, MeOH); ^{1}H-NMR (250 MHz, D$_2$O): δ 4.40 (d, 1 H, *J* 9.5 Hz, H-1), 3.89 (dd, 1 H, *J* 11.3, 5.2 Hz, H-5a), 3.59-3.49 (m, 1 H, H-4), 3.38-3.17 (m, 3 H, H-2,3,5b), 2.71-2.54 (m, 2 H, SCH$_2$CH$_3$), 1.18 (t, 3 H, J 7.4 Hz, SCH$_2$CH$_3$); ^{13}C-NMR (250 MHz, CDCl$_3$): δ 86.4 (C-1), 77.7, 72.6, 69.6, 69.2, 24.7 (SCH$_2$CH$_3$), 15.0 (SCH$_2$CH$_3$); m/z (FAB): 379 (M+Na)$^+$.

Ethyl 1-thio-(β-D-galactopyranosyl)-(1-4)-*O*-β-D-glucopyranoside 15 and Ethyl 1-thio-(β-D-galactopyranosyl)-(1-6)-*O*-β-D-glucopyranoside 16. To a solution of ethyl 1-thio-β-D-glucopyranoside **3** (0.49 g, 2.17 mmol) in buffer (pH 5.0) was added *p*-nitrophenyl β-D-galactopyranoside (0.11 g, 0.35 mmol) and β-galactosidase (2.7 U) according to the general method described above. After 8.5 h the reaction was stopped by heating and the mixture was purified by carbon-Celite chromatography to give **15** (49.5 mg, 36%) and **16** (4.6 mg, 3%). Data for compound **15**: Rf 0.51 (silica, *i*-PrOH/CH$_3$NO$_2$/H$_2$O [10 : 9 : 2]); $[\alpha]_D^{28}$ = -20.2 (c 0.56, H$_2$O); ^{1}H-NMR (400 MHz, D$_2$O): δ 4.53 (d, 1 H, *J* 9.9 Hz, H-1), 4.42 (d, 1 H, *J* 7.7 Hz, H-1'), 3.93 (dd, 1 H, *J* 12.4, 2.0 Hz, H-6b), 3.89 (appd, 1 H, *J* 3.3 Hz, H-4'), 3.79-3.55 (m, 8 H, H-3,3',4,5,5',6a,6'a,6'b), 3.51 (dd, 1 H, *J* 9.6, 7.7 Hz, H-2'), 3.34 (dd, 1 H, *J* 9.4, 8.9 Hz, H-2), 2.78-2.65 (m, 2 H, SCH$_2$CH$_3$), 1.24 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH$_3$); ^{13}C-NMR (400 MHz, CDCl$_3$): δ 103.5 (C-1'), 85.6 (C-1), 79.3 (C-5), 78.8 (C-4), 76.4 (C-3), 76.0 (C-5'), 73.2 (C-3'), 72.6 (C-2), 71.6 (C-2'), 69.2 (C-4'), 61.7 (C-6'), 60.8 (C-6), 24.8 (SCH$_2$CH$_3$), 15.1 (SCH$_2$CH$_3$); m/z found (M+Na)$^+$ 409.1150. $C_{14}H_{26}O_{10}S$ requires (M+Na) 409.1144. Data for compound **16**: Rf 0.42 (silica, *i*-PrOH/CH$_3$NO$_2$/H$_2$O [10 : 9 : 2]); $[\alpha]_D^{23}$ = -28.0 (c 0.2, H$_2$O); ^{1}H-NMR (400 MHz, D$_2$O): δ 4.53 (d, 1 H, *J* 9.9 Hz, H-1), 4.41 (d, 1 H, *J* 7.7 Hz, H-1'), 4.19 (dd, 1 H, *J* 11.6, 1.8 Hz, H-6b), 3.89 (appd, 1 H, *J* 3.4 Hz, H-4'), 3.81 (dd, 1 H, *J* 11.6, 5.4 Hz, H-6a), 3.77-3.57 (m, 5 H, H-3',5,5',6'a,6'b), 3.52 (dd, 1 H, *J* 10.0, 7.8 Hz, H-2'), 3.48-3.44 (m, 2 H, H-3,4), 3.32-3.26 (m, 1 H, H-2), 2.81-2.66 (m, 2 H, SCH$_2$CH$_3$), 1.25 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH$_3$); ^{13}C-NMR (400 MHz, CDCl$_3$): δ 103.9 (C-1'), 85.9 (C-1), 79.4 (C-5), 77.8 (C-3), 75.8 (C-5'), 73.3 (C-3'), 72.8 (C-2), 71.4 (C-2'), 69.9 (C-4), 69.3 (C-4'), 69.2 (C-6), 61.6 (C-6'), 25.0 (SCH$_2$CH$_3$), 15.1 (SCH$_2$CH$_3$); m/z found 409.1148 (M+Na)$^+$. $C_{14}H_{26}O_{10}S$ requires (M+Na) 409.1144.

Ethyl 1-thio-(β-D-galactopyranosyl)-(1-4)-*O*-α-D-glucopyranoside 19. To a solution of ethyl 1-thio-α-D-glucopyranoside **11** (0.51 g, 2.27 mmol) in buffer (pH 5.0) was added *p*-nitrophenyl β-D-galactopyranoside (0.11 g, 0.37 mmol) and β-galactosidase (2.7 U) according to the general method described above. After 3.5 h the reaction was stopped by heating and the mixture was purified by carbon-Celite chromatography to give **19** (43.1 mg, 30%) Rf 0.55 (silica, *i*-PrOH/CH$_3$NO$_2$/H$_2$O [10 : 9 : 2]); $[\alpha]_D^{28}$ = +158.4 (c 0.49, H$_2$O); ^{1}H-NMR (400 MHz, D$_2$O): δ 5.40 (d, 1 H, *J* 5.5 Hz, H-1), 4.41 (d, 1 H, *J* 7.7 Hz, H-1'), 4.15-4.11 (m, 1 H, H-5'), 3.89 (d, 1 H, *J* 3.4 Hz, H-4'), 3.87-3.59 (m, 9 H, H-2',3,3',4,5,6a,6b,6'a,6'b), 3.51 (dd 1 H, *J* 9.9, 7.7 Hz, H-2), 2.65-2.54 (m, 2 H, SCH$_2$CH$_3$), 1.24 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH$_3$); ^{13}C-NMR (400 MHz, CDCl$_3$): δ 103.5 (C-1'), 85.4 (C-1), 79.3 (C-4), 76.0 (C-5), 75.8 (C-3), 73.2 (C-3'), 72.9 (C-2), 71.6 (C-5'), 71.3 (C-2'), 69.2 (C-4'), 61.6 (C-6'), 60.6 (C-6), 24.7 (SCH$_2$CH$_3$), 14.7 (SCH$_2$CH$_3$); m/z found (M+Na)$^+$ 409.1109. $C_{14}H_{26}O_{10}S$ requires (M+Na) 409.1144 .

Ethyl 1-thio-(β-D-galactopyranosyl)-(1-4)-*O*-β-D-galactopyranoside 17. To a solution of ethyl 1-thio-β-D-galactopyranoside **6** (0.48 g, 2.15 mmol) in buffer (pH 5.0) was added *p*-nitrophenyl β-D-galactopyranoside (0.11 g, 0.36 mmol) and β-galactosidase (2.7 U) according to the general method described above. After 32 h and 48 h more enzyme was added (twice 2.7 U) and finally the reaction was stopped by heating after 70 h. The mixture was purified by carbon-Celite chromatography to give **17** (85.2 mg, 60%) Rf 0.34 (silica, *i*-PrOH/CH_3NO_2/H_2O [10 : 9 : 2]); $[\alpha]_D^{28}$ = +1.9 (c 0.55, H_2O); ^{1}H-NMR (400 MHz, D_2O): δ 4.54 (d, 1 H, *J* 7.7 Hz, H-1'), 4.48 (d, 1 H, *J* 9.8 Hz, H-1), 4.16 (appd, 1 H, *J* 3.1Hz, H-4), 3.85 (appd, 1 H, *J* 3.3 Hz, H-4'), 3.77-3.50 (m, 10 H, H-2,2',3,3',5,5',6a,6b,6'a,6'b), 2.78-2.65 ((m, 2 H, S$\underline{CH_2}$$CH_3$), 1.22 (t, 3 H, *J* 7.4 Hz, SCH$_2$$\underline{CH_3}$); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 104.9 (C-1'), 86.3 (C-1), 78.6 (C-3), 78.1 (C-4), 75.7 (C-5'), 74.9 (C-5), 73.4 (C-3'), 72.0 (C-2'), 70.8 (C-2), 69.2 (C-4'), 61.6 (C-6'), 61.1 (C-6), 25.1 (S$\underline{CH_2}$$CH_3$), 15.1 (SCH$_2$$\underline{CH_3}$); m/z found $(M+Na)^+$ 409.1120. $C_{14}H_{26}O_{10}S$ requires (M+Na) 409.1144.

Ethyl 1-thio-(β-D-galactopyranosyl)-(1-4)-(2-Acetamido-2-deoxy)-*O*-β-D-glucopyranoside 20 and ethyl 1-thio-(β-D-galactopyranosyl)-(1-4)-(β-D-galactopyranosyl))-(1-4)-(2-Acetamido-2-deoxy)-*O*-β-D-glucopyranoside 21. To a solution of ethyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside **14** (0.51 g, 1.96 mmol) in buffer (pH 5.0) was added *p*-nitrophenyl β-D-galactopyranoside (0.109 g, 0.36 mmol) and β-galactosidase (2.7 U) according to the general method described above. After 7.5 h the reaction was stopped by heating and the mixture was purified by carbon-Celite chromatography to give **20** (79.6 mg, 51%) and **21** (13.3 mg, 9%). Data for compound **20**: Rf 0.69 (silica, *i*-PrOH/CH_3NO_2/H_2O [10 : 9 : 2]); $[\alpha]_D^{23}$ = -25.9 (c 0.47, H_2O); ^{1}H-NMR (400 MHz, D_2O): δ 4.59 (d, 1 H, *J* 10.3 Hz, H-1), 4.42 (d, 1 H, *J*7.7 Hz, H-1'), 3.92 (dd, 1 H, *J* 12.4, 2.0 Hz, H-6b), 3.87 (appd, 1 H, *J* 3.3 Hz, H-4'), 3.78-3.64 (m, 7 H, H-2,3,4,5',6a,6'a,6'b), 3.61 (dd, 1 H, *J* 10.1, 3.3 Hz, H-3'), 3.56-3.52 (m, 1 H, H-5), 3.48 (dd, 1 H, *J* 9.9, 7.7 Hz, H-2'), 2.75-2.59 (m, 2 H, S$\underline{CH_2}$$CH_3$), 1.18 (t, 3 H, *J* 7.4 Hz, SCH$_2$$\underline{CH_3}$); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 175.0 (NH$\underline{C}$OCH$_3$), 103.3 (C-1'), 84.6 (C-1), 79.3 (C-5), 78.9 (C-4), 76.0 (C-5'), 74.3 (C-3), 73.1 (C-3'), 71.6 (C-2'), 69.2 (C-4'), 61.6 (C-6'), 60.8 (C-6), 54.9 (C-2), 25.1 (S$\underline{CH_2}$$CH_3$), 22.8 (NHCO$\underline{CH_3}$), 14.9 (SCH$_2$$\underline{CH_3}$); m/z found $(M+Na)^+$ 450.1409. $C_{16}H_{29}O_{10}NS$ requires (M+Na) 450.1410. Data for compound **21**: Rf 0.58 (silica, *i*-PrOH/CH_3NO_2/H_2O [10 : 9 : 2]); $[\alpha]_D^{23}$ = -22.1 (c , 0.41, H_2O); ^{1}H-NMR (400 MHz, D_2O): δ 4.59 (d, 1 H, *J* 10.3 Hz, H-1), 4.54 (d, 1 H, *J* 7.7 Hz, H-1'), 4.45 (d, 1 H, *J*7.9 Hz, H-1"), 4.13 (d, 1 H, *J* 3.1 Hz, H-4'), 3.92 (dd, 1 H, *J* 12.5, 2.1 Hz, H-6"b), 3.84 (d, 1 H, *J* 3.4 Hz, H-4"), 3.79-3.99 (m, 15 H), 2.73-2.60 (m, 2 H, S$\underline{CH_2}$$CH_3$), 1.18 (t, 3 H, *J* 7.4 Hz, SCH$_2$$\underline{CH_3}$); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 175.1 (NH$\underline{C}$OCH$_3$), 104.9 (C-1'), 103.6 (C-1"), 84.7 (C-1), 79.5, 79.2 (C-4'), 77.9, 75.9, 75.2, 74.5, 73.5, 73.4, 72.1, 72.1, 69.2 (C-4"), 61.8 (C-6"), 61.4 (C-6'), 60.8 (C-6), 55.0 (C-2), 25.1 (S$\underline{CH_2}$$CH_3$), 23.0 (NHCO$\underline{CH_3}$), 15.1 (SCH$_2$$\underline{CH_3}$); m/z found $(M+Na)^+$ 613.6138. $C_{22}H_{40}O_{15}NS$ requires (M+Na) 613.6140.

Ethyl 1-thio-(β-D-galactopyranosyl)-(1-3)-*O*-β-D-xylopyranoside 18. To a solution of ethyl 1-thio-β-D-xylopyranoside **9** (0.37 g, 1.94 mmol) in buffer (pH 5.0) was added *p*-nitrophenyl β-D-galactopyranoside (0.111 g, 0.37 mmol) and β-galactosidase (2.7 U) according to the general method described above. After 24 h more enzyme (2.7 U) was added and the reaction was stopped by heating after 28 h. The mixture was purified by carbon-Celite chromatography to give **18** (32.1 mg, 25%) Rf 0.55 (silica, *i*-PrOH/CH_3NO_2/H_2O [10 : 9 : 2]); $[\alpha]_D^{24}$ = -42.8 (c 0.14, H_2O); ^{1}H-NMR (400 MHz, D_2O): δ 4.63 (d, 1 H, *J* 7.7 Hz, H-1'), 4.45 (d, 1 H, *J* 9.9 Hz, H-1), 3.97 (dd, 1 H, *J* 11.3, 5.0 Hz, H-5a), 3.85 (appd, 1 H, *J* 3.2 Hz, H-4'), 3.76-3.60 (m, 6 H, H-3,3',4,5',6'a,6'b), 3.52 (dd, 1 H, *J* 9.9, 7.7 Hz, H-2'), 3.45 (dd, 1 H, *J* 9.8, 8.6Hz, H-2), 3.30 (dd, 1 H, *J* 11.3, 10.1 Hz, H-5b), 2.74-2.60 (m, 2 H, S$\underline{CH_2}$$CH_3$), 1.20 (t, 3 H, *J* 7.4 Hz, SCH$_2$$\underline{CH_3}$); ^{13}C-NMR (400 MHz,

$CDCl_3$): δ 103.8 (C-1'), 86.4 (C-1), 85.9 (C-3), 75.9 (C-5'), 73.2 (C-3'), 72.3 (C-2), 71.9 (C-2'), 69.3 (C-4), 69.0 (C-5), 68.6 (C-4), 61.7 (C-6), 24.8 ($S\underline{CH_2}CH_3$), 15.2 ($SCH_2\underline{CH_3}$); m/z found $(M+Na)^+$ 369.1131. $C_{13}H_{24}O_9S$ requires (M+Na) 369.1132.

ACKNOWLEDGMENTS

We thank Mr I. Katyal for determining mass spectra and the SERC/EPSRC for financial support.

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(*Received in UK* 20 *November* 1995; *accepted* 31 *January* 1996)