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Chemical Synthesis of ^{13}C-Labelled Ganglioside Gb$_3$ Trisaccharide from [U-^{13}C]-D-Glucose[1]

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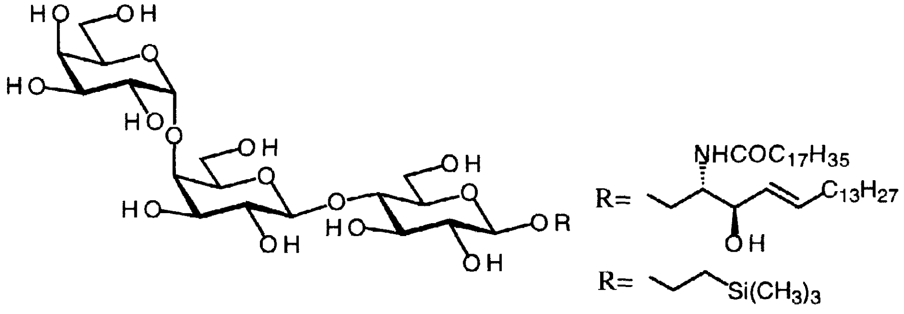
Abstract: The interaction of bacterial toxins with their cell surface glycolipid receptors offers scope for therapeutic intervention. Whilst crystal and solution structures of *Escherichia coli* verotoxin-1 have been reported, the precise nature of the interaction of the glycolipid binding domain of the toxin with its natural ligand, ganglioside Gb$_3$ (Galα1→4Galβ1→4Glcβ1→Cer), remain to be confirmed. To this end we now report the synthesis of the (2-trimethylsilyl)ethyl glycoside of the Gb$_3$ trisaccharide in isotopically enriched form from [U-^{13}C]-D-glucose.

Introduction

The numerous recent worldwide outbreaks of food-poisoning attributable to the enteropathogenic 0157 strain of *Escherichia coli*, and related organisms, are a cause for concern.[2] The toxins produced by these organisms give rise to debilitating diarrhoea, and can cause death; for patients that recover, there is still a risk of toxin-induced kidney damage. The toxins concerned, the verotoxins (VTs) or Shiga-like toxin (SLTs), are related to the Shiga toxins produced by *Shigella dysenteriae*.[3] These proteins also possess structurally and functionally similar AB$_5$ structures, in common with the heat-labile, diphtheria and cholera toxins.[4] The B-subunit is responsible for initial adhesion to epithelial cells of the gut wall, through specific recognition of key cell surface glycolipids, and once inside the cell the catalytically active A-subunit causes damage. In the case of the verotoxins, the B-subunits recognise globo-series gangliosides [VT-1 recognises ganglioside Gb$_3$: Galα1→4Galβ1→4Glcβ→Cer (**1**)] on target endothelial cells, and the A-subunit serves as an *N*-glycosidase that degrades ribosomal RNA and hence shuts down protein synthesis.



Gb$_3$-Cer (**1**)

Gb$_3$-OSE (**2**)

Figure 1. Ganglioside Gb$_3$: Ligand for Verotoxin-1

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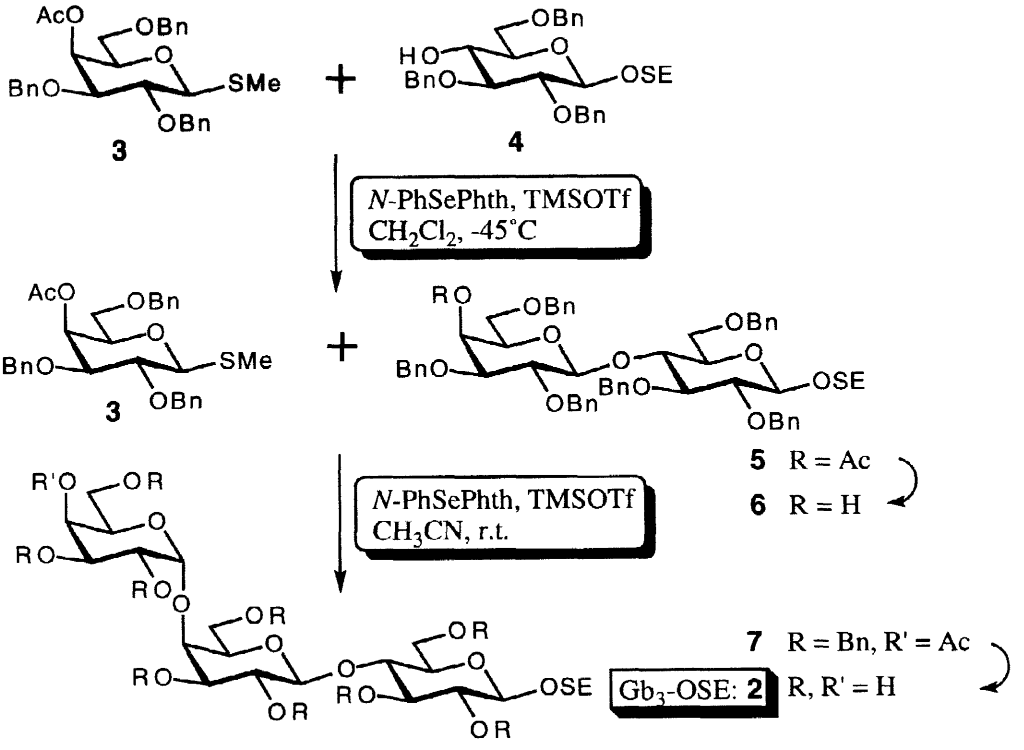
Crystal structures of the VT-1 B_5 pentamer[5] and of the related enzymatically active Shiga toxin AB_5 holotoxin have been reported.[6] The solution structure of VT-1 B_5 pentamer, determined by NMR spectroscopy with the aid of ^{13}C,^{15}N-enriched protein, has also recently been reported.[7] All of these structures show a 'doughnut' arrangement of the B_5 system, with the proposed carbohydate binding sites in the vicinity of the subunit interfaces. For the VT : Gb_3 complex, extensive syntheses of ligand analogues[8] have enabled identification of the key polar contacts between saccharide and protein, and calorimetric studies[9] in conjunction with site-directed mutagenesis[10] have suggested a key role for Phe-30 in defining the five independent sugar binding sites (one binding site per B sub-unit monomer). This mode of binding is further supported by molecular modelling.[11] However, the recent crystal structure of VT-1 complexed with an analogue of Gb_3 surprisingly showed three distinct carbohydrate binding sites per B sub-unit monomer.[12] Further details of the mode of interaction of VT-1 with Gb_3 in solution are clearly required. We have recently been investigating the use of ^{13}C-enriched carbohydrate ligands to define protein : carbohydrate interactions.[13] To this end, we now report the synthesis of the Gb_3-OSE trisaccharide (**2**) in ^{13}C-enriched form from [U-^{13}C]-D-glucose.

Results and Discussion

The synthesis of Gb_3 trisaccharide (otherwise known as P_k trisaccharide) has previously been reported by several groups.[14-16] In our case, the principle consideration was that [U-^{13}C]-D-glucose is the only suitable readily available building block. Our synthetic strategy therefore needed to provide access to suitable galactose and glucose building blocks, preferrably from a common precursor. The initially chosen synthetic route to the trimethylsilylethyl (SE)[17] glycoside of Gb_3 trisaccharide (**2**)[15] is outlined in Scheme 1. This proposed route

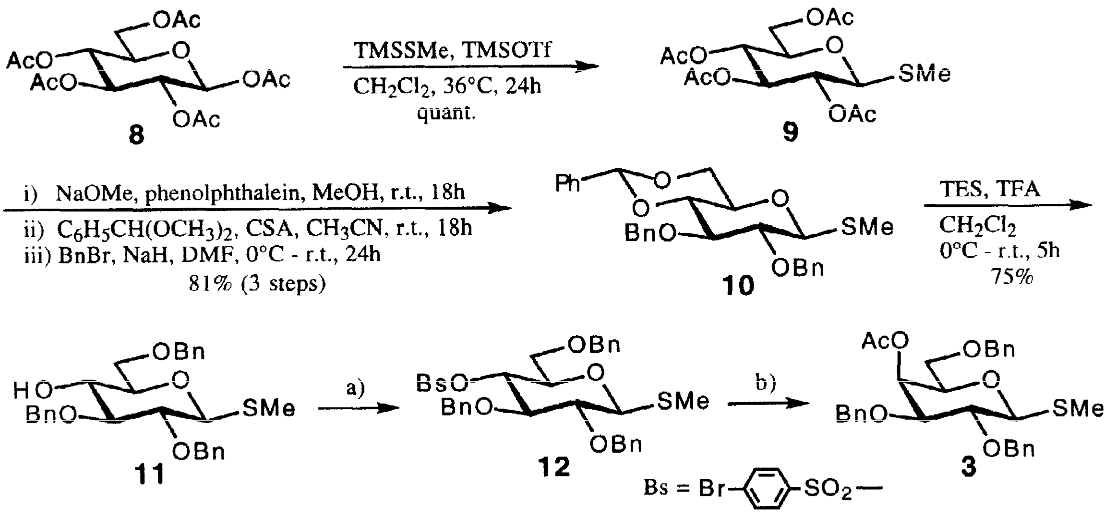


Scheme 1. Proposed Synthetic Approach to Gb_3-OSE Trisaccharide (**2**)

makes use of stereoselective glycosylation reactions, controlled by solvents and temperature, using *N*-PhSePhth-TMSOTf as a promoter for donor **3**, in a similar manner to that reported previously.[18] That is, our expectation was that for formation of the β-linkage the reaction would be carried out at low temperature in a non-polar solvent such as CH_2Cl_2 or toluene. The subsequent α-glycosylation reaction would then be carried out at room temperature in a polar solvent such as CH_3CN (Scheme 1).

Key points in the synthesis of Gb$_3$-OSE trisaccharide (2). All of the synthetic procedures outlined were optimised with unlabelled material prior to embarking on the synthesis of the ^{13}C-enriched version of Gb$_3$-OSE (**2**). In this synthesis there were two the key considerations: 1) inversion of glucose C-4 stereochemistry to give *galacto*-configured building blocks, and 2) stereoselective introduction of α- and β-galactopyranosyl linkages.

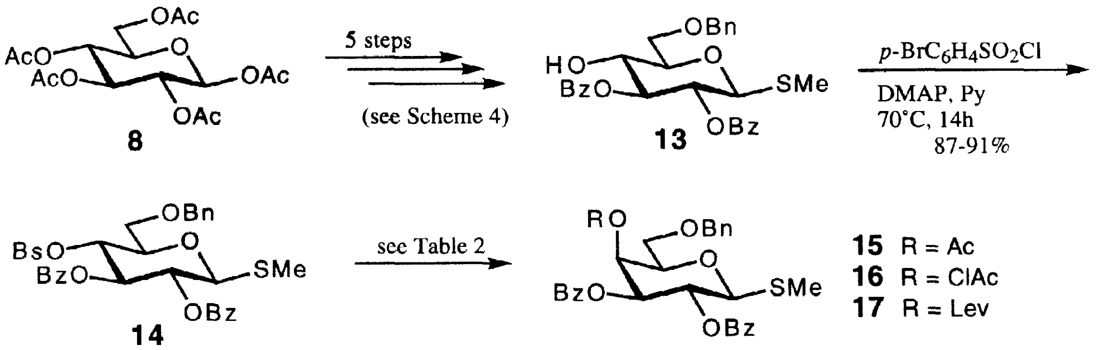
Known thioglycoside (**11**)[19] was prepared in 5 steps from glucose pentaacetate (**8**) (Scheme 2). The free 4-OH group of (**11**) was sulfonylated for an extended period to give 4-bromobenzenesulfonate (**12**) in only 26% yield. Shorter reaction times gave higher yields, together with unreacted starting material (**11**), but the reaction proved impractical, largely due to the lability of sulfonate (**12**) and the somewhat forcing conditions required to produce it. In addition, attempts to displace the sulfonate group of (**12**) with cesium acetate[20] in DMF, to give *galacto*-configured acetate (**3**), required overnight reaction at 110°C and proceeded in only 49% yield. Reaction with tetraethylammonium acetate[21] in DMF at 90°C for 5h gave an improved 71% yield, but again decomposition of sulfonate (**11**) was apparent.



Conditions	Reagents	Solvent	Temperature	Time	Yield
a)	*p*-$BrC_6H_4SO_2Cl$ (5eq.) DMAP (cat.)	Py	70°C	11h	26%
				7h	45% + 29% recovered **11**
b)	CsOAc	DMF	110°C	18h	53%
	$Et_4NOAc{\bullet}4H_2O$		90°C	5h	71%

Scheme 2. Synthesis of Key Glycosyl Donor (3)

Since the difficulties noted above in the prepartion of galactoside (3) are likely to be attributable to electronic factors associated with the benzyl ether protecting groups, an alternative strategy was adopted that employed glucoside (**13**), which contains electron withdrawing benzoyl groups that will stabilise the sulfonate ester group (Scheme 3). Alcohol (**13**) was treated with 4-bromobenzenesulfonyl chloride and DMAP in pyridine at 70°C gave the relatively robust sulfonate (**14**) in 87% yield. In light of the change to ester protecting groups in key intermediate (**14**), it became necessary to consider displacement at C-4 with a nucleophile that offered scope for selective deprotection of the resulting C-4 substituent. Initially the introduction of an acetoxy group at C-4 was investigated using commercially available reagents (Table 1). This met with a degree of success, but subsequent selective removal of the C-4 acetyl group with methanolic HCl[22] proved troublesome. Reaction with tetraethylammonium chloracetate was ineffective. Cesium levulinoate was subsequently investigated since the levulinoyl group (Lev) can be selectively removed with hydrazine in the presence of other ester protecting groups.[23] However, the two preferred reagents, Et_4NOLev and CsOLev, are not commercially available and were therefore prepared as outlined in the experimental section [see experimental section under compound (**17**)]. Et_4NOLev proved ineffective in both toluene and DMF, but reaction of sulfonate (**14**) with CsOLev in the presence of 18-crown-6 worked well, giving levulinoate (**17**) in near quantitative yield (Table 1).



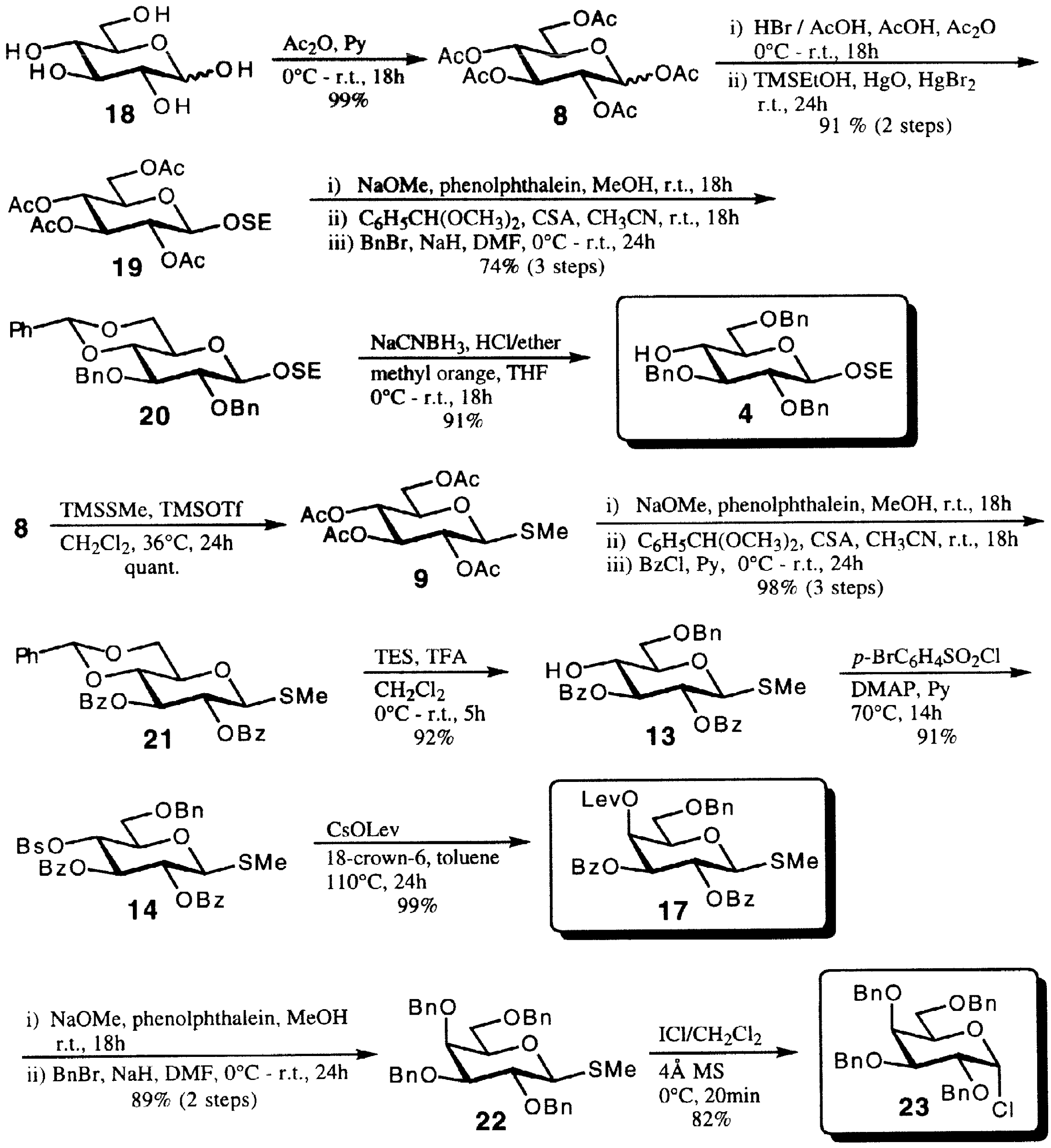
Scheme 3. Study of the C-4 Inversion Reaction

Entry	R	Reagents	Solvent	Temperature	Time	Compounds	Yield
1	Ac	CsOAc 18-crown-6	toluene	reflux	18h	**15**	74%
2	Ac	$Et_4NOAc{\bullet}4H_2O$	toluene	reflux	18h	**15**	90%
3	ClAc	$Et_4NOClAc$	toluene	reflux	18h	**16**	no reaction
4	Lev	Et_4NOLev	toluene	reflux	18h	**17**	no reaction
5	Lev	Et_4NOLev	DMF	110°C	18h	**17**	no reaction
6	Lev	CsOLev 18-crown-6	toluene	reflux	18h	**17**	93 -99%

Table 1. Study of the C-4 Inversion Reaction of Benzoyl-Protected Glucoside (**14**)

Synthesis of Gb_3 trisaccharide in isotopically enriched form.

The actual synthesis of ^{13}C-labelled Gb_3 trisaccharide (**3**) was carried as reported in Schemes 4 and 5. **Monosaccharide buliding blocks** (Scheme 4) - [$^{13}C_6$]-D-Glucose (**18**) was converted to peracetate (**8**), and subsequently via a number of standard steps to (trimethylsilyl)ethyl (SE)[17] glycoside (**19**). Deacetylation, 4,6-*O*-benzylidenation and 2,3-di-*O*-benzylation gave (**20**) in 74% yield. Subsequent reductive benzylidene acetal opening with $NaHCN_3$ and HCl[24] then gave the desired glucoside (**4**)[17] in 91% yield.



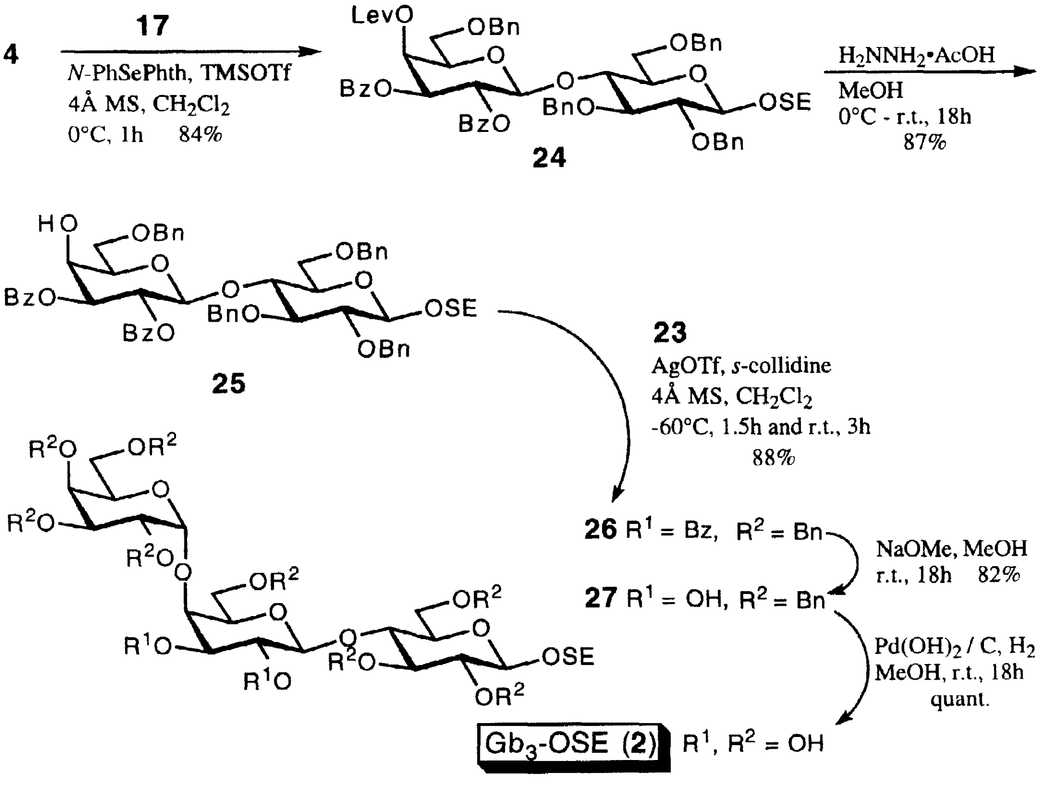
Scheme 4. Preparation of ^{13}C-Labelled Monosaccharide Building Blocks

Glucose pentaacetate (**8**) was also converted into methyl thioglucoside (**9**), which on deacetylation, 4,6-*O*-benzylidation and 2,3-di-*O*-benzoylation gave thioglycoside (**21**).[25] Treatment with triethylsilane and trifluoroacetic acid[26] gave alcohol (**13**), which was sulfonylated to give *gluco*-configured 4-bromobenzenesulfonate (**14**). Treatment with CsOLev and 18-crown-6 in refluxing toluene then gave C-4 levulinoate-protected thiogalactoside (**17**) in good yield.

Our initial aim was to use thiogalactoside (**3**) as the building block for introduction of the α-galactosyl unit (Scheme 1). We also noted that the straightforward introduction of this moiety into Gb_3 had been reported by

Magnusson and co-workers,[8] who routinely employ 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**23**) in conjunction with silver triflate for this purpose. Thioglycoside (**17**) was therefore also converted to per-*O*-benzylated thioglycoside (**22**) (Scheme 4), and some of this material was subsequently coverted to the desired α-galactosyl halide building block (**23**) by treatment with ICl.[27]

Coupling chemistry (Scheme 5) - Glycosylation of SE glucoside (**4**) with thiogalactoside (**17**) was carried out with *N*-PhSePhth-TMSOTf, as reported by Shimizu *et al.*[18] The resulting lactose unit (**24**) was treated with hydrazine acetate in methanol overnight to effect selective de-*O*-levulinoylaton,[23] giving the lactoside acceptor (**25**). Initial attempts to glycosylate this acceptor with thiogalactoside (**22**), as origianlly planned (Scheme 1), proved fruitless; the stereoselectivity of the reaction was poor and the yield uncomforatbly low (α:β, approx. 3:2; yield approx. 14%). We therefore resorted to using galactosyl chloride (**23**) as a glycosyl donor in the presence of silver triflate, which gave protected SE Gb_3 trisaccharide (**26**) in 88% yield; only α-glycosylation was observed. De-*O*-benzoylation followed by de-*O*-benzylation of (**26**) gave the desired ^{13}C-labelled Gb_3-OSE, (**2**).



Scheme 5. Glycosylation and Deprotection

Conclusions

In summary, we have accomplished the synthesis of the ^{13}C-enriched trisaccharide component of ganglioside Gb_3 [Galα1→4Galβ1→4Gluβ→OSE, (**2**)] from [U-^{13}C]-D-glucose in 22 steps and an overall yield of 19%. Structural studies on the interaction of ^{13}C-enriched Gb_3-OSE (**2**) with the glycolipid-binding B-subunit of verotoxin-1 will be reported in due course.

Experimental

General. All reagents were used as purchased without further purification. Solvents used for reactions were dried by distillation and stored over suitable activated molecular sieves. [U-^{13}C]-Labelled glucose (98% enrichment) was produced by Martek Biosciences Corporation (Columbia, MD, U.S.A.). Reactions were monitored by TLC, which was performed with 0.25 mm Macherey-Nagel precoated silica gel SIL G-25 on glass. Compounds were detected by dipping the TLC plates in an ethanolic solution of sulfuric acid (5% v/v) and heating. Sorbsil C60 40/60 A (Sorbsil Chromatography Media) was used for silica gel chromatography. Melting points were determined on a Gallenkamp Melting Point Apparatus and are uncorrected. Optical rotations were recorded on an Optical Activity Ltd. AA-1000 Polarimeter at room temperature (approx. 18 to 23°C). All NMR data are reported in parts per million downfield shift from tetramethylsilane. ^{1}H NMR spectra were routinely recorded at 300 MHz on a Varian Gemini 2000 spectrometer, or at 500MHz on a Varian Unity plus with broadband carbon decoupling for ^{13}C-enriched compounds. Chemical shifts are expressed relative to that of the residual proton in the NMR solvents [δ 7.26 ppm and 4.70 ppm for residual $C\mathit{H}Cl_3$ and $\mathit{H}OD$, respectively]. ^{13}C NMR spectra were recorded at 75.47 MHz on a Varian Gemini 2000 spectrometer, and chemical shifts are expressed relative to that of the deuterated solvents [δ 77.0 ppm and 29.8 ppm for $\mathit{C}DCl_3$ and $(\mathit{C}D_3)_2C{=}O$, respectively]. Microanalyses were performed by the in-house analytical service of this department. FAB-mass data were recorded by the in-house mass spectrometry service of this department, and at the EPSRC Mass Spectrometry Service Centre, Swansea.

For simplicity, experimental details are reported for the synthesis of unlabelled compounds due to complications arising from strong C-H and C-C coupling in ^{1}H and ^{13}C NMR spectra, respectively, of enriched compounds. Only partial NMR data are reported for protected compounds; other spectral features were in accord with proposed structures. The structures of ^{13}C-labelled synthetic intermediates were confirmed by comparison of chromatographic data for all compounds. Carbon-decoupled ^{1}H NMR data for some compounds were also compared with that obtained for the corresponding unlabelled compounds, and selected intermediates were characterised by mass spectrometry. Compounds designated as [$^{13}C_6$]-labelled are derived from [U-$^{13}C_6$]-D-glucose.

Experimental details are reported in the same order as the compounds appear in the text except where compounds have been subsequently re-made in ^{13}C-labelled form. In this case the compounds appear in the order that they are referred to in Schemes 4 and 5.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4-bromobenzenesulfonyl)-1-thio-β-D-glucopyranoside (12) To a solution of **11**[19] (166 mg, 345 μmol) in pyridine (12 ml) was added 4-bromobenzenesulfonyl chloride (640 mg, 2.51 mmol) and 4-dimethylaminopyridine (cat.). The mixture was stirred for 11h at 70°C, cooled to room temperature and diluted with EtOAc and water. The aqueous layer was extracted three times with EtOAc and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (430 mg) was purified by column chromatography [silica gel (22 g); toluene/EtOAc (15/1-15/1)] to give **12** (62 mg, 26% yield). Reaction of 4-bromobenzenesulfonyl chloride (570 mg, 2.23 mmol) and 4-dimethylaminopyridine (cat.) with **7** (215 mg, 446μmol) for 7h at 70°C gave **12** (157 mg, 45% yield) and recovered **7** (63 mg, 29 %); **12**: Rf 0.68 [toluene/EtOAc (7/1)]; m.p. 83-84 °C (*n*-hexane/Et_2O); $[\alpha]_D$ +10.7 (c 3.0, $CHCl_3$); Found: C, 58.56; H, 5.15. $C_{34}H_{35}O_7S_2$ requires: C, 58.37; H, 5.04%; ^{1}H NMR ($CDCl_3$) δ: 2.24, (s, 3H, $SC\mathit{H_3}$), 3.52 (t, 1H, J = 9.6 Hz, H-2), 3.63 (dd, 1H, J = 9.6 and 8.7 Hz, H-3), 4.35 (d, 1H, J

= 9.6 Hz, H-1), 4.47 (d, 1H, *J* = 9.6 Hz, $C_6H_5CH_2O$), 4.52 (s, 2H, $C_6H_5CH_2O$), 4.64 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.77 (d, 1H, *J* = 11.4 Hz, $C_6H_5CH_2O$), 4.82 (t, 1H, *J* = 8.7 Hz, H-4), 4.85 (d, 1H, *J* = 10.2 Hz, $C_6H_5CH_2O$), 7.19-7.65 (m, 19 H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: 12.65, 68.62, 73.41, 74.92, 75.42, 77.57, 78.33, 81.15, 83.15, 85.19, 126.98, 127.54, 127.62, 128.04, 128.38, 128.42, 128.80, 129.20, 132.24, 136.20, 137.43, 137.89, 138.12.

Methyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (3)

Reaction with Cesium Acetate - To a stirred solution of **12** (82 mg, 117 μmol) in DMF (2 ml) was added cesium acetate (140 mg, 729 μmol) and the mixture was stirred at 110°C overnight. On cooling to room temperature the reaction mixture was diluted with diethyl ether and water. The aqueous layer was extracted three times with diethyl ether, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (200 mg) was purified by column chromatography [silica gel (10 g); toluene/EtOAc (30/1)] to give **3** (32 mg, 53 % yield).

Reaction with Tetraethylammonium Acetate - To a stirred solution of **12** (108 mg, 154 μmol) in DMF (2 ml) was added $Et_4NOAc \bullet 4H_2O$ (232 mg, 888 μmol) and the mixture was stirred for 5 h at 90°C. On cooling to room temperature the reaction mixture was diluted with diethyl ether and water. The aqueous layer was extracted three times with diethyl ether, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (100 mg) was purified by column chromatography [silica gel (5 g); toluene/EtOAc (30/1)] to give **3** (58 mg, 71 % yield); **3**: Rf 0.59 [toluene/EtOAc (7/1)]; m.p. 79-80 °C (*n*-hexane/Et_2O); $[\alpha]_D$ +11.3 (c 2.3, $CHCl_3$); Found: C, 68.92; H, 6.99. $C_{30}H_{34}O_6S$ requires: C, 68.94; H, 6.56%; ^1H NMR ($CDCl_3$) δ: 2.09, (s, 3H, CH_3C=O or SCH_3), 2.24, (s, 3H, CH_3CO or SCH_3), 3.74 (t, 1H, *J* = 6.6 Hz, H-5), 4.39 (d, 1H, *J* = 8.4 Hz, H-1), 4.46 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.51 (d, 1H, *J* = 11.1 Hz, $C_6H_5CH_2O$), 4.56 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.74 (s, 2H, $C_6H_5CH_2O$), 4.78 (d, 1H, *J* = 11.1 Hz, $C_6H_5CH_2O$), 5.65 (d, 1H, *J* = 2.4 Hz, H-4), 7.28-7.39 (m, 15 H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: 12.97, 20.83, 66.92, 68.08, 71.87, 73.72, 75.84, 77.20, 81.05, 85.88, 127.85, 127.88, 127.97, 128.14, 128.25, 128.41, 128.54, 137.71, 137.81, 138.17, 170.48.

Methyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside (15)

Reaction with Cesium Acetate - To a stirred solution of **14** (75 mg, 104 μmol) in toluene (2 ml) were added cesium acetate (242 mg, 1.26 mmol) and 18-crown-6 (30 mg, 114 μmol) and the mixture was refluxed overnight. On cooling to room temperature the reaction mixture was diluted with EtOAc and water. The aqueous layer was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (300 mg) was purified by column chromatography [silica gel (15 g); toluene/EtOAc (15/1)] to give **15** (42 mg, 74 % yield).

Reaction with Tetraethylammonium Acetate - To a stirred solution of **14** (74 mg, 102 μmol) in toluene (2 ml) were added tetraethyl ammonium acetate (130 mg, 497 μmol) and the mixture was refluxed overnight. On cooling to room temperature the reaction mixture was diluted with EtOAc and water. The aqueous layer was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (200 mg) was purified by column chromatography [silica gel (10 g); toluene/EtOAc (15/1-7/1)] to give **15** (51 mg, 90 % yield); **15**: Rf 0.38 [toluene/EtOAc (7/1)]; m.p. 145-146 °C (*n*-hexane/Et_2O); $[\alpha]_D$ +16.6 (c 0.75, $CHCl_3$); Found: C, 65.37; H, 5.77. $C_{30}H_{30}O_8S$

requires: C, 65.44; H, 5.49%; ^{1}H NMR ($CDCl_3$) δ: 2.06, (s, 3H, CH_3CO or SCH_3), 2.26, (s, 3H, $CH_3C{=}O$ or SCH_3), 3.54 (dd, 1H, *J* = 9.9 and 6.3 Hz, H-6), 3.65 (dd, 1H, *J* = 9.9 and 6.3 Hz, H-6), 4.07 (t, 1H, *J* = 6.3 Hz, H-5), 4.46 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.59 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.65 (d, 1H, *J* = 9.9 Hz, H-1) 5.47 (dd, 1H, *J* = 9.9 and 3.0 Hz, H-3), 5.71 (t, 1H, *J* = 9.9 Hz, H-2), 5.76 (d, 1H, *J* = 3.0 Hz, H-4), 7.16-7.54 (m, 11 H, aromatic-*H*), 7.87-7.98 (m, 4 H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: 11.68, 20.93, 67.59, 67.63, 68.02, 72.83, 73.57, 76.23, 76.81, 83.77, 127.92, 128.04, 128.28, 128.45, 128.53, 129.14, 129.31, 129.69, 129.89, 133.36, 165.59, 169.95, 171.27.

Synthesis of ^{13}C-Labelled Gb$_3$ Trisaccharide.

1,2,3,4,6-Penta-*O*-acetyl-α,β-D-glucopyranose (8)[28]

*Synthesis of Unlabelled **8**.* To a stirred, ice-cold solution of acetic anhydride (10.0 ml, 106 mmol) and pyridine (14.0 ml, 174 mmol) was added β-D-glucose (2.00 g, 11.1 mmol). Once the starting material had dissolved, the mixture was allowed to gradually warm to room temperature and was stirred overnight. The reaction mixture was poured into iced-water (40 ml) and stirred for 1h. The resulting precipitate was removed by filtration, washed twice with iced-water and air dried to give β-**8** (3.08 g, 71 %). The filtrate was extracted three times with EtOAc, the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (4.4 g) was purified by column chromatography [silica gel (220 g); toluene/EtOAc (2/1)] to give further α- and β-**8** (1.02 g, 24%; total 95 % yield); β-**8**: Rf 0.44 [toluene/EtOAc (2/1)]; ^{1}H NMR ($CDCl_3$) δ: 2.01 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 3.84 (ddd, 1H, *J* = 9.6, 4.5 and 2.1 Hz, H-5), 4.11 (dd, 1H, *J* = 12.6 and 2.1 Hz, H-6), 4.28 (dd, 1H, *J* = 12.6 and 4.5 Hz, H-6), 5.13 (dd, 1H, *J* = 9.6 and 9.3 Hz, H-4), 5.14 (dd, 1H, *J* = 9.3 and 8.1 Hz, H-2), 5.25 (t, 1H, *J* = 9.3 Hz, H-3), 5.72 (d, 1H, *J* = 8.1 Hz, H-1).

Synthesis of [$^{13}C_6$]-8. Using the procedure described above, reaction of [U-^{13}C]-glucose (2.50 g, 13.5 mmol) with acetic anhydride (12.5 ml, 132 mmol) and pyridine (17.5 ml, 216 mmol) gave β-[^{13}**C**$_6$]-**8** (4.36 g, 82 %) and α- and β-[^{13}**C**$_6$]-**8** (899 mg, 17 %; total 99 % yield); TLC mobility and carbon decoupled ^{1}H NMR data in agreement with that of the unlabelled compounds. Found: m/z(FAB), 419 [M+Na]$^+$. $^{13}C_6{}^{12}C_{10}H_{22}O_{11}$ requires: 396.

(2-Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (19)[17]

*Synthesis of Unlabelled **19**.* To a stirred, ice-cold solution of **8** (6.00g, 15.1 mmol) in dichrolomethane (18 ml), acetic acid (6 ml) and acetic anhydride (6 ml) was added dropwise HBr in acetic acid (45 % w/v, 18 ml, 100 mmol). The mixture was allowed to gradually warm to room temperature and was stirred overnight. The reaction mixture was neutralised by careful addition of $NaHCO_3$ (100 g) and iced-water, and the aqueous solution was extracted three times with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was dissolved in dry CH_2Cl_2 (70 ml), and (2-trimethylsilyl)ethanol (7 ml, 48.8 mmol), mercury (II) oxide (3.60 g, 16.6 mmol) and mercury (II) bromide (900 mg, 2.50 mmol) were added. The resulting suspension was stirred for 24h at room temperature. The reaction mixture was then diluted with $CHCl_3$, stirred with KI and $NaHCO_3$ solution for 2h at room temperature, and filtered through a pad of Celite. The aqueous eluate was extracted four times with $CHCl_3$, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (12 g) was purified by column chromatography [silica gel (360 g); toluene/EtOAc (5/1)] to give **19** (5.92g, 86 % yield); Rf 0.58

[toluene/EtOAc (2/1)]; ^{1}H NMR ($CDCl_3$) δ: -0.01 (s, 9H, $OCH_2CH_2SiMe_3$), 0.83-1.02 (m, 2H, $OCH_2CH_2SiMe_3$), 1.99 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 3.56 (dt, 1H, J = 9.9 and 6.9 Hz, $OCH_2CH_2SiMe_3$), 3.68 (ddd, 1H, J = 9.6, 4.8 and 2.4 Hz, H-5), 3.97 (dt, 1H, J = 9.9 and 6.0 Hz, $OCH_2CH_2SiMe_3$), 4.12 (dd, 1H, J = 12.3 and 2.4 Hz, H-6), 4.26 (dd, 1H, J = 12.3 and 4.8 Hz, H-6), 4.51 (d, 1H, J = 7.8 Hz, H-1), 4.97 (dd, 1H, J = 9.6 and 7.8 Hz, H-2), 5.08 (t, 1H, J = 9.6 Hz, H-4), 5.19 (t, 1H, J = 9.6 Hz, H-3).

*Synthesis of [$^{13}C_6$]-**19**.* Using the procedure described above, reaction of **[$^{13}C_6$]-8** (899 mg, 2.27 mmol) with acetic acid (3 ml), acetic anhydride (1 ml) and HBr in acetic acid (45 % w/v, 3 ml, 16.7 mmol) gave the corresponding glycosyl bromide which on reaction with (2-trimethylsilyl)ethanol (1 ml, 6.98 mmol), mercury (II) oxide (543 mg, 2.51 mmol) and mercury (II) bromide (137 mg, 380 μmol) in dry CH_2Cl_2 (10 ml) gave **[$^{13}C_6$]-19** (935 mg, 91 % yield); TLC mobility in agreement with that of the unlabelled compound. Found: m/z(FAB), 477 $[M+Na]^+$. $^{13}C_6{}^{12}C_{13}H_{32}O_{10}Si$ requires: 454.

(2-Trimethylsilyl)ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (20)[17]

*Synthesis of Unlabelled **20**.* To a solution of **19** (1.19 g, 2.65 mmol) in MeOH (25 ml) containing phenolphthalein (cat.) was added sodium methoxide (cat.) and the mixture was stirred overnight at room temperature. The reaction mixture was neutralised with Amberlite IRC-50 (H^+) ion-exchange resin, filtered and concentrated *in vacuo*. The residue was dissolved in dry CH_3CN (15 ml), benzaldehyde dimethyl acetal (660 μl, 4.40 mmol) and camphorsulfonic acid (126 mg, 542 μmol) were added, and the mixture was stirred overnight at room temperature. The reaction mixture was neutralised with sat. $NaHCO_3$ solution and the resulting aqueous solution was extracted three times with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in dry DMF (12 ml), and benzyl bromide (450 μl, 3.78 mmol) and sodium hydride (250 mg, 60% dispersion in mineral oil, 6.25 mmol) were added successively at 0°C. The mixture was stirred for 24h at room temperature, quenched by careful addition of MeOH, and diluted with diethyl ether and water. The aqueous layer was extracted four times with diethyl ether. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (0.8 g) was purified by column chromatography [silica gel (40 g); toluene/EtOAc (15/1)] to give **20** (749 mg, 51 % yield); Rf 0.60 [toluene/EtOAc (9/1)]; ^{1}H NMR ($CDCl_3$) δ: 0.03 (s, 9H, $OCH_2CH_2SiMe_3$), 1.04, (dd, 2H, J = 9.6 and 7.8 Hz, $OCH_2CH_2SiMe_3$), 4.36 (dd, 1H, J = 10.5 and 4.8 Hz, H-6), 4.51 (d, 1H, J = 7.8 Hz, H-1), 4.78 (d, 1H, J = 11.1 Hz, $C_6H_5CH_2O$), 4.80 (d, 1H, J = 11.1 Hz, $C_6H_5CH_2O$), 4.91 (d, 2H, J = 11.1 Hz, $C_6H_5CH_2O$ X 2), 5.57 (s, 1H, C_6H_5CH), 7.25-7.51 (m, 15H, aromatic-*H*).

*Synthesis of [$^{13}C_6$]-**20*** Using the procedure described above, reaction of **[$^{13}C_6$]-19** (757 mg, 1.67 mmol) with sodium methoxide (cat.) in MeOH (3 ml) conating phenolphthalein (cat.) gave the deprotected sugar. Subsequent reaction with benzaldehyde dimethyl acetal (270 μl, 1.80 mmol) and camphorsulfonic acid (45 mg, 0.194 mmol) in CH_3CN (30 ml) gave the benzylidene acetal, which on benzylation with benzyl bromide (700 μl, 5.89 mmol) and sodium hydride (290 mg, 60% dispersion in mineral oil, 7.25 mmol) in dry DMF (15 ml) gave **[$^{13}C_6$]-20** (682 mg, 74 % yield); TLC mobility in agreement with that of the unlabelled compound.

(2-Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4)[17]

*Synthesis of Unlabelled **4**.* To an ice-cold, stirred mixture of **20** (273 mg, 498 μmol) and sodium cyanoborohydride (250 mg, 3.98 mmol) in dry THF (10 ml) containing methyl orange (cat.) and 4Å molecular sieves (1 g) was added dropwise HCl-saturated diethyl ether until a pink colouration persisted in the reaction mixture. The mixture was allowed to gradually warm to room temperature and was stirred overnight. The reaction mixture was then diluted with EtOAc, neutralised with sat. $NaHCO_3$ solution and filtered through a pad of Celite. The aqueous eluate was extracted three times with EtOAc and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (650 mg) was purified by column chromatography [silica gel (30 g); toluene/EtOAc (15/1)] to give **4** (141 mg, 51 % yield); Rf 0.49 [toluene/EtOAc (7/1)]; ^1H NMR ($CDCl_3$) δ: 0.03 (s, 9H, $OCH_2CH_2Si\mathit{Me}_3$), 1.01-1.07 (m, 2H, $OCH_2C\mathit{H}_2SiMe_3$), 2.55 (d, 1H, J = 1.8 Hz, O*H*), 4.43 (d, 1H, J = 7.2 Hz, H-1), 4.59 (d, 1H, J = 12.3 Hz, $C_6H_5C\mathit{H}_2O$), 4.59 (d, 1H, J = 12.3 Hz, $C_6H_5C\mathit{H}_2O$), 4.73 (d, 2H, J = 11.1 Hz, $C_6H_5C\mathit{H}_2O$ X 2), 4.94 (d, 1H, J = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 4.97 (d, 1H, J = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 7.24-7.35 (m, 15H, aromatic-*H*).

Synthesis of $[^{13}C_6]$*-4*. Using the procedure described above, reaction of $[^{13}C_6]$-**20** (323 mg, 583 μmol) in dry THF (9 ml) with sodium cyanoborohydride (346 mg, 5.51 mmol), methyl orange (cat.), 4Å molecular sieves (1.17 g) and HCl-saturated diethylether gave $[^{13}C_6]$-**4** (295 mg, 91% yield); TLC mobility and carbon-decoupled ^1H NMR data in agreement with that of the unlabelled compound.

Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (9)[29]

*Synthesis of Unlabelled **9***. To a stirred solution of **8** (10.0 g, 25.6 mmol) and (methylthio)trimethylsilane (5.00 ml, 35.3 mmol) in dry CH_2Cl_2 (100 ml) was added trimethylsilyl trifuluoromethanesulfonate (4.50 ml, 24.9 mmol) dropwise at room temperature and the mixture was subsequently stirred for 24h at 36°C. The reaction mixture was then diluted with EtOAc and neutralised with sat. $NaHCO_3$ solution. The aqueous layer was extracted three times with EtOAc and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (8.3 g) was purified by column chromatography [silica gel (250 g); toluene/EtOAc (9/1-7/1)] to give **9** (6.60 g, 68 % yield); Rf 0.33 [toluene/EtOAc (2/1)] : ^1H-NMR ($CDCl_3$) δ: 2.01 (s, 3H, $SC\mathit{H}_3$ or $COC\mathit{H}_3$), 2.03 (s, 3H, $SC\mathit{H}_3$ or $COC\mathit{H}_3$), 2.06 (s, 3H, $SC\mathit{H}_3$ or $COC\mathit{H}_3$), 2.08 (s, 3H, $SC\mathit{H}_3$ or $OC\mathit{H}_3$), 2.17 (s, 3H, $SC\mathit{H}_3$ or $COC\mathit{H}_3$), 3.73 (ddd, 1H, J = 12.3, 4.8 and 2.4 Hz, H-5), 4.15 (dd, 1H, J = 12.3 and 2.4 Hz, H-6), 4.25 (dd, 1H, J = 12.3 and 4.8 Hz, H-6), 4.39 (d, 1H, J = 9.6 Hz, H-1), 5.07 (t, 1H, J = 9.6 Hz, H-3 or H-4), 5.08 (t, 1H, J = 9.6 Hz, H-3 or H-4), 5.25 (t, 1H, J = 9.6 Hz, H-2).

Synthesis of $[^{13}C_6]$*-9*. Using the procedure described above, reaction of $[^{13}C_6]$-**8** (1.02 g, 2.57 mmol) with (methylthio)trimethylsilane (1.00 ml, 7.07 mmol) and trimethylsilyl trifuluoromethanesulfonate (300 μl, 1.66 mmol) in dry CH_2Cl_2 (10 ml) gave recovered $[^{13}C_6]$-**8** (70 mg) and product $[^{13}C_6]$-**9** (921 mg, 93 % yield based on isolated product; quantitative yield based on recovered $[^{13}C_6]$-**8**); TLC mobility in agreement with that of the unlabelled compound.

Methyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (21)[24]

*Synthesis of Unlabelled **21***. To a solution of **9** (1.25 g, 3.30 mmol) in MeOH (10 ml) containing phenolphthalein (cat.) was added sodium methoxide (cat.) and the mixture was stirred overnight at room temperature. The reaction mixture was neutralised with Amberlite IRC-50 (H^+) ion-exchange resin, filtered and concentrated *in vacuo*. The resulting residue was dissolved in dry CH_3CN (150 ml), and benzaldehyde dimethylacetal (1.00 ml, 6.66 mmol) and camphorsulfonic acid (150 mg, 646 μmol) were added. The mixture was stirred overnight at room temperature and neutralised with sat. $NaHCO_3$ solution. The aqueous solution was extracted four times with EtOAc and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was dissolved with pyridine (2 ml) and benzoyl chloride (2.00 ml, 17.2 mmol) in dichloromethane (40 ml) was added dropwise at 0°C. The mixture was stirred for 24h at room temperature, diluted with EtOAc and water, and the aqueous layer was extracted four times with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The resulting residue (4.3 g) was purified by column chromatography [silica gel (200 g); toluene/EtOAc (10/1)] to give **21** (1.51 g, 90 % yield); Rf 0.72 [toluene/EtOAc (7/1)]; ^1H NMR ($CDCl_3$) δ: 2.25 (s, 1H, SCH_3), 4.72 (d, 1H, J = 9.9 Hz, H-1), 5.55 (dd, 1H, J = 9.9 and 9.6 Hz, H-2), 5.56 (s, 1H, C_6H_5CH), 5.82 (t, 1H, J = 9.6 Hz, H-3).

Synthesis of ***$[^{13}C_6]$-21***. Using the procedure described above, deprotection of **$[^{13}C_6]$-9** (920 mg, 2.93 mmol) with sodium methoxide (cat.) and phenolphthalein (cat.) in MeOH (10 ml), followed by reaction with benzaldehyde dimethylacetal (500 µl, 3.33 mmol) and camphorsulfonic acid (108 mg, 0.465 mmol) in dry CH_3CN (30 ml), and subsequent esterification with benzoyl chloride (3.00 ml, 25.8 mmol) and pyridine (15 ml) gave **$[^{13}C_6]$-21** (1.20 g, 98 % yield); TLC mobility in agreement with that of the unlabelled compound.

Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (13)

Synthesis of Unlabelled ***13***. To a stirred, ice-cold solution of **21** (1.51g, 2.98 mmol) and triethylsilane (3.60 ml, 22.5 mmol) in CH_2Cl_2 (18 ml) was added dropwise trifluoroacetic acid (2.00 ml, 22.6 mmol). The mixture was stirred for 5h as it was allowed to slowly warm to room temperature. The reaction mixture was then diluted with EtOAc and neutralised with sat. $NaHCO_3$ solution. The aqueous layer was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (3 g) was purified by column chromatography [silica gel (150 g); toluene/EtOAc (7/1)] to give **13** (1.34 g, 88 % yield); Rf 0.30 [toluene/EtOAc (2/1)]; $[\alpha]_D$ +69.3 (c 1.2, $CHCl_3$); Found: C, 65.94; H, 5.80. $C_{28}H_{28}O_7S$ requires: C, 66.13; H, 5.55%; ^1H NMR ($CDCl_3$) δ: 2.22 (s, 3H, SCH_3), 3.21 (d, 1H, J = 3.3 Hz, OH), 3.98 (dt, 1H, J = 9.0 and 3.3 Hz, H-4), 4.60 (d, 1H, J = 9.6 Hz, H-1), 4.61 (d, 1H, J = 12.3 Hz, $C_6H_5CH_2O$), 4.64 (d, 1H, J = 12.3, $C_6H_5CH_2O$), 5.44-5.53 (m, 2H, H-2 and H-3), 7.29-7.98 (m, 15 H, aromatic-H); ^{13}C NMR ($CDCl_3$) δ: 11.31, 69.53, 69.79, 73.67, 77.45, 78.94, 82.94, 127.77, 127.87, 128.38, 128.50, 129.14, 129.24, 129.83, 129.89, 133.27, 133.39, 137.75, 165.51, 167.04.

Synthesis of ***$[^{13}C_6]$-13***. Using the procedure described above, reaction of **$[^{13}C_6]$-21** (1.05 g, 2.05 mmol) with triethylsilane (3.00 ml, 18.8 mmol) and trifluoroacetic acid (1.60 ml, 18.1 mmol) in CH_2Cl_2 (18 ml) gave **$[^{13}C_6]$-13** (973 mg, 92 % yield); TLC mobility in agreement with that of the unlabelled compound.

Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(4-bromobenzenesulfonyl)-1-thio-β-D-glucopyranoside (14)

Synthesis of Unlabelled ***14***. To a solution of **13** (124 mg, 244 µmol) in pyridine (12 ml) was added 4-bromobenzenesulfonyl chloride (310 mg, 1.21 mmol) and 4-dimethylaminopyridine (cat.). The mixture was stirred for 14h at 70°C, cooled to room temperature and diluted with EtOAc and water. The aqueous layer was extracted three times with EtOAc and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (320 mg) was purified by column chromatography [silica gel (15 g); toluene/EtOAc (20/1-15/1)] to give **14** (154 mg, 87 % yield); Rf 0.56 [toluene/EtOAc (15/1)];

m.p. 145-146 °C (toluene/EtOAc); $[\alpha]_D$ +24.6 (c 2.0, $CHCl_3$); Found: C, 55.88; H, 4.20. $C_{34}H_{31}O_9BrS_2$ requires: C, 56.12; H, 4.29%; ^{1}H NMR ($CDCl_3$) δ: 2.22 (s, 3H, SC*H*3), 3.76 (dd, 1H, *J* = 11.1 and 3.9 Hz, 6-H), 4.58 (d, 1H, *J* = 9.6 Hz, H-1), 4.61 (d, 1H, *J* = 12.3 Hz, $C_6H_5CH_2O$), 4.66 (d, 1H, *J* = 12.3 Hz, $C_6H_5CH_2O$), 5.15 (t, 1H, *J* = 9.6 Hz, H-2, H-3 or H-4), 5.42 (t, 1H, *J* = 9.6 Hz, H-2, H-3 or H-4), 5.71 (t, 1H, *J* = 9.6 Hz, H-2, H-3 or H-4), 7.19-7.86 (m, 19 H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: 11.30, 68.15, 69.89, 73.47, 73.59, 77.99, 82.97, 127.75, 127.85, 128.40, 128.44, 128.78, 128.93, 129.80, 129.85, 132.27, 133.37, 133.56, 135.71, 138.01, 165.19.

Synthesis of [$^{13}C_6$]-14. Using the procedure described above, reaction of **[$^{13}C_6$]-13** (727 mg, 1.41 mmol) with 4-bromobenzenesulfonyl chloride (2.08 g, 8.14 mmol), 4-dimethylaminopyridine (cat.) and pyridine (12 ml) gave **[$^{13}C_6$]-14** (943 mg, 91 % yield); TLC mobility in agreement with that of the unlabelled compound; Found: C, 56.58; H, 4.32%. $^{13}C_6C_{28}H_{31}O_9BrS_2$ requires: C, 56.47; H, 4.26%; Found: m/z(FAB), 757 $[M+Na]^+$. $^{13}C_6{}^{12}C_{28}H_{31}O_9BrS_2$ requires 734.

Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-levulinoyl-1-thio-β-D-galactopyranoside (17)

Synthesis of Unlabelled 17. Levulinic acid (860 μl, 8.40 mmol) was added dropwise to a stirred solution of cesium hydroxide monohydrate (1.37 g, 8.18 mmol) in MeOH (2 ml) and the mixture was stirred for 1h at room temperature. The reaction mixture was concentrated *in vacuo* to give cesium levulinoate (2.03 g, quant.) which was used directly in the next step. To a stirred solution of **14** (197 mg, 271 μmol) in toluene (2 ml) were added cesium levulinoate (280 mg, 1.13 mmol) and 18-crown-6 (66 mg, 250 μmol) and the mixture was refluxed overnight. On cooling to room temperature the reaction mixture was diluted with EtOAc and water. The aqueous layer was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (220 mg) was purified by column chromatography [silica gel (11 g); toluene/EtOAc (15/1-7/1)] to give recovered **14** (85 mg) and product **17** (93 mg, 52 % yield based on isolated product; quantitative yield based on recovered **14**); Rf 0.20 [toluene/EtOAc (15/1)]; $[\alpha]_D$ +61.0 (c 1.5, $CHCl_3$); Found: C, 65.53; H, 5.79. $C_{33}H_{34}O_9S$ requires: C, 65.33; H, 5.65%; ^{1}H NMR ($CDCl_3$) δ: 2.11 (s, 3H, COC*H*3 or SC*H*3), 2.25 (s, 3H, COC*H*3 or SC*H*3), 2.58-2.69 (m, 4H, $OCH_2CH_2COCH_3$), 3.57 (dd, 1H, *J* = 9.6 and 6.9 Hz, H-6), 3.68 (dd, 1H, *J* = 9.6 and 5.7 Hz, H-6), 4.05 (dd, 1H, *J* = 6.9 and 5.7 Hz, H-5), 4.50 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.55 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2O$), 4.64 (d, 1H, *J* = 9.9 Hz, H-1), 5.47 (dd, 1H, *J* = 9.9 and 3.3 Hz, H-3), 5.69 (t, 1H, *J* = 9.9 Hz, H-2), 5.77 (d, 1H, *J* = 3.3 Hz, H-4), 7.16-7.98 (m, 15H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: 11.94, 28.06, 29.99, 38.06, 67.96, 68.05, 68.64, 73.16, 73.95, 76.66, 77.85, 84.03, 128.22, 128.44, 128.81, 129.50, 129.70, 130.18, 130.25, 133.69, 133.73, 138.17, 165.92, 172.12, 206.43.

Synthesis of [$^{13}C_6$]-17. Using the procedure described above, reaction of **[$^{13}C_6$]-14** (867 mg, 1.18 mmol) with cesium levulinoate (1.80 g, 7.26 mmol) and 18-crown-6 (1.00 g, 3.78 mmol) in toluene (20 ml) gave **[$^{13}C_6$]-17** (717 mg, 99 % yield); TLC mobility in agreement with that of the unlabelled compound; Found: m/z(FAB), 635 $[M+Na]^+$. $^{13}C_6{}^{12}C_{27}H_{34}O_9S$ requires: 612.

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (22)[16]

Synthesis of Unlabelled 22. To a stirred solution of **17** (92 mg, 152 μmol) in MeOH (5 ml) containing phenolphthalein (cat.) was added sodium methoxide (cat.) and the mixture was stirred overnight at room temperature. The reaction mixture was neutralised with Amberlite resin IRC-50 (H^+) ion-exchange resin, filtered and concentrated *in vacuo*. The residue was dissolved in dry DMF (30 ml), and benzyl bromide (330 µl, 2.77 mmol) and sodium hydride (120 mg, 60% dispersion in mineral oil, 3.00 mmol) were added successively at 0°C. The mixture was stirred for 24h at room temperature, quenched by careful addition of MeOH, and diluted with diethyl ether and water. The aqueous layer was extracted four times with diethyl ether, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (350 mg) was purified by column chromatography [silica gel (18 g); toluene/EtOAc (20/1-10/1)] to give **22** (70 mg, 81 % yield); Rf 0.41 [*n*-hexane/EtOAc (5/1)]; ^1H NMR ($CDCl_3$) δ: 2.01 (s, 3H, $SC\mathit{H}_3$), 3.85 (t, 1H, *J* = 9.6 Hz, H-2), 3.97 (d, 1H, *J* = 2.7 Hz, H-4), 4.34 (d, 1H, *J* = 9.6 Hz, H-1), 4.61 (d, 1H, *J* = 11.7 Hz, $C_6H_5C\mathit{H}_2O$), 4.73 (s, 2H, $C_6H_5C\mathit{H}_2O$), 4.82 (d, 1H, *J* = 10.2 Hz, $C_6H_5C\mathit{H}_2O$), 4.82 (d, 1H, *J* = 10.2 Hz, $C_6H_5C\mathit{H}_2O$), 4.86 (d, 1H, *J* = 10.2 Hz, $C_6H_5C\mathit{H}_2O$), 4.95 (d, 1H, *J* = 11.7 Hz, $C_6H_5C\mathit{H}_2O$), 7.38-7.25 (m, 20H, aromatic-H).

Synthesis of [$^{13}C_6$]-22. Using the procedure described above, reaction of **[$^{13}C_6$]-17** (515 mg, 841 µmol) with phenolphthalein (cat.) and sodium methoxide (cat.) in MeOH (5 ml), followed by benzylation with benzyl bromide (2.00 ml, 16.8 mmol), sodium hydride (700 mg, 60% dispersion in mineral oil, 17.5 mmol) in dry DMF (30 ml) gave **[$^{13}C_6$]-22** (429 mg, 89 % yield); TLC mobility in agreement with that of the unlabelled compound.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl chloride (23)[16]

Synthesis of Unlabelled 23. To a stirred, ice-cold mixture of **22** (60 mg, 109 µmol) in CH_2Cl_2 (1 ml) containing 4Å molecular sieves (75 mg) was added dropwise iodine monochloride (300 µl, 1.0M in CH_2Cl_2, 300 µmol) and the mixture was stirred for 20 min. The reaction mixture was diluted with ice-cold CH_2Cl_2, quenched with cold aq. $Na_2S_2O_3$ solution (15% w/v) and filtered. The aqueous layer was quickly extracted twice with CH_2Cl_2, the combined organic extracts were back-washed with cold sat. $NaHCO_3$ solution and ice-cold brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (100 mg) was purified by column chromatography [silica gel (5 g); toluene/EtOAc (100/1-50/1)] to give **23** (56 mg, 92 % yield); Rf 0.84 [toluene/EtOAc (7/1)]; ^1H NMR ($CDCl_3$) δ: 4.73 (d, 1H, *J* = 11.7 Hz, $C_6H_5C\mathit{H}_2O$), 4.77 (d, 1H, *J* = 11.7 Hz, $C_6H_5C\mathit{H}_2O$), 4.86 (d, 1H, *J* = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 4.95 (d, 1 H, *J* = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 6.19 (d, 1 H, *J* = 3.9 Hz, H-1), 7.25-7.39 (m, 20 H, aromatic-H).

Synthesis of [$^{13}C_6$]-23. Using the procedure described above, reaction of **[$^{13}C_6$]-22** (77 mg, 140 µmol) with iodine monochloride (140 µl, 1.0M in CH_2Cl_2, 140 µmol) and 4Å molecular sieves (150 mg) in CH_2Cl_2 (1.5 ml) gave **[$^{13}C_6$]-23** (62 mg, 82 % yield); TLC mobility in agreement with that of the unlabelled compound.

(2-Trimethylsilyl)ethyl *O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-levulinoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (24)

Synthesis of Unlabelled 24. To a stirred, ice-cold mixture of **4** (60 mg, 109 µmol), **17** (83 mg, 137 µmol), *N*-(phenylseleno)phthalimide (52 mg, 172 µmol) in CH_2Cl_2 (1.5 ml) containing 4Å molecular sieves (180 mg) under nitrogen was added dropwise trimethylsilyl trifluoromethanesulfonate (27 µl, 149 µmol). The mixture was stirred for 1h at 0°C, diluted with EtOAc, neutralised with $NaHCO_3$ solution, and filtered through a pad of Celite. The aqueous layer was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (140 mg) was purified by column chromatography [silica gel (7 g); toluene/EtOAc (7/1)] to give **24** (101 mg, 84 % yield); Rf 0.56 [toluene/EtOAc (7/1)]; $[\alpha]_D$ +10.2 (c 1.6, $CHCl_3$); Found: C, 68.01; H, 6.74. $C_{64}H_{72}O_{15}Si{\bullet}H_2O$ requires: C, 68.18; H, 6.62%; ^1H NMR ($CDCl_3$) δ: 0.00 (s, 9H, Si*Me3*), 0.86-1.03 (m, 2H, $OCH_2C\mathit{H}_2SiMe_3$), 2.05, (s, 3H, $COC\mathit{H}_3$), 2.48-2.66 (m, 4H, $OC\mathit{H}_2C\mathit{H}_2COCH_3$), 3.76 (dd, 1H, *J* = 6.6 and 6.9 Hz, H-5), 4.31 (d, 1H, *J* = 7.8 Hz, H-1), 4.32 (d, 1H, *J* = 12.0 Hz, $C_6H_5C\mathit{H}_2O$), 4.34 (d, 1H, *J* = 11.7 Hz, $C_6H_5C\mathit{H}_2O$), 4.44 (d, 1H, *J* = 11.7 Hz, $C_6H_5C\mathit{H}_2O$), 4.64 (d, 1H, *J* = 12.0 Hz, $C_6H_5C\mathit{H}_2O$), 4.71 (d, 1H, *J* = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 4.82 (d, 1H, *J* = 10.8 Hz, $C_6H_5C\mathit{H}_2O$), 4.89 (d, 1H, *J* = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 4.90 (d, 1H, *J* = 8.1 Hz, H-1'), 5.01 (d, 1H, *J* = 10.8 Hz, $C_6H_5C\mathit{H}_2O$), 5.25 (dd, 1H, *J* = 10.5 and 3.3 Hz, H-3'), 5.58 (dd, 1H, *J* = 10.5 and 8.1 Hz, H-2'), 5.68 (d, 1H, *J* = 3.3 Hz, H-4'), 7.89-7.17 (m, 30H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: -1.59, 18.30, 27.65, 29.53, 37.61, 66.86, 67.27, 67.72, 67.90, 70.43, 71.96, 72.06, 73.40, 74.30, 74.78, 75.28, 81.90, 82.72, 100.44, 103.08, 127.38, 127.52, 127.76, 127.99, 128.17, 128.28, 128.38, 128.48, 128.58, 129.28, 129.77, 133.20, 133.31, 137.96, 138.18, 138.81, 139.20, 165.13, 165.49, 171.73, 205.90.

Synthesis of $[^{13}C_6]$*-24*. Using the procedure described above, reaction of $[^{13}C_6]$**-4** (117 mg, 210 μmol) and $[^{13}C_6]$**-17** (140 mg, 229 μmol) with *N*-(phenylseleno)phthalimide (90 mg, 297 μmol) and 4Å molecular sieves (500 mg) in CH_2Cl_2 (5 ml) gave $[^{13}C_6]_2$**-24** (204 mg, 84 % yield); TLC mobility in agreement with that of the unlabelled compound; Found: m/z(FAB), 1143 $[M+Na]^+$. $^{13}C_{12}{}^{12}C_{52}H_{72}O_{15}Si$ requires: 1120.

(2-Trimethylsilyl)ethyl *O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (25)

Synthesis of Unlabelled 25. To a stirred, ice-cold solution of **24** (97 mg, 87.4 μmol) in MeOH (3 ml) was added hydrazine acetate (150 mg, 134 mmol) and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and sat. $NaHCO_3$ solution. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with dilute HCl (2 N), water, sat. $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (130 mg) was purified by column chromatography [silica gel (6 g); toluene/EtOAc (7/1)] to give **25** (79 mg, 90 % yield); Rf 0.61 [toluene/EtOAc (7/1)]; m.p. 93-94 °C (*n*-hexane/Et_2O); $[\alpha]_D$ +28.1 (c 0.78, $CHCl_3$); Found: C, 69.95; H, 6.50. $C_{59}H_{66}O_{13}Si$ requires: C, 70.08; H, 6.58%; ^1H NMR ($CDCl_3$) δ: 0.00 (s, 9H, Si*Me3*), 0.96-1.02 (m, 2H, $OCH_2C\mathit{H}_2SiMe_3$), 4.39 (d, 1H, *J* = 12.0 Hz, $C_6H_5C\mathit{H}_2O$), 4.42 (d, 1H, *J* = 12.3 Hz, $C_6H_5C\mathit{H}_2O$), 4.58 (d, 1H, *J* = 12.0 Hz, $C_6H_5C\mathit{H}_2O$), 4.70 (d, 1H, *J* = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 4.85 (d, 1H, *J* = 11.4 Hz, $C_6H_5C\mathit{H}_2O$), 4.88 (d, 1H, *J* = 8.1 Hz, H-1'), 4.89 (d, 1H, *J* = 11.4 Hz, $C_6H_5C\mathit{H}_2O$), 5.00 (d, 1H, *J* = 11.1 Hz, $C_6H_5C\mathit{H}_2O$), 5.13 (dd, 1 H, *J* = 10.2 and 3.0 Hz, H-3'), 5.72 (dd, 1 H, *J* = 10.2 and 8.1 Hz, H-2'), 7.24-7.98 (m, 30 H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: -1.58, 18.33, 67.30, 67.63, 68.12, 68.41, 70.45, 72.87, 73.33, 73.54, 74.33, 74.48, 74.75, 75.18, 76.86, 81.99, 82.97, 100.74, 103.08, 127.22, 127.55, 127.75, 127.84, 128.04, 128.17, 128.30, 128.50, 129.29, 129.50, 129.74, 129.92, 133.24, 133.37, 137.82, 138.31, 138.80, 139.41, 165.25, 165.91.

Synthesis of $[^{13}C_6]$*-25*. Using the procedure described above, reaction of $[^{13}C_6]_2$**-24** (171 mg, 153 μmol) with hydrazine acetate (260 mg,2.82 mmol) in MeOH (5 ml) gave $[^{13}C_6]_2$**-25** (136 mg, 87 % yield); TLC mobility in agreement with that of the unlabelled compound; Found: m/z(FAB), 1046 $[M+Na]^+$. $^{13}C_{12}{}^{12}C_{52}H_{72}O_{15}Si$ requires: 1022.

(2-Trimethylsilyl)ethyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (26)

Synthesis of Unlabelled ***26****.* A stirred mixture of **25** (53 mg, 52.4 μmol) and **23** (100 mg, 179 μmol) in CH_2Cl_2 (4.5 ml) containing *s*-collidine (26 μl, 197 μmol) and 4Å molecular sieves (400 mg) under nitrogen was cooled to -60°C and light was excluded. Silver trifluoromethanesulfonate (105 mg, 409 μmol) was added and the mixture was stirred for 1.5h, followed by a further 3h at room temperature. The reaction mixture was diluted with EtOAc, neutralised with sat. $NaHCO_3$ solution, and filtered through a pad of Celite. The aqueous eluate was extracted three times with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (200 mg) was purified by column chromatography [silica gel (10 g); toluene/EtOAc (15/1)] to give protected trisaccharide **26** (69 mg, 86 % yield); Rf 0.76 [toluene/EtOAc (7/1)]; $[\alpha]_D$ +43.0 (c 1.1, $CHCl_3$); Found: C, 72.13; H, 7.16. $C_{93}H_{100}O_{18}Si{\bullet}H_2O$ requires: C, 72.82; H, 6.57%; ^1H NMR ($CDCl_3$) δ: -0.01 (s, 9H, Si*Me*$_3$), 0.96-1.02 (m, 2H, $OCH_2CH_2SiMe_3$), 5.07 (d, 1H, *J* = 10.8 Hz, H-3'), 5.76 (dd, 1H, *J* = 10.8 and 8.1 Hz, H-2'), 7.18-7.91 (m, 50H, aromatic-*H*); ^{13}C NMR ($CDCl_3$) δ: -1.58, 18.36, 67.27, 67.83, 68.32, 69.50, 70.75, 72.37, 72.90, 73.01, 73.30, 73.76, 74.12, 74.36, 74.76, 74.91, 75.64, 79.11, 82.04, 82.54, 100.71, 101.00, 103.01, 127.32, 127.53, 127.59, 127.78, 127.89, 128.01, 128.09, 128.27, 128.40, 128.50, 129.40, 129.67, 129.95, 133.16, 133.21, 138.39, 138.50, 138.76, 138.81, 138.86, 139.05, 139.37, 165.33, 166.59.

Synthesis of [$^{13}C_6$]-26. Using the procedure described above, reaction of **[$^{13}C_6$]$_2$-25** (35 mg, 34.1 μmol) and **[$^{13}C_6$]-23** (45 mg, 80.0 μmol) with silver trifluoromethanesulfonate (45 mg, 175 μmol), *s*-collidine (11 μl, 83.2 μmol) and 4Å molecular sieves (100 mg) in dry CH_2Cl_2 (1 ml) gave **[$^{13}C_6$]$_3$-26** (47 mg, 88 % yield); TLC mobility in agreement with that of the unlabelled compound; Found: m/z(FAB), 1573 $[M+Na]^+$. $^{13}C_{18}{}^{12}C_{75}H_{100}O_{18}Si$ requires: 1550.

(2-Trimethylsilyl)ethyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (27)

Synthesis of Unlabelled 27. A solution of **26** (15 mg, 9.78 μmol) in MeOH (4 ml) containing sodium methoxide (cat.) was stirred overnight at room temperature. The reaction mixture was then neutralised with Amberlite IRC-50 (H^+) ion-exchange resin, filtered and concentrated *in vacuo*. The residue was partitioned between EtOAc and water, and the aqueous layer was extracted three times with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue (40 mg) was purified by column chromatography [silica gel (2 g); toluene/EtOAc (3/1)] to give **27** (13 mg, quantative yield); Rf 0.60 [toluene/EtOAc = (2/1)]; $[\alpha]_D$ +22.5 (c 0.61, $CHCl_3$); Found: m/z(FAB), 1347.7069 $[M+Na]^+$. $C_{79}H_{92}O_{16}Si$ requires: 1347.7053 [M]; ^1H NMR ($CDCl_3$) δ: 0.00 (s, 9 H, Si*Me*$_3$); ^{1}HNMR data were complicated, but it was clear that the reaction had taken place since the H-2' and H-3' signals were shifted to higher field [from 5.07ppm (H-3' on **26**) and 5.76ppm (H-2' on **26**) to over 4.97ppm]; ^{13}C NMR ($CDCl_3$) δ: 1.40, 18.54, 67.38, 68.48, 69.62, 70.94, 72.96, 73.28, 73.70, 74.04, 74.55, 74.59, 74.81, 74.94, 75.18, 79.21, 80.45, 82.24, 83.42, 100.67, 103.26, 103.60, 127.37, 127.75, 128.23, 138.51.

Synthesis of [$^{13}C_6$]-27. Using the procedure described above, reaction of **[$^{13}C_6$]$_3$-26** (44 mg, 28.4 μmol) with sodium methoxide (cat.) and MeOH (10 ml) gave **[$^{13}C_6$]$_3$-27** (31 mg, 82 % yield); TLC mobility in agreement with that of the unlabelled compound; Found: m/z(FAB), 1366 [M+Na]$^+$. $^{13}C_{18}{}^{12}C_{61}H_{92}O_{16}Si$ requires: 1343.

(2-Trimethylsilyl)ethyl *O*-α-D-galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2)[15]

Synthesis of Unlabelled **2**. To a stirred solution of **27** (5 mg, 3.77 μmol) in MeOH (2 ml) was added palladium hydroxide on carbon (20 % Pd, 13 mg) and the mixture was stirred overnight at room temperature under hydrogen. The reaction mixture was filtered and concentrated *in vacuo*. The resulting residue (5 mg) was purified by column chromatography (Sephadex LH-20; methanol) to give **2** (2 mg, 88 %); **2**: Rf 0.18 [EtOAc/EtOH/water (9/2/1)]; $[\alpha]_D$ +42.4 (c 0.76, MeOH); Found: m/z(FAB), 627.2291 [M+Na]$^+$. $C_{23}H_{44}O_{16}SiNa$ requires: 627.2297; ^1H NMR (D_2O) δ: 0.03 (s, 9 H, $OCH_2CH_2Si\mathit{Me}_3$), 4.33 (t, 2H, J = 6.0 Hz, H-5'''), 4.47 (d, 1H, J = 8.1 Hz, H-1), 4.49 (d, 1H, J = 7.5 Hz, H-1'), 4.93 (d, 1H, J = 3.9 Hz, H-1''); ^{13}C NMR (D_2O) δ: -2.61, 17.53, 59.65, 59.99, 60.14, 68.04, 68.20, 68.59, 68.76, 70.43, 70.57, 71.81, 72.56, 74.24, 74.42, 75.06, 77.02, 78.30, 100.00 (C-1''), 101.13 (C-1), 102.96 (C-1').

Synthesis of [$^{13}C_6$]-2. Using the procedure described above, reaction of **[$^{13}C_6$]$_3$-27** (13 mg, 9.68 μmol) with palladium hydroxide on carbon (20% Pd, 18 mg) in MeOH (1 ml) under hydrogen gave **[$^{13}C_6$]$_3$-2** (6 mg, quantitative yield); TLC mobility and carbon-decoupled ^1H NMR data in agreement with that of the unlabelled compound; Found: m/z(FAB), 645.2941 [M+Na]$^+$. $^{13}C_{18}{}^{12}C_5H_{44}O_{16}SiNa$ requires: 645.2901.

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