Pergamon

Tetrahedron 58 (2002) 9403–9411

TETRAHEDRON

Total synthesis of the Glc_3Man *N*-glycan tetrasaccharide

S. C. Ennis,[a] I. Cumpstey,[a] A. J. Fairbanks,[a,*] T. D. Butters,[b] M. Mackeen[b] and M. R. Wormald[b]

[a]*Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, UK*
[b]*Department of Biochemistry, Oxford Glycobiology Institute, University of Oxford, South Parks Road, Oxford OX1 3QU, UK*

Received 20 June 2002; revised 5 September 2002; accepted 26 September 2002

Abstract—The total synthesis of the tetrasaccharide Glcα(1→2)Glcα(1→3)Glcα(1→3)ManαOMe, which corresponds to the terminal tetrasaccharide portion of the glucose terminated arm of the *N*-glycan tetradecasaccharide, was achieved by the use of differentially protected selenoglycosides and thioglycosides as glycosyl donors, all of which possessed non-participating protection of the 2-hydroxyl group. Favourable anomeric stereoselectivity was achieved for the glycosylation reactions by the use of ether as solvent, or co-solvent. Global deprotection by catalytic hydrogenation with palladium acetate in a mixture of ethanol and acetic acid yielded the target tetrasaccharide. © 2002 Published by Elsevier Science Ltd.

1. Introduction

The construction of the oligosaccharide portion of *N*-linked glycoproteins, and the process by which the oligosaccharide is linked to the protein are now well understood.[1] Transfer of a dolichol bound tetradecasaccharide ($Glc_3Man_9GlcNAc_2$) to certain asparagine residues of the nascent glycoprotein is mediated by the enzyme oligosaccharyl transferase (OST) during protein synthesis in the endoplasmic reticulum. Subsequent trimming of this tetradecasaccharide is initially mediated by a series of glycosidases, which remove all the glucose and some of the mannose residues sequentially. Further elaboration to one of the three standard types of *N*-glycan (high mannose, complex or hybrid) is then controlled by a series of glycosyltransferases in the Golgi apparatus.

The glucose residues of the $Glc_3Man_9GlcNAc_2$ tetradecasaccharide have been shown to be part of the recognition motif for OST,[2] as well as forming the substrate for glucosidase I. The solution conformation of the glucose portion of the $Glc_3Man_7GlcNAc_2$ oligosaccharide in water has been determined by NMR analysis.[3] In addition the conformation of the tetrasaccharide $Glc_3ManOPr$ in DMSO has also been determined by NMR and molecular modeling.[4] However, these two experimentally derived conformations are significantly different. Therefore in order to determine whether this difference is (i) due to an incorrect analysis of one of the compounds, (ii) a result of the different solvents used or (iii) a result of the different primary sequences of the saccharides studied, the synthesis of the Glc_3Man tetrasaccharide [Glcα(1→2)Glcα(1→3)Glcα(1→3)ManαOMe **1**] was undertaken. Following successful synthesis, the aim is to study the conformation of the tetrasaccharide in a variety of solvent systems and compare the results to those obtained for the full-length glycan.

This paper details one of the synthetic routes undertaken to allow access to this biologically important tetrasaccharide.[5] The key synthetic consideration was control of anomeric stereochemistry during the respective glycosylation reactions, which necessitated that all glycosyl donors possessed non-participating protecting groups at the 2-position. It was therefore envisaged that all hydroxyl groups would either be protected in the vast majority of cases as benzyl ethers, or in the case of two hydroxyls on the mannose unit as 4,6-benzylidene. This strategy had the advantage that a final global deprotection step by catalytic hydrogenation would allow access to the deprotected tetrasaccharide. Such considerations revealed four potential building blocks which were needed to complete the synthesis of **1** (Fig. 1). Detailed herein are the synthetic routes taken to access these building blocks, the subsequent glycosylation reactions undertaken to construct the tetrasaccharide, and finally the global deprotection step.

2. Results and discussion

2.1. Synthesis of glycosyl donors

Firstly the required *manno* glycosyl acceptor **2** was accessed from methyl mannopyranoside **6**, via a two step reaction sequence by way of the *endo* dibenzylidene derivative **7**, following a modification of the original literature procedure.[6] Thus treatment of **6** with benzaldehyde dimethyl

Keywords: carbohydrates; glycosidation; thioglycosides; glycoproteins.
* Corresponding author. Tel.: +44-1865-275-647; fax: +44-1865-275-674; e-mail: antony.fairbanks@chem.ox.ac.uk

0040–4020/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4020(02)01221-8

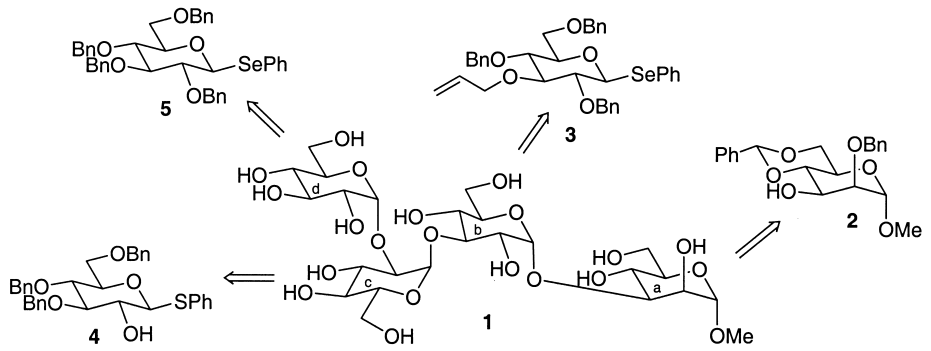
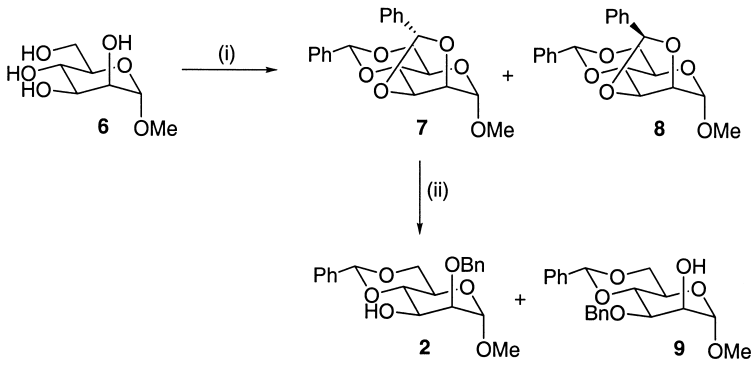


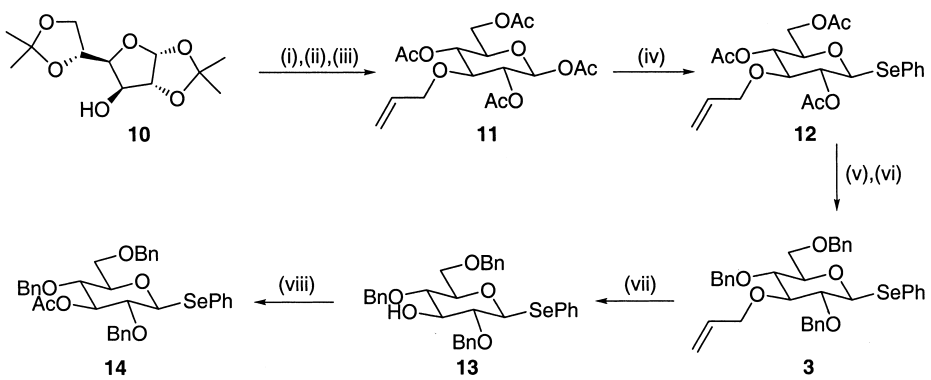
Figure 1.



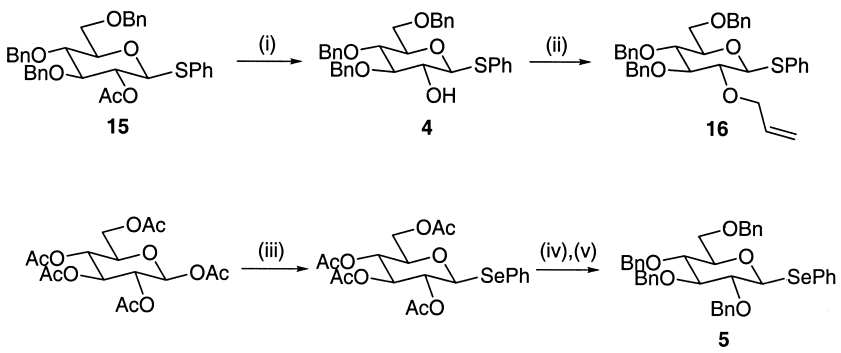
Scheme 1. (i) Benzaldehyde dimethyl acetal, CSA, DMF, 60°C, **7** 53%, **8** 35%, (ii) DIBAL, $PhCH_3$, −40°C, **2** 85%, **9** 10%.

acetal gave a mixture of *endo* and *exo* dibenzylidene derivatives **7** and **8** which were readily separable on a large scale by crystallization. Attempted treatment of the *endo* isomer **7** with $LiAlH_4$ and $AlCl_3$ did indeed give the desired 2-*O*-benzyl product **2** but only in moderate yield, since competitive reduction of the 4,6-benzylidene was also observed. However, treatment of **7** with di-*iso*-butylaluminium hydride (DIBAL)[7] in toluene at −40°C resulted in the formation of the desired product **2** in an excellent yield of 85%, together with a small amount of the undesired regioisomer **9** (10%, Scheme 1).

The required *gluco* glycosyl donor **3** in which the 3-hydroxyl was selectively protected was synthesized from diacetone glucose **10** as follows. Allylation of **10** by treatment with sodium hydride and allyl bromide in DMF was subsequently followed by heating in aqueous sulfuric acid, which effected removal of both acetonide protecting groups and reversion to the pyranose form. Finally complete acetylation by treatment with acetic anhydride and sodium acetate gave the 3-*O*-allyl tetraacetate **11** (45% yield over three steps). Introduction of selenium at the anomeric centre was achieved by treatment with selenophenol and BF_3 etherate to yield selenoglycoside **12** (81% yield). A protecting group swap of acetates for non-participating benzyls, necessary to avoid formation of β-linkages during subsequent glycosylation, was achieved by Zemplen deacetylation followed by complete benzylation with benzyl bromide and sodium hydride in DMF (76% yield over two steps) to yield the desired allyl protected selenoglycoside **3**. In addition, as later glycosylation reactions using **3** as a donor proved to be only moderately successful and displayed poor stereoselectivity (vide infra) the allyl protecting group at OH-3 was also exchanged for



Scheme 2. (i) Allyl bromide, NaH, DMF, rt, (ii) aq. H_2SO_4, Δ, (iii) Ac_2O, NaOAc, 45% over three steps, (iv) PhSeH, $BF_3{\cdot}OEt_2$, DCM, rt, 81%, (v) NaOMe/MeOH, (vi) BnBr, NaH, DMF, rt, 76%, (vii) Ti(*O*-*iso*-Pr)$_4$, $C_6H_{11}MgCl$, THF, rt, 85%, (viii) Ac_2O, pyridine, rt, 91%.



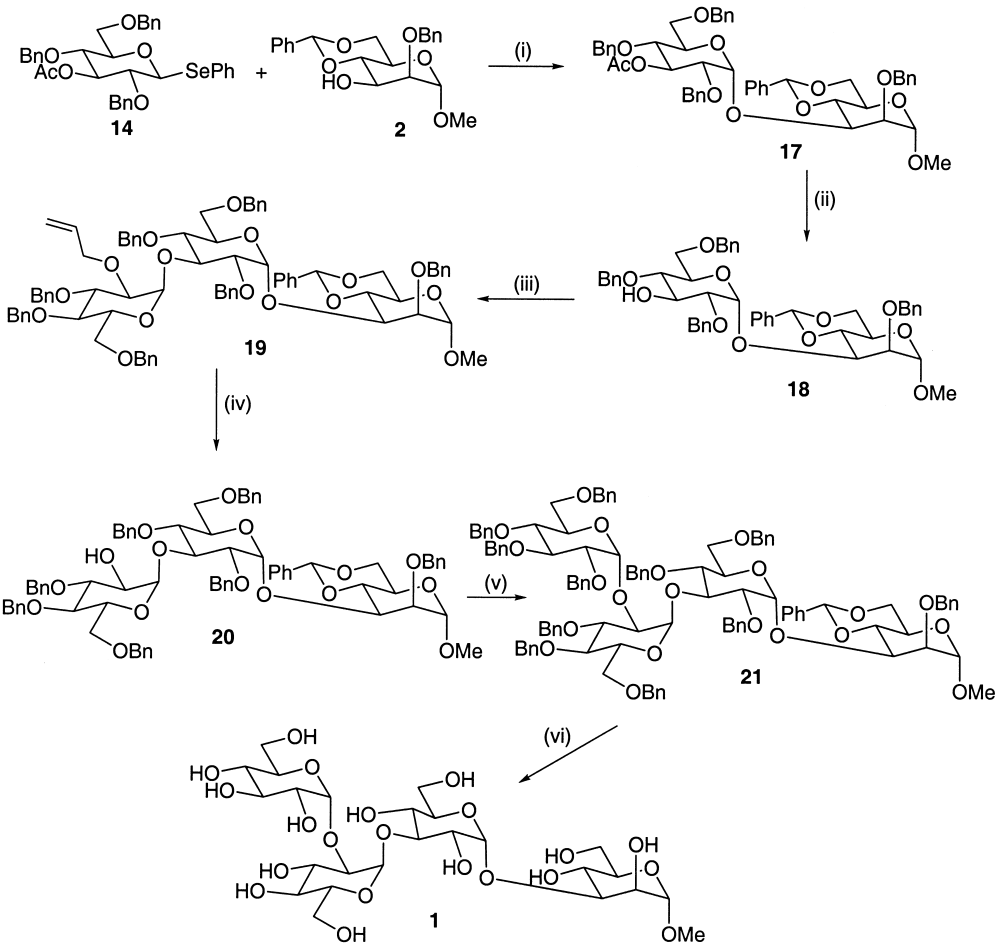
Scheme 3. (i) NaOMe, MeOH, rt, 75%, (ii) allyl bromide, NaH, DMF, 0°C to rt, 95%, (iii) PhSeH, $BF_3{\cdot}OEt_2$, DCM, 74%, (iv) NaOMe, MeOH, (v) BnBr, NaH, DMF, 62% over two steps.

an acetate; removal by treatment of **3** with titanium tetra-*iso*-propoxide and cyclohexyl magnesium chloride[8] produced alcohol **13** (85% yield) and subsequent acetylation with acetic anhydride in pyridine yielded the desired acetate **14** (91% yield, Scheme 2).

The known *gluco* 2-*O*-acetyl thiophenyl glycoside **15** (synthesized from glucose pentacetate via a 1,2-orthoester in five steps as previously described)[9] was deacetylated by treatment with sodium methoxide in methanol to yield the thiophenyl glycosyl acceptor **4**. In addition **4** was subsequently allylated to yield the allyl protected thioglycoside **16**. Finally the perbenzylated selenoglycoside **5** was accessed from glucose pentaacetate in two steps following standard procedures (Scheme 3).[10]

2.2. Glycosylation reactions

With all building blocks in hand construction of the glycosidic linkages was undertaken. The initial convergent strategy involved glycosylation of allyl protected donor **3** with the *manno* glycosyl acceptor **2**. Attempted glycosylation of **2** mediated by *N*-iodosuccinimide (NIS) with catalytic triflic acid in a mixture of dichloroethane (DCE)



Scheme 4. (i) NIS, AgOTf, DCE: diethyl ether, 1:1, 4 Å molecular sieves, rt, 88%, 4:1, α/β, (ii) NaOMe, MeOH, rt, 83%, (iii) **16**, MeOTf, 4 Å molecular sieves, diethyl ether, rt, 80%, 3:1, α/β, (iv) $Ti(O\text{-}iso\text{-}Pr)_4$, $C_6H_{11}MgCl$, THF, rt, 83%, (v) **5**, NIS, AgOTf, 4 Å molecular sieves, DCE/diethyl ether, 1:1, rt, 73%, 3:1, α/β, (vi) $Pd(OAc)_2$, H_2, EtOH/acetic acid, 9:1, rt, 86%.

Table 1. ^{1}H Assignment of Glcα(1→2)Glcα(1→3)Glcα(1→3)ManαOMe **1** in CD_3OD

Sugar residue	H-1	H-2	H-3	H-4	H-5	H-6/H-6′		OMe
d	5.027 (3.7 Hz)	3.436	3.713	3.313	3.981	3.667	3.808	
c	5.391 (3.7 Hz)	3.582	3.794	3.365	4.066	3.698	3.831	
b	5.093 (4.4 Hz)	3.488	3.812	{3.343}	{3.637}	3.728	{3.866}	
a	4.617	4.039	3.758	{*3.521*}	{*3.839*}	{3.769}	{3.816}	3.373

The peaks for $Glc_3ManOMe$ were assigned via COSY unless stated otherwise. Italics indicate peaks assigned via RELAY spectra (unresolved in COSY) and braces ({ }) indicate peaks assigned via TOCSY spectra.

Table 2. ^{1}H Assignment of Glcα(1→2)Glcα(1→3)Glcα(1→3)ManαOMe **1** in D_2O

Sugar residue	H-1	H-2	H-3	H-4	H-5	H-6/H-6′	OMe
d	5.175 (3.7 Hz)	3.602	3.782	3.449	3.965	3.781[a]	
c	5.524 (3.7 Hz)	3.688	*3.835*	3.503	4.058	3.801[a]	
b	5.237 (4.4 Hz)	3.643	*3.908*	3.620	3.810	3.749[a]	
a	4.735	4.086	3.852	{3.656}[b]	{3.775}	{3.832}, {3.907}	3.410

The peaks for $Glc_3ManOMe$ were assigned via COSY unless stated otherwise. Italics indicate peaks assigned via RELAY spectra (unresolved in COSY) and braces ({ }) indicate peaks assigned via TOCSY spectra.
[a] H-6 and H-6′ indistinguishable.
[b] { } from H-2.

and ether as solvent only produced a moderate (53%) yield of disaccharide product, and in addition unfortunately proceeded with no stereoselectivity (α/β mixture, 1:1). However, glycosylation of the acetate protected selenoglycoside **14** with **2** not only produced the desired disaccharide **17** in a much improved 87% yield, but also with the desired anomeric selectivity (α/β mixture, 4:1, Scheme 4).

The convergent synthetic strategy next required glycosylation of the fully armed[11] selenoglycoside **5** by the armed thioglycoside **4**, and therefore relied upon the selective activation of one of the two anomeric leaving groups. Although selective activation of selenoglycosides in the presence of thioglycosides is well precedented,[12] several attempted glycosylation reactions of **4** with **5** under a range of conditions (IDCP, silver triflate/K_2CO_3 and NIS/TfOH) all met with failure. Thin layer chromatography (tlc) indicated the formation of many reaction products, presumably due to competitive activation of **4** and self-condensation. In the face of this disappointment several alternative strategies were considered,[13] and in the light of several possibilities a linear approach was adopted.

Deprotection of the acetate protected disaccharide **17** with sodium methoxide in methanol yielded the disaccharide glycosyl acceptor **18** (83% yield). This was followed by methyl triflate mediated glycosylation of **18** with the allyl protected thioglycoside **16** in ether[14] as the reaction solvent to yield the desired trisaccharide **19** in 80% yield (α/β ratio, 4:1; unfortunately this anomeric mixture was inseparable and therefore the mixture was carried on as such for subsequent transformations). Removal of the allyl protecting group was again achieved by treatment of **19** with titanium tetra-*iso*-propoxide and cyclohexyl magnesium chloride, to yield the trisaccharide acceptor **20** (83% yield). Glycosylation of **20** with the selenoglycoside **5** was achieved by activation with NIS and silver triflate in DCE/ether to yield the protected tetrasaccharide **21** in 73% yield, again as a mixture of anomers but with favourable stereoselectivity (α/β ratio, 3:1). Finally complete deprotection of tetrasaccharide **21** was achieved by catalytic hydrogenation in the presence of palladium acetate in a mixture of ethanol/acetic acid (9:1) to yield the desired deprotected tetrasaccharide **1** (Scheme 4). This completely deprotected material could satisfactorily be purified to homogeneity by high performance anion exchange chromatography (HPAEC PAD). Detailed two-dimensional ^{1}H NMR studies revealed the following assignments (Tables 1 and 2).

3. Summary and conclusion

The total synthesis of the deprotected tetrasaccharide Glcα(1→2)Glcα(1→3)Glcα(1→3)ManαOMe **1** has been successfully achieved. Although a convergent strategy was precluded by difficulties encountered during the selective activation of a selenoglycoside in the presence of a thioglycoside, reversion to a linear approach allowed access to the required tetrasaccharide in good yield. Although favourable ratios of the desired α products could be obtained by the use of ether a solvent or as a co-solvent for the glycosylation reaction, some β products were invariably produced, which were difficult to separate. However, the completely deprotected tetrasaccharide could be purified by high performance anion exchange chromatography.

The solution NMR conformation of the tetrasaccharide is currently being investigated in a number of different solvents and these findings will be compared with those already published for the $Glc_3Man_7GlcNAc_2$ and Glc_3-ManOPr oligosaccharides. In addition, an alternative and completely stereoselective route to this tetrasaccharide using an iterative intramolecular glycosylation strategy is currently under investigation. All of these results will be published in due course.

4. Experimental

4.1. General methods

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Bruker DPX 400 (400 MHz) or on a Bruker AMX 500 (500 MHz) spectrometer. Multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), triplet (t), apparent triplet (at) or doublet of doublet of triplets (ddt). Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Bruker AC 200 (50.3 MHz), or on a Bruker DPX 400 (100.6 MHz) spectrometer. Multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ-scale in parts per million (ppm). All NMR experiments were performed at a probe temperature of 30°C. Two-dimensional phase-sensitive COSY, RELAY and TOCSY spectra, referenced to internal acetone at 2.217 ppm, were used to assign the ^{1}H resonances of **1**. Sequence-specific assignments were made by comparison with reported assignments for glucosylated oligomannose-type oligosaccharides.[3] Infrared spectra were recorded on a Perkin–Elmer 150 Fourier Transform spectrophotometer. Low resolution mass spectra were recorded on a Micromass Platform 1 APCI using atmospheric pressure chemical ionisation (APCI). High resolution mass spectra (electrospray) were performed on a Waters 2790-Micromass LCT electrospray ionisation mass spectrometer, or by the EPSRC Mass Spectrometry Service Centre, Department of Chemistry, University of Wales, Swansea on a MAT900 XLT electrospray ionisation mass spectrometer. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalytical services of the Inorganic Chemistry Laboratory, Oxford. TLC was carried out on Merck Kieselgel 0.22–0.25 mm thickness glass-backed sheets, pre-coated with $60F_{254}$ silica. Plates were developed using 5% w/v ammonium molybdate in 2 M sulfuric acid. Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and reagents were dried and purified before use according to standard procedures; methanol was distilled from sodium hydride, dichloromethane (DCM) was distilled from calcium hydride, pyridine was distilled from calcium hydride and stored over potassium hydroxide and tetrahydrofuran was distilled from a solution of sodium benzophenone ketyl immediately before use. Petrol was distilled between 40 and 60°C before use to remove involatile fractions. HPAEC was carried out on a Dionex BioLC system (Dionex, USA) with pulsed amperometric detection (PAD) on a CarboPac PA-10 column (4×250 mm) connected to a guard column (4×50 mm). The detector settings used were $E1=+0.05$ V, $t_1=0-400$ ms, $E2=+0.75$ V, $t_2=410-600$ ms and $E3=-0.15$ V, $t_3=610-1000$ ms. Elution was performed isocritically using 70 mM NaOH as the solvent at a flow rate of 1 ml/min at 30°C. Before sample injection, the column was eluted with the solvent until detector readings stabilized. The solution of crude $Glc_3ManOMe$ sample was centrifuged for 10 min at 13000 rpm to remove particulate matter which settled to the bottom of the centrifuge tube. Multiple injections (25 μl each) of crude $Glc_3ManOMe$ were performed. The duration of each run was 40 min. Eluates of each run were collected in fractions of 200 μl per micro eppendorf tube. Combined fractions of the product of interest were passed through a desalting column of Dowex AG50W-X12 (H^+) resin and subsequently, concentrated in vacuo.

4.1.1. Methyl (*S*),(*R*)-2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside 7 and methyl(*R*),(*R*)-2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside 8. Benzaldehyde dimethyl acetal (40 ml, 266 mmol) was added to a solution of methyl-α-D-mannopyranoside **6** (20 g, 103 mmol) and camphor sulfonic acid (300 mg, 1.29 mmol) in DMF (220 ml). The resulting solution was heated to 60°C on a rotary evaporator under a pressure of 250 mbar. After 3 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.0) to two products (R_f 0.50 and 0.80). Further benzaldehyde dimethyl acetal (20 ml, 133 mmol) and camphor sulfonic acid (150 mg, 0.65 mmol) was added to the reaction mixture. After 2 h, TLC (petrol/ethyl acetate, 3:1) indicated the formation of a single product (R_f 0.80). The solvent was removed in vacuo, the residue co-evaporated with toluene (50 ml), then dissolved in DCM (300 ml), and washed with saturated sodium bicarbonate solution (150 ml) and brine (150 ml). The organic phase was then dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by recrystallization (ethyl acetate/methanol) to give the *exo*-dibenzylidene compound **8** (13.2 g, 35%) as a white crystalline solid; mp 171–173°C; $[\alpha]_D^{24}=-7.3$ (*c*, 1.1, $CHCl_3$) [lit.[15] mp 176–180°C; $[\alpha]_D^{20}=-1.9$ (*c*, 0.76, $CHCl_3$)]; δ_H (400 MHz, $CDCl_3$) 3.42 (3H, s, OMe), 3.83–3.94 (3H, m, H-4, H-5, H-6), 4.16 (1H, d, $J_{2,3}=5.4$ Hz, H-2), 4.37 (1H, d, $J_{5,6'}=5.4$ Hz, H-6′), 4.64 (1H, dd, $J_{2,3}=5.4$ Hz, $J_{3,4}=7.8$ Hz, H-3), 5.03 (1H, s, H-1), 5.66 (1H, s, O_2CHPh), 6.31 (1H, s, O_2CHPh), 7.26–7.57 (10H, m, Ar-*H*); the mother liquor was concentrated in vacuo and the resulting residue was purified by recrystallization (ethanol) to give the desired *endo*-dibenzylidene derivative **7** (19.5 g, 53%) as a white crystalline solid; mp 94–95°C; $[\alpha]_D^{24}=-58$ (*c*, 1, $CHCl_3$) [lit.[15] mp 95–97°C; $[\alpha]_D^{20}=-63$ (*c*, 1, $CHCl_3$)]; δ_H (400 MHz, $CDCl_3$) 3.44 (3H, s, OMe), 3.73–3.86 (3H, m, H-4, H-5, H-6), 4.31 (1H, d, $J_{2,3}=6.2$ Hz, H-2), 4.33 (1H, dd, $J_{5,6}=4.2$ Hz, $J_{6,6'}=9.8$ Hz, H-6′), 4.49 (1H, at, $J=6.6$ Hz, H-3), 5.10 (1H, s, H-1), 5.54 (1H, s, O_2CHPh), 5.99 (1H, s, O_2CHPh), 7.27–7.57 (10H, m, Ar-*H*).

4.1.2. Methyl 2-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside 2 and methyl 3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside 9. The *endo*-dibenzylidene derivative **7** (100 mg, 0.27 mmol) was dissolved in freshly distilled toluene (5 ml). The solution was stirred under an atmosphere of argon and cooled to −40°C. Di-*iso*-butyl aluminium hydride (0.20 ml, 0.30 mmol of a 1.5 M solution in toluene) was added and the reaction was allowed to reach room temperature. After 20 min TLC (petrol/ethyl acetate, 3:1) indicated partial conversion of starting material (R_f 0.8) to a product (R_f 0.4). Additional di-*iso*-butyl aluminium hydride (0.20 ml, 0.30 mmol of a 1.5 M solution in toluene) was added to the reaction mixture. After 30 min TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.8) to two products (R_f 0.4 and 0.3). Methanol (2 ml) was added dropwise to the reaction

mixture, followed by DCM (50 ml). The organic phase was washed with water (25 ml), brine (25 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by column chromatography (petrol/ethyl acetate, 3:1) to give the 2-*O*-benzyl alcohol **2** (87 mg, 85%) as a white crystalline solid; mp 44–46°C; $[\alpha]_D^{24}=+1.1$ (*c*, 1.0, $CHCl_3$) [lit.[15] mp 42–44°C; $[\alpha]_D^{20}=+2$ (*c*, 1, $CHCl_3$)]; δ_H (400 MHz, $CDCl_3$) 2.40 (1H, d, $J_{3,OH}=7.7$ Hz, O*H*), 3.37 (3H, s, OMe), 3.79–3.87 (3H, m, H-2, H-5, H-6), 3.92 (1H, at, $J=9.7$ Hz, H-4), 4.06–4.11 (1H, m, H-3), 4.27 (1H, dd, $J_{5,6'}=4.1$ Hz, $J_{6,6'}=9.5$ Hz, H-6′), 4.70 (1H, d, $J=11.7$ Hz, OCH_2Ph), 4.76 (1H, d, $J_{1,2}=1.1$ Hz, H-1), 4.77 (1H, d, $J=11.7$ Hz, OCH_2Ph), 5.59 (1H, s, O_2C*H*Ph), 7.27–7.51 (10H, m, Ar-*H*); and the undesired 3-*O*-benzyl alcohol **9** (10 mg, 10%) as a colourless oil; $[\alpha]_D^{24}=+30$ (*c*, 0.4, EtOH) [lit.[15] $[\alpha]_D^{20}=+33$ (*c*, 2, EtOH)]; δ_H (400 MHz, $CDCl_3$) 2.67 (1H, s, O*H*), 3.39 (3H, s, OMe), 3.79–3.93 (3H, m, H-3, H-5, H-6), 4.06 (1H, d, $J_{2,3}=3.0$ Hz, H-2), 4.10 (1H, at, $J=9.2$ Hz, H-4), 4.29 (1H, dd, $J_{5,6'}=4.1$ Hz, $J_{6,6'}=9.5$ Hz, H-6′), 4.72 (1H, d, $J=11.6$ Hz, OCH_2Ph), 4.78 (1H, s, H-1), 4.86 (1H, d, $J=11.6$ Hz, OCH_2Ph), 5.62 (1H, s, O_2C*H*Ph), 7.27–7.53 (10H, m, Ar-*H*).

4.1.3. Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-1-seleno-β-D-glucopyranoside 12. Selenophenol (4.1 ml, 38.7 mmol) and boron trifluoride etherate (2.5 ml, 19.4 mmol) were added to a stirred solution of peracetate **11** (5.0 g, 12.9 mmol) in DCM (30 ml), under argon at room temperature. After 3 h, TLC (petrol/ethyl acetate, 1:1) indicated complete conversion of starting material (R_f 0.5) to a major product (R_f 0.6). DCM (100 ml) was added to the reaction mixture, which was then washed with water (100 ml), brine (100 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by recrystallization from ether/petrol to give the selenoglycoside **12** (5.1 g, 81%) as a white crystalline solid; (found C, 51.90; H, 5.28. $C_{21}H_{26}O_8Se$ requires C, 51.84; H, 5.39%); mp 113–115°C; $[\alpha]_D^{20}=-28.3$ (*c*, 1.0, $CHCl_3$); ν_{max} (KBr Disc) 1740 (CO_2CH_3), 1641 (C=C) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.12, 2.18 (9H, 2×s, 3×CH_3CO_2), 3.61–3.66 (2H, m, H-3, H-5), 4.10–4.13 (2H, m, CH_2CH=CH$_2$), 4.20–4.21 (2H, m, H-6, H-6′), 4.87 (1H, d, $J_{1,2}=10.2$ Hz, H-1), 5.04 (1H, dd, $J_{2,3}=8.4$ Hz, H-4), 5.08 (1H, dd, $J_{1,2}=10.2$ Hz, $J_{2,3}=8.4$ Hz, H-2), 5.18 (1H, dd, $J_{gem}=2.7$ Hz, $J_Z=10.3$ Hz, CH=CH$_E$H$_Z$), 5.24 (1H, dd, $J_{gem}=2.9$ Hz, $J_E=17.2$ Hz, CH=CH$_E$H$_Z$), 5.80 (1H, ddt, $J=4.8$ Hz, $J_Z=10.3$ Hz, $J_E=17.2$ Hz, C*H*=CH$_2$), 7.31–7.67 (5H, m, Ar-H); δ_C (50.3 MHz, $CDCl_3$) 20.6, 20.7, 21.0 (3×q, 3×CH_3CO_2), 69.5, 72.2, 77.4, 81.2, 81.7 (5×d, C-1, C-2, C-3, C-4, C-5), 62.5, 73.3 (2×t, C-6, OCH$_2$CH=CH$_2$), 117.2 (t, OCH$_2$CH=*C*H$_2$), 128.1, 128.5, 129.4, 134.4, 134.9 (1×s, 4×d, Ar-C), 169.6 (s, 2×Me*C*O$_2$), 171.0 (s, Me*C*O$_2$); *m*/*z* (APCI$^+$) 509 (M+Na$^+$, 80%), 293 (50), 169 (50), 109 (100).

4.1.4. Phenyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-seleno-β-D-glucopyranoside 3. Sodium (430 mg) was dissolved in methanol (20 ml) and the mixture was added to a stirred solution of acetylated selenoglycoside **12** (4.4 g, 9.0 mmol) in methanol (80 ml) at 0°C under argon. After 1 h 45 min, TLC (petrol/ethyl acetate, 1:2) indicated complete conversion of starting material (R_f 0.8) to a major product (R_f 0.4). 1 M HCl (20 ml) was added to the reaction mixture, the product was then extracted with DCM (2×200 ml), organic phase dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was dissolved in DMF (80 ml), benzyl bromide (4.8 ml, 40.5 mmol) and sodium hydride (60% in mineral oil, 2.16 g, 54 mmol) were then added. An air condenser was attached to the flask and the reaction mixture was stirred at room temperature. After 18 h, TLC (petrol/ethyl acetate, 4:1) indicated complete conversion of starting material (R_f 0.0) to a major product (R_f 0.6). Methanol (50 ml) was added portionwise in order to quench the reaction. DCM (200 ml) was added, the organic phase was washed with water (150 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 6:1) to give the benzylated selenoglycoside **3** (4.3 g, 76%) as a white crystalline solid; (found C, 68.70; H, 6.07. $C_{36}H_{38}O_5Se$ requires C, 68.55; H, 6.08%); mp 62–63°C (petrol/ether); $[\alpha]_D^{20}=-16.5$ (*c*, 1.2, $CHCl_3$); ν_{max} (KBr Disc) 1641 (C=C) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 3.49–3.52 (1H, m, H-5), 3.52 (1H, at, $J=9.5$ Hz, H-2), 3.59 (1H, at, $J=8.8$ Hz, H-4), 3.66 (1H, at, $J=9.2$ Hz, H-3), 3.79 (1H, dd, $J_{5,6}=4.4$ Hz, $J_{6,6'}=10.9$ Hz, H-6), 3.83 (1H, dd, $J_{5,6'}=1.9$ Hz, $J_{6,6'}=10.9$ Hz, H-6′), 4.37–4.44 (2H, m, CH_2-CH=CH$_2$), 4.60 (1H, d, $J=12.5$ Hz, OCH_2Ph), 4.64 (1H, d, $J=11.4$ Hz, OCH_2Ph), 4.67 (1H, d, $J=12.5$ Hz, OCH_2Ph), 4.78 (1H, d, $J=10.2$ Hz, OCH_2Ph), 4.87 (1H, d, $J=11.4$ Hz, OCH_2Ph), 4.89 (1H, d, $J=10.2$ Hz, OCH_2Ph), 4.88 (1H, d, $J_{1,2}=9.7$ Hz, H-1), 5.23 (1H, dd, $J_Z=10.4$ Hz, $J_{gem}=1.3$ Hz, CH=CH$_E$H$_Z$), 5.36 (1H, dd, $J_E=17.2$ Hz, $J_{gem}=1.5$ Hz, CH=CH$_E$H$_Z$), 6.02 (1H, ddt, $J=5.7$ Hz, $J_Z=10.8$ Hz, $J_E=17.2$ Hz, C*H*=CH$_2$), 7.24–7.76 (20H, m, Ar-H); δ_C (50.3 MHz, $CDCl_3$) 69.0, 73.5, 74.6, 75.2, 75.4 (5×t, C-6, O*C*H$_2$CH=CH$_2$, 3×O*C*H$_2$Ph), 77.7, 80.2, 81.3, 83.1, 86.6 (5×d, C-1, C-2, C-3, C-4, C-5), 117.2 (t, OCH$_2$CH=*C*H$_2$), 127.8, 127.9, 128.1, 128.3, 128.7, 129.3, 134.8, 135.2 (8×d, Ar-CH), 138.3, 138.4, 138.6 (3×s, Ar-*C*); *m*/*z* (APCI$^+$) 654 (M+Na$^+$, 100%).

4.1.5. Phenyl 2,4,6-tri-*O*-benzyl-1-seleno-β-D-glucopyranose 13. Titanium tetra-*iso*-propoxide (1.2 ml, 4.0 mmol) was added to a stirred solution of the allyl protected selenoglycoside **3** (2.5 g, 4.0 mmol) in THF (50 ml) at room temperature under argon. Cyclohexylmagnesium chloride (8.0 ml, 16.0 mmol) was added to the reaction mixture over a period of 1 h (via a syringe pump). After a further 1 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.5), to a major product (R_f 0.4). Water (50 ml) was added to the reaction mixture, the product was then extracted with ether (3×150 ml), the organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 4:1) to give the alcohol **13** (2.0 g, 85%) as a colourless oil; (found C, 66.72; H, 6.02. $C_{33}H_{34}O_5Se$ requires C, 67.10; H, 5.81%); $[\alpha]_D^{26}=-13.6$ (*c*, 0.96, $CHCl_3$); ν_{max} (film, $CHCl_3$) 3435 (OH) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.41 (1H, at, $J=9.7$ Hz, H-2), 3.47 (1H, ddd, $J_{4,5}=9.7$ Hz, $J_{5,6}=4.2$ Hz, $J_{5,6'}=1.9$ Hz, H-5), 3.56 (1H, at, $J=9.6$ Hz, H-4), 3.74–3.78 (2H, m, H-3, H-6), 3.82 (1H, dd, $J_{5,6'}=1.8$ Hz, $J_{6,6'}=11.0$ Hz, H-6′), 4.55 (1H, d, $J=12.0$ Hz, OCH_2Ph), 4.63 (1H, d, $J=11.2$ Hz, OCH_2Ph), 4.65 (1H, d, $J=12.0$ Hz, OCH_2Ph), 4.69 (1H, d, $J=10.8$ Hz, OCH_2Ph), 4.79 (1H, d,

J=11.2 Hz, OCH_2Ph), 4.85 (1H, d, $J_{1,2}$=9.8 Hz, H-1), 4.93 (1H, d, J=10.8 Hz, OCH_2Ph), 7.20–7.76 (20H, m, Ar-H); δ_C (100.6 MHz, $CDCl_3$) 69.0, 73.4, 74.7, 75.0 (4×t, C-6, 3×OCH_2Ph), 77.4, 78.7, 79.9, 81.2, 82.6 (5×d, C-1, C-2, C-3, C-4, C-5), 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 128.5, 128.6, 129.0 (10×d, Ar-*C*H), 128.7, 138.1, 138.2 (3×s, Ar-*C*); *m/z* (ES^+) 608 ($M+NH_4^+$, 100%).

4.1.6. Phenyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-1-seleno-β-D-glucopyranoside 14. Acetic anhydride (6 ml, 64 mmol) was added to a stirred solution of the alcohol **13** (1.9 g, 3.2 mmol) in pyridine (50 ml) at room temperature, under argon. After 20 h, TLC (toluene/acetone, 9:1) indicated complete conversion of starting material (R_f 0.6) to a major product (R_f 0.7). The reaction mixture was concentrated in vacuo. The resulting residue was dissolved in DCM (150 ml), washed with 1 M HCl (100 ml), the organic phase dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 4:1) to give the acetate **14** (1.8 g, 91%) as a white crystalline solid; (found C, 66.29; H, 5.65. $C_{35}H_{36}O_6Se$ requires C, 66.40; H, 5.74%); mp 78–79°C (ethanol); $[\alpha]_D^{20}$=−21.2 (*c*, 1.3, $CHCl_3$); ν_{max} (KBr disc) 1740 (CH_3CO_2) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.83 (3H, s, CH_3CO$_2$), 3.48–3.54 (2H, m, H-2, H-5), 3.69 (1H, at, J=9.6 Hz, H-4), 3.72–3.81 (2H, m, H-6, H-6′), 4.52 (1H, d, J=10.5 Hz, OCH_2Ph), 4.54 (2H, s, OCH_2Ph), 4.55 (1H, d, J=11.9 Hz, OCH_2Ph), 4.65 (1H, d, J=11.9 Hz, OCH_2Ph), 4.82 (1H, d, J=10.5 Hz, OCH_2Ph), 4.90 (1H, d, $J_{1,2}$=9.8 Hz, H-1), 5.27 (1H, at, J=9.3 Hz, H-3), 7.18–7.71 (20H, m, Ar-H); δ_C (50.3 MHz, $CDCl_3$) 20.9 (q, *C*H_3CO$_2$), 68.6, 73.5, 74.5, 74.6 (4×t, C-6, 3×OCH_2Ph), 75.9, 77.2, 79.5, 80.0, 82.8 (5×d, C-1, C-2, C-3, C-4, C-5), 128.0, 128.1, 128.2, 128.4, 128.7, 129.3, 134.7 (7×d, Ar-*C*H), 138.0, 138.1, 138.4 (3×s, Ar-*C*), 170.2 (s, CH_3CO_2); *m/z* (probe CI^+) 650 ($M+NH_4^+$, 15%), 525 (15), 324 (15), 216 (20), 181 (25), 108 (55), 91 (100).

4.1.7. Phenyl 3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside 4. Sodium (184 mg) was dissolved in methanol (20 ml) and the mixture was added to a stirred solution of the acetate **15** (3.5 g, 6.0 mmol) in methanol (60 ml) at 0°C under argon. The reaction was allowed to reach room temperature. After 1.5 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.8) to a major product (R_f 0.7). DCM (150 ml) was added to the reaction mixture, which was then washed with 1 M HCl (100 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by recrystallization (petrol/ether) to give the alcohol **4** (2.4 g, 75%) as a white crystalline solid; mp 72–74°C; $[\alpha]_D^{22}$=−9.8 (*c*, 1.12, $CHCl_3$) [lit.[16] mp 71–73°C; $[\alpha]_D^{25}$=−11.5 (*c*, 1.4, $CHCl_3$)]; δ_H (500 MHz, $CDCl_3$) 2.41 (1H, d, $J_{OH,2}$=2.1 Hz, O*H*), 3.48–3.56 (2H, m, H-2, H-5), 3.58–3.64 (2H, m, H-3, H-4), 3.75 (1H, dd, $J_{5,6}$=4.5 Hz, $J_{6,6'}$=11.0 Hz, H-6), 3.80 (1H, dd, $J_{5,6'}$=1.9 Hz, $J_{6,6'}$=11.0 Hz, H-6′), 4.51 (1H, d, $J_{1,2}$=9.6 Hz, H-1), 4.56 (1H, d, J=12.1 Hz, OCH_2Ph), 4.60 (1H, d, J=9.6 Hz, OCH_2Ph), 4.62 (1H, d, J=12.1 Hz, OCH_2Ph), 4.83 (1H, d, J=9.6 Hz, OCH_2Ph), 4.85 (1H, d, J=10.6 Hz, OCH_2Ph), 4.91 (1H, d, J=10.6 Hz, OCH_2Ph), 7.12–7.60 (20H, m, Ar-H).

4.1.8. Phenyl 2-*O*-allyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside 16. A solution of the alcohol **4** (2.6 g, 4.8 mmol) and allyl bromide (0.83 ml, 9.6 mmol) in DMF (40 ml) was stirred at 0°C under argon. Sodium hydride (383 mg, 9.58 mmol, 60% in mineral oil) was added and the reaction was allowed to reach room temperature. After 2 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.7) to a major product (R_f 0.8). Methanol (5 ml) was added portionwise and the solution was co-evaporated with toluene in vacuo. The resulting residue was dissolved in DCM (200 ml), washed with water (50 ml) and brine (50 ml). The organic phase dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (petrol/ethyl acetate, 4:1) to give the allyl protected thioglycoside **16** (2.7 g, 95%) as a white crystalline solid; (found C, 74.59; H, 6.55. $C_{36}H_{38}O_5S$ requires C, 74.20; H, 6.57%; HRMS+ NH_4^+: 600.2784. $C_{36}H_{38}O_5S$ requires: 600.2793); mp 51–52°C (petrol/ethyl acetate); $[\alpha]_D^{25}$=−17.3 (*c*, 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.39 (1H, at, J=9.5 Hz, H-2), 3.50–3.53 (1H, m, H-5), 3.62 (1H, at, J=9.2 Hz, H-4), 3.67 (1H, at, J=8.6 Hz, H-3), 3.72 (1H, dd, $J_{5,6}$=4.7 Hz, $J_{6,6'}$=10.8 Hz, H-6), 3.79 (1H, dd, $J_{5,6'}$=1.8 Hz, $J_{6,6'}$=10.9 Hz, H-6′), 4.26 (1H, dd, J=6.0 Hz, J_{gem}=11.9 Hz, C*H*H′CH=CH$_2$), 4.39 (1H, dd, J=5.6 Hz, J_{gem}=11.8 Hz, CH*H*′CH=CH$_2$), 4.54 (1H, d, J=11.9 Hz, OCH_2Ph), 4.59 (1H, d, J=11.7 Hz, OCH_2Ph), 4.61 (1H, d, J=11.9 Hz, OCH_2Ph), 4.62 (1H, d, $J_{1,2}$=10.0 Hz, H-1), 4.83 (1H, d, J=11.7 Hz, OCH_2Ph), 4.84 (1H, d, J=10.8 Hz, OCH_2Ph), 4.92 (1H, d, J=10.8 Hz, OCH_2Ph), 5.20 (1H, dd, J_{gem}=1.2 Hz, J_Z=10.5 Hz, CH=CH$_E$*H*$_Z$), 5.30 (1H, dd, J_{gem}=1.6 Hz, J_E=17.2 Hz, CH=CH$_E$*H*$_Z$), 5.99 (1H, ddt, J=5.8 Hz, J_Z=10.5 Hz, J_E=17.1 Hz, C*H*=CH$_2$), 7.20–7.60 (20H, m, Ar-H); δ_C (50 MHz, $CDCl_3$) 69.5, 71.6, 73.9, 74.7, 75.0 (5×t, C-6, 3×OCH_2Ph, OCH_2CH=CH$_2$), 75.3, 78.1, 79.5, 87.2, 87.9 (5×d, C-1, C-2, C-3, C-4, C-5), 117.9 (t, OCH$_2$CH=*C*H$_2$), 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.7, 128.8, 128.9, 129.4, 132.5, 135.1 (12×d, Ar-CH, OCH$_2$*C*H=CH$_2$), 136.6, 138.5, 138.7, 138.9 (4×s, Ar-C); *m/z* ($APCI^+$) 606 ($M+Na^+$, 80%), 181 (45), 132 (100), 121 (80).

4.1.9. Methyl (3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidine-α-D-mannopyranoside 17. A solution of the selenoglycoside **14** (140 mg, 0.22 mmol), the alcohol **2** (60 mg, 0.16 mmol) and 4 Å molecular sieves (powdered, 400 mg) in DCE/ether (1:1, 1.5 ml) was stirred at room temperature, under argon, for 2 h. NIS (107 mg, 0.44 mmol) and silver triflate (5 mg, 0.02 mmol) were then added to the reaction mixture. After 5 min, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting materials (R_f 0.7 and 0.3) to a major product (R_f 0.3). Collidine (1 ml) was then added in order to quench the reaction. Ether (150 ml) was added, the reaction mixture was then filtered through Celite®, and the filtrate was washed with 10% aq. sodium thiosulfite (50 ml). The organic phase was then dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 4:1) to give the disaccharide **17** (117 mg, 88%, 4:1 α/β mixture) as a colourless oil; (HRMS+NH_4^+: 864.3959. $C_{50}H_{54}O_{12}$ requires: 864.3965); α anomer (small quantity separated by flash column chromatography (toluene/acetone, 20:1);

$[\alpha]_D^{20}$=+44.3 (*c*, 1.13, $CHCl_3$); ν_{max} (thin film) 1741 (CH_3CO_2) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.98 (3H, s, O_2CH_3), 3.34 (1H, s, OMe), 3.36 (1H, dd, $J_{1,2}$=3.7 Hz, $J_{2,3}$=10.2 Hz, H-2_b), 3.58–3.89 (7H, m), 4.12 (1H, d, *J*=13.0 Hz, OCH_2Ph), 4.22–4.28 (1H, m, H-5_a), 4.34–4.35 (2H, m, H-3_a, H-$6'_a$), 4.38 (1H, d, *J*=11.3 Hz, OCH_2Ph), 4.43 (1H, d, *J*=12.0 Hz, OCH_2Ph), 4.49 (1H, d, *J*=13.0 Hz, OCH_2Ph), 4.52 (1H, d, *J*=11.3 Hz, OCH_2Ph), 4.61 (1H, d, *J*=12.0 Hz, OCH_2Ph), 4.73 (1H, d, $J_{1,2}$=1.5 Hz, H-1_a), 4.87 (1H, d, *J*=12.5 Hz, OCH_2Ph), 4.92 (1H, d, *J*=12.5 Hz, OCH_2Ph), 5.47 (1H, s, O_2CHPh), 5.53 (1H, d, $J_{1,2}$=3.6 Hz, H-1_b), 5.62 (1H, at, *J*=9.7 Hz, H-3b), 6.82–7.47 (25H, m, Ar-*H*); δ_C (100.6 MHz, $CDCl_3$) 21.1 (q, CH_3CO_2), 45.8 (q, OCH_3), 63.9, 70.4, 72.8, 73.1, 75.5, 75.8, 76.5, 76.9 (8×d, C-2_a, C-3_a, C-4_a, C-5_a, C-2_b, C-3_b, C-4_b, C-5_b), 68.2, 68.9, 69.9, 76.7, 77.0, 77.3 (6×t, C-6_a, C-6_b, 4×OCH_2Ph), 96.6, 100.5, 102.4 (3×d, C-1_a, C-1_b, O_2CHPh), 126.4, 127.0, 127.2, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.5, 129.3 (12×d, Ar-*C*H), 137.7, 137.9, 138.0 (3×s, Ar-*C*), 169.9 (s, CH_3CO_2); *m*/*z* (ES^+) 864 ($M+NH_4^+$, 100%).

4.1.10. Methyl (2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidine-α-D-mannopyranoside 18. Sodium (12 mg) was dissolved in methanol (2 ml) and the mixture was added to a stirred solution of the acetylated disaccharide **17** (235 mg, 0.3 mmol, α/β 4:1) in methanol (3 ml) at 0°C, under argon. The reaction was then allowed to reach room temperature. After 4 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.5) to a major product (R_f 0.45). DCM (100 ml) was then added. The organic phase was washed with 1 M HCl (20 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 3:1) to give the alcohol **18** (201 mg, 83%, α/β 4:1) as a colourless oil; (α anomer) (HRMS+NH_4^+: 822.3838. $C_{48}H_{52}O_{11}$ requires: 822.3853); $[\alpha]_D^{26}$=+73.4 (*c*, 0.41, $CHCl_3$); ν_{max} (thin film) 3492 (OH) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 3.32 (1H, dd, $J_{1,2}$=3.7 Hz, $J_{2,3}$=9.5 Hz, H-2_b), 3.33 (3H, s, OMe), 3.55 (1H, at, *J*=9.5 Hz, H-4_b), 3.67–3.79 (2H, m, H-6_b, H-$6'_b$), 3.80–3.87 (4H, m, H-2_a, H-5_a, H-6_a, H-5_b), 4.13 (1H, at, *J*=9.1 Hz, H-3_b), 4.19 (1H, d, *J*=12.0 Hz, OCH_2Ph), 4.22–4.24 (1H, m, H-$6'_a$), 4.31 (1H, at, *J*=9.7 Hz, H-4_a), 4.38 (1H, dd, $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.0 Hz, H-3_a), 4.46 (1H, d, *J*=12.1 Hz, OCH_2Ph), 4.51 (1H, d, *J*=11.3 Hz, OCH_2Ph), 4.54 (1H, d, *J*=12.0 Hz, OCH_2Ph), 4.59 (1H, d, *J*=12.0 Hz, OCH_2Ph), 4.70 (1H, s, H-1_a), 4.75 (1H, d, *J*=12.0 Hz, OCH_2Ph), 4.90 (1H, d, *J*=11.3 Hz, OCH_2Ph), 4.91 (1H, d, *J*=11.9 Hz, OCH_2Ph), 5.41 (1H, s, O_2CHPh), 5.52 (1H, d, $J_{1,2}$=3.5 Hz, H-1_b), 6.99–7.43 (25H, m, Ar-*H*); δ_C (100.6 MHz, $CDCl_3$) 55.8 (q, OMe), 63.9, 70.4, 73.0, 73.4, 77.2, 75.6, 79.5, 79.6 (8×d, C-2_a, C-3_a, C-4_a, C-5_a, C-2_b, C-3_b, C-4_b, C-5_b), 68.7, 68.9, 70.3, 73.5, 73.8, 74.4 (5×t, C-6_a, C-6_b, 4×OCH_2Ph), 96.4, 100.6, 102.5 (3×d, C-1_a, C-1_b, O_2CHPh), 126.3, 126.4, 127.4, 127.5, 127.6, 127.8, 127.9, 128.0, 128.1, 128.3, 128.5, 128.6, 129.3 (13 x d, Ar-*C*H), 137.4, 137.8, 138.0, 138.1, 138.6 (5×s, Ar-*C*); *m*/*z* (ES^+) 822 ($M+NH_4^+$, 100%).

4.1.11. Methyl (2-*O*-allyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidine-α-D-mannopyranoside 19. A solution of the thioglycoside **16** (175 mg, 0.30 mmol), the disaccharide **18** (118 mg, 0.15 mmol) and 4 Å molecular sieves (powdered, 200 mg) in ether (4 ml) was stirred at room temperature, under argon. After 30 min, methyl triflate (0.03 ml, 0.30 mmol) was added to the reaction mixture. After 18 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting materials (R_f 0.8 and 0.3) to a major product (R_f 0.4). Triethylamine (0.5 ml) was then added to the reaction and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was then filtered through Celite® and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 3:1) to give the trisaccharide **19** (155 mg, 80%, inseparable 3:1 α/β mixture) as a colourless oil; (HRMS+NH_4^+: 1294.6098. $C_{78}H_{84}O_{16}$ requires: 1294.6103); (α anomer) δ_H (400 MHz, $CDCl_3$) 3.12–3.21 (3H, m), 3.34 (3H, s, OCH_3), 3.43 (1H, dd, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.8 Hz, H-2_c), 3.48 (1H, dd, $J_{1,2}$=3.7 Hz, $J_{2,3}$=9.9 Hz, H-2_b), 3.62–5.05 (31H, m), 4.73 (1H, d, $J_{1,2}$=1.7 Hz, H-1_a), 5.35 (1H, s, O_2CHPh), 5.53 (1H, d, $J_{1,2}$=3.6 Hz, H-1_c), 5.62 (1H, d, $J_{1,2}$=3.7 Hz, H-1_b), 5.62–5.68 (1H, m, C*H*=CH_2), 7.03–7.48 (40H, m, Ar-*H*); δ_C (100 MHz, $CDCl_3$) 54.8 (q, OCH_3), 64.0, 69.8, 70.5, 71.9, 75.5, 76.4, 76.7, 77.9, 78.6, 79.2, 80.0, 82.4 (12×d, C-2_a, C-3_a, C-4_a, C-5_a, C-2_b, C-3_b, C-4_b, C-5_b, C-2_c, C-3_c, C-4_c, C-5_c), 68.9, 73.1, 73.4, 73.5, 73.6, 74.4, 77.2, 77.3 (8×t, C-6_a, C-6_b, C-6_c, OCH_2CH=CH_2, 7×OCH_2Ph), 96.0, 97.8, 100.8, 102.4 (4×d, C-1_a, C-1_b, C-1_c, O_2CHPh), 117.6 (t, OCH$_2$CH=CH_2), 126.3, 126.6, 127.3, 127.4, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.6, 129.1 (13×d, Ar-CH), 134.5, 137.8, 138.0, 138.9 (4×s, Ar-C); *m*/*z* (ES^+) 1295 ($M+NH_4^+$, 100%).

4.1.12. Methyl (3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidine-α-D-mannopyranoside 20. Titanium tetra-*iso*-propoxide (0.05 ml, 0.18 mmol) was added to a stirred solution of allylated trisaccharide **19** (225 mg, 0.18 mmol) in THF (10 ml) at room temperature under argon. Cyclohexylmagnesium chloride (0.4 ml, 0.81 mmol) was added to the reaction mixture over a period of 1 h (via a syringe pump). After a further 1 h, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting material (R_f 0.4), to a major product (R_f 0.3). Water (50 ml) was added to the reaction mixture, the product was then extracted with ether (3×50 ml), the organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (petrol/ethyl acetate, 3.5:1) to give the deprotected trisaccharide **20** (189 mg, 83%, as 3:1 α/β mixture) as a colourless oil; (HRMS+NH_4^+: 1254.5798. $C_{75}H_{80}O_{16}$ requires: 1254.5790); $[\alpha]_D^{25}$=+73.1 (*c*, 1.1, $CHCl_3$); ν_{max} (film) 3446 (OH) cm^{-1}; (α anomer) δ_H (400 MHz, $CDCl_3$) 2.37 (1H, d, $J_{OH,2}$=7.1 Hz, O*H*), 3.13–3.23 (2H, m), 3.36 (3H, s, OCH_3), 3.42 (1H, dd, $J_{1,2}$=3.7 Hz, $J_{2,3}$=9.7 Hz, H-2_b), 3.44–4.96 (30H, m), 5.36 (1H, s, O_2CHPh), 5.51 (1H, d, $J_{1,2}$=2.9 Hz, H-1_c), 5.61 (1H, d, $J_{1,2}$=3.7 Hz, H-1_b), 7.06–7.47 (40H, m, Ar-H); (β anomer) (400 MHz, $CDCl_3$) 5.10 (1H, d, $J_{1,2}$=10.2 Hz, H-1_c); δ_C (125 MHz, $CDCl_3$) 54.8 (q, OCH_3), 63.8, 67.9, 68.3, 68.9, 70.1, 70.3, 70.5, 72.0, 72.8, 73.2, 73.6, 74.2, 74.3, 75.3, 76.1, 76.5, 77.5, 78.6, 79.9, 83.3 (12×*C*H, 10×CH_2), 96.1, 98.6, 100.7, 102.4 (4×d, C-1_a, C-1_b, C-1_c, O_2CHPh), 126.3, 126.4, 127.1, 127.2, 127.3, 127.4, 127.5, 127.7, 127.8, 127.9, 128.0, 128.1,

128.2, 128.3, 128.4, 128.5 (16×d, Ar-CH), 137.2, 137.6, 137.8, 137.9, 137.9, 138.0, 138.7, 138.8 (8×s, Ar-C); *m/z* (ES^+) 1255 ($M+NH_4^+$, 100%).

4.1.13. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidine-α-D-mannopyranoside 21. A solution of the selenoglycoside **5** (129 mg, 0.19 mmol), the trisaccharide **20** (120 mg, 0.097 mmol) and 4 Å molecular sieves (powdered, 200 mg) in DCE/ether (1:1, 2 ml) was stirred at room temperature, under argon, for 30 min. NIS (51 mg, 0.21 mmol) and silver triflate (5 mg, 0.02 mmol) was then added to the reaction mixture. After 5 min, TLC (petrol/ethyl acetate, 3:1) indicated complete conversion of starting materials (R_f 0.8 and 0.3) to a major product (R_f 0.4). Collidine (0.5 ml) was then added in order to quench the reaction. DCM (50 ml) was added, the reaction mixture filtered through Celite® and the filtrate washed with 10% aq. sodium thiosulfite (30 ml). The organic phase was then dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by column chromatography (petrol/ethyl) acetate, 4:1) to give the protected tetrasaccharide **21** (127 mg, 73% as a 3:1 α/β inseparable mixture) as a colourless oil; (α anomer selected data) (400 MHz, $CDCl_3$) 3.32 (3H, s, OCH_3), 5.15 (1H, d, $J_{1,2}$=3.6 Hz, H-1), 5.35 (1H, s, O_2CHPh), 5.60 (1H, d, $J_{1,2}$=3.8 Hz, H-1), 5.78 (1H, d, $J_{1,2}$=3.2 Hz, H-1), 7.00–7.45 (60H, m, Ar*H*); *m/z* (electrospray) Isotope distribution $M+NH_4^+$: 1781.0 (18), 1779.9 (32), 1778.9 (72), 1777.9 (100), 1776.9 (98). $C_{109}H_{114}O_{21}$ requires: 1780.8 (11), 1779.8 (29), 1778.8 (67), 1777.8 (100), 1776.8 (83%).

4.2. Methyl (α-D-glucopyranosyl)-(1→2)-(α-D-glucopyranosyl)-(1→3)-(α-D-glucopyranosyl)-(1→3)-α-D-mannopyranoside 1

Palladium acetate (20 mg) was added to a solution of the protected tetrasaccharide **21** (40 mg, 0.022 mmol) in ethanol (2.25 ml) and glacial acetic acid (0.25 ml). The resulting solution was degassed and stirred at room temperature under an atmosphere of hydrogen. After 18 h, TLC (CMAW) indicated complete conversion of starting material (R_f 0.9) to a major product (R_f 0.1). The reaction mixture was filtered through Celite® and concentrated in vacuo to give the deprotected tetrasaccharide **1** (13 mg, 86%) as a crude colourless oil; (HRMS+NH_4^+: 698.2716. $C_{25}H_{44}O_{21}$ requires: 698.2719); $[\alpha]_D^{25}$=+84 (*c*, 0.2, MeOH); *m/z* (electrospray) 698 ($M+NH_4^+$, 100%). See Tables 1 and 2 for NMR data.

Acknowledgements

We gratefully acknowledge the EPSRC (Quota Awards to S. C. E. and I. C.) for financial support. We also gratefully acknowledge the use of the EPSRC Mass Spectrometry Service (Swansea, UK) and the Chemical Database Service (CDS) at Daresbury, UK.

References

1. See: Roth, J. *Chem. Rev.* **2002**, *102*, 285–303, and references contained therein.
2. Breuer, W.; Bause, E. *Eur. J. Biochem.* **1995**, *228*, 689–696.
3. Petrescu, A. J.; Butters, T. D.; Reinkensmeier, G.; Petrescu, S.; Platt, F.; Dwek, R. A.; Wormald, M. R. *EMBO J.* **1997**, *16*, 4302–4310.
4. Alvarado, E.; Nukada, T.; Ogawa, T.; Ballou, C. E. *Biochemistry* **1991**, *30*, 881–886.
5. For a previous approach to the *n*-propyl glycoside of this tetrasaccharide see: Ogawa, T.; Nukada, T.; Kitajima, T. *Carbohydr. Res.* **1983**, *123*, C12–C15.
6. Liptak, A.; Czegeny, I.; Harangi, J.; Nanasi, P. *Carbohydr. Res.* **1974**, *73*, 327–331.
7. Ohnishi, Y.; Ando, H.; Kawai, T.; Nakahara, Y.; Ito, Y. *Carbohydr. Res.* **2000**, *328*, 263–276.
8. Lee, J.; Cha, J. K. *Tetrahedron Lett.* **1996**, *37*, 3663–3666.
9. de Pouilly, P.; Chénedé, A.; Mallet, J.-M.; Sinaÿ, P. *Bull. Chem. Soc. Fr.* **1993**, *130*, 256–265.
10. Mehta, S.; Pinto, B. M. *J. Org. Chem.* **1993**, *58*, 3269–3276.
11. Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583–5584.
12. See for example: (a) Mehta, S.; Pinto, B. M. *Tetrahedron Lett.* **1991**, *32*, 4435–4438. (b) Zuurmond, H. M.; van der Klein, P. A. M.; van der Meer, P. H.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 365–366.
13. Alternative strategies such as the use of an orthogonal glycosyl donor in place of **5** to allow a convergent approach, and the use of an iterative IAD-based linear approach were also investigated, and the results of these studies will be published in due course.
14. Wulff, G.; Röhle, G. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 157–170.
15. Szurmai, Z.; Balatoni, L.; Liptak, A. *Carbohydr. Res.* **1994**, *254*, 301–309.
16. Gordon, D. M.; Danishefsky, S. M. *Carbohydr. Res.* **1990**, *206*, 361–366.