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Synthesis of the Glc_3Man N-glycan tetrasaccharide by iterative allyl IAD

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Abstract—The synthesis of the tetrasaccharide α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Man*p*-OMe, corresponding to the terminal tetrasaccharide portion of the glucose terminated arm of the N-glycan tetradecasaccharide, was achieved with complete stereocontrol by the use of iterative allyl protecting group mediated intramolecular aglycon delivery (allyl IAD) demonstrating the utility of intramolecular glycosylation for the stereocontrolled construction of multiple glycosidic linkages during the synthesis of an oligosaccharide.

Keywords: Carbohydrates; Intramolecular; Glycosylation; Allyl; Intramolecular aglycon delivery; Thioglycosides; Glycoproteins

1. Introduction

Post-translational modification of proteins by glycosylation[1] is well known to have an important role in protein folding,[2] is able to modulate protein stability and enzymatic activity,[3] and in addition can also affect other important protein properties, such as circulatory lifetime.[4] The most common form of protein glycosylation is N-linked glycosylation, in which the oligosaccharides (N-glycans) are attached to the side chains of asparagine residues. The attachment of N-glycans to a protein is initiated by the enzyme oligosaccharyl transferase (OST), which mediates the transfer of a 14-membered dolichol phosphate bound oligosaccharide ($Glc_3Man_9GlcNAc_2$) to particular asparagine residues (consensus sequence Asn-X-Ser/The, where X ≠ Pro).[5] Following this transfer sequential trimming of the oligosaccharide chain occurs, which is initiated by two glycoprotein-processing enzymes, glucosidases I and II, in the endoplasmic reticulum (ER). Diversification of N-glycan structures then occurs following the action of a whole variety of other glycosidases and glycosyl transferases, leading to the eventual production of glycoproteins bearing the familiar variety of mature N-glycan structures (high mannose, complex, hybrid type).

At first glance it may appear that the terminal three glucose residues are somewhat redundant as these residues are invariably cleaved during glycoprotein processing. However, it is now known that the terminal glucose trisaccharide part of the $Glc_3Man_9GlcNAc_2$ oligosaccharide is important for OST recognition,[6] and it has even been suggested that the Glc_3 trisaccharide portion has a well-defined conformation which functions as a recognition epitope. Moreover, it has also been demonstrated that correct folding of the glycoprotein is dependent on the binding of the lectin chaperones calnexin and calreticulin to a monoglucosylated $GlcMan_9GlcNAc_2$ moiety[7] produced by sequential removal of two of the three terminal glucose units from the tetradecasaccharide: the first glucose being removed by glucosidase I[8] and the second by glucosidase II.[9] These chaperones do not bind to the nonglucosylated $Man_9GlcNAc_2$ glycan, so cleavage of the third glucose residue by glucosidase II actually stops protein folding. Indeed, if a protein has not achieved its native conformation at this point, it is recognised and re-glucosylated by a glucosyltransferase[10] as part of a quality control mechanism to complete the folding process.[11] Conversely if correct folding has occurred prior to cleavage of the last glucose residue by glucosidase II,

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the protein is not re-glucosylated and it then leaves the ER to continue maturation.

The interesting and diverse roles played by residues present in the glucose terminated arm have therefore initiated significant interest from the synthetic community. The synthesis of the complete α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Man*p*-OMe tetrasaccharide has been reported both by Ogawa[12] and ourselves,[13] and in addition we have previously published[14] the synthesis of fluorescence labeled disaccharides corresponding to portions of the tetrasaccharide as substrates for glucosidase II. Moreover, Ito and co-workers[15] have most recently completed the total synthesis of the complete $Glc_3Man_9GlcNAc_2$ tetradecasaccharide itself, whilst other research groups have synthesised other truncated portions of the glucose terminated arm.[16]

Our previous efforts were directed at accessing significant pure quantities of the Glc_3Man tetrasaccharide to allow extensive conformational studies. However, our previous synthesis was not completely stereoselective, and inseparable mixtures of anomers were formed during the attempted formation of the 1,2-*cis*-α-*gluco* linkages, necessitating laborious final product purification by high performance anion exchange chromatography (HPAEC). This paper details a completely stereoselective approach to the Glc_3Man tetrasaccharide in which two of the terminal α-*gluco* linkages are formed by successive intramolecular glycosylation reactions using the allyl IAD approach.[17,18] The complete stereocontrol achieved during these two reactions greatly simplifies the whole synthesis and facilitates product isolation and purification.

2. Results and discussion

Allyl IAD[19,20] is a particular intramolecular glycosylation strategy[21] based on the use of allyl protecting groups to allow linking of glycosyl donor and acceptor via the 2-hydroxyl group of the donor and the hydroxyl of the acceptor which is to be glycosylated. The basic reaction sequence involves isomerisation[22] of the double bond of the allyl group to produce a vinyl ether, which then undergoes mixed acetal formation with an alcohol, usually mediated by the addition of a source of iodonium ions. Subsequent activation of the glycosyl donor is then followed by a completely stereoselective intramolecular glycosylation reaction, in which the acceptor alcohol is delivered *syn* to the 2-hydroxyl of the donor enforcing the formation of a 1,2-cis linkage.

In principle, mixed acetal formation may be performed either way around, and so either 2-*O*-allyl protected glycosyl donors and deprotected acceptors (the 'forward' approach), or donors which possess a free hydroxyl at the 2-position and acceptors in which the hydroxyl to be glycosylated is allyl protected (the 'reverse' approach), may be used as the partners for this reaction sequence (Fig. 1).

A notable feature of the IAD approach is that the product formed after the intramolecular glycosylation step possesses a free hydroxyl group at the 2-position of the nonreducing terminus. Because the terminal linkage of the target Glc_3Man tetrasaccharide is an α-(1→2) linkage, it was therefore envisaged that both of the two terminal glucose residues could be added sequentially using the same basic glycosyl donor in an iterative IAD process. The strategy chosen involved the use of a first IAD reaction between an allyl protected disaccharide acceptor and a thioglycoside donor with OH-2 free, and a second IAD reaction between the newly formed trisaccharide as acceptor and a 2-*O*-allyl protected version of the same thioglycoside donor. Access was therefore required to both an allyl protected disaccharide glycosyl acceptor for the first IAD reaction, and a glycosyl donor with a free 2-hydroxyl group, which would act

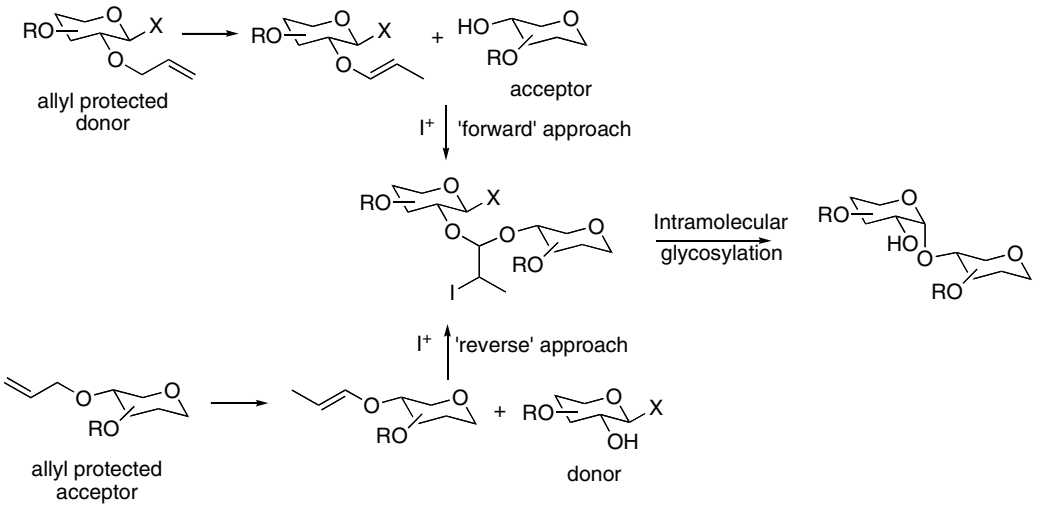
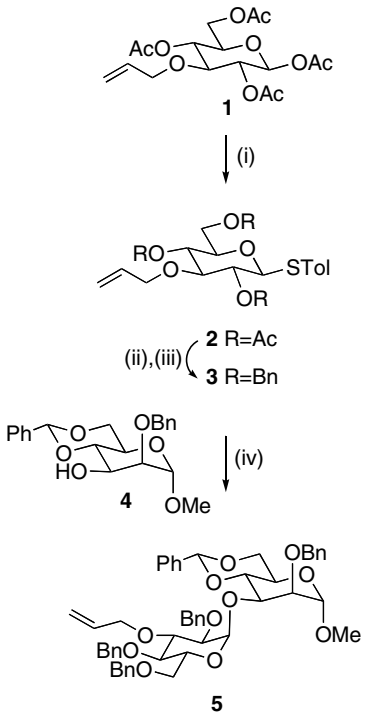


Figure 1. Allyl IAD: 'Forward' and 'Reverse' approaches.

as a divergent donor; OH-2 free for the first IAD sequence, OH-2 allyl protected for the second.

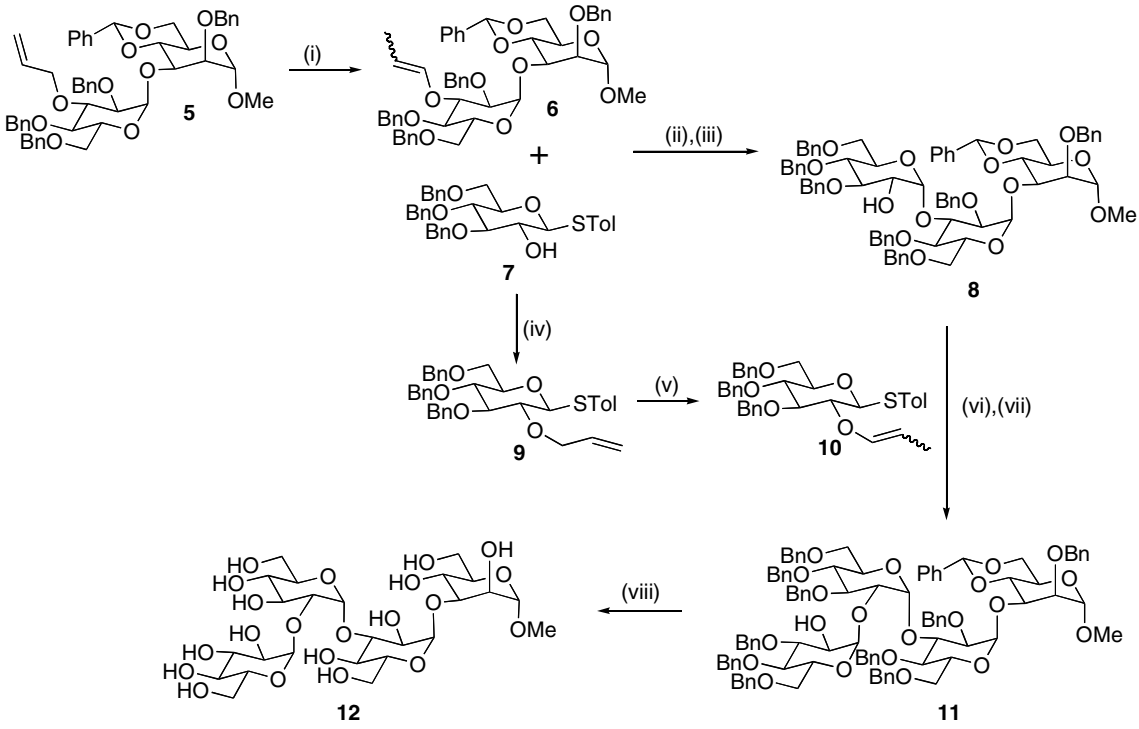
The synthesis of the allyl protected disaccharide acceptor was undertaken first. The 3-*O*-allyl protected *gluco* tetraacetate **1** was accessed as described previously,[13] and was then converted into the β-thioglycoside acceptor **2** by treatment with *p*-thiocresol and boron trifluoride etherate in dichloromethane. Subsequent deacetylation and immediate benzylation gave the required thioglycoside donor **3**, possessing nonparticipating protection at the 2-position. The known *manno* glycosyl acceptor **4** was accessed as previously described,[23] and glycosylation of this acceptor **4** with donor **3** gave the desired disaccharide **5** as expected. However, it was necessary to investigate a range of reaction conditions to maximise both product yield and α-stereoselectivity for this step. Thioglycoside activation in ether, using either NIS with catalytic triflic acid or methyl triflate, only produced the desired disaccharide in low to moderate yield (45% and 34% yields, respectively), though in both cases reasonably good stereoselectivity was observed (α:β ratio >3:1). However, glycosylation in ether mediated with iodine, silver triflate and 2,6-di-*tert*-butyl-4-methyl pyridine (DTBMP), produced the desired disaccharide **5** in excellent yield and with a high level of stereocontrol (87% yield, α:β ratio, 23:1, Scheme 1).



Scheme 1. Reagents and conditions: (i) TolSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C, 78%; **2β**:**2α**, 35:1; (ii) Na, MeOH, rt; (iii) NaH, BnBr, DMF, 0 °C, 89% over two steps; (iv) I_2, AgOTf, DTBMP, CH_2Cl_2, −78 °C, 87%, **5α**:**5β**, 23:1.

With the allyl protected disaccharide acceptor in hand, attention then turned to the required donors. Thus the thioglycoside acceptor **7**[24] was accessed as previously described as the donor for the first IAD sequence. With the second IAD reaction in mind, a portion of the thioglycoside **7** was then allylated by treatment with sodium hydride and allyl bromide in DMF to yield the allyl protected donor **9**, and subsequent Wilkinson's catalyst mediated double bond isomerisation then produced enol ethers **10**. With all key building blocks in hand, the two IAD reactions were investigated in turn (Scheme 2). Thus, isomerisation of the double bond of allyl protected disaccharide acceptor **5** yielded enol ethers **6**. Previous reports on allyl IAD have mainly focussed on a two-step approach, in which tethering (i.e., mixed acetal formation) and glycosylation are performed as separate steps. However, it has also been demonstrated that both operations may be performed sequentially in a single reaction vessel (the one-pot approach)[18a,20m] provided that an excess of glycosyl donor is present; the caveat being that any excess alcohol remaining after tethering can lead to competitive and nonstereoselective intermolecular glycosylation. In this instance it was decided to investigate both approaches for the first IAD reaction and apply the most efficient and practical for the second.

Mixed acetal formation was achieved by treatment of a mixture of **6** and **7** with iodine, silver triflate and DTBMP (78% yield). Intramolecular glycosylation of the purified tethered intermediates was then undertaken. Several methods of thioglycoside activation were investigated, including NIS/TfOH, NIS/TMSOTf, NIS/TMSOTf/DTBMP and I_2/AgOTf/DTBMP. However, none proved particularly satisfactory and all either led to the formation of multiple products as observed by TLC, or to very slow reaction. Attention then turned to the use of a variant on DMTST activation recently reported by Fügedi and Tatai,[25] involving thioglycoside activation by treatment with a mixture of Me_2S_2 and triflic anhydride. In concurrence with Fügedi, this reagent combination proved to be much more potent than the use of DMTST, but unfortunately substantial decomposition was also observed. The reported conditions were therefore modified by adding the base DTBMP to the activation mixture and pleasingly this produced a highly efficient and clean reaction yielding trisaccharide **8** in 65% yield, and, as expected, with complete control of anomeric stereochemistry. Attention then turned to the one-pot approach as a possibly more efficient alternative to the two-step procedure above (yield 51% over the two steps). A mixture of donor alcohol **7** and acceptor **6** (1.1 equiv) was stirred with iodine, silver triflate and DTBMP and once mixed acetal formation was complete (as monitored by TLC) the activation mixture of Me_2S_2/Tf_2O/DTBMP was added. However, this approach proved less satisfactory; following work-up, trisaccharide



Scheme 2. Reagents and conditions: (i) Wilkinson's catalyst, *n*-BuLi, THF, 70 °C, 97%; (ii) **7**, I_2, AgOTf, DTBMP, CH_2Cl_2, 0 °C→rt, 78%; (iii) Me_2S_2, Tf_2O, DTBMP, CH_2Cl_2, 0 °C→rt, 65%; (iv) allyl bromide, NaH, DMF, 0 °C→rt, 98%; (v) Wilkinson's catalyst, *n*-BuLi, THF, 70 °C, 95%; (vi) **10**, I_2, AgOTf, DTBMP, CH_2Cl_2, 0 °C→rt, 77%; (vii) Me_2S_2, Tf_2O, DTBMP, CH_2Cl_2, 0 °C→rt, 58%; (viii) H_2, $Pd(OAc)_2$, AcOH, EtOH, 98%.

8 was isolated in only 35% yield, although again glycosylation had occurred with complete control of anomeric stereochemistry. In light of this, the two-step approach was therefore applied to the second IAD reaction. Thus tethering of trisaccharide acceptor **8** (1.0 equiv) with enol ethers **10** (1.1 equiv) by treatment with iodine, silver triflate and DTBMP gave intermediate mixed acetals (77% yield), which then underwent intramolecular glycosylation, again using the modified Fügedi conditions (Me_2S_2/Tf_2O/DTBMP), to yield desired tetrasaccharide **11** (58% yield), again with complete control of anomeric stereochemistry. Finally, global deprotection of benzyl ethers and benzylidene acetal by catalytic hydrogenation in the presence of a palladium acetate catalyst in a mixture of acetic acid and ethanol gave the deprotected tetrasaccharide **12** (98% yield, Scheme 2). In contrast to our previous synthesis, tetrasaccharide was easily purified as no other stereoisomeric products had been formed during either of the two intramolecular glycosylation reactions.

3. Summary and conclusion

The stereoselective synthesis of the tetrasaccharide α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Man*p*-OMe **12** has been successfully achieved using two successive intramolecuar glycosylation reactions. To the best of our knowledge, this represents the first synthesis in which IAD has been used in an iterative manner for the completely stereocontrolled formation of two successive glycosidic linkages in the same oligosaccharide. Moreover, it has been demonstrated that allyl IAD may be efficiently achieved either in the forward (allyl protected donor) or reverse (allyl protected acceptor) senses. Finally, it has also been demonstrated that the addition of the base DTBMP to the Fügedi thioglycoside activation conditions (Me_2S_2, Tf_2O) produces a highly reactive activation system for thioglycosides, which in particular leads to high yielding clean intramolecular glycosylation reactions; a process that none of the other commonly used thioglycoside activators that were investigated was capable of achieving. Further investigations into the use of these conditions in combination with the IAD approach for the synthesis of more complex biologically important oligosaccharides are currently in progress, and the results will be reported in due course.

4. Experimental

4.1. General methods

Melting points were recorded on a Kofler hot block and are uncorrected. ^1H NMR spectra were recorded on a

Bruker DPX 400 (400 MHz) or on a Bruker AMX 500 (500 MHz) spectrometer. ^{13}C NMR 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 the DEPT sequence. All chemical shifts are quoted on the δ-scale in parts per million (ppm). The abbreviations at, dat, etc, refer to apparent triplet, double apparent triplet, etc. All NMR experiments were performed at a probe temperature of 30 °C. 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. 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. Thin layer chromatography (TLC) was carried out on Merck Kieselgfel 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; CH_3OH was distilled from sodium hydride, CH_2Cl_2 was distilled from calcium hydride, pyridine was distilled from calcium hydride and stored over potassium hydroxide and THF was distilled from a solution of sodium benzophenone ketyl immediately before use. Petrol was distilled between 40 and 60 °C before use to remove nonvolatile fractions.

4.2. *para*-Tolyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-1-thio-β-D-glucopyranoside (2β) and *para*-tolyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-1-thio-α-D-glucopyranoside (2α)

Tetraacetate **1** (2.00 g, 5.15 mmol) was suspended in freshly distilled CH_2Cl_2 (10 mL) under Ar in a flame-dried flask. *p*-Thiocresol (766 mg, 6.18 mmol) was added and the mixture cooled to 0 °C. $BF_3{\cdot}OEt_2$ (0.95 mL, 7.73 mmol) was added and the reaction mixture was stirred under Ar. After 1.5 h, TLC (3:2, petrol–EtOAc) indicated formation of a major product ($R_f = 0.3$) and a minor product ($R_f = 0.35$). Triethylamine (2 mL) was then added, the mixture was diluted with ether (100 mL), and washed with water (100 mL). The aqueous layer was re-extracted with ether (50 mL), and the combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (3:2, petrol–EtOAc) to afford the desired β-thioglycoside **2β** (1.81 g, 78%) as a white solid, mp 127–128 °C (ether/petrol); $[\alpha]_D^{21}$ −14.4 (*c* 0.9, $CHCl_3$); IR: 1744 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.08, 2.08, 2.15 (9H, 3 × s, 3 × $COCH_3$), 2.34 (3H, s, $ArCH_3$), 3.57–3.63 (1H, m, H-5), 3.59 (1H, at, $J = 9.2$ Hz, H-3), 4.02–4.11 (2H, m, $OCH_2CH{=}CH_2$), 4.13–4.20 (2H, m, H-6, H-6′), 4.56 (1H, d, $J_{1,2} = 10.1$ Hz, H-1), 4.96 (1H, at, $J = 9.5$ Hz, H-2), 5.00 (1H, at, $J = 9.8$ Hz, H-4), 5.13 (1H, d, $J_Z = 10.4$ Hz, $CH{=}CH_EH_Z$), 5.19 (1H, dd, $J_{gem} = 1.6$ Hz, $J_E = 17.2$ Hz, $CH{=}CH_EH_Z$), 5.75 (1H, ddat, $J = 5.5$ Hz, $CH{=}CH_2$), 7.11, 7.40 (4H, 2 × d, $J = 8.1$ Hz, Ar–H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.2, 21.3, 21.5, 21.6 (4 × q, 3 × $COCH_3$, $ArCH_3$), 63.0, 73.5 (2 × t, C-6, $CH_2CH{=}CH_2$), 69.9, 71.7, 76.4, 81.7 (4 × d, C-2, C-3, C-4, C-5), 86.8 (d, C-1), 117.5 (t, $CH{=}CH_2$), 129.0, 138.8 (2 × s, Ar–C) 130.0, 133.7 (2 × d, Ar–CH), 134.6 (d, $CH{=}CH_2$), 169.6, 169.7, 171.2 (3 × s, 3 × C=O); *m*/*z* (ES^+) 475 ($M+Na^+$, 100), 470 ($M+NH_4^+$, 77%); ESIMS *m*/*z* calcd for $C_{22}H_{32}NO_8S$ $[M+NH_4]^+$: 470.1849. Found 470.1850. Anal Calcd for $C_{22}H_{28}O_8S$: C, 58.39; H, 6.24. Found: C, 58.50; H, 6.27. In addition a small quantity of the corresponding α-thioglycoside **2α** was also produced (50 mg, 2%) as a colourless oil; $[\alpha]_D^{21}$ +163.3 (*c* 1.8, $CHCl_3$); IR: 1748 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.03, 2.11, 2.16, 2.32 (12H, 4 × s, 3 × $COCH_3$, $ArCH_3$), 3.80 (1H, at, $J = 9.6$ Hz, H-3), 4.04 (1H, dd, $J_{5,6} = 2.2$ Hz, $J_{6,6'} = 12.3$ Hz, H-6), 4.11 (1H, dd, $J_{gem} = 13.1$ Hz, $J_{vic} = 5.7$ Hz, $OCHH'CH{=}CH_2$), 4.20–4.26 (2H, m, H-6′, $OCHH'CH{=}CH_2$), 4.48 (1H, ddd, $J_{4,5} = 10.1$ Hz, $J_{5,6'} = 5.6$ Hz, H-5), 5.02 (1H, dd, $J_{1,2} = 5.7$ Hz, $J_{2,3} = 10.0$ Hz, H-2), 5.04 (1H, at, $J = 9.8$ Hz, H-4), 5.17 (1H, d, $J_Z = 10.4$ Hz, $CH{=}CH_EH_Z$), 5.25 (1H, d, $J_E = 17.2$ Hz, $CH{=}CH_EH_Z$), 5.78–5.88 (1H, m, $CH{=}CH_2$), 5.82 (1H, d, H-1), 7.11, 7.33 (4H, 2 × d, $J = 8.0$ Hz, Ar–H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.7, 20.8, 20.9, 21.0 (4 × q, 3 × $COCH_3$, $ArCH_3$), 62.2, 73.8 (2 × t, C-6, $CH_2CH{=}CH_2$), 68.4, 69.7, 73.1, 77.3 (4 × d, C-2, C-3, C-4, C-5), 85.6 (d, C-1), 116.9 (t, $CH{=}CH_2$), 129.8, 132.4, 134.3 (3 × d, Ar–CH, $CH{=}CH_2$), 128.8, 138.0 (2 × s, Ar–C), 169.4, 169.8, 170.7 (3 × s, 3 × C=O); *m*/*z* (ES+) 475 ($M+Na^+$, 43), 470 ($M+NH_4^+$, 64%). ESIMS *m*/*z* calcd for $C_{22}H_{32}NO_8S$ $[M+NH_4]^+$: 470.1849. Found 470.1854.

4.3. *para*-Tolyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (3)

Acetylated thioglycoside **2** (1.77 g, 3.92 mmol) was suspended in CH_3OH (10 mL). Sodium (5 mg, 0.20 mmol) was dissolved in CH_3OH (5 mL) and the solution then added to the reaction mixture, which was then stirred under Ar. After 2 h, TLC (EtOAc) indicated formation of a single product ($R_f = 0.6$) and complete consumption of the starting material ($R_f = 0.7$). The mixture was concentrated in vacuo, and the resulting residue

was then dissolved in DMF (30 mL) and the solution cooled to 0 °C. Benzyl bromide (2.09 mL, 17.6 mmol) and sodium hydride (940 mg, 23.5 mmol) were added and the reaction mixture was stirred under Ar. After 2 h, TLC (5:1, petrol–EtOAc) indicated the formation of two products ($R_f = 0.5$ and 0.7) and complete consumption of starting material ($R_f = 0.0$). Further benzyl bromide (0.35 mL, 2.94 mmol) and sodium hydride (156 mg, 3.91 mmol) were added, and the reaction mixture was stirred under Ar. After a further 14 h, TLC (5:1, petrol–EtOAc) indicated the formation of a single product ($R_f = 0.7$). CH_3OH (8 mL) was added slowly, and the mixture was then diluted with ether (200 mL), and washed with water (200 mL). The organic extracts were dried ($MgSO_4$), filtered, concentrated in vacuo and the residue was purified by flash column chromatography (4:1, petrol–ether) to afford benzylated thioglycoside **3** (2.08 g, 89%) as a white, crystalline solid, mp 75 °C (EtOH); $[\alpha]_D^{21}$ −6.5 (*c*, 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.32 (3H, s, $ArCH_3$), 3.41–3.51 (2H, m, H-2, H-5), 3.53–3.61 (2H, m, H-3, H-4), 3.73 (1H, dd, $J_{5,6} = 4.8$ Hz, $J_{6,6'} = 10.9$ Hz, H-6), 3.79 (1H, dd, $J_{5,6'} = 2.1$ Hz, H-6′), 4.33 (1H, ddat, $J_{gem} = 12.3$ Hz, J_{vic} 5.7 Hz, $J = 1.4$ Hz, OC*H*H′CH=CH_2), 4.38 (1H, ddat, $J_{vic} = 5.7$ Hz, $J = 1.5$ Hz, OC*H*H′CH=CH_2), 4.55, 4.62 (2H, ABq, $J_{AB} = 12.1$ Hz, PhCH_2), 4.58 (1H, d, $J_{1,2} = 9.7$ Hz, H-1), 4.59, 4.83 (2H, ABq, $J_{AB} = 11.1$ Hz, PhCH_2), 4.74, 4.88 (2H, ABq, $J_{AB} = 10.1$ Hz, PhCH_2), 5.18 (1H, daq, $J_Z = 10.4$ Hz, $J = 1.4$ Hz, CH=CH$_E$H$_Z$), 5.30 (1H, daq, $J = 1.7$ Hz, $J_E = 17.1$ Hz, CH=CH_EH$_Z$), 5.98 (1H, ddat, C*H*=CH_2), 7.04–7.52 (19H, m, Ar–H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.1 (q, Ar*C*H$_3$), 69.0, 73.4, 74.5, 75.1, 75.5 (5 × t, C-6, *C*H$_2$CH=CH_2, 3 × Ph*C*H$_2$), 77.7, 79.0, 80.7, 86.4, 87.6 (5 × d, C-1, C-2, C-3, C-4, C-5), 116.9 (t, CH=*C*H$_2$), 127.5, 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 129.6, 132.7, 134.9 (11 × d, Ar–CH, *C*H=CH_2), 137.7, 138.1, 138.1, 138.4 (4 × s, Ar–C); *m*/*z* (ES$^+$) 619 (M+Na$^+$, 32), 614 (M+NH$_4^+$, 100%); ESIMS *m*/*z* calcd for $C_{37}H_{44}NO_5S$ [M+NH$_4$]$^+$: 614.2940. Found 614.2935. Anal Calcd $C_{37}H_{40}O_5S$: C, 74.47; H, 6.76. Found: C, 74.45; H, 6.79.

4.4. Methyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (5)

Iodine (62 mg, 0.17 mmol), silver trifluoromethanesulfonate (63 mg, 0.25 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (70 mg, 0.34 mmol) and 4 Å molecular sieves were suspended in freshly distilled ether (2 mL) and cooled to −78 °C whilst stirring under Ar. Thioglycoside **3** (100 mg, 0.168 mmol) and methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside **4** (62 mg, 0.17 mmol) were dissolved in freshly distilled ether (3 mL) and this solution was then added to the reaction vessel via cannula. The reaction was then allowed to warm to rt. After 24 h, TLC (3:1, petrol–EtOAc) indicated the formation of a major product ($R_f = 0.4$) and consumption of starting materials ($R_f = 0.6$ and 0.3). The reaction was quenched by the addition of sodium thiosulfate (40 mL of a 10% aqueous solution), diluted with ether (40 mL), and then filtered through Celite®. The organic phase was washed with sodium thiosulfate (2 × 100 mL of a 10% aqueous solution), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (5:1, petrol–EtOAc then 15:1, toluene–EtOAc) to afford pure α-disaccharide **5** (118 mg, 83%) as a colourless oil; $[\alpha]_D^{21}$ +93.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.33 (3H, s, OCH_3), 3.46 (1H, dd, $J_{1,2} = 3.6$ Hz, $J_{2,3} = 9.6$ Hz, H-2$_b$), 3.54 (1H, at, $J = 9.4$ Hz, H-4$_b$), 3.64–3.71 (2H, m, H-6$_b$, H-6′$_b$), 3.78–4.15 (5H, m, H-2$_a$, H-5$_a$, H-6$_a$, H-3$_b$, H-5$_b$), 4.18–4.26 (2H, m, H-6′$_a$, OC*H*H′CH=CH_2), 4.31, 4.54 (2H, ABq, $J_{AB} = 12.5$ Hz, PhCH_2), 4.31–4.35 (1H, m, H-4$_a$), 4.39 (1H, dd, $J_{2,3} = 2.9$ Hz, $J_{3,4} = 10.0$ Hz, H-3$_a$), 4.42–4.49 (1H, m, OCH*H*′CH=CH_2), 4.45, 4.58 (2H, ABq, $J_{AB} = 12.2$ Hz, PhCH_2), 4.47, 4.88 (2H, ABq, $J_{AB} = 11.0$ Hz, PhCH_2), 4.71 (1H, d, $J_{1,2} = 1.3$ Hz, H-1$_a$), 4.78, 4.93 (2H, ABq, $J_{AB} = 11.9$ Hz, PhCH_2), 5.09 (1H, dd, $J_Z = 10.4$ Hz, $J_{gem} = 1.0$ Hz, CH=CH$_E$H$_Z$), 5.22 (1H, dd, $J_{gem} = 1.6$ Hz, $J_E = 17.2$ Hz, CH=CH_EH$_Z$), 5.47 (1H, s, PhC*H*) 5.50 (1H, d, H-1$_b$), 5.96 (1H, ddat, $J = 5.5$ Hz, C*H*=CH_2), 7.00–7.47 (25H, m, Ar–H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 54.8 (q, OCH_3), 63.9 (d, C-5$_a$), 68.6 (t, C-6$_b$), 68.9 (t, C-6$_a$), 70.5 (t, Ph*C*H$_2$), 70.8 (d, C-5$_b$), 72.7 (d, C-3$_a$), 73.4, 73.8, 74.3, 75.0 (4 × t, O*C*H$_2$CH=CH_2, 3 × Ph*C*H$_2$), 77.2, 77.3 (2 × d, C-2$_a$, C-4$_b$), 78.6 (d, C-2$_b$), 79.6 (d, C-4$_a$), 81.0 (d, C-3$_b$), 96.9 (d, C-1$_b$), 100.6 (d, C-1$_a$), 102.3 (d, Ph*C*H), 116.6 (t, CH=*C*H$_2$), 126.4, 127.0, 127.1, 127.6, 127.6, 127.8, 127.8, 127.9, 128.1, 128.2, 128.2, 128.3, 128.4, 129.2 (14 × d, Ar–CH), 135.4 (d, *C*H=CH_2), 137.4, 138.0, 138.0, 138.3, 138.5 (5 × s, Ar–C); *m*/*z* (ES$^+$) 867 (M+Na$^+$, 27), 862 (M+NH$_4^+$, 100%); ESIMS *m*/*z* calcd for $C_{51}H_{60}NO_{11}$ [M+NH$_4$]$^+$: 862.4166. Found 862.4160.

4.5. Methyl 2,4,6-tri-*O*-benzyl-3-*O*-prop-1′-enyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (6)

Wilkinson's catalyst (86 mg, 0.093 mmol) was dissolved in distilled THF (4 mL) and degassed. *n*-Butyl lithium (0.09 mL, 0.14 mmol, 1.6 M solution in hexanes) was added and the mixture stirred for 10 min under Ar. Allyl protected disaccharide **5** (786 mg, 0.93 mmol) was dissolved in distilled THF (4 mL) and the mixture heated to 70 °C. The catalyst solution prepared above was then added via cannula under Ar. After 2 h, 30 min, TLC (4:1, petrol–EtOAc) indicated the presence of a single compound ($R_f = 0.3$). The reaction mixture was allowed

to cool to rt, diluted with CH_2Cl_2 (20 mL) and then concentrated in vacuo. The residue was purified by flash column chromatography (4:1, petrol–EtOAc) to afford vinyl ethers **6** (745 mg, 95%) as a pale yellow oil; partial data: ^{1}H NMR (400 MHz, $CDCl_3$) (*E*:*Z*, 1:2.6): δ 1.51 (3H, dd, $J = 1.6$, 6.8 Hz, OCH=CHCH_3–*E*), 1.64 (3H, dd, $J = 1.6$, 6.8 Hz, OCH=CHCH_3–*Z*); *m*/*z* (ES^+) 867 ($M+Na^+$, 21), 862 ($M+NH_4^+$, 100%); ESIMS *m*/*z* calcd for $C_{51}H_{60}NO_{11}$ $[M+NH_4]^+$: 862.4166. Found 862.4161.

4.6. 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*-benzylidene-α-D-mannopyranoside (8)

Iodine (138 mg, 0.54 mmol), silver trifluoromethanesulfonate (142 mg, 0.55 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (284 mg, 1.38 mmol) and 4 Å molecular sieves were added to freshly distilled CH_2Cl_2 (5 mL) in a flame-dried flask. The mixture was cooled to −78 °C and stirred for 20 min under an atmosphere of Ar. Enol ethers **6** (390 mg, 0.46 mmol) and thioglycoside **7**[24] (308 mg, 0.55 mmol) were dissolved in freshly distilled CH_2Cl_2 (5 mL) and added to the reaction vessel via cannula under an atmosphere of Ar. The reaction was allowed to warm to rt whilst stirring. After 15 h, TLC (10:1, toluene–EtOAc) indicated complete conversion of starting materials ($R_f = 0.48$ and 0.44) to a major product ($R_f = 0.52$). The reaction mixture was diluted with CH_2Cl_2 (20 mL) and quenched by addition of sodium thiosulfate (20 mL of a 10% aqueous solution). The mixture was filtered and the two phases separated. The aqueous layer was extracted with CH_2Cl_2 (2×20 mL), and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (20:1, toluene–EtOAc) to afford intermediate mixed acetals (548 mg, 77%) as a mixture of diastereomers as a colourless oil, a portion of which was used directly for the next step; *m*/*z*(ES^+) 1549 ($M+Na^+$, 38), 1544 ($M+NH_4^+$, 100%); *m*/*z* (isotopic distribution) expected mass 1547 (MNH_4^+, 23%), 1546 (MNH_4^++2, 55%), 1545 (MNH_4^++1, 97%), 1544 (MNH_4^+, 100%), found 1547 (MNH_4^++3, 14%), 1546 (MNH_4^++2, 48%), 1545 (MNH_4^++1, 94%), 1544 (MNH_4^+, 100%).

Mixed acetals prepared above (50 mg, 0.0327 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (27 mg, 0.0655 mmol) and 4 Å molecular sieves were added to freshly distilled CH_2Cl_2 (2 mL) in a flame-dried flask under an atmosphere of Ar and the mixture was cooled to 0 °C whilst stirring. Dimethyldisulfide (6.0 μL, 0.0655 mmol) and triflic anhydride (11.0 μL, 0.0655 mmol) were dissolved in freshly distilled CH_2Cl_2 (0.25 mL) and after 5 min the mixture was added to the reaction vessel under an atmosphere of Ar. The reaction mixture was allowed to warm to rt and after 90 min, TLC (10:1, toluene–EtOAc) indicated the complete consumption of starting material ($R_f = 0.52$), and the formation of three main products ($R_f = 0.42$, 0.28 and 0.20). The reaction mixture was cooled to 0 °C, then dioxane (5 mL) and an aqueous NaH_2PO_4/Na_2HPO_4 buffer (pH 6.0, 5 mL) were added, and the mixture was stirred for a further 10 min. The mixture was filtered, diluted with CH_2Cl_2 (20 mL) and the two phases separated. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was dissolved in acetone (5 mL) and water (0.5 mL). NIS (11 mg, 0.049 mmol) was added and the mixture was stirred overnight at rt. The reaction mixture was then treated with sodium thiosulfate (5 mL of a 10% aqueous solution), and diluted with CH_2Cl_2 (20 mL). The aqueous phase was washed with CH_2Cl_2 (10 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (40:1→2:1, CH_2Cl_2–ether) to afford trisaccharide **8** (26 mg, 65%) as a colourless oil; $[\alpha]_D^{22}$ +73.1 (*c* 1.1, $CHCl_3$); IR: 3374 (br, OH) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (1H, br s, OH-2_c), 3.19 (2H, br s, H-6, H-6′), 3.36 (3H, s, OCH_3), 3.42 (1H, dd, $J_{1,2} = 3.7$ Hz, $J_{2,3} = 9.8$ Hz, H-2_b), 3.65–3.79 (8H, m), 3.82–3.89 (2H, m), 4.13 (1H, d, $J = 12.3$ Hz, PhC*H*H′), 4.16, 4.47 (2H, ABq, $J_{AB} = 11.1$ Hz, PhCH_2), 4.21–4.25 (3H, m, H-3_b, H-5, H-6), 4.34–4.44 (5H, m), 4.48, 4.67 (2H, ABq, $J_{AB} = 11.8$ Hz, PhCH_2), 4.72 (1H, d, $J = 11.0$ Hz, PhC*H*H′), 4.75 (1H, br s, H-1_a), 4.82, 4.95 (2H, ABq, $J_{AB} = 12.0$ Hz, PhCH_2), 4.83 (2H, s, PhCH_2), 4.91 (1H, d, $J = 10.4$ Hz, PhC*H*H′), 5.36 (1H, s, PhC*H*), 5.51 (1H, d, $J_{1,2} = 2.9$ Hz, H-1_c), 5.62 (1H, d, H-1_b), 7.07–7.44 (40H, m, Ar–H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 54.8 (q, OCH_3), 63.8, 70.1, 70.5, 72.1, 72.8, 76.1, 76.5, 76.8, 77.4, 78.6, 79.9, 83.3 ($12 \times$ 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), 67.9, 68.3, 68.9 ($3 \times$ t, C-6_a, C-6_b, C-6_c), 70.3, 73.2, 73.6, 74.2, 74.3, 75.2 ($6 \times$ t, $7 \times$ PhCH_2), 96.1 (d, C-1_b), 98.6 (d, C-1_c), 100.7 (d, C-1_a), 102.4 (d, PhC*H*), 126.3, 127.3, 127.4, 127.4, 127.5, 127.7, 127.7, 127.8, 127.8, 127.8, 128.0, 128.1, 128.1, 128.1, 128.2, 128.3, 128.3, 128.4, 128.5, 129.2 ($20 \times$ d, Ar–CH), 137.2, 137.6, 137.8, 137.9, 137.9, 138.0, 138.7, 138.8 ($8 \times$ s, Ar–C); *m*/*z* (ES^+) 1275 ($M+K^+$, 11), 1259 ($M+Na^+$, 100), 1254 ($M+NH_4^+$, 64%); ESIMS *m*/*z* calcd for $C_{75}H_{84}NO_{16}$ $[M+NH_4]^+$: 1254.5790. Found 1254.5782.

4.7. *para*-Tolyl 2-*O*-allyl-3,4,6-*O*-benzyl-1-thio-β-D-glucopyranose (9)

Alcohol **7**[24] (0.393 g, 0.707 mmol) was dissolved in anhydrous DMF (20 mL) in a flame-dried flask under an atmosphere of Ar. The solution was cooled to 0 °C whilst stirring and sodium hydride (53 mg, 1.3 mmol, 60% dispersion in mineral oil) was added portion-wise

over a 10 min period. Allyl bromide (0.1 mL, 2 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 38 h, TLC (3:1, petrol–EtOAc) indicated the formation of a single product ($R_f = 0.6$) and complete consumption of starting material ($R_f = 0.4$). The reaction was quenched by the addition of CH_3OH (10 mL), and then partitioned between ether (50 mL) and brine (50 mL of a saturated aqueous solution). The organic extracts were washed with brine (2×50 mL of a saturated aqueous solution), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (5:1, petrol–EtOAc) to afford allyl protected thioglycoside **9** (0.412 g, 98%) as a white crystalline solid, mp 59–61 °C (EtOAc/petrol); $[\alpha]_D^{21}$ −18.6 (*c* 1.0, $CHCl_3$); IR: (KBr) 1648 (w, C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.31 (3H, s, $ArCH_3$), 3.35 (1H, at, $J = 9.2$ Hz, H-2), 3.57–3.81 (5H, m, H-3, H-4, H-5, H-6, H-6′) 4.25 (1H, dat, $J_{gem} = 12.5$ Hz, $J_{vic} = 5.8$ Hz, $J = 1.1$ Hz, $OCHH'CH{=}CH_2$), 4.39 (1H, dat, $J = 1.5$ Hz, $OCHH'CH{=}CH_2$), 4.52, 4.62 (2H, ABq, $J_{A,B} = 11.6$ Hz, OCH_2Ph), 4.54 (1H, d, $J_{1,2} = 9.9$ Hz, H-1), 4.77, 4.83 (2H, ABq, $J_{A,B} = 12.2$ Hz, OCH_2Ph), 4.58, 4.82 (2H, ABq, $J_{A,B} = 10.5$ Hz, OCH_2Ph), 5.19 (1H, daq, $J_Z = 10.4$ Hz, $J = 0.9$ Hz, $CH{=}CH_EH_Z$), 5.30 (1H, daq, $J_E = 17.1$ Hz, $J = 1.3$ Hz, $CH{=}CH_EH_Z$), 5.99 (1H, ddat, $CH{=}CH_2$), 7.04 (2H, d, $J = 7.5$ Hz, Ar–H), 7.27–7.38 (15H, m, Ar–H), 7.46 (2H, d, $J = 1.9$ Hz, Ar–H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.1 (q, $ArCH_3$), 69.0 (t, C-6), 73.4, 74.2, 75.0, 75.8 ($4 \times$ t, $3 \times PhCH_2$, $CH_2CH{=}CH_2$), 77.0 (d, C-2), 77.3, 79.1 ($2 \times$ d, C-4, C-5), 86.7 (d, C-3), 87.6 (d, C-1), 117.3 (t, $CH{=}CH_2$) 127.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 128.4, 129.6, 132.6, 134.7 ($11 \times$ d, $CH{=}CH_2$, Ar–CH), 137.6, 138.3, 138.4 ($3 \times$ s, Ar–C); *m*/*z* (ES^+) 614 ($M+NH_4^+$, 100%); ESIMS *m*/*z* calcd for $C_{37}H_{44}NO_5S$ $[M+NH_4]^+$: 614.2940. Found 614.2933. Anal calcd for $C_{37}H_{40}O_5S$: C, 74.47; H, 6.76. Found: C, 74.49; H, 6.78.

4.8. *para*-Tolyl 3,4,6-*O*-benzyl-2-*O*-prop-1′-enyl-1-thio-β-D-glucopyranose (10)

Wilkinson's catalyst (93 mg, 0.10 mmol) was dissolved in freshly distilled THF (5 mL) in a flame-dried flask and degassed. *n*-Butyl lithium (0.10 mL, 0.16 mmol, 1.6 M solution in hexanes) was added and the mixture was stirred for 20 min under an atmosphere of Ar. Allyl protected thioglycoside **9** (152 mg, 0.26 mmol) was dissolved in freshly distilled THF (5 mL) in a flame-dried flask and heated to 70 °C under an atmosphere of Ar. The catalyst solution prepared above was then added via cannula. After 5 h, TLC (3:1, petrol–EtOAc) indicated the formation of a single product ($R_f = 0.65$) and the complete consumption of the starting material ($R_f = 0.60$). The reaction mixture was allowed to cool to rt and was then diluted with CH_2Cl_2 (50 mL) and concentrated in vacuo. The resulting residue was purified by flash column chromatography (5:1, petrol–EtOAc) to afford enol ethers **10** (144 mg, 95%) as an off-white crystalline solid, mp 50–53 °C (EtOAc/petrol); IR: (KBr) 1456 (w, C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (*E*:*Z*, 1:2.9): δ 1.58 (1H, dd, $J = 1.5$ Hz, $J = 6.8$ Hz, $OCH{=}CHCH_3$-*E*), 1.67 (3H, dd, $J = 1.6$ Hz, $J = 6.8$ Hz, $OCH{=}CHCH_3$−*Z*), 2.32 (3H, s, $ArCH_3$), 3.46–3.59 (2H, m, H-4, H-5), 3.59 (1H, at, $J = 9.6$ Hz, H-3), 3.70 (1H, at, $J = 8.7$ Hz, H-2), 3.72–3.82 (2H, m, H-6, H-6′), 4.46 (1H, aquint, $J = 6.7$ Hz, $OCH{=}CHCH_3$–*Z*), 4.55, 4.62 (2H, ABq, $J_{A,B} = 12.1$ Hz, OCH_2Ph), 4.58 (1H, d, $J_{1,2} = 10.4$ Hz, H-1), 4.58, 4.83 (2H, ABq, $J_{A,B} = 10.7$ Hz, OCH_2Ph), 4.72, 4.83 (2H, ABq, $J_{A,B} = 10.2$ Hz, OCH_2Ph), 5.03 (1H, m, $OCH{=}CHCH_3$–*E*), 6.04 (1H, dd, J_Z 6.2 Hz, $OCH{=}CHCH_3$–*Z*), 6.10 (1H, dd, J_E 12.1 Hz, $OCH{=}CHCH_3$–*E*), 7.04 (2H, d, *J* 8.5 Hz, Ar–H), 7.20-7.38 (15H, m, Ar–H), 7.48 (2H, d, *J* 8.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) (*Z* isomer): δ 9.4 (q, $CH{=}CHCH_3$), 21.1 (q, $ArCH_3$), 69.0 (t, C-6), 73.4, 75.1, 75.6 ($3 \times$ t, $3 \times PhCH_2$), 79.2, 79.2, 82.3 ($3 \times$ d, C-3, C-4, C-5), 86.0, 86.3 ($2 \times$ d, C-1, C-2), 100.2 (d, $CH{=}CHCH_3$), 127.5, 127.6, 127.8, 127.9, 128.3, 128.3, 128.4, 129.6, 133.7, 133.7 (10 X d, Ar–CH), 138.0, 138.3 ($2 \times$ s, Ar–C), 146.5 (d, $CH{=}CHCH_3$); *m*/*z* (ES^+) 619 ($M+Na^+$, 100), 614 ($M+NH_4^+$, 57%); ESIMS *m*/*z* calcd for $C_{37}H_{44}NO_5S$ $[M+NH_4]^+$: 614.2940. Found 614.2938. Anal Calcd for $C_{37}H_{40}O_5S{\cdot}1/2H_2O$: C, 73.36; H, 6.82. Found: C, 73.47; H, 6.76.

4.9. Methyl 3,4,6-tri-*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*-benzylidene-α-D-mannopyranoside (11)

Iodine (11.6 mg, 0.0457 mmol), silver trifluoromethanesulfonate (12 mg, 0.0485 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (20 mg, 0.097 mmol) and 4 Å molecular sieves were added to freshly distilled CH_2Cl_2 (2 mL) in a flame-dried flask. The mixture was cooled to −78 °C and stirred for 20 min under an atmosphere of Ar. Enol ethers **10** (23.5 mg, 0.0388 mmol) and trisaccharide **8** (40 mg, 0.0323 mmol) were dissolved in freshly distilled CH_2Cl_2 (1 mL) and added to the reaction vessel via cannula under an atmosphere of Ar. The reaction was allowed to warm to rt whilst stirring. After 17 h, TLC (10:1, toluene–EtOAc) indicated the complete consumption of the trisaccharide ($R_f = 0.2$) and enol ethers ($R_f = 0.64$). The reaction mixture was diluted with CH_2Cl_2 (5 mL) and quenched by addition of sodium thiosulfate (5 mL of a 10% aqueous solution). The mixture was filtered and the two phases separated. The aqueous layer was extracted with CH_2Cl_2 (10 mL) and the combined organic extracts were dried ($MgSO_4$), fil-

tered and concentrated in vacuo. The residue was purified by flash column chromatography (20:1→10:1, toluene–EtOAc) to afford intermediate mixed acetals (48 mg, 77%) as a pale yellow foam as a mixture of diastereomers; *m*/*z* (ES^+) 1983 ($M+Na^+$, 17) 1978 ($M+NH_4^+$, 7%); *m*/*z* (isotopic distribution) expected mass 1985 (MNa^++2, 36%), 1984 (MNa^++1, 70%), 1983 (MNa^+, 100%), 1982 (MNa^+−1, 78%), found 1985 (MNa^++2, 37%), 1984 (MNa^++1, 62%), 1983 (MNa^+, 100%), 1982 (MNa^+−1, 88%).

Mixed acetals prepared above (42 mg, 0.0216 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (9 mg, 0.0438 mmol) and 4 Å molecular sieves were added to freshly distilled CH_2Cl_2 (1 mL) in a flame-dried flask under an atmosphere of Ar, and the mixture was cooled to 0 °C whilst stirring. Dimethyldisulfide (4.0 μL, 0.044 mmol) and triflic anhydride (7.0 μL, 0.041 mmol) were dissolved in freshly distilled CH_2Cl_2 (0.25 mL) and after 5 min the mixture was added to the reaction vessel under an atmosphere of Ar. The reaction was allowed to reach rt and stirred. After 90 min, TLC (10:1, toluene–EtOAc) indicated the complete consumption of starting material ($R_f = 0.49$) and the formation of several products ($R_f = 0.15$–0.49). The reaction mixture was cooled to 0 °C, then dioxane (5 mL) and an aqueous NaH_2PO_4/Na_2HPO_4 buffer (pH 6.0, 3 mL) were added and the mixture stirred for a further 10 min. The mixture was filtered and diluted with CH_2Cl_2 (20 mL). The organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuo. The crude residue was dissolved in acetone (3 mL) and water (0.3 mL). NIS (7 mg, 0.032 mmol) was added and the mixture stirred overnight at rt. The reaction mixture was treated with sodium thiosulfate (5 mL of a 10% aqueous solution) and diluted with CH_2Cl_2 (10 mL). The aqueous extracts were washed with CH_2Cl_2 (10 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (10:1, toluene–EtOAc) to afford tetrasaccharide **11** (21 mg, 58%) as a colourless foam; $[\alpha]_D^{25}$ +94 (*c* 0.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$):[†] δ 3.15–3.16 (1H, br m, H-2_d), 3.26 (1H, dd, $J_{5,6} = 1.6$ Hz, $J_{6,6'} = 11.6$ Hz, H-6_d), 3.30 (1H, dd, $J_{5,6} = 3.1$ Hz, H-$6'_d$), 3.34 (3H, s, OCH_3), 3.35 (1H, at, $J = 9.6$ Hz, H-3_d), 3.43 (1H, at, $J = 9.5$ Hz, H-4_d), 3.48 (1H, dd, $J_{1,2} = 3.7$ Hz, $J_{2,3} = 9.5$ Hz, H-2_x), 3.52 (1H, d, $J_{6,6'} = 10.0$ Hz, H-6_y), 3.65–3.76 (6H, m, H-4_x, H-6_x, H-$6'_x$, H-2_y, H-4_y, H-$6'_y$), 3.82–3.87 (4H, m, H-2_a, H-5_a, H-6_a, H-5_d), 3.91 (1H, dat, $J = 9.9$ Hz, H-5_x), 3.96 (1H, at, $J = 9.4$ Hz, H-3_y), 4.18–4.24 (4H, m, H-$6'_a$, H-5_y, 2 × PhC*H*H′), 4.30–4.58 (15H, m, H-3_a, H-4_a, H-3_x, H-1_d, 11 × PhC*H*H′), 4.68 (1H, d, $J = 11.2$ Hz, PhC*H*H′), 4.70 (1H, d, $J_{1,2} = 1.3$ Hz, H-1_a), 4.70 (1H, d, $J = 10.6$ Hz, PhC*H*H′), 4.73 (1H, d, $J = 11.3$ Hz, PhC*H*H′), 4.82 (1H, d, $J = 10.8$ Hz, PhC*H*H′), 4.85 (1H, d, $J = 10.5$ Hz, PhC*H*H′), 5.02 (1H, d, $J = 11.4$ Hz, PhC*H*H′), 5.07 (1H, d, $J = 11.8$ Hz, PhC*H*H′), 5.38 (1H, s, PhC*H*), 5.46 (1H, d, $J_{1,2} = 3.2$ Hz, H-1_y), 5.66 (1H, d, H-1_x), 6.99–7.53 (55H, m, Ar–H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 54.7 (q, OCH_3), 63.6 (d, C-5_a), 68.0, 68.3, 68.5, 68.7 (4 × t, C-6_a, C-6_x, C-6_y, C-6_d), 69.8, 70.0 (2 × d, C-5_x, C-5_d), 71.0, 73.0, 73.4, 73.4, 74.4, 74.6, 74.8, 74.8, 75.8 (9 × t, 9 × Ph*C*H_2), 71.0 (d, C-5_y), 71.9 (d, C-3_a), 75.5 (d, C-2_y), 76.8 (d, C-4_d), 77.1 (d, C-2_a), 77.7, 78.0, 78.3 (3 × d, C-3_x, C-4_x, C-4_y), 78.7 (d, C-2_x), 80.0 (d, C-4_a), 80.3 (d, C-3_y), 83.8 (d, C-3_d), 95.9 (d, C-1_x, C-1_y, C-1_d), 100.9 (d, C-1_a), 102.0 (d, Ph*C*H), 126.2, 127.1, 127.1, 127.2, 127.3, 127.5, 127.6, 127.6, 127.7, 127.7, 127.9, 127.9, 127.9, 128.0, 128.1, 128.1, 128.2, 128.2, 128.2, 128.3, 128.3, 128.4, 129.0 (24 × d, Ar–CH), 137.2, 137.6, 137.8, 138.0, 138.1, 138.1, 138.3, 138.3, 138.9, 139.1 (9 × s, Ar–C); *m*/*z* (ES^+) Isotope Distribution calcd for $C_{102}H_{108}O_{21}Na$ (MNa^+) 1694.7 (26), 1693.7 (61), 1692.7 (100), 1691.7 (88%). Found 1694.9 (28), 1693.9 (61), 1692.9 (100), 1691.9 (95%).

4.10. Methyl α-D-glucopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-α-D-mannopyranoside (12)

Protected tetrasaccharide **11** (10 mg, 5.9 μmol) was dissolved in ethanol (2.25 mL) and acetic acid (0.25 mL), and palladium(II) acetate (5 mg 0.022 mmol) was added. The mixture was degassed and stirred under an atmosphere of H_2. After 22 h, TLC (CMAW) indicated the formation of a single major product ($R_f = 0.1$). The reaction mixture was filtered through Celite and concentrated in vacuo. The residue purified by size-exclusion chromatography on Sephadex G-25 (water) to afford deprotected tetrasaccharide **12** (4 mg, 98%); $[\alpha]_D^{25}$ +84 (*c* 0.2, MeOH); ^1H NMR (500 MHz, D_2O)[13]: δ 3.41 (3H, s, OCH_3), 3.46 (1H, H-4_d), 3.53 (1H, H-4_c), 3.60 (1H, H-2_d), 3.62 (1H, H-4_b), 3.65 (1H, H-2_b), 3.66 (1H, H-4_a), 3.69 (1H, H-2_c), 3.76 (2H, H-6_b, H-$6'_b$), 3.78 (1H, H-5_a), 3.79 (1H, H-3_d), 3.79 (2H, H-6_c, H-$6'_c$), 3.82 (1H, H-5_b), 3.82 (2H, H-6_d, H-$6'_d$), 3.83 (1H, H-6_a), 3.84 (1H, H-3_c), 3.86 (1H, H-3_a), 3.91 (1H, H-3_b), 3.91 (1H, H-$6'_a$), 3.96 (1H, H-5_d), 4.06 (1H, H-5_c), 4.09 (1H, H-2_a), 4.74 (1H, H-1_a), 5.17 (1H, d, $J_{1,2} = 3.7$ Hz, H-1_d), 5.24 (1H, d, $J_{1,2} = 4.4$ Hz, H-1_b), 5.52 (1H, d, $J_{1,2} = 3.7$ Hz, H-1_c); ^{13}C NMR (100.6 MHz, D_2O): δ 54.6 (q, OCH_3), 60.0 (t, C-6_c), 60.1 (t, C-6_d), 60.1 (t, C-6_b), 60.7 (t, C-6_a), 65.9 (d, C-3_c), 69.1 (d, C-4_d), 69.1 (d, C-4_c), 69.6 (d, C-2_a), 69.7 (d, C-4_b), 70.1 (d, C-2_b), 71.0 (d, C-5_b), 71.1 (d, C-2_d), 71.3 (d, C-5_c), 71.6 (d, C-5_d), 71.7 (d, C-3_d), 72.4 (d, C-4_a), 72.7 (d, C-5_a), 75.2 (d, C-2_c), 78.4 (d, C-3_a),

[†] It was not possible to distinguish glucose residues b and c from the NMR data and consequently, they have been arbitrarily denoted as *x* and *y*.

79.6 (d, C-3_b), 95.7 (d, C-1_d), 96.4 (d, C-1_c), 100.5 (d, C-1_b), 100.6 (d, C-1_a); *m/z* (ES^+) 703 ($M+Na^+$, 100%). ESIMS *m/z* calcd for $C_{25}H_{48}NO_{21}$ $[M+NH_4]^+$: 698.2719. Found 698.2716.

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