Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1937–1949
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Synthesis of fluorescence-labelled disaccharide substrates of glucosidase II

Ian Cumpstey,[a] Terry D. Butters,[b] Richard J. Tennant-Eyles,[a] Antony J. Fairbanks,[a,*] Robert R. France,[a] Mark 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 February 2003; received in revised form 23 May 2003; accepted 31 May 2003

Abstract

The fluorescence-labelled disaccharides Glcα(1→3)GlcαOR and Glcα(1→3)ManαOR, both substrates for the glycoprotein-processing enzyme glucosidase II, were synthesised via the use of a *n*-pentenyl-derived linker at the anomeric position. This allowed incorporation of a pyrenebutyric acid label, via a sequence of oxidative hydroboration, mesylation, azide displacement, reduction with concomitant global deprotection, and peptide coupling. Selective activation of a fully armed thioglycoside in the presence of *n*-pentenyl glycosides was readily achieved by the use of methyl triflate as promoter.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Glucosidase II; Glycoprotein processing; *n*-Pentenyl glycosides; Fluorescence labels

1. Introduction

N-Glycoprotein biosynthesis is initiated by the oligosaccharyl transferase (OST)-mediated transfer of a 14-membered dolichol phosphate bound oligosaccharide ($Glc_3Man_9GlcNAc_2$) to particular asparagine residues (Asn-X-Ser/The).[1] Following this transfer sequential trimming of the oligosaccharide chain occurs that is initiated by two glycoprotein-processing enzymes, glucosidases I and II, in the endoplasmic reticulum (ER) of the cell. Subsequently, the action of several other glycosidases and glycosyl transferases leads to the eventual production of glycoproteins bearing the familiar variety of *N*-glycan structures.

Glucosidase I[2] is a type II protein found in the ER, and is responsible for cleavage of the terminal α-(1→2)-linked glucose residue from the $Glc_3Man_9GlcNAc_2$ glycan. Glucosidase II,[3] a heterodimeric enzyme found in the ER, then sequentially cleaves the remaining two α-linked glucose residues from the precursor oligosaccharide $Glc_2Man_9GlcNAc_2$ (Fig. 1). In particular, cleavage of the first glucose residue as catalysed by glucosidase II releases the glycan $Glc_1Man_9GlcNAc_2$. The correct folding of the glycoprotein is dependent on the binding of the lectin chaperones calnexin and calreticulin to this monoglucosylated moeity.[4] The chaperones do not bind to the non-glucosylated $Man_9GlcNAc_2$ glycan, so cleavage of the final glucose residue by glucosidase II stops protein folding. Indeed, if a protein has not achieved its native conformation at this point, it is recognized and re-glucosylated by a glucosyl transferase[5] as part of a quality control mechanism to complete the folding process.[6] Conversely, if correct folding has occurred prior to cleavage of the last glucose residue by glucosidase II, the protein is not re-glucosylated and it then leaves the ER to continue maturation.

Previous work investigating the kinetics of glucosidase II from rat liver using α-*para*nitrophenyl glucopyranoside as an artificial substrate, has led to the proposal that the enzyme contains two active sites.[7] Moreover, these authors have suggested that the $Glc_2Man_9GlcNAc_2$ substrate may bind to these two sites simultaneously, resulting in the cleavage of both glucose linkages before release of the $Man_9GlcNAc_2$ glycan.

* Corresponding author. Tel.: +44-1865-275647; fax: +44-1865-275674.
E-mail address: antony.fairbanks@chem.ox.ac.uk (A.J. Fairbanks).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00255-6

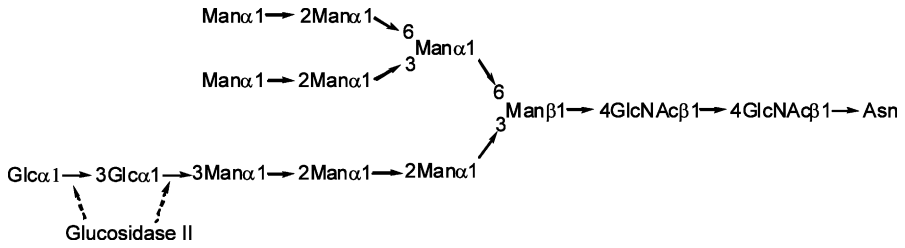


Fig. 1. Enzymatic cleavages of N-linked glycans by Glucosidase II.

However, the isolation of substantial quantities of the $Glc_1Man_9GlcNAc_2$ glycan during treatment of the $Glc_2Man_9GlcNAc_2$ substrate with catalytic quantities of glucosidase II indicated that for the natural substrates cleavage of the Glcα(1→3)Glc linkage was faster than cleavage of the Glcα(1→3)Man linkage.[8] This rate differential, which may be important to allow the opportunity for chaperone binding before the removal of the final glucose unit, could be entirely due to the different stereochemistry of the respective *endo* residues (i.e., *gluco* vs. *manno*), or alternatively, could at least in part be effected by the rest of the oligosaccharide chain. In order to further investigate the kinetics of glucosidase II, and whether the rest of the oligosaccharide chain plays a role in determining this differential rate of cleavage, the synthesis of two disaccharides representing the two α-gluco linkages in question, each with a fluorescence label attached to the terminal anomeric position via a short α-linker, was undertaken (Fig. 2). It was also envisaged that these substrates might also be used for the development of an assay for glucosidase II.

Fluorescence-labelling of enzyme substrates is a valuable tool, as it enables the sensitive detection of the small quantities of material involved in an enzymatic assay.[9] When considering the means of attachment of such a label to the carbohydrate several factors must be considered; the most important being that the linker should be long and flexible enough so that the presence of the label should not affect the kinetics of enzymatic cleavage. Moreover, direct attachment of a label to the terminal residue, for example via a reductive amination strategy[10] would necessarily involve destruction of the terminal pyranose ring, and clearly in this case meaningless kinetic results would ensue.

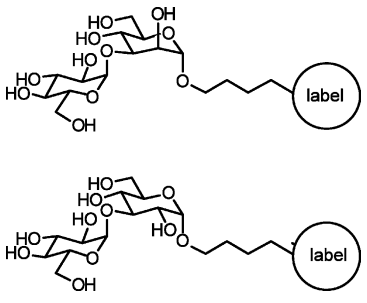
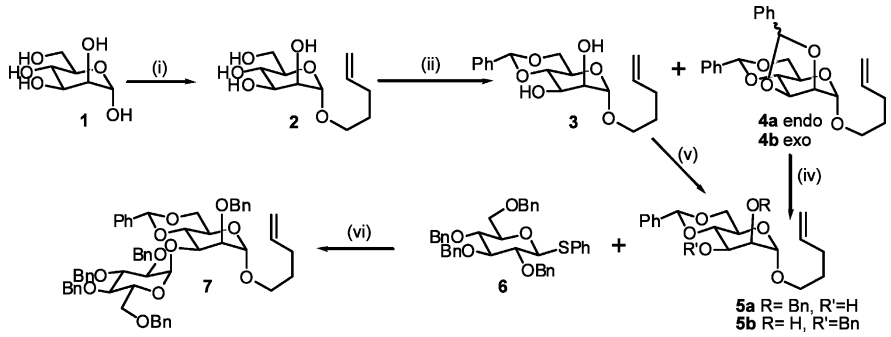


Fig. 2. Fluorescence labelled substrates for glucosidase II.

As such the use of an *n*-pentenyl glycoside to furnish a sterically undemanding and flexible linker between the disaccharides and the label seemed ideal.[11] Indeed we recently reported a protocol whereby *n*-pentenyl glycosides, which themselves are readily available and commonly used glycosyl donors—thus allowing the possibility of extending the synthesis to tri- and higher oligosaccharides if desired, may be derivatised and attached to the fluorescent label 2-aminobenzamide (2-AB) via a reductive amination strategy.[12] Herein we report the synthesis of the two disaccharide substrates for glucosidase II[13] and their fluorescence-labelling via *n*-pentenyl-derived linkers.

2. Results and discussion

The labelling strategy adopted involved the synthesis of the two required disaccharides as their α-*n*-pentenyl glycosides, and subsequent installation of the fluorescence label by manipulation of the alkene function of the pentenyl group. Such a strategy is flexible in that further monosaccharide units may be easily added if desired, allowing access to higher oligosaccharide substrates of glucosidase II. Synthesis of the Glcα(1→3)Man disaccharide proceeded as follows. Heating mannose **1** with camphor sulfonic acid (CSA) in penten-1-ol produced the α-pentenyl mannoside **2**, which was then treated with benzaldehyde dimethyl acetal to yield a mixture of monobenzylidene derivative **3** (52% yield) as well as the *endo* and *exo* dibenzylidene derivatives **4a** and **4b** (46% yield, *endo*–*exo* ratio 1:1, Scheme 1). Selective reductive cleavage of the *endo* dibenzylidene derivative **4a** with DIBAL at −40 °C in toluene produced the desired 2-O-benzylated glycosyl acceptor (**5a**) (40% yield). Alternatively, benzylation of the mono-benzylidene derivative **3** under phase-transfer conditions also gave the required alcohol **5a** (45% yield) along with a minor amount of the undesired 3-O-benzylated sugar (**5b**) (20% yield). Therefore, in summary the most efficient synthesis of **5a** could be achieved by selective mono-benzylidination of **2** to yield **3** and subsequent selective benzylation. Mindful of the fact that *n*-pentenyl glycosides are readily activated under many of the same sets of conditions as thioglycosides (for example with sources of I^+) glycosylation of the

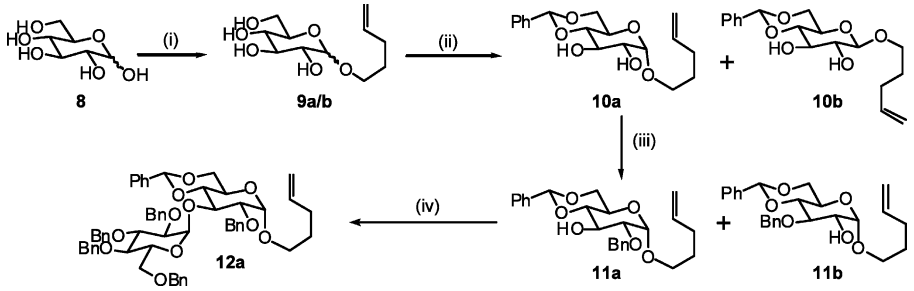


Scheme 1. Reagents and conditions: (i) penten-1-ol, CSA, 100 °C, 61%; (ii) $PhCH(OMe)_2$, CSA, DMF, 65 °C, 260–280 mbar; **3**, 52%; **4a**:**4b** (endo:exo, 1:1), 46%; (iv) DIBAL, toluene, −40 °C, 40%; (v) BnBr, NaOH, Bu_4NHSO_4, CH_2Cl_2, H_2O, 41 °C; **5a**, 45%; **5b**, 20%; (vi) MeOTf, 4 Å sieves, Et_2O, RT, 58%.

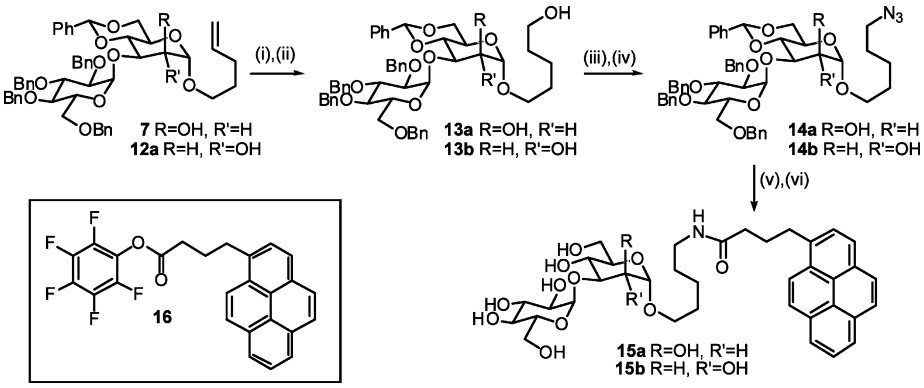
alcohol **5a** was then attempted with the fully armed *gluco* thioglycoside **6**.[14] It was found that selective activation of the thioglycoside could be achieved by the use of methyl triflate. Thus glycosylation of **5a** with **6** was achieved by treatment with methyl triflate, using diethyl ether as solvent in order to ensure good α-selectivity, the desired disaccharide **7** was produced in an acceptable 58% yield, along with a small quantity of the *O*-methylated acceptor; no β-disaccharide was isolated. To the best of our knowledge, this represents only the second report of the selective activation of a thioglycoside in the presence of an *n*-pentenyl glycoside.[15]

A similar reaction sequence was employed for the synthesis of the required Glcα(1→3)Glc disaccharide (Scheme 2). Thus treatment of D-glucose **8** with penten-1-ol and CSA produced an inseparable mixture of α- and β-pentenyl glucosides **9a/b** (63% yield), which were treated with benzaldehyde dimethyl acetal to yield the mono-benzylidene derivatives **10a** and **10b** (46 and 20% yields, respectively) which could easily be separated by column chromatography. Selective benzylation of the required α-glucoside **10a** under phase-transfer conditions yielded the desired 2-O-benzyl alcohol **11a** as the major product, together with some of the undesired 3-O-benzylated regioisomer **11b**. Glycosylation of the required alcohol **11a** using thioglycoside **6** as the glycosyl donor was again achieved by the use of methyl triflate as activator, with ether as solvent, and proceeded with good stereoselectivity to yield the desired α-disaccharide **12a** (80% yield) along with a small quantity of the undesired β-linked disaccharide (**12b**, 15% yield).

Previous studies on the attachment of the commonly used fluorescence label 2-aminobenzamide (2-AB) to an *n*-pentenyl glycoside had been achieved via a procedure of dihydroxylation, periodate cleavage and subsequent reductive amination.[12] Although this procedure had worked on a monosaccharide, problems had been encountered therein, particularly with attempted removal of benzyl protecting groups by hydrogenation. Indeed disappointingly several attempts to use similar reductive amination methodology with aldehydes derived from the pentenyl disaccharides all met with failure. Consequently, a different methodology was sought. Various possibilities were investigated, including linkage of methylumbellieryl, pyrenebutyryl and dansyl labels via ether, ester and sulfonamide linkages, respectively. However, as in the 2-AB case, it was found that the coumarin and pyrene labels were incompatible with the hydrogenation conditions necessary to remove the benzyl ether protecting groups. Consequently, it was seen to be advantageous to attach the fluorescent label after debenzylation, and so it was necessary to find a way to attach a label to the deprotected sugar. It was envisaged that this could be achieved by installation of



Scheme 2. Reagents and conditions: (i) penten-1-ol, CSA, 140 °C, 63%; (ii) $PhCH(OMe)_2$, CSA, DMF, 65 °C, 260–280 mbar; **10a**, 46%; **10b**, 20%; (iii) BnBr, NaOH, Bu_4NHSO_4, CH_2Cl_2, H_2O, 41 °C, 66%, **11a**:**11b**, 1.6:1; (iv) **6**, MeOTf, 4 Å sieves, Et_2O, RT; **12a**, 80%; **12b**, 15%.



Scheme 3. Reagents and conditions: (i) 9-BBN, THF or toluene, 50 °C; (ii) H_2O_2, NaOH, H_2O; **13a**, 52%; **13b**, 60%; (iii) MsCl, Et_3N, DCM, 0 °C; (iv) NaN_3, DMF, RT; **14a**, 81%; **14b**, 80%; (v) MeOH, AcOH, H_2, Pd (10% on carbon), RT; (vi) **16**, Et_3N, DMF; **15a**, 44%; **15b**, 79%.

an azide functionality at the terminus of the pentenyl group which could be reduced to an amine concomitant with the removal of the benzyl and benzylidene protecting groups; peptide coupling to a fluorescent carboxylic acid would then yield the desired deprotected labelled disaccharides.

To this end, the pentenyl glycosides **7** and **12a** were converted to the alcohols **13a** and **13b** by regioselective hydroboration in 52 and 60% yields, respectively. Surprisingly this reaction required elevated temperatures, and also in the case of the Glcα(1→3)Glc disaccharide **12a**, a change of solvent from THF to toluene, in order to proceed efficiently. Mesylation of these primary alcohols, followed by displacement by azide proceeded smoothly in both cases to yield the desired azides **14a** and **14b** (81 and 80% yields, respectively). Finally, global deprotection with concomitant reduction of the azides was achieved by catalytic hydrogenation, to give the desired deprotected disaccharide amines. These were then directly coupled to the pentafluorophenyl ester of 1-pyrenebutyric acid **16**[†] to furnish the desired labelled deprotected disaccharides **15a** (44% yield) and **15b** (79% yield), respectively (Scheme 3).

The deprotected pyrene labelled disaccharides were purified using normal phase HPLC and then assessed as substrates for glucosidase II (Fig. 3). Incubation for short times at 37 °C with linkage-specific α-glucosidase II showed rapid cleavage of the Glcα1,3Glc-pyrene **15b** to Glc-pyrene (Fig. 3A). Following a 1 h incubation, 65% of the fluorescent substrate had been hydrolysed to Glc-pyrene as indicated by the shift in retention time. The rate at which glucosidase II hydrolysed the Glcα1,3Man-pyrene **15a** was significantly reduced (7.6%) as indicated by the appearance of the hydrolysis product at an earlier retention time (Fig. 3D). The differential rates of hydrolysis of the two linkages have

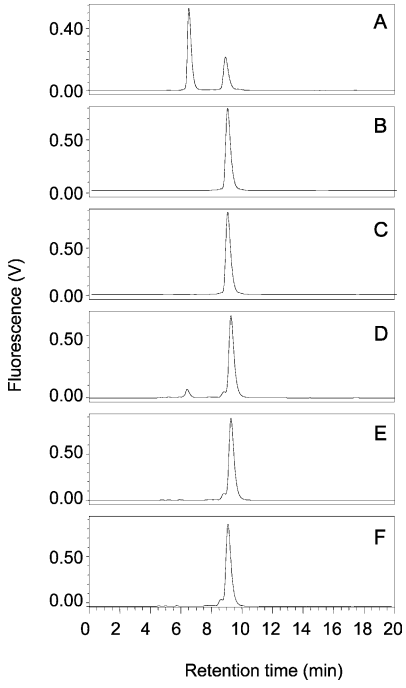


Fig. 3. Normal Phase HPLC Analysis of Fluorescent Glucosidase II Substrates. Pyrene disaccharide **15b** (Glcα1,3Glc, 2 mM) was incubated for 1 h at 37 °C with A, α-glucosidase II; B, α-glucosidase I; C, buffer alone. Pyrene disaccharide **15a** (Glcα1,3Man, 2 mM) was incubated for 1 h at 37 °C with D, α-glucosidase II; E, α-glucosidase I; F, buffer alone. Hydrolysis products were separated at 0.5 mL/min on a 4.6 × 250 mm TSK gel amide-80 column at 30 °C using a solvent composed of 80% acetonitrile, 20% ammonium acetate (100 mM), pH 3.85 and detected at Ex λ 345 nm, Ex λ 380 nm.

[†] Made by treatment of 1-pyrene butyric acid and pentafluorophenol (4 equiv) with dicyclohexylcarbodiimide (DCC, 2 equiv) in dichloromethane at room temperature for 1 h 30 min, and subsequent purification by flash column chromatography.

been observed using glucosylated oligosaccharides[8,16], and is in support of the data presented here. Importantly, no hydrolysis of the fluorescent disaccharides was seen using purified α-glucosidase I or buffer alone (Fig. 3B, C, E, F).

In summary, two fluorescence-labelled disaccharide substrates for glucosidase II have been successfully synthesized by means of selective activation of a thioglycoside donor in the presence of a pentenyl glycoside; in both cases installation of a fluorescence label was achieved by derivatisation of the anomeric pentenyl chain and peptide coupling with an activated ester of pyrene butyric acid. Both deprotected disaccharides have been demonstrated to be substrates for glucosidase II, and differential rates of cleavage observed; the GlcGlc disaccharide **15b** being cleaved more rapidly than the GlcMan disaccharide **15a**. Detailed investigation of the relative rates of cleavage of each by glucosidase II is currently underway, and the results will be reported in due course.

3. Experimental

3.1. 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 Varian Gemini 200 (200 MHz) spectrometer. 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. Infrared spectra were recorded on a Perkin–Elmer 150 Fourier Transform spectrophotometer. Low resolution mass spectra were recorded a VG Micromass Platform using either atmospheric pressure chemical ionisation (APCI), or negative ion electrospray (ES^-) or positive ion electrospray (ES^+). 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. Thin layer chromatography (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 sulphuric acid. Flash column chromatography was carried out using Sorbsil C60 40/60 silica. CMAW ($CHCl_3$–MeOH–AcOH–water) used as an eluant was prepared in the following ratio ($CHCl_3$–MeOH–AcOH–H_2O, 60:30:3:5). Solvents and reagents were dried and purified before use according to standard procedures under an atmosphere of argon; MeOH was distilled from sodium hydride, dichloromethane (DCM) and toluene were distilled from CaH_2, Py was distilled from CaH_2 and stored over potassium hydroxide, and Et_2O and THF were distilled from a solution of sodium benzophenone ketyl immediately before use.

3.2. Pent-4′-enyl α-D-mannopyranoside (2)

Camphor sulfonic acid (110 mg, 0.47 mmol) was added to a stirred suspension of D-mannose **9** (8.5 g, 47.2 mmol) in pent-4-en-1-ol (40 g) and the reaction mixture stirred at 100 °C for 20 h. After this time TLC (EtOAc–MeOH, 4:1) indicated the formation of a major product (R_f 0.4). Pentenol (30 g) was recovered by vacuum distillation using a dry ice condenser, and the remaining brown residue was partitioned between water (200 mL) and CH_2Cl_2 (200 mL). The aqueous layer was concentrated in vacuo and the resulting foam purified by flash chromatography (EtOAc then EtOAc–MeOH, 9:1) to give α-pentenyl glycoside **2** (7.64 g, 61%) as a pale yellow oil; $[\alpha]_D^{22}$ +64.2 (*c*, 0.5 in $CHCl_3$) [Lit. +54.4 (*c*, 1.0 in $CHCl_3$)][17]; δ_H (400 MHz, $CDCl_3$) 1.66 (2 H, m, OCH_2CH_2), 2.08 (2 H, m, $OCH_2CH_2CH_2$), 3.41 (1 H, dt, J_{gem} 9.6 Hz, *J* 6.4 Hz, $OCHH'CH_2$), 3.51 (1 H, br, H-5), 3.65 (1 H, dt, *J* 6.7 Hz, $OCHH'CH_2$), 3.76 (1 H, br, H-6) 3.84 (1 H, br, H-3) 3.89–3.97 (3 H, m, H-2, H-4, H-6′), 4.81 (1 H, s, H-1), 4.96–5.05 (2 H, m, $CH=CH_2$), 5.80 (1 H, ddt, J_E 17.0 Hz, J_Z 10.2 Hz, *J* 6.6 Hz, $CH=CH_2$).

3.3. Pent-4′-enyl 4,6-O-benzylidene-α-D-mannopyranoside (3), pent-4′-enyl *endo*-2,3:4,6-di-O-benzylidene-α-D-mannopyranoside (4a) and pent-4′-enyl *exo*-2,3:4,6-di-O-benzylidene-α-D-mannopyranoside (4b)

Tetrol **2** (5.4 g, 20.5 mmol), benzaldehyde dimethyl acetal (7.1 mL, 47.0 mmol) and CSA (95 mg, 0.41 mmol) were dissolved in DMF (30 mL) and the mixture was put onto a rotary evaporator at 65 °C and 260–280 mbar. After 5 h 30 min, TLC (petrol–EtOAc, 3:1) indicated the formation of a major product (R_f 0.9) and small amounts of starting material (baseline) and a minor product (R_f 0.1). The mixture was concentrated in vacuo, and the residue dissolved in CH_2Cl_2 (400 mL). This solution was washed with water (2 × 150 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (petrol–EtOAc, 8:1) to afford a 1:1

mixture of *endo* and *exo* dibenzylidene derivatives **4a/4b** (4.0 g, 46%) as a waxy solid. Recrystallisation from EtOH gave the *exo* dibenzylidene **4b** as a white, crystalline solid, mp 131–136 °C; $[\alpha]_D^{22}$ +11.5 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr disk) 1641 (w, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.67–1.74 (2 H, m, OCH_2CH_2), 2.11–2.17 (2 H, m, $OCH_2CH_2CH_2$), 3.45 (1 H, dt, J_{gem} 9.7 Hz, *J* 6.4 Hz, $OCHH'CH_2$), 3.75 (1 H, dt, *J* 6.6 Hz, $OCHH'CH_2$), 3.81–3.93 (3 H, m, H-4, H-5, H-6), 4.16 (1 H, d, $J_{2,3}$ 5.4 Hz, H-2), 4.35 (1 H, dd, $J_{5,6'}$ 3.0 Hz, $J_{6,6'}$ 8.3 Hz, H-6′), 4.66 (1 H, dd, $J_{3,4}$ 7.6 Hz, H-3), 5.00 (1 H, dd, J_{gem} 1.7 Hz, J_Z 10.2 Hz, $CH=CH_EH_Z$) 5.04 (1 H, dd, J_E 17.1 Hz, $CH=CH_EH_Z$, 5.11 (1 H, s, H-1), 5.65 (1 H, s, PhC*H*), 5.81 (1 H, ddt, *J* 6.7 Hz, $CH=CH_2$), 6.30 (1 H, s, PhC*H*), 7.34–7.55 (10 H, m, Ar–H); δ_C ($CDCl_3$) 28.4, 30.2 (2 × t, $OCH_2CH_2CH_2$), 60.4, 75.5, 75.6, 77.6 (4 × d, C-2, C-3, C-4, C-5), 67.4, 68.9 (22 × t, C-6, $OCH_2CH_2CH_2$), 97.5, (d, C-1), 102.1, 103.1 (2 × d, 2 × Ph*C*H), 115.4 (t, $CH=CH_2$), 126.3, 126.5, 128.5, 128.7, 129.4 (5 × d, 10 × Ar–CH), 137.4, 138.9 (2 × s, 2 × Ar–C), 138.0 (d, $CH=CH_2$); *m/z* ($APCI^+$) 425 ($M+H^+$, 100), 339 ($M-OC_5H_9$, 6%). (Found: C, 70.43; H, 6.78. $C_{25}H_{28}O_6$ requires C, 70.73; H, 6.65%). The mother liquor was a concentrated to give colourless oil which had a ^1H NMR spectrum consistent with a 6:1 *endo–exo* mixture of dibenzylidenes **4a** and **4b**. Partial NMR data for **4a** follows: δ_H (200 MHz, $CDCl_3$) 1.73 (2 H, m, $OCH_2CH_2CH_2$), 2.15 (2 H, m, $OCH_2CH_2CH_2$), 3.48 (1 H, dt, $OCHH'CH_2$), 4.28–4.33 (2 H, m), 4.50 (1 H, at), 4.98–5.11 (2 H, m, $CH=CH_2$), 5.18 (1 H, s, H-1), 5.54 (1 H, s, PhC*H*), 5.83 (1 H, ddt, $CH=CH_2$), 5.98 (1 H, s, PhC*H*), 7.37–7.58 (10 H, m, Ar–H). Further elution of the flash column afforded the monobenzylidene derivative **3** (3.8 g, 52%) as a gelatinous solid; $[\alpha]_D^{22}$ +44.2 (*c*, 1.25 in $CHCl_3$) [Lit. +53.5 (*c*, 1.1 in $CHCl_3$)][18]; δ_H (200 MHz, $CDCl_3$) 1.65–1.79 (2 H, m, $OCH_2CH_2CH_2$), 2.09–2.21 (2 H, m, $OCH_2CH_2CH_2$), 2.60 (2 H, br s, 2 × OH), 3.45 (1 H, dt, J_{gem} 9.6 Hz, *J* 6.3 Hz, $OCHH'CH_2$), 3.73 (1 H, dt, *J* 6.5 Hz, $OCHH'CH_2$), 3.81–4.26 (5 H, m, H-2, H-3, H-4, H-5 and H-6), 4.29 (1 H, d, *J* 5.6 Hz, H-6′), 4.88 (1 H, s, H-1), 4.99–5.11 (2 H, m, $CH=CH_2$) 5.60 (1 H, s, PhC*H*), 5.83 (1 H, ddt, J_E 16.8 Hz, J_Z 10.1 Hz, *J* 6.4 Hz, $CH=CH_2$), 7.34–7.55 (5 H, m, Ar–H).

3.4. Pent-4′-enyl 2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (5a) and pent-4′-enyl 3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (5b)

3.4.1. Method 1 selective reduction. The ~6:1 *endo–exo* mixture of dibenzylidene derivatives **4a/4b** (as detailed above, 300 mg, 0.71 mmol) was dissolved in freshly distilled toluene (3 mL) in a flame-dried flask. The solution was stirred under Ar and cooled to −40 °C. Diisobutyl aluminium hydride (0.52 mL of 1.5 M solution in toluene, 0.78 mmol) was added and the reaction allowed to warm to room temperature (rt). After 2 h, TLC (EtOAc–petrol, 1:2) indicated formation of two products (R_f 0.45 and 0.6) and remaining starting material (R_f 0.8). The reaction was cooled to −40 °C and further diisobutyl aluminium hydride (0.52 mL of 1.5 M solution in toluene, 0.78 mmol) was added. After a further 2 h, TLC indicated the formation of a major product (R_f 0.6). The reaction was quenched with MeOH (2 mL) and the mixture partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (petrol–EtOAc, 6:1) to afford the desired 2-O-benzylated compound **5a** (120 mg, 40% (43% over recovered starting material)) as a colourless oil, which crystallised from petrol to give a white, crystalline solid, mp 68–71 °C (petrol); $[\alpha]_D^{22}$ +16.0 (*c*, 0.5 in $CHCl_3$); ν_{max} (KBr disk) 3392 (b, OH), 1641 (w, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.64–1.71 (2 H, m, $OCH_2CH_2CH_2$), 2.10–2.16 (2 H, m, $OCH_2CH_2CH_2$), 2.39 (1 H, d, *J* 8.0 Hz, OH-3), 3.39 (1 H, dt, J_{gem} 9.6 Hz, *J* 6.4 Hz, $OCHH'CH_2$), 3.70 (1 H, dt, *J* 6.6 Hz, $OCHH'CH_2$), 3.79–3.86 (3 H, m, H-2, H-5, H-6), 3.92 (1 H, m, H-4), 4.12 (1 H, m, H-3), 4.26 (1 H, m, H-6′), 4.71, 4.76 (2 H, ABq, J_{AB} 11.7 Hz, $PhCH_2$), 4.83 (1 H, d, $J_{1,2}$ 1.1 Hz, H-1), 5.00 (1 H, dd, J_{gem} 1.7 Hz, J_Z 11.1 Hz, $CH=CH_EH_Z$ 5.04 (1 H, dd, J_E 17.1 Hz, $CH=CH_EH_Z$, 5.59 (1 H, s, PhC*H*), 5.81 (1 H, ddt, $CH=CH_2$), 7.29–7.55 (10 H, m, Ar–H); δ_C ($CDCl_3$) 28.4, 30.2 (2 × t, $OCH_2CH_2CH_2$), 63.5, 68.8, 78.7, 79.6 (4 × d, C-2, C-3, C-4, C-5), 67.1, 68.8, 73.8 (3 × t, C-6, $PhCH_2$, $OCH_2CH_2CH_2$), 98.5, 102.2 (2 × d, C-1, Ph*C*H), 115.3 (t, $CH=CH_2$), 126.5, 128.2, 128.5, 128.8, 129.3 (5 × d, Ar–CH), 137.0 (2 × s, Ar–C), 138.1 (d, $CH=CH_2$); *m/z* ($APCI^+$) 875 ($2M+Na^+$, 5), 449 ($M+Na^+$, 9), 427 ($M+H^+$, 9), 425 (M−H, 100), 341 ($M-OC_5H_9$, 8%). (Found: C, 70.26; H, 7.31. $C_{25}H_{30}O_6$ requires C, 70.40; H, 7.09); together with undesired 3-O-benzylated material **5b** (15 mg, 5%) as a colourless oil; $[\alpha]_D^{22}$ +45.9 (*c*, 1.25 in $CHCl_3$); ν_{max} (film) 3468 (OH), 1641 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.66–1.73 (2 H, m, $OCH_2CH_2CH_2$), 2.10–2.16 (2 H, m, $OCH_2CH_2CH_2$), 2.66 (1 H, s, OH-2), 3.44 (1 H, dt, J_{gem} 9.7 Hz, *J* 6.4 Hz, $OCHH'CH_2$), 3.71 (1 H, dt, *J* 6.6 Hz, $OCHH'CH_2$), 3.84–3.90 (2 H, m, H-5, H-6), 3.94 (1 H, dd, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 9.6 Hz, H-3), 4.07 (1 H, m, H-2), 4.11 (1 H, m, H-4), 4.28 (1 H, m, H-6′), 4.74, 4.88 (2 H, ABq, J_{AB} 11.8 Hz, $PhCH_2$), 4.87 (1 H, s, H-1), 4.99, (1 H, dd, J_Z 10.2 Hz, J_{gem} 1.5 Hz, $CH=CH_EH_Z$ 5.04 (1 H, dd, J_E 17.1 Hz, $CH=CH_EH_Z$, 5.63 (1 H, s, PhC*H*), 5.81 (1 H, ddt, *J* 6.8 Hz, $CH_2CH=CH_2$), 7.29–7.52 (10 H, m, 10 × Ar–H); δ_C ($CDCl_3$) 28.4, 30.2 (2 × t, $OCH_2CH_2CH_2$), 63.3, 70.0, 75.9, 79.0 (4 × d, C-2, C-3, C-4, C-5), 67.1, 68.9, 73.2 (3 × t, C-6, $PhCH_2$, $OCH_2CH_2CH_2$), 100.1, 101.7 (2 × d, C-1, Ph*C*H), 115.3 (t, $CH=CH_2$), 126.3, 128.1, 128.5, 128.7, 129.1 (5 × d, Ar–CH), 137.8, 138.2 (2 × s,

Ar–C), 138.1 (d, CH=CH_2); *m/z* (APCI$^-$) 425 (M−H, 6), 335 (M−Bn, 77), 317 (44), 121 (100); (Found: C, 69.90; H, 7.32; $C_{25}H_{30}O_6$ requires C, 70.40; H, 7.09).

3.4.2. Method 2 selective benzylation. Monobenzylidene derivative **3** (200 mg, 0.60 mmol) was dissolved in CH_2Cl_2 (10 mL) and tetrabutyl ammonium hydrogensulfate (41 mg, 0.12 mmol) and benzyl bromide (0.11 mL, 0.96 mmol) were added. An aq soln of sodium hydroxide (50 mg in 1 mL) was added and the mixture was refluxed at 41 °C. After 24 h, TLC (EtOAc–petrol, 1:2) indicated formation of two products (R_f 0.45 and 0.6) and starting material (R_f 0.1). The reaction mixture was cooled and partitioned between CH_2Cl_2 (20 mL) and water (20 mL). The organic layer was washed with water (2 × 20 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography (30–40 petrol–EtOAc, 8:1) to afford the desired 2-O-benzylated material **5a** (115 mg, 45%) as a white crystalline solid together with the undesired 3-O-benzylated material **5b** (52 mg, 20%) as a colourless oil, identical to the materials previously described.

3.5. Pent-4′-enyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (7)

Pentenyl glycoside **3** (300 mg, 0.70 mmol) and thioglycoside **6**[14] (456 mg, 0.72 mmol) were dissolved in freshly distilled ether (15 mL) in a flame-dried flask. The mixture was stirred under Ar with activated 4 Å molecular seives for 30 min. Methyl triflate (0.4 mL, 3.52 mmol) was added and the mixture stirred at rt. After 19 h, TLC (petrol–EtOAc, 4:1) indicated the formation of a major product (R_f 0.45) and very little remaining starting materials (R_f 0.25 and 0.5). The reaction was quenched with Et_3N (1 mL) and stirred for a further 30 min. The mixture was filtered through Celite® and concentrated in vacuo. The residue was purified by flash chromatography (petrol–EtOAc, 7:1) to afford the desired α-disaccharide **7** (385 mg, 58%) as a colourless oil; $[\alpha]_D^{22}$ +76.9 (*c*, 0.32 in $CHCl_3$); ν_{max} (film) 1640 (w, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.61–1.68 (2 H, m, $OCH_2CH_2CH_2$), 2.07–2.12 (2 H, m, $OCH_2CH_2CH_2$), 3.37 (1 H, dt, J_{gem} 9.6 Hz, *J* 6.5 Hz, $OCHH'CH_2$), 3.52 (1 H, dd, $J_{1,2}$ 3.3 Hz, $J_{2,3}$ 9.6 Hz, H-2_b) 3.58–3.70 (4 H, m, $OCHH'CH_2$, H-4_b, H-6_b, H-$6'_b$), 3.78–3.81 (2 H, m, H-2_a, H-5_b) 3.84–3.89 (2 H, m, H-5_a, H-6_a) 3.99 (1 H, at, *J* 9.3 Hz, H-3_b), 4.20 (1 H, m, H-$6'_a$), 4.31, 4.55 (2 H, ABq, J_{AB} 12.3 Hz, PhCH_2), 4.35 (1 H, at, *J* 8.2 H-4_a), 4.37 (1 H, m, H-3_a), 4.42, 4.84 (2 H, ABq, J_{AB} 10.9 Hz, PhCH_2), 4.43, 4.60 (2 H, ABq, J_{AB} 12.1 Hz, PhCH_2), 4.73, 4.96 (2 H, ABq, J_{AB} 10.9 Hz, PhCH_2), 4.77, 4.92 (2 H, ABq, J_{AB} 11.9 Hz, PhCH_2), 4.80 (1 H, s, H-1_a), 4.98–5.04 (2 H, m, CH=CH_2), 5.47 (1 H, s, PhC*H*), 5.54 (1 H, d, H-1_b), 5.78 (1 H, ddt, J_E 16.9 Hz, J_Z 10.2 Hz, *J* 6.6 Hz, C*H*=CH_2), 6.97–7.52 (30 H, m, Ar–H); δ_C ($CDCl_3$) 28.4, 30.2 (2 × t, $OCH_2CH_2CH_2$), 64.0, 70.8, 72.9, 77.4, 77.5, 78.9, 79.8, 81.4 (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), 67.1, 68.4, 69.0, 70.7, 73.5, 73.9, 75.0, 75.6 (8 × t, C-6_a, C-6_b, 5 × PhCH_2, OCH_2CH_2), 97.1, 99.7, 102.5 (3 × d, C-1_a, C-1_b, PhCH), 115.3 (t, CH=CH_2), 126.6, 127.4, 127.7, 128.0, 128.2, 128.4, 128.6, 128.7, 129.5 (9 × d, 30 × Ar–CH), 137.7, 138.4, 138.5, 138.7 (4 × s, 6 × Ar–C), 138.2 (d, CH=CH_2); *m/z* (APCI$^+$) 966 (M+NH_4^+, 68), 949 (M+H$^+$, 9%), (APCI$^-$) 983 (M+Cl$^-$, 98%). (HRMS Calcd. For $C_{59}H_{68}NO_{11}$ (MNH_4^+) 966.479238. Found 966.478812).

3.6. Pent-4′-enyl 4,6-O-benzylidene-α-D-glucopyranoside (10a) and pent-4′-enyl 4,6-O-benzylidene-β-D-glucopyranoside (10b)

Camphor sulfonic acid (200 mg, 0.86 mmol) was added to a stirred suspension of D-glucose **8** (10.0 g, 55.5 mmol) in 4-penten-1-ol (40 mL) and the mixture was refluxed at 140 °C for 48 h. After this time, TLC (EtOAc–MeOH, 4:1) indicated the formation of a major product (R_f 0.4) and the absence of starting material (R_f 0). Pentenol (~30 g) was recovered by vacuum distillation using a dry ice condenser. Triethylamine (10 drops) was added to the remaining brown residue, and the mixture concentrated in vacuo. The resulting brown oil was purified by flash chromatography (EtOAc then EtOAc–MeOH, 9:1) to give an α/β mixture of pentenyl glycosides **9a/b** (9.2 g, 63%) as a brown oil, which was used directly without further characterisation. The mixture of glycosides **9a/b** (9.2 g, 34.8 mmol), benzaldehyde dimethyl acetal (6.00 mL, 40.0 mmol) and CSA (160 mg, 0.70 mmol) were dissolved in DMF (30 mL) and the mixture was put onto the rotary evaporator at 65 °C and 260–280 mbar. After 7 h, TLC (EtOAc) indicated the formation of two products (R_f 0.5 and 0.6) and small amounts of starting material (R_f 0.1). Et_3N (10 drops) was added, and the mixture concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with water (2 × 100 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (petrol–EtOAc, 1:1) to afford the desired α-pentenyl glycoside **10a** as a white solid (5.3 g, 46% (62% over recovered starting material)) which was recrystallised from ether/petrol; mp 87 °C [Lit. 90 °C][19]; $[\alpha]_D^{22}$ +105.3 (*c*, 1.0 in $CHCl_3$) [Lit. +96.5 (*c*, 1.13 in $CHCl_3$)];[19] δ_H (400 MHz, $CDCl_3$) 1.74–1.81 (2 H, m, $OCH_2CH_2CH_2$), 2.14–2.20 (3 H, m, $OCH_2CH_2CH_2$, OH-2), 2.62 (1 H, d, *J* 2.0 Hz, OH-3), 3.49 (1 H, dt, $OCHH'CH_2$), 3.52 (1 H, at, *J* 9.3 Hz, H-4), 3.64 (1 H, m, H-2), 3.76 (1 H, at, *J* 10.2 Hz, H-6), 3.77–3.87 (2 H, m, H-5, $OCHH'CH_2$), 3.95 (1 H, at, *J* 9.2 Hz, H-3), 4.29 (1 H, dd, $J_{5,6'}$ 4.6 Hz, $J_{6,6'}$ 9.9 Hz, H-6′), 4.89 (1 H, d, $J_{1,2}$ 4.0 Hz, H-1), 5.00–5.09 (2

H, m, CH=CH_2), 5.55 (1 H, s, PhCH), 5.83 (1 H, ddt, J_E 16.9 Hz, J_Z 10.2 Hz, J 6.6 Hz, CH=CH$_2$), 7.36–7.52 (5 H, m, Ar–H); and the undesired β-pentenyl glycoside **10b** as a white solid (2.3 g, 20% (27% over recovered starting material)) which was recrystallised from ether–EtOAc; mp 148 °C [Lit. 144–145 °C];[19] $[\alpha]_D^{22}$ −51.9 (*c*, 1.0 in $CHCl_3$) [Lit. −43.8 (*c*, 1.16 in $CHCl_3$)];[19] δ_H (400 MHz, $CDCl_3$) 1.72–1.80 (2 H, m, OCH$_2$CH_2CH$_2$), 2.14–2.19 (2 H, m, OCH$_2$CH$_2$CH_2), 2.54 (1 H, d, J 2.4 Hz, OH-2), 2.72 (1 H, d, J 2.2 Hz, OH-3), 3.44–3.62 (4 H, m, H-5, H-2, H-4, OCHH′CH$_2$), 3.80 (1 H, at, J 10.2 Hz, H-6), 3.84 (1 H, m, H-3), 3.94 (1 H, dt, J_{gem} 9.5 Hz, J 6.6 Hz, OCHH'CH$_2$), 4.36 (1 H, dd, $J_{5,6'}$ 4.9 Hz, $J_{6,6'}$ 10.5 Hz, H-6′), 4.40 (1 H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.99–5.08 (2 H, m, CH=CH_2), 5.55 (1 H, s, PhCH), 5.83 (1 H, ddt, J_E 16.9 Hz, J_Z 10.2 Hz, J 6.7 Hz, CH=CH$_2$), 7.37–7.51 (5 H, m, Ar–H).

3.7. Pent-4′-enyl 2-O-benzyl-4,6-benzylidene-α-D-glucopyranoside (11a) and pent-4′-enyl 3-O-benzyl-4,6-benzylidene-α-D-glucopyranoside (11b)

The α-diol **10a** (1.55 g, 4.6 mmol) was dissolved in CH_2Cl_2 (50 mL), and tetrabutylammonium hydrogensulfate (310 mg, 0.92 mmol) and benzyl bromide (0.88 mL, 7.4 mmol) were added. An aq soln of sodium hydroxide (250 mg in 5 mL) was added and the mixture was refluxed at 41 °C. After 46 h, TLC (EtOAc–petrol, 1:2) indicated the formation of a major product (R_f 0.4) and remaining starting material (R_f 0.1). The reaction mixture was cooled, and partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The organic layer was washed with water (50 mL) and brine (20 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography (petrol–EtOAc, 4:1) to afford a mixture of the desired 2-O-benzylated material **11a** and undesired 3-O-benzylated material **11b** (1.30 g, 66%) (**11a**–**11b**, 1.6:1 as shown by ^{1}H NMR spectroscopy). Fractional crystallisation from ether–petrol gave the pure desired 2-O-benzylated material **11a** as a white, crystalline solid, mp 47–49 °C (ether/petrol); $[\alpha]_D^{22}$ +65.2 (*c*, 0.25 in $CHCl_3$); ν_{max} (film) 3400 (br, OH), 1641 (w, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.72–1.79 (2 H, m, OCH$_2$CH_2CH$_2$), 2.15–2.21 (2 H, m, OCH$_2$CH$_2$CH_2), 2.53 (1 H, d, J 2.0 Hz, OH-3), 3.37 (1 H, dt, J_{gem} 9.7 Hz, OCHH′CH$_2$), 3.47 (1 H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.52 (1 H, at, J 9.4 Hz, H-4), 3.68 (1 H, dt, J 6.5 Hz, OCHH'CH$_2$), 3.71 (1 H, at, J 10.2 Hz, H-6), 3.85 (1 H, adt, $J_{5,6'}$ 4.8 Hz, J 10.0 Hz, H-5), 4.18 (1 H, dt, H-3), 4.25 (1 H, dd, $J_{6,6'}$ 10.1 Hz, H-6′), 4.69, 4.77 (2 H, ABq, PhCH_2), 4.75 (1 H, d, H-1), 4.99–5.08 (2 H, m, CH=CH_2), 5.53 (1 H, s, PhCH), 5.83 (1 H, ddt, J_E 16.9 Hz, J_Z 10.2 Hz, J 6.6 Hz, CH=CH$_2$), 7.30–7.53 (10 H, m, Ar–H); δ_C ($CDCl_3$) 28.5, 30.2 (2 × t, OCH$_2$CH_2CH$_2$), 62.2, 70.1, 79.9, 81.4 (4 × d, C-2, C-3, C-4, C-5), 67.7, 69.0, 73.1 (3 × t, C-6, PhCH_2, OCH_2CH$_2$CH$_2$), 97.6, 102.0 (2 × d, C-1, PhCH), 115.3 (t, CH=CH_2), 126.6, 127.9, 128.3, 128.5, 128.8, 129.2, 129.4 (7 × d, Ar–CH), 137.4, 138.2 (2 × s, Ar–C), 138.2 (d, CH=CH$_2$); *m/z* (APCI$^-$) 851 (2M−H, 42), 425 (M−H, 59), 335 (M−Bn, 32), 207 (62), 113 (100%); (HRMS Calcd. For $C_{25}H_{31}O_6$ (MH$^+$) 427.212064. Found 427.211859); together with pure 3-O-benzylated material **11b** as a white, crystalline solid, mp 107–108 °C (ether/petrol); $[\alpha]_D^{22}$ +59.4 (*c*, 0.88 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.73–1.80 (2 H, m, OCH$_2$CH_2CH$_2$), 2.13–2.19 (2 H, m, OCH$_2$CH$_2$CH_2), 2.24 (1 H, d, J 8.4 Hz, OH-2), 3.50 (1 H, dt, J_{gem} 9.7 Hz, J 6.5 Hz, OCHH′CH$_2$), 3.65 (1 H, at, J 9.2 Hz, H-4), 3.70–3.89 (5 H, m, OCHH'CH$_2$, H-2, H-3, H-5, H-6), 4.29 (1 H, dd, $J_{5,6'}$ 4.6 Hz, $J_{6,6'}$ 10.0 Hz, H-6′), 4.83, 4.97 (2 H, ABq, J_{AB} 11.6 Hz, PhCH_2), 4.90 (1 H, d, $J_{1,2}$ 3.9 Hz, H-1), 4.99–5.08 (2 H, m, CH=CH_2), 5.58 (1 H, s, PhCH), 5.81 (1 H, ddt, J_E 17.0 Hz, J_Z 10.2 Hz, J 6.6 Hz, CH=CH$_2$), 7.28–7.52 (10 H, m, 10 × Ar–H); δ_C ($CDCl_3$) 28.5, 30.3 (2 × t, OCH$_2$CH_2CH_2), 62.7, 72.6, 79.2, 82.0 (4 × d, C-2, C-3, C-4, C-5), 67.9, 69.0, 74.9 (3 × t, C-6, PhCH_2, OCH_2CH$_2$), 99.0, 101.3 (2 × d, C-1, PhCH), 115.4 (t, CH=CH_2), 126.2, 127.9, 128.2, 128.5, 128.6, 129.0, 129.2 (7 × d, Ar–CH), 137.6, 138.8 (2 × s, Ar–C), 138.1 (d, CH=CH$_2$); *m/z* (APCI$^-$) 425 (M−H, 7), 317 (32), 113 (100%). (Found: C, 70.25; H, 6.99; $C_{25}H_{30}O_6$ requires C, 70.40; H, 7.09%).

3.8. Pent-4′-enyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (12a) and pent-4′-enyl 2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (12b)

The alcohol **11a** (50 mg, 0.117 mmol) and thioglycoside **6**[14] (74 mg, 0.117 mmol) were dissolved in freshly distilled ether (3 mL) in a flame-dried flask. The mixture was stirred under Ar with activated 4 Å molecular seives for 30 min. Methyl triflate (0.066 mL, 0.585 mmol) was added and the mixture stirred at rt. After 17 h 30 min, TLC (petrol–EtOAc, 4:1) indicated the formation of a major product (R_f 0.2) and a minor product (R_f 0.3) and no remaining starting materials (R_f 0.15, 0.5). The reaction was quenched with Et_3N (0.16 mL) and stirred for a further 30 min. The mixture was filtered through Celite® and concentrated in vacuo. The residue was purified by flash chromatography (petrol–EtOAc, 6:1) to afford the desired α-disaccharide **12a** (89 mg, 80%) as a colourless oil; $[\alpha]_D^{22}$ +98.2 (*c*, 0.11 in $CHCl_3$); ν_{max} (film) 1640 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.74–1.81 (2 H, m, OCH$_2$CH_2CH$_2$), 2.17–2.23 (2 H, m, OCH$_2$CH$_2$CH_2), 3.38–3.47 (3 H, m, OCHH′CH$_2$, H-6$_b$, H-6′$_b$), 3.49 (1 H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.7 Hz, H-2$_b$) 3.61–3.76 (4 H, m, OCHH'CH$_2$, H-4$_b$, H-2$_a$, H-6$_a$), 3.80 (1 H, at, J 9.4 Hz, H-4$_a$) 3.91 (1 H, m, $J_{5,6}$ 10.0 Hz, $J_{5,6'}$ 4.8 Hz, H-5$_a$), 3.97 (1 H, at, J 9.4 Hz, H-3$_b$), 4.20–4.26 (2

H, m, H-5_b, H-$6'_a$), 4.29, 4.33, 4.38 (3 H, 3 × d, 3 × PhC*H*H′), 4.41 (1 H, at, *J* 9.3 Hz, H-3_a), 4.55–4.64 (4 H, m, 4 × PhC*H*H′), 4.79, 4.81 (2 H, 2 × d, 2 × PhC*H*H′), 4.87 (1 H, d, $J_{1,2}$ 3.7 Hz, H-1_a), 4.99–5.10 (2 H, m, CH=CH_2), 5.48 (1 H, s, PhC*H*), 5.59 (1 H, d, H-1_b), 5.85 (1 H, ddt, J_E 16.9 Hz, J_Z 10.2 Hz, *J* 6.6 Hz, C*H*=CH_2), 6.91–7.42 (30 H, m, Ar–H); δ_C ($CDCl_3$) 28.5, 30.2 (2 × t, $OCH_2CH_2CH_2$), 62.0, 69.6, 72.8, 75.6, 77.6, 78.8, 81.8, 83.0 (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), 67.7, 68.0, 69.2, 71.0, 73.0, 73.3, 74.8, 75.6 (8 × t, C-6_a, C-6_b, 5 × Ph*C*H_2, O*C*H_2CH_2), 96.3, 97.5, 102.5 (3 × d, C-1_a, C-1_b, Ph*C*H), 115.2 (t, CH=*C*H_2), 126.4, 126.6, 127.4, 127.6, 127.8, 128.0, 128.3, 128.5, 128.6, 129.6 (10 × d, Ar–CH), 137.3, 137.9, 138.0, 139.1, 139.2 (5 × s, 6 × Ar–C), 138.3 (d, *C*H=CH_2); *m/z* ($APCI^+$) 971 ($M+Na^+$, 16), 966 ($M+NH_4^+$, 18), 307 (31), 121 (100%). (HRMS Calcd. For $C_{59}H_{68}NO_{11}$ (MNH_4^+) 966.479238. Found 966.481418); together with undesired β-disaccharide **12b** (29 mg, 15%) as a colourless oil; $[\alpha]_D^{22}$ +29.5 (*c*, 1.95 in $CHCl_3$); ν_{max} (film) 1640 (w, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.72–1.79 (2 H, m, $OCH_2CH_2CH_2$), 2.15–2.21 (2 H, m, $OCH_2CH_2CH_2$), 3.24 (1 H, adt, $J_{4,5}$ 9.2 Hz, *J* 3.0 Hz, H-5_b), 3.36 (1 H, dt, J_{gem} 9.8 Hz, *J* 6.6 Hz, OC*H*H′CH_2), 3.50 (1 H, at, *J* 3.82 Hz, H-2_b), 3.55–3.66 (6 H, m, OCH*H*′CH_2, H-2_a, H-3_b, H-4_b, H-6_b, H-$6'_b$), 3.68 (1 H, at, *J* 9.4 Hz, H-4_a), 3.69 (1 H, at, *J* 10.2 Hz, H-6_a), 3.86 (1 H, adt, $J_{5,6'}$ 4.7 Hz, *J* 9.9 Hz, H-5_a), 4.21 (1 H, dd, $J_{6,6'}$ 10.1 Hz, H-$6'_a$), 4.38 (1 H, at, *J* 9.1 Hz, H-3_a), 4.47, 4.70 (2 H, ABq, J_{AB} 11.7 Hz, PhCH_2), 4.49 (2 H, s, PhCH_2), 4.53, 4.77 (2 H, ABq, J_{AB} 10.7 Hz, PhCH_2), 4.64 (1 H, d, *J* 3.8 Hz, H-1_a), 4.75, 5.08 (2 H, ABq, J_{AB} 10.9 Hz, PhCH_2), 4.79, 4.93 (2 H, ABq, J_{AB} 10.9 Hz, PhCH_2), 4.89 (1 H, d, $J_{1,2}$ 7.7 Hz, H-1_b), 4.99–5.08 (2 H, m, CH=CH_2), 5.48 (1 H, s, PhC*H*), 5.83 (1 H, ddt, J_E 17.0 Hz, J_Z 10.2 Hz, *J* 6.6 Hz, C*H*=CH_2), 7.13–7.43 (30 H, m, Ar–H); δ_C (100 MHz, $CDCl_3$) 28.5, 30.2 (2 × t, $OCH_2CH_2CH_2$), 62.2, 74.6, 76.2, 77.9, 80.3, 80.7, 82.9, 84.9 (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), 67.7, 68.6, 69.1, 73.5, 74.8, 74.9, 75.6, 77.3 (8 × t, C-6_a, C-6_b, 5 × Ph*C*H_2, O*C*$H_2CH_2CH_2$), 97.6, 101.5, 102.5 (3 × d, C-1_a, C-1_b, Ph*C*H), 115.1 (t, CH=*C*H_2), 126.1, 127.4, 127.5, 127.7, 127.8, 127.8, 127.9, 128.0, 128.1, 128.1, 128.1, 128.2, 128.3, 128.3, 128.9, (15 × d, 30 × Ar–CH), 137.4, 138.2, 138.4, 138.7, 138.7 (5 × s, 6 × Ar–C), 137.9 (d, *C*H=CH_2); *m/z* ($APCI^+$) 971 ($M+Na^+$, 15), 966 ($M+NH_4^+$, 28), 307 (39), 121 (100%), ($APCI^-$) 983 ($M+Cl^-$, 100), 893 (12), 857 (M−Bn, 13), 317 (22%).

3.9. 5′-Hydroxypentyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (13a)

Disaccharide **7** (36 mg, 0.039 mmol) was dissolved in toluene (2 mL) and 9-BBN (0.5 M solution in THF, 0.62 mL) was added. The reaction mixture was heated to 50 °C and stirred under Ar. After 18 h, the reaction mixture was cooled to rt and ice-cold water (1 mL) was added. Then aq H_2O_2 (35% v/v, 0.5 mL) and aq NaOH (2 M, 0.75 mL) were added dropwise. The reaction mixture was stirred for a further 12 h after which time TLC (cyclohexane–EtOAc, 1:1) indicated no further consumption of starting material (R_f 0.7) and the formation of a major product (R_f 0.3). The mixture was dissolved in ether (30 mL) and washed with water (30 mL). The aqueous layer was re-extracted with ether (30 mL) and the combined organic extracts dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane–EtOAc, 3:1) to afford alcohol **13a** (19 mg, 52, 57% over recovered starting material) as a clear oil; $[\alpha]_D^{25}$ +67.9 (*c*, 0.68 in $CHCl_3$); ν_{max} (thin film) 3409 (br, OH/NH) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.37–1.45 (2 H, m, $OCH_2CH_2CH_2$), 1.53–1.60 (4 H, m, $OCH_2CH_2CH_2CH_2$), 3.38 (1 H, m, OC*H*H′CH_2), 3.53 (1 H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.7 Hz, H-2_b), 3.57–3.71 (6 H, m, H-6_b, H-$6'_b$, H-4_b, OCH*H*′CH_2, CH_2CH_2O), 3.82–3.84 (2 H, m, H-5_b, H-2_a), 3.86–3.88 (2 H, m, H-5_a, H-6_a), 4.00 (1 H, at, *J* 9.3 Hz, H-3_b), 4.22 (1 H, d, $J_{5,6'}$ 5.3 Hz, H-$6'_a$), 4.31, 4.57 (2 H, ABq, J_{AB} 12.0 Hz, PhCH_2), 4.36–4.41 (2 H, m, H-3_a, H-4_a), 4.43, 4.60 (2 H, ABq, J_{AB} 12.2 Hz, PhCH_2), 4.43, 4.86 (2 H, ABq, J_{AB} 11.0 Hz, PhCH_2), 4.75, 4.98 (2 H, ABq, J_{AB} 10.4 Hz, PhCH_2), 4.80, 4.94 (2 H, ABq, J_{AB} 12.3 Hz, PhCH_2), 4.82 (1 H, d, $J_{1,2}$ 1.4 Hz, H-1_a), 5.49 (1 H, s, PhC*H*), 5.55 (1 H, d, H-1_b), 7.00 (2 H, ad, *J* 6.7 Hz, Ar–H), 7.14–7.39 (26 H, m, Ar–H), 7.47 (2 H, ad, *J* 6.7 Hz, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 22.9 (t, $OCH_2CH_2CH_2$), 29.0, 32.4 (2 × t, $OCH_2CH_2CH_2CH_2$), 62.6 (t, C-6_b), 64.0 (d, C-5_a), 67.7, 68.5, 68.9 (3 × t, C-6_a, 2 × CH_2CH_2O), 70.6, 73.3, 73.8, 74.9, 75.5 (5 × t, 5 × Ph*C*H_2), 70.8 (d, C-5_b), 73.3 (d, C-3_a), 77.3, 77.5 (2 × d, C-4_b, C-2_a), 79.1 (d, C-2_b), 79.7 (d, C-4_a), 81.3 (d, C-3_b), 96.9 (d, C-1_b), 99.4 (d, C-1_a), 102.4 (d, Ph*C*H), 126.2, 126.4, 127.1, 127.3, 127.4, 127.5, 127.6, 127.8, 127.9, 128.1, 128.2, 128.2, 128.3, 128.4, 129.2 (15 × d, Ar–CH), 137.4, 137.9, 138.0, 138.2, 138.5, 138.7 (6 × s, Ar–C); *m/z* (ES^+) 990 (55), 984 ($M+NH_4^+$, 100%), HRMS Calcd. for $C_{59}H_{70}O_{12}N$ (MNH_4^+) 984.4898. Found 984.4915.

3.10. 5′-Hydroxypentyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (13b)

Disaccharide **12a** (39 mg, 0.041 mmol) was dissolved in toluene (2 mL) and 9-BBN (0.5 M solution in THF) (0.66 mL, 0.33 mmol) was added. The reaction mixture was heated to 50 °C and stirred under Ar. After 18 h, the reaction mixture was cooled to rt and ice-cold water (1 mL) was added. Then aq H_2O_2 (35% v/v, 0.5 mL) and aq

NaOH (2 M, 0.75 mL) were added dropwise. The reaction mixture was stirred for a further 4 h at which point TLC (cyclohexane–EtOAc, 1:1) indicated no further consumption of starting material (R_f 0.7) and the formation of a major product (R_f 0.3). The mixture was dissolved in ether (30 mL) and washed with water (30 mL). The aqueous layer was re-extracted with ether (30 mL) and the combined organics dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (cyclohexane–EtOAc, 3:1) to afford alcohol **13b** (24 mg, 60%) as a clear oil; $[\alpha]_D^{25}$ +58.9 (*c*, 3.0 in $CHCl_3$); ν_{max} (thin film) 3418 (br, OH/NH) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.48–1.53 (2 H, m, $OCH_2CH_2CH_2$), 1.58–1.66, 1.67–1.74 (4 H, 2 × m, $OCH_2CH_2CH_2CH_2$), 3.39–3.51 (4 H, m, H-2_b, $CH_2CHH'O$, CH_2CH_2O), 3.60–3.67 (4 H, m, H-2_a, H-4_b, H-6_b, H-$6'_b$), 3.70–3.77 (2 H, m, $CH_2CHH'O$, H-6_a), 3.80 (1 H, at, *J* 9.5 Hz, H-4_a), 3.90 (1 H, dd, $J_{5,6}$ 4.8 Hz, *J* 9.6 Hz, H-5_a), 3.97 (1 H, at, *J* 9.3 Hz, H-3_b), 4.21 (1 H, m, H-5_b), 4.25 (1 H, dd, $J_{6,6'}$ 10.2 Hz, H-$6'_a$), 4.30 (1 H, d, *J* 12.2 Hz, PhC*H*H′), 4.34 (1 H, d, *J* 12.4 Hz, PhC*H*H′), 4.39, 4.79 (2 H, ABq, J_{AB} 11.0 Hz, $PhCH_2$), 4.41 (1 H, at, *J* 9.3 Hz, H-3_a), 4.56–4.61 (3 H, m, 3 × PhC*H*H′), 4.64 (1 H, d, *J* 11.1 Hz, PhCH*H*′), 4.81, 5.01 (2 H, ABq, J_{AB} 10.8 Hz, $PhCH_2$), 4.86 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1_a), 5.49 (1 H, s, PhC*H*), 5.59 (1 H, d, $J_{1,2}$ 3.6 Hz, H-1_b), 6.92–6.94 (2 H, ad, *J* 7.1 Hz, Ar–H), 7.08–7.43 (28 H, m, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 22.4 (t, $OCH_2CH_2CH_2$), 29.0, 32.4 (2 × t, $OCH_2CH_2CH_2CH_2$), 61.9 (d, C-5_a), 62.6 (t, C-6_b), 68.0, 68.4, 69.2 (3 × t, C-6_a, 2 × CH_2CH_2O), 69.6 (d, C-5_b), 71.0 (t, $PhCH_2$), 72.7 (d, C-3_a), 72.9, 73.2, 74.7, 75.5 (4 × t, 4 × $PhCH_2$), 77.5, 78.1 (2 × d, C-2_a, C-4_b), 78.7 (d, C-2_b), 81.7 (d, C-3_b), 82.9 (d, C-4_a), 96.1 (d, C-1_b), 97.4 (d, C-1_a), 102.1 (s, *C*HAr), 126.4, 127.3, 127.4, 127.5. 127.6, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.4 (16 × d, CH–Ar), 137.1, 137.6, 137.8, 138.1, 138.8, 138.9 (6 × s, Ar–C); *m/z* (ES^+) 989 ($M+Na^+$, 100), 984 ($M+NH_4^+$, 75%); HRMS Calcd. for $C_{59}H_{70}NO_{12}$ (MNH_4^+) 984.4898. Found 984.4902.

3.11. 5′-Azidopentyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (14a)

Methanesulfonyl chloride (3 μL, 0.040 mmol) was added dropwise to a stirred solution of alcohol **13a** (32 mg, 0.033 mmol) and Et_3N (7 μL, 0.050 mmol) in an DCM (1 mL) at 0 °C under Ar. After 20 min, TLC (cyclohexane–EtOAc, 1:1) indicated the absence of starting material (R_f 0.4) and the formation of a major product (R_f 0.5). The reaction mixture was diluted with DCM (40 mL) and washed with distilled water (40 mL), 1 M HCl (40 mL), $NaHCO_3$ (40 mL of a saturated aqueous solution) and brine (40 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was dissolved in DMF (0.5 mL), and NaN_3 (6.5 mg, 0.099 mmol) added. After 48 h, TLC (cyclohexane–EtOAc, 1:1) indicated the absence of starting material (R_f 0.5) and the formation of a major product (R_f 0.7). The residue was concentrated in vacuo and purified by flash column chromatography (cyclohexane–EtOAc, 3:1) to afford azide **14a** (27 mg, 81%) as a clear oil; $[\alpha]_D^{25}$ +64.3 (*c*, 1.52 in $CHCl_3$); ν_{max} (thin film) 2090 (N_3) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.43 (2 H, m, $OCH_2CH_2CH_2$), 1.57–1.62 (4 H, m, $OCH_2CH_2CH_2CH_2$), 3.24–3.28 (2 H, at, *J* 6.8 Hz, CH_2N_3), 3.37 (1 H, m, $OCHH'CH_2$), 3.54 (1 H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.7 Hz, H-2_b), 3.59–3.72 (4 H, m, H-4_b, H-6_b, H-$6'_b$, $OCHH'CH_2$), 3.81–3.87 (4 H, m, H-5_b, H-5_a, H-2_a, H-6_a), 4.01 (1 H, at, *J* 9.3 Hz, H-3_b), 4.22 (1 H, d, $J_{5,6'}$ 5.4 Hz, H-$6'_a$), 4.31–4.33 (2 H, m, H-3_a, H-4_a) 4.33, 4.58 (2 H, ABq, J_{AB} 12.2 Hz, $PhCH_2$), 4.44, 4.61 (2 H, ABq, J_{AB} 12.0 Hz, $PhCH_2$), 4.44, 4.86 (2 H, ABq, J_{AB} 10.8 Hz, $PhCH_2$), 4.75, 4.98 (2 H, ABq, J_{AB} 11.0 Hz, $PhCH_2$), 4.78, 4.95 (2 H, ABq, J_{AB} 12.4 Hz, $PhCH_2$), 4.82 (1 H, d, $J_{1,2}$ 1.6 Hz, H-1_a), 5.48 (1 H, s, PhC*H*), 5.56 (1 H, d, H-1_b), 7.01 (2 H, ad, *J* 6.7 Hz, Ar–H), 7.14–7.37 (26 H, m, Ar–H), 7.47 (2 H, ad, *J* 6.7 Hz, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 23.3 (t, $OCH_2CH_2CH_2$), 28.6, 28.9 (2 × t, $OCH_2CH_2CH_2CH_2$), 51.2, 67.5 (2 × t, CH_2N_3, CH_2CH_2O), 64.1 (d, C-5_a), 68.5, 68.9 (2 × t, C-6_b, C-6_a), 70.6, 73.3, 73.8, 74.9, 75.5 (5 × t, 5 × CH_2Ar), 70.8 (d, C-5_b), 72.8 (d, C-3_a), 77.3, 77.5 (2 × d, C-2_a, C-4_b), 78.9 (d, C-2_b), 79.7 (d, C-4_a), 81.3 (d, C-3_b), 96.9 (d, C-1_b), 99.5 (d, C-1_a), 102.3 (d, Ph*C*H), 126.4, 127.1, 127.2, 127.5, 127.5, 127.7, 127.8, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 129.2 (14 × d, Ar–CH), 137.9, 137.9, 138.2, 138.5, 138.7 (5 × s, Ar–C); *m/z* (ES^+) 1037 (30), 1013 ($M+Na^+$, 55), 1009 ($M+NH_4^+$, 18), 153 (100%); Isotope distribution Calcd. for $C_{59}H_{65}N_3NaO_{11}$ (MNa^+) 1017.5 (7), 1016.5 (25), 1015.5 (68), 1014.5 (100%). Found 1017.5 (5), 1016.6 (24), 1015.5 (71), 1014.5 (100%).

3.12. 5′-Azidopentyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (14b)

Methanesulfonyl chloride (3 μL, 0.035 mmol) was added dropwise to a stirred solution of alcohol **13b** (28 mg, 0.029 mmol) and Et_3N (6 μL, 0.044 mmol) in an DCM (1 mL) at 0 °C under Ar. After 20 min, TLC (cyclohexane–EtOAc, 1:1) indicated the absence of starting material (R_f 0.2) and the formation of a major product (R_f 0.3). The reaction mixture was diluted with DCM (20 mL) and washed with distilled water (20 mL), 1 M HCl (20 mL), $NaHCO_3$ (20 mL of a saturated aqueous solution) and brine (20 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was dissolved in DMF (0.5 mL), and NaN_3 (6 mg, 0.087 mmol) added. After 48 h, TLC (cyclohexane–

EtOAc, 1:1) indicated the absence of starting material (R_f 0.3) and the formation of a major product (R_f 0.7). The residue was concentrated in vacuo and purified by flash column chromatography (cyclohexane–EtOAc, 3:1) to afford azide **14b** (23 mg, 80%) as a clear oil; $[\alpha]_D^{25}$ +64.9 (*c*, 2.32 in $CHCl_3$); ν_{max} (thin film) 2096 (N_3) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.47–1.53 (2 H, m, $OCH_2CH_2CH_2$), 1.62–1.71 (4 H, m, OCH_2CH_2-CH_2CH_2), 3.26 (2 H, at, *J* 6.8 Hz, CH_2N_3), 3.39–3.47 (3 H, m, H-6_b, H-$6'_b$, $OCHH'CH_2$), 3.49 (1 H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.7 Hz, H-2_b), 3.61–3.68 (2 H, m, H-2_a, H-4_b), 3.70–3.76 (2 H, m, H-6_a $OCHH'CH_2$), 3.80 (1 H, at, *J* 9.3 Hz, H-4_a), 3.89 (1 H, dd, $J_{5,6}$ 4.5 Hz, H-5_a), 3.97 (1 H, at, *J* 9.3 Hz, H-3_b), 4.20–4.27 (2 H, m, H-5_b, H-$6'_a$), 4.29, 4.59 (2 H, ABq, J_{AB} 12.0 Hz, $PhCH_2$), 4.34, 4.57 (2 H, ABq, J_{AB} 12.3 Hz, $PhCH_2$), 4.36–4.43 (2 H, m, H-3_a, $PhCHH'$), 4.57, 4.63 (2 H, ABq, J_{AB} 11.2 Hz, $PhCH_2$), 4.78 (1 H, d, *J* 11.2 Hz, $PhCHH'$), 4.81, 5.01 (2 H, ABq, J_{AB} 10.8 Hz, $PhCH_2$), 4.87 (1 H, d, $J_{1,2}$ 3.7 Hz, H-1_a), 5.48 (1 H, s, PhC*H*), 5.59 (1 H, d, H-1_b), 6.92 (2 H, ad, *J* 7.0 Hz, Ar–H), 7.09–7.41 (28 H, m, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 21.0 (t, $OCH_2CH_2CH_2$), 28.6, 28.9 (2 × t, $OCH_2CH_2CH_2CH_2$), 62.0 (d, C-5_a), 51.3, 68.0, 68.2 (3 × t, C-6_b, OCH_2CH_2, CH_2N_3), 69.2 (t, C-6_a), 69.6 (d, C-5_b), 71.1, 72.9, 73.2, 74.7, 75.5 (5 × t, 5 × $PhCH_2$), 72.7 (d, C-3_a), 76.7 (d, C-4_b), 77.0 (d, C-2_a), 78.1 (d, C-2_b), 81.7 (d, C-3_b), 82.9 (d, C-4_a), 96.1 (d, C-1_b), 97.4 (d, C-1_a), 102.1 (d, PhCH), 126.4, 127.3, 127.4, 127.5, 127.6, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 129.4 (12 × d, Ar–CH), 137.1, 137.6, 137.8, 138.1, 138.8, 138.9 (6 × s, Ar–C); *m*/*z* (ES^+) 1036 (65), 1009 ($M+NH_4^+$, 100%); Isotope distribution Calcd. for $C_{59}H_{65}N_3NaO_{11}$ (MNa^+) 1017.5 (7), 1016.5 (25), 1015.5 (68), 1014.5 (100%). Found 1017.5 (6), 1016.5 (21), 1015.5 (68), 1014.5 (100%).

3.13. 5′-(1″-Pyr-1‴enylbutyramido)pentyl α-D-glucopyranosyl-(1→3)-α-D-mannopyranoside (15a)

Azide **14a** (29 mg, 0.029 mmol) and palladium on carbon (10% Pd/C, 45 mg) were stirred in MeOH–AcOH (99:1, 3 mL) under an atmosphere of hydrogen. After 46 h, TLC (CMAW) indicated complete conversion of starting material (R_f 0.95) to a single product (R_f 0.05). The mixture was filtered through Celite® (MeOH) and concentrated in vacuo. The residue was dissolved in DMF (1 mL) and PFP ester **16** (14 mg, 0.047 mmol) and Et_3N (41 μL, 0.293 mmol) were added. After 23 h TLC (CMAW) indicated complete conversion of starting material (R_f 0.05) to a major product (R_f 0.2). The reaction mixture was concentrated in vacuo and purified by flash column chromatography (MeOH–$CHCl_3$, 1:4) to afford labelled disaccharide **15a** (9 mg, 44%) as a clear oil; $[\alpha]_D^{25}$ +46.0 (*c*, 0.4 in MeOH); ν_{max} (thin film) 3349 (br, OH), 1642 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 1.41–1.46 (2 H, m, $OCH_2CH_2CH_2$), 1.51–1.55, 1.60–1.64 (4 H, m, $OCH_2CH_2CH_2CH_2$), 2.16–2.19 (2 H, m, $NC(O)CH_2CH_2$), 2.36–2.39 (2 H, at, *J* 7.3 Hz, $NC(O)CH_2$), 3.19–3.23 (2 H, m, CH_2N), 3.25 (1 H, dd, *J* 10.0 Hz, *J* 9.0 Hz), 3.37–3.45 (4 H, m, H-2_b, $CHH'O$, CH_2Ar), 3.58 (1 H, ddd, *J* 2.6 Hz, *J* 5.7 Hz, *J* 8.1 Hz), 3.65 (1 H, dd, *J* 6.4 Hz, *J* 11.6 Hz), 3.69–3.76 (3 H, m, $CHH'O$, H-3_b), 3.78 (1 H, dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.6 Hz, H-3_a), 3.82–3.87 (3 H, m), 3.89 (1 H, m), 4.07 (1 H, dd, $J_{1,2}$ 1.9 Hz, H-2_a), 4.73 (1 H, d, H-1_a), 5.09 (1 H, d, $J_{1,2}$ 4.3 Hz, H-1_b), 7.92 (1 H, d, *J* 7.8 Hz, Ar–H), 8.01 (1 H, t, *J* 7.7 Hz, Ar–H), 8.05, 8.06 (2 H, 2 × s, Ar–H), 8.15–8.22 (4 H, m, Ar–H), 8.36 (1 H, d, *J* 9.5 Hz, Ar); δ_C (100.6 MHz, CD_3OD) 23.8 (t, $OCH_2CH_2CH_2$), 28.1, 29.2 (2 × t, $NC(O)CH_2CH_2$, $OCH_2CH_2CH_2CH_2$), 32.8 (t, CH_2Ar), 35.8 (t, $NC(O)CH_2$), 39.3 (t, CH_2N), 61.7, 61.8 (2 × t, C-6_b, C-6_a), 67.5 (t, CH_2CH_2O), 66.4, 70.4, 70.8, 73.0, 73.1, 73.6, 74.2 (7 × d, C-2_b, C-3_b, C-4_b, C-5_b, C-3_a, C-4_a, C-5_a), 81.2 (d, C-2_a),100.6 (d, C-1_a), 101.1 (d, C-1_b), 123.5, 124.9, 125.0, 126.0, 126.7, 127.4, 127.6 (7 × d, Ar–CH), 135.1, 128.9, 130.4, 131.3, 131.8, 136.4, 174.8 (7 × s, Ar–C); *m*/*z* (ES^-) 696.46 ($M-H^+$, 52%); HRMS Calcd. for $C_{37}H_{46}NO_{12}$ ($M-H^+$) 696.3020. Found 696.3038.

3.14. 5′-(1″-Pyr-1‴enylbutyramido)pentyl α-D-glucopyranosyl-(1→3)-α-D-glucopyranoside (15b)

Azide **14b** (47 mg, 0.047 mmol) and palladium on carbon (10% Pd/C, 60 mg) were stirred in MeOH–AcOH (99:1, 4 mL) under an atmosphere of hydrogen. After 63 h, TLC (CMAW) indicated complete conversion of starting material (R_f 0.95) to a single product (R_f 0.05). The mixture was filtered through Celite® (MeOH) and concentrated in vacuo. The residue was dissolved in DMF (2 mL) and PFP ester **16** (23 mg, 0.047 mmol) added. After 28 h Et_3N (65 μL, 0.474 mmol) was added. After a further 16 h TLC (CMAW) indicated complete conversion of starting material (R_f 0.05) to a major product (R_f 0.2). The reaction mixture was concentrated in vacuo and purified by flash column chromatography (MeOH–$CHCl_3$, 1:4) to afford labelled disaccharide **15b** (26 mg, 79%) as a clear oil; $[\alpha]_D^{25}$ +73.0 (*c*, 1.45 in MeOH); ν_{max} (thin film) 3359 (br, OH), 1652 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 1.40–1.46 (2 H, m, $OCH_2CH_2CH_2$), 1.49–1.55, 1.60–1.67 (4 H, 2 × m, $OCH_2CH_2CH_2CH_2$), 2.14 (2 H, at, *J* 7.7 Hz, $NC(O)CH_2CH_2$), 2.36 (2 H, at, *J* 7.1 Hz, $NC(O)CH_2$), 3.19–3.21 (2 H, m, CH_2N), 3.27 (1 H, dd, *J* 10.1 Hz, *J* 8.9 Hz), 3.36 (2 H, s, $PhCH_2$), 3.43–3.55 (4 H, m, H-2_b, H-2_a, $OCHH'CH_2$), 3.58 (1 H, dd, *J* 2.1 Hz, *J* 5.2 Hz), 3.62–3.77 (5 H, m, $OCHH'CH_2$), 3.79 (1 H, dd, *J* 11.9 Hz, *J* 2.0 Hz), 3.86 (1 H, dd, *J* 2.2 Hz, *J* 11.6 Hz), 3.95 (1 H, m), 4.79 (1 H, d, $J_{1,2}$ 4.0 Hz, H-1_a), 5.15 (1 H, d, $J_{1,2}$ 3.7 Hz, H-1_b), 7.87 (1 H, d, *J* 7.8 Hz, Ar–H), 7.96–8.02 (3 H, m, Ar–H), 8.12–8.18 (4 H, m, Ar–H), 8.31 (1 H, d, *J* 9.2 Hz, Ar–H); δ_C (100.6 MHz, CD_3OD) 25.1 (t,

$OCH_2CH_2CH_2$), 29.6 (t, $NC(O)CH_2CH_2$), 30.6 (2 × t, $OCH_2CH_2CH_2CH_2$), 34.2 (t, CH_2Ar), 37.2 (t, $NC(O)CH_2$), 40.7 (t, CH_2N), 62.9, 63.2 (2 × t, C-6_b, C-6_a), 69.4 (t, OCH_2CH_2), 71.9, 72.2, 72.6, 73.8, 74.4, 74.6, 75.6, 85.3 (8 × d, C-2_b, C-3_b, C-4_b, C-5_b, C-2_a, C-3_a, C-4_a, C-5_a), 100.6, 102.2 (2 × d, C-1_b, C-1_a), 124.8, 126.2, 126.4, 127.4, 128.1, 128.8, 128.9, 129.0 (8 × d, Ar–CH), 130.3, 131.7, 132.7, 133.2, 137.8, 176.2 (6 × s, Ar–C); *m/z* (ES^+) 720 ($M+Na^+$, 12), 698 ($M+H^+$, 3), 152.91 (100%); HRMS Calcd. for $C_{37}H_{47}NNaO_{12}$ (MNa^+) 720.2996. Found 720.3010.

3.15. α-Glucosidase hydrolysis of pyrene labelled disaccharides

Rat liver α-glucosidase I was extracted from microsomes in buffer containing 0.8% Lubrol PX and affinity purified using *N*-(5-carboxypentyl)-deoxynojirimycin coupled to agarose. The activity was measured using a (^{3}H)-glucose radiolabelled $Glc_3Man_7GlcNAc_2$ substrate as described.[20] Rat liver α-glucosidase II was extracted from microsomes in buffer containing 0.5% Triton X-100 and purified using anion-exchange and size exclusion chromatography. The activity was measured using a (^{3}H)-glucose radiolabelled $Glc_2Man_7GlcNAc_2$ substrate. Rates of hydrolysis were similar to previously published data[21] and no cross contamination of glucosidase activity could be detected. Pyrene disaccharides were solubilised in MeOH and a 1 μL aliquot (2 mM final concentration) taken for hydrolysis in a final volume of 20 μL of α-glucosidase I in 0.1 M sodium phosphate buffer, pH 6.8 containing 0.8% Lubrol PX and 1 mg/mL bovine serum albumin, or 20 μL of α-glucosidase II in 80 mM Et_3N buffer, pH 7 containing 0.5% Triton X-100, 0.1 M NaCl, 10% glycerol and 1 mg/mL bovine serum albumin. Control digestions were perormed using glucosidase buffers and disaccharides alone. Following incubation at 37 °C for 1 h, MeCN (80 μL) was added and protein removed by filtration using Millipore 5000 NMWL Ultrafree filters. Aliquots were analysed by HPLC using a Waters Alliance 2695 separations module and an in-line fluorescence detector set at Ex λ 345, Ex λ 380 nm. Fluorescently derivatised disaccharides were separated isocratically at 0.5 mL/min on a 4.6 × 250 mm TSK gel amide-80 column at 30 °C using a solvent composed of 80% MeCN, 20% ammonium acetate (100 mM), pH 3.85.

Acknowledgements

We gratefully acknowledge financial support from the Leverhulme Trust (Postdoctoral Fellowship to R.J.T.E.) and the use of the EPSRC Mass Spectrometry Service (Swansea, UK) and the Chemical Database Service (CDS) at Daresbury, UK. We are also grateful to Dr David Neville for his advice and use of HPLC equipment.

References

1. Kornfield, R.; Kornfield, S. *Annu. Rev. Biochem.* **1985**, *54*, 631–664.
2. Kalz-Fuller, B.; Bieberich, E.; Bause, E. *Eur. J. Biochem.* **1995**, *231*, 344–351.
3. Pelletier, M. F.; Marcil, A.; Sevigny, G.; Jakob, C. A.; Tessier, D. C.; Chevet, E.; Menard, R.; Bergeron, J. J. M.; Thomas, D. Y. *Glycobiology* **2000**, *10*, 815–827.
4. (a) Zapun, A.; Petrescu, S. M.; Rudd, P. M.; Dwek, R. A.; Thomas, D. Y.; Bergeron, J. J. M. *Cell* **1997**, *88*, 29–38;
   (b) Rodan, A. R.; Simons, J. F.; Trombetta, S. E.; Helenius, A. *EMBO J.* **1996**, *15*, 6921–6930.
5. Tessier, D. C.; Dignadr, D.; Zapun, A.; Radominska, A.; Parodi, A. J.; Bergeron, J. J. M.; Thomas, D. Y. *Glycobiology* **2000**, *10*, 403–412.
6. Lellgaard, L.; Molinari, M.; Helenius, A. *Science* **1999**, *286*, 1882–1888.
7. Alonso, J. M.; Santa-Cecilia, A.; Calvo, P. *Biochem. J.* **1991**, *278*, 721–727.
8. Petrescu, A. J.; Butters, T. D.; Reinkensmeier, G.; Petrescu, S.; Platt, F. M.; Dwek, R. A.; Wormald, M. R. *EMBO J.* **1997**, *16*, 4302–4310.
9. Shibaev, V. N.; Veselovsky, V. V.; Lozanova, A. V.; Maltsev, S. D.; Danilov, L. L.; Forsee, W. T.; Xing, J.; Cheung, H. C.; Jedrzejas, M. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 189–192.
10. (a) Kotani, N.; Takasaki, S. *Anal. Biochem.* **1998**, *264*, 66–73;
    (b) Kinoshita, A.; Sugahara, K. *Anal. Biochem.* **1999**, *269*, 367–378;
    (c) Watanabe, T.; Inoue, N.; Kutsukake, T.; Matsuki, S.; Takeuchi, M. *Biol. Pharm. Bull.* **2000**, *23*, 269–273.
11. For some examples of the use of *n*-pentenyl glycosides for the attachment of linkers or labels see:(a) Iyer, S.S.; Rele, S.M.; Baskaran, S.; Chaikof, E.L. *Tetrahedron*, **2003**, *59*, 631–638;
    (b) Buskas, T.; Söderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.*, **2000**, *65*, 958–963.
12. France, R. R.; Cumpstey, I.; Butters, T. D.; Fairbanks, A. J.; Wormald, M. R. *Tetrahedron: Asymmetry* **2000**, *11*, 4985–4994.
13. For the synthesis of a fluorescence labelled substrate for glucosidase I see: Scaman, C. H.; Hindsgaul, O.; Palcic, M. M.; Srivastava, O. P. *Carbohydr. Res.* **1996**, *296*, 203–213.
14. Ferrier, R. J.; Furneaux, R. H. *Carbohydr. Res.* **1973**, *52*, 63–68.
15. Demchenko, A. V.; De Meo, C. *Tetrahedron Lett.* **2002**, *43*, 8819–8822.
16. Kaushal, G. P.; Pastuszak, I.; Hatanaka, K.; Elbein, A. D. *J. Biol. Chem.* **1990**, *265*, 16271–16279.
17. Fraser-Reid, B.; Udodong, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927–942.

18. Madsen, R.; Udodong, U. E.; Roberts, C.; Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1554–1565.
19. Andrews, C. W.; Rodebaugh, R.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 5280–5289.
20. Butters, T. D.; van den Broek, L. A. G. M.; Fleet, G. W. J.; Krulle, T. M.; Wormald, M. R.; Dwek, R. A.; Platt, F. M. *Tetrahedron: Asymmetry* **2000**, *11*, 113–124.
21. Karlsson, G. B.; Butters, T. D.; Dwek, R. A.; Platt, F. M. *J. Biol. Chem.* **1993**, *268*, 570–576.