Synthesis of a truncated bi-antennary complex-type *N*-glycan oxazoline; glycosylation catalysed by the endohexosaminidases Endo A and Endo M†

Thomas B. Parsons,[a] James W. B. Moir[b] and Antony J. Fairbanks*[c]

Received 9th April 2009, Accepted 13th May 2009
First published as an Advance Article on the web 17th June 2009
DOI: 10.1039/b907273j

The synthesis of a truncated complex *N*-glycan hexasaccharide oxazoline was achieved producing a substrate that was assayed as an activated donor for glycosylation catalysed by the endohexosaminidases Endo A and Endo M. For Endo M competitive product hydrolysis was seen to limit synthetic efficiency. In spite of its natural hydrolytic selectivity wild type Endo A was able to process the truncated complex *N*-glycan oxazoline, albeit with limited synthetic efficiency; notably the product was not a substrate for Endo A catalysed hydrolysis. Two Endo A mutants, E173Q and E173H, were also assayed, but were unable to process this oxazoline.

Introduction

Glycosylation of proteins plays significant roles in protein folding,[1] and in the modulation of important protein properties.[2] Protein glycosylation is sub-divided into two categories; *N*-linked, in which the oligosaccharide chains are attached to asparagine residues, and *O*-linked, in which the carbohydrates are attached to serine or threonine residues. *N*-Linked glycosylation, the more common of the two, 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)[3] as the protein is assembled. Following this initial transfer sequential trimming of the oligosaccharide chain(s) occurs, and 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 which are classified into three groups; high mannose, complex, and hybrid. Due to the action of numerous enzymes in competing processes glycoproteins are produced intracellularly as heterogeneous mixtures of different 'glycoforms'; proteins in which different oligosaccharide structures are linked to the same peptide chain.

Access to pure homogeneous single glycoforms of glycoproteins has now become a major scientific objective.[4] Not only is this a prerequisite to facilitate more precise biological investigation into the effects that different glycans have on protein properties, but it is also an important commercial goal in the field of glycoprotein therapeutics, which are currently marketed as heterogeneous mixtures.

A particularly attractive approach, which may provide general access to homogenous glycoproteins bearing defined glycan structures, involves the combined use of synthesis and biocatalysis.[5] The oligosaccharide chains of heterogeneous mixtures of glycoforms produced intracellularly may be 'trimmed' back to single GlcNAc residues, and synthetic carbohydrates then added to these 'GlcNAc handles' using enzymatic catalysis. Enzymes which display considerable synthetic potential in this respect are the endohexosaminidases[6] which specifically cleave the chitobiose core [GlcNAcβ(1-4)GlcNAc] of *N*-linked glycans between the two *N*-acetyl glucosamine residues. The two members of this class that have shown the most useful *synthetic* glycosylation activity are Endo M from *Mucor Hiemalis*[7] and Endo A from *Arthrobacter protophormiae*,[8] both of which are members of the glycohydrolase (GH) family 85.[9] However since these enzymes naturally operate hydrolytically, Endo M cleaves both high mannose and complex *N*-glycans, whilst Endo A specifically cleaves high mannose glycans, then competitive product hydrolysis greatly reduces achievable product yield. Early studies into their use as catalysts for transglycosylations using un-activated donors were quite inefficient.

In 2001 a paradigm shift occurred when Shoda[10] and co-workers demonstrated that carbohydrate oxazolines could be used as activated glycosyl donor substrates for these enzymes, leading to substantial increases in synthetic efficiency. Not only did this report stimulate our interest in the area,[11] but also that of other groups, most prolifically Wang and co-workers, who have published[12] extensively on the application and further development of this methodology. Indeed numerous demonstrations of the utility of both Endo A and Endo M for the transfer of high mannose oligosaccharides to amino acids,[11a–c] peptides[12a–d] and proteins,[11d,12e,f,j] (including monoclonal antibodies)[12i] have now been reported.

Although the use of oxazolines as donor substrates is extremely beneficial, the issue of hydrolytic activity remains, and with more extended glycan structures such activity can seriously compromise synthetic efficiency. Two approaches have recently been developed to counter this limitation. The first is the use of slightly modified oxazoline donor substrates; herein the product formed by glycosylation is not hydrolysable by the enzyme. For example exchanging the central mannose for a glucose unit has in certain cases been demonstrated to be beneficial in this respect;[11b,c]

[a]*Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK*
[b]*Department of Biology, University of York, YO10 5YW, UK*
[c]*Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand*

† Electronic supplementary information (ESI) available: Spectra for compounds 1c–13. See DOI: 10.1039/b907273j

 This journal is © The Royal Society of Chemistry 2009

oxazolines can still be processed as 'privileged' activated donors, but the enzyme is not able to hydrolyse the glycopeptide product. Furthermore Wang has in particular demonstrated the ability of Endo A to transfer novel oxazolines, which correspond to non-natural glycans, to RNase B.[12e,12j] Notably Endo A was not able to hydrolyse the products of these reactions.

A second approach is to engineer the enzyme. Both Wang[12l] and we[11d] have independently applied this approach to produce 'glycosynthase'[13] mutants of Endo A, whilst Wang and Yamamoto[12f] have also reported the engineering of Endo M. As an aid to enzyme engineering we recently solved the X-ray crystal structure of an E173Q glycosynthase mutant of Endo A; this being the first crystal structure solved for a member of the GH 85 family.[14] In the few months since deposition of this data at the Protein Data Bank (PDB) structures for WT Endo A[15] and Endo D,[16] another family 85 glycohydrolase, have also been solved, including as their complexes with inhibitors and acceptor substrates, indicating the intense current interest in this class of enzymes. Such structural information will undoubtedly be invaluable for the future design and engineering of more efficient endohexosaminidase catalysts.

With respect to the potential application of endohexosaminidases for re-engineering of glycoprotein therapeutics, access to homogenous glycoproteins bearing defined complex *N*-glycans is a topic of particular interest. For example it has been demonstrated that the circulatory lifetime, and therefore the *in vivo* bioactivity, of glycoprotein therapeutics such as erthyropoietin (EPO) is highly dependent on the number and type of complex *N*-glycans attached to *N*-linked glycosylation sites.[17] Indeed a commercial variant of EPO that has had extra *N*-linked glycosylation sites 'engineered-in' to improve its therapeutic profile is now marketed under the trade name Aranesp.®[18] The next objective in the field of endohexosaminidase mediated glycoform engineering is therefore an investigation of the structural requirements and tolerances of endohexosaminidase enzymes and their mutants for the transfer of complex *N*-glycans to glycopeptides and proteins using oxazolines as privileged donor substrates. The successful development of such methodology will facilitate the production of bespoke glycoproteins bearing defined complex-type *N*-glycans.

A representative bi-antennary complex *N*-glycan structure is shown in Fig. 1, including the endohexosaminidase cleavage site between the two GlcNAc residues of the chitobiose core. Since Endo M is capable of cleaving complex-type glycans, it should be able to catalyse the transfer of complex-type oxazoline donors to acceptors bearing GlcNAc residues. However wild type (WT) Endo M will also hydrolyse the products of such reactions, and

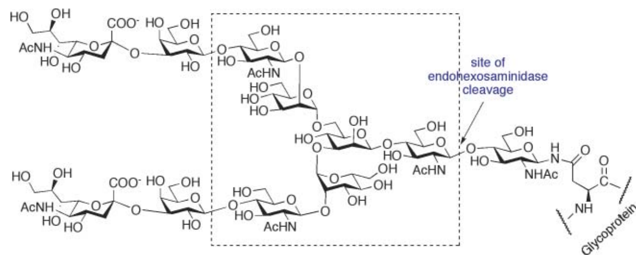


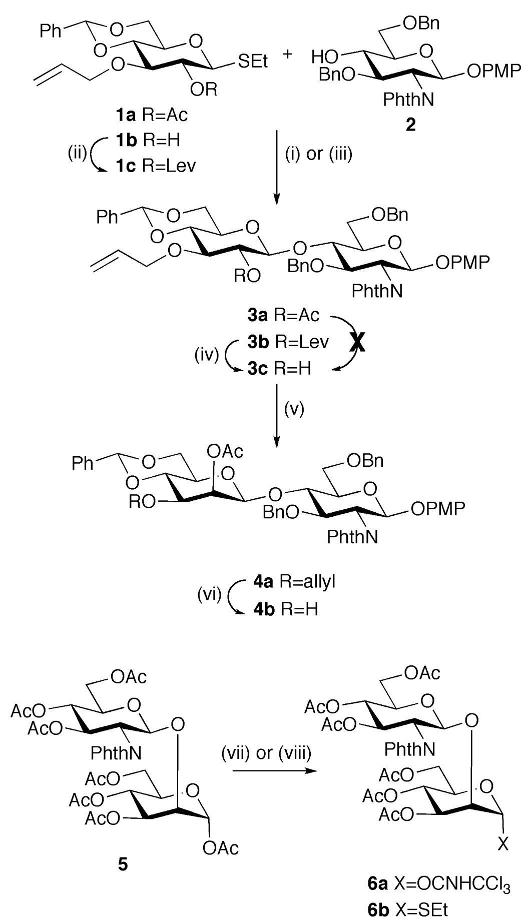
Fig. 1 Representative structure of a typical bi-antennary complex *N*-glycan of an *N*-linked glycoprotein showing the site of endohexosaminidase cleavage.

thus synthetic efficiency may be compromised. On the other hand Endo A, which specifically cleaves high mannose glycans, will not be expected to display hydrolytic activity towards such reaction products. However, will Endo A be able to accept truncated complex *N*-glycan oxazolines as donor substrates? Pertinently Wang has previously demonstrated the ability of Endo A to process non-natural glycan oxazolines and to transfer them to RNase B.[12e,j] So, if Endo A is indeed able to process such oxazoline donors then it may represent a useful catalyst for the synthesis of neoglycoconjugates bearing complex *N*-glycans. In order to investigate this synthetic potential the synthesis of a truncated bi-antennary complex *N*-glycan oxazoline, in which the terminal sialic acid and galactose residues have been removed (Fig. 1, area within dotted line) was undertaken. This material was then used as a donor substrate in a series of glycosylation reactions catalysed by both Endo M and Endo A. In addition, two available mutants of Endo A were also investigated as catalysts.

Results and discussion

Synthesis

A convergent strategy was applied to access the hexasaccharide oxazoline **11** in which the same GlcNAcβ(1→2)Man disaccharide building blocks were used for sequential glycosylation of the 3- and 6-positions of a central Manβ(1→4)GlcNAc disaccharide core. With respect to the synthesis of this disaccharide core, formation of the key Manβ(1→4)GlcNAc linkage by the use of *gluco* configured donor followed by epimerization at the 2-position was again considered to be the most practicable route. This *gluco* donor would require orthogonal protection at positions 2 and 3, and moreover a participating protecting group was required at the 2-position in order to ensure formation of the β-linkage during glycosylation with a glucosamine acceptor. An allyl ether was selected as the method for protection of the 3-position and initially the thioglycoside donor **1a**[19] possessing acetate protection at the 2-postion, was used in conjunction with the known glucosamine acceptor **2**.[20] However although glycosylation proceeded smoothly to give the desired β(1→4) disaccharide **3a** in good yield, it was found that removal of the 2-*O* acetate was not possible without partial cleavage of the phthalamide protection of the nitrogen of the glucosamine unit. Therefore protection of the 2-hydroxyl of the donor was undertaken using a levulinic ester, the method of protection originally employed by Boons.[21] Dicyclohexylcarbodiimide (DCC) mediated coupling of the alcohol **1b**[19] with levulinic acid in the presence of *N,N*-dimethylaminopyridine (DMAP) gave the fully protected *gluco* thioglycoside donor **1c** in 96% yield (Scheme 1). Glycosylation of **1c** with the glucosamine acceptor **2**[20] produced the desired β(1→4) disaccharide **3b** in 91% yield. Removal of the levulinoyl ester with hydrazine acetate gave alcohol **3c**, with no problems of partial phthalamide cleavage. The *gluco* alcohol **3c** was then epimerised by triflation and subsequent S_N2 displacement with tetra-*n*-butylammonium acetate with sonication,[21] to afford the β-*manno* disaccharide **4a**. Removal of the 3-*O*-allyl ether proved substantially more problematic than was expected. Direct cleavage with either palladium on carbon,[22] palladium dichloride,[23] bis(benzonitrile) palladium(II) chloride, or bis(acetonitrile) palladium(II) chloride all proved unsuccessful; the latter three resulting in simultaneous cleavage of the 4,6-benzylidene group.



Scheme 1 Reagents and conditions: (i) **1a**, **2**, MeOTf, *t*-TBP, 3 Å mol. sieves, DCM, 0 °C-rt, 15 h, 80%; (ii) Levulinic acid, DCC, DMAP, DCM, rt, 14 h, 96%; (iii) **1c**, **2**, MeOTf, *t*-TBP, 3 Å mol. sieves, DCM, 0 °C-rt, 14 h, 91%; (iv) hydrazine acetate, MeOH, DCM, rt, 14 h, 100%; (v) (a) Tf_2O, py, DCM, 0 °C-rt, 3h; (b) *n*-Bu_4NOAc, toluene, rt, sonication, 16 h, 91% over two steps; (vi) (a) (1,5-cyclooctadiene) bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate, THF, rt, 14 h; (b) NIS, H_2O, rt, 18 h, 86% over two steps; (vii) (a) hydrazine acetate, DMF, rt; (b) Cl_3CCN, DCM, DBU, 0 °C, 61% over 2 steps; (viii) EtSH, $BF_3{\cdot}OEt_2$, $CHCl_3$, reflux, 1 h, 70%.

Methods of cleavage involving two-step isomerisation and hydrolysis were therefore investigated. Isomerisation using either KO^{t}Bu in DMSO,[24] tris(triphenylphosphine) ruthenium(II) chloride with Hunig's base,[25] Grubbs' second generation catalyst, or Wilkinson's catalyst with DABCO[26] all met with failure. However finally a successful procedure was arrived upon; reaction with a (1,5-cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate catalyst activated with hydrogen[27] resulted in the smooth formation of the corresponding enol ether with no competing side reactions; this enol ether was immediately treated with *N*-iodosuccinimide (NIS) and water to afford the disaccharide alcohol **4b** in 86% yield.

Disaccharide donors **6a** and **6b** were accessed from the known GlcNAcβ(1→2)Man disaccharide **5**.[28] Formation of the trichloroacetimidate donor **6a** was achieved by selective anomeric de-acetylation and subsequent reaction with trichloroacetonitrile and DBU, as previously described.[28] Thioglycoside donor **6b** was formed by treatment of acetate **5** with ethanethiol and $BF_3{\cdot}OEt_2$ in refluxing chloroform.

Assembly of the target hexasaccharide structure was achieved in a stepwise manner (Scheme 2). Glycosylation of the disaccharide acceptor **4b** with the trichloracetimidate donor **6a** which resulted in formation of tetrasaccharide **7** in a modest 50% yield (84% yield based on recovered acceptor), though complete control of anomeric stereochemistry was observed. In this instance use of the thioglycoside donor **6b** proved to be superior and glycosylation of **4b** with thioglycoside donor **6b**, activated with NIS and AgOTf, gave the tetrasaccharide **7** in an improved 79% yield; again the reaction produced solely the desired α-product, but did not go quite to completion (93% yield based on recovered acceptor). Regioselective reductive ring opening of the 4,6-benzylidene was best achieved by treatment with triethylsilane and dichlorophenylborane, and afforded the tetrasaccharide acceptor **8** in which the 6-hydroxyl of the branching mannose unit was revealed for the addition of further carbohydrate units. Notably glycosylation of **8** with branched mannose trisaccharides would allow the synthesis of hybrid *N*-glycan oxazolines for study as substrates of endohexosaminidase catalysed glycosylation. However in this instance the truncated bi-antennary complex-type structure was targeted, so glycosylation of **8** was undertaken with the GlcNAcβ(1→4)Man thioglycoside donor **6b** and afforded the desired hexasaccharide **9a** in 79% yield.

Conversion of **9a** to the final de-protected product required a series of protecting group manipulations as well as oxazoline formation at the reducing terminus. A prime consideration when considering the sequence of reactions to be employed was the acid sensitive nature of the final glycosyl oxazoline product. Thus, as previously, a synthetic scheme that involved firstly conversion of all the alcohol protecting groups to acetates and the phthalamides to the required acetamides, subsequent oxazoline formation, and then final global de-protection by de-acetylation under basic conditions was considered to be a suitable procedure. Firstly removal of the three benzyl groups was undertaken by catalytic hydrogenation with Pearlman's catalyst. Although two of the benzyl ethers of **9a** were rapidly removed, cleavage of the third, presumably that on position-4 of the central branching mannose unit, was slow even under conditions of increased pressure or temperature. It was presumed that this reduced rate of cleavage was primarily due to a problem of steric accessibility. However, the use of stoichiometric quantities of 'catalyst', and extended reaction times did eventually effect complete benzyl ether removal; immediate acetylation of the crude resulting triol then produced **9b**. Conversion of the three phthalimide protecting groups of **9b** to the required acetamides was accomplished by reaction with ethylene diamine in refluxing methanol followed by immediate re-acetylation of the crude product with acetic anhydride in pyridine, which yielded **9c**. Removal of the anomeric *para*-methoxyphenyl (PMP) protection with ceric ammonium nitrate (CAN) and subsequent acetylation then yielded the peracetylated hexasaccharide **9d**, as the α-glycosyl acetate. Conversion to oxazoline **10** was achieved in an excellent 88% yield by treatment of **9d** with TMS bromide, $BF_3{\cdot}OEt_2$, and tri-*tert*-butyl pyrimidine (*t*-TBP) in dichloroethane at 40 °C for 6 h; the use of the hindered non-nucleophilic base *t*-TBP instead of collidine led to a considerable improvement in the yield for this step. Finally, global de-protection by treatment of **10** with sodium methoxide in methanol gave the target hexasaccharide oxazoline **11**.

 This journal is © The Royal Society of Chemistry 2009

Transglycosylation experiments

Endohexosaminidase-catalysed glycosylations were carried out using oxazoline **11** as the donor substrate and the previously detailed glycosyl amino acid **12**[11a] as a model acceptor (Scheme 3, Fig. 2). Both Endo M, which naturally cleaves both high mannose and complex glycans, and Endo A, which cleaves only high mannose *N*-glycans, were investigated. The ability of Endo A to process oxazoline **11** was a primary focus for investigation. Two salient points in this respect are firstly that the bi-antennary complex structure is truncated, *i.e.* **11** does not posses the usual galactose or sialic acid extensions at the terminal GlcNAc residues and secondly that endohexosaminidases such as Endo A have previously been demonstrated to accept oxazoline substrates that correspond to oligosaccharide structures that they are not able to cleave.[12e,j] The successful application of Endo A in a synthetic process with a donor such as **11** could therefore provide a

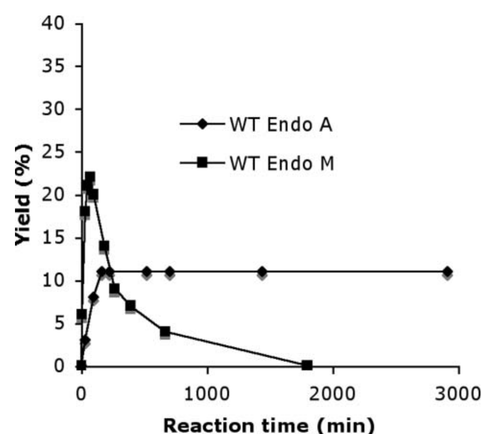
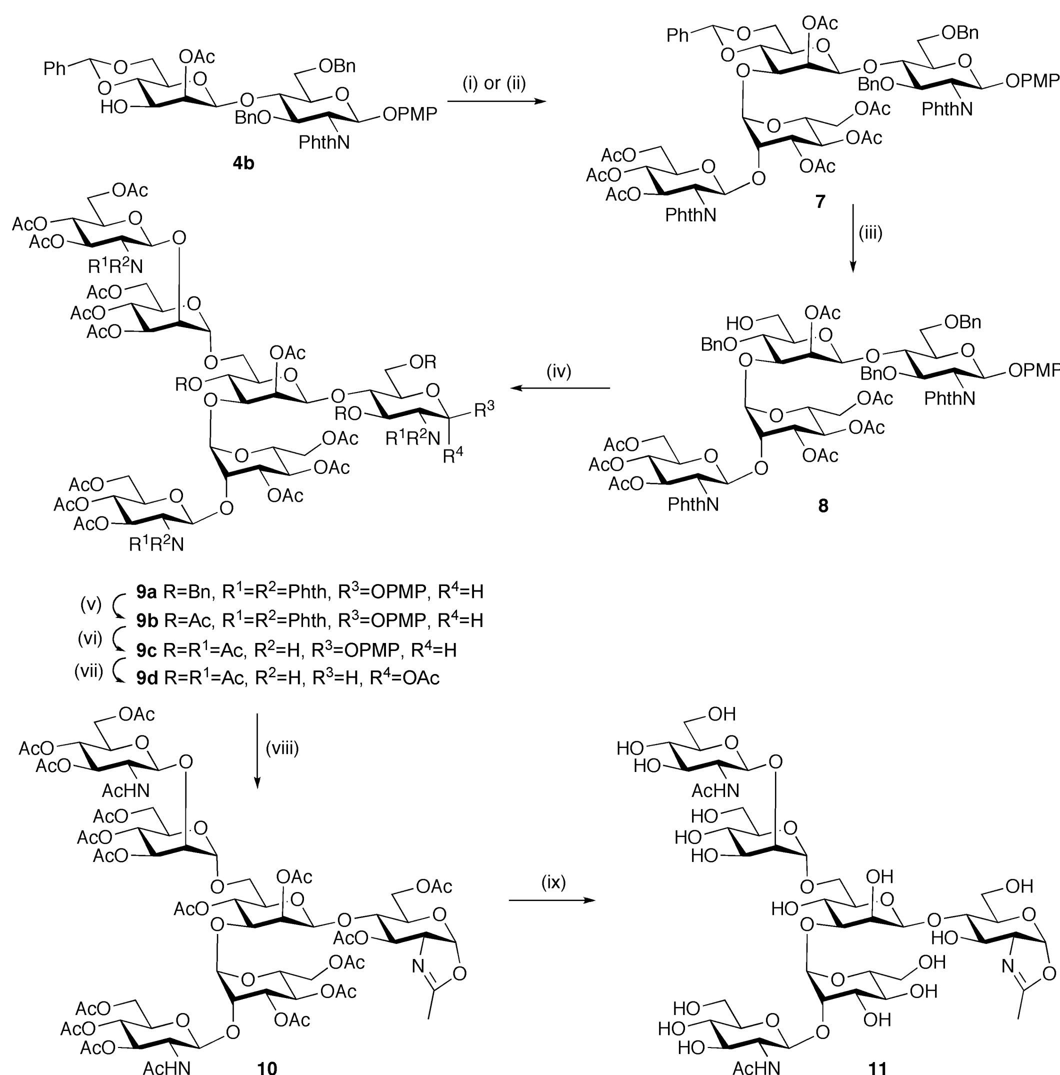
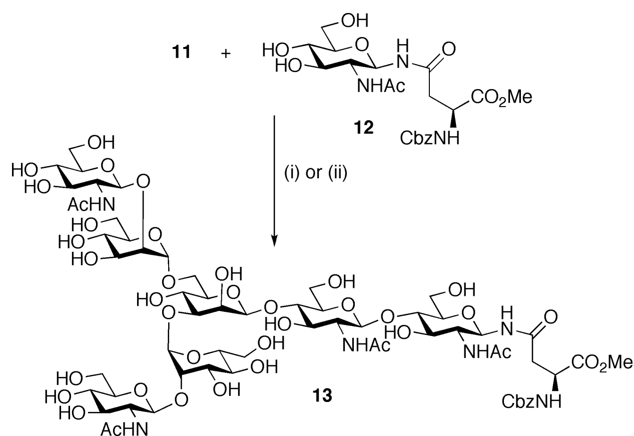


Fig. 2 Time course of glycosylation of acceptor **12** with oxazoline **11** catalysed by Endo M and WT Endo A.



Scheme 2 Reagents and conditions: (i) **6a**, $BF_3{\cdot}OEt_2$, 3 Å mol. sieves, DCM, −20 °C, 2 h, 50% (84% based on recovered **4b**); (ii) **6b**, AgOTf, NIS, 3 Å mol. sieves, DCM, 0 °C, 1 h, 79% (93% based on recovered **4b**); (iii) Et_3SiH, $PhBCl_2$, 3 Å mol. sieves, DCM, −78 °C, 45 min, 91%; (iv) **6b**, AgOTf, NIS, 3 Å mol. sieves, DCM, 0 °C, 1 h, 79% (89% based on recovered **8**); (v) (a) H_2, $Pd(OH)_2$, MeOH, THF, rt, 10 days; (b) Ac_2O, py, rt, 16 h, 86% over two steps; (vi) (a) $NH_2CH_2CH_2NH_2$, MeOH, reflux, 16 h; (b) Ac_2O, py, rt, 24 h, 76% over two steps; (vii) (a) CAN, MeCN, H_2O, rt, 18 h; (b) Ac_2O, py, rt, 16 h, 69% over two steps; (viii) TMSBr, $BF_3{\cdot}OEt_2$, tri-*t*-butylpyrimidine, DCE, 40 °C, 6 h, 88%; (ix) NaOMe, MeOH, rt, 40 h, 100%.



Scheme 3 Reagents and conditions: (i) Endo M, Na_2HPO_4 (100 mM), pH 6.5, 23 °C, 70 min, 22%; (ii) WT Endo A, Na_2HPO_4 (100 mM), pH 6.5, 23 °C, 180 min, 11%.

solution to the problem of competitive product hydrolysis, which curtails the utility of WT enzymes for the construction of neo-glycoconjugates bearing more extended *N*-glycans.

Reaction of oxazoline **11** with **12** catalysed by Endo M proceeded reasonably rapidly; the yield of heptasaccharide **13** increased with time to reach a rather modest maximal value of 22% after 70 min. However, product yield then decreased with time, albeit at a slightly slower rate, as competitive product hydrolysis dominated. Indeed the use of extended reaction times led to complete hydrolysis of **13**, and after 30 h the acceptor **12** was entirely re-formed (Fig. 2).

Investigation of the ability of Endo A to effect glycosylation using **11** entailed the use of the wild-type (WT) enzyme, and also two mutants, E173H and E173Q; these had been previously designed and engineered as enzymes for which hydrolytic activity had been curtailed by exchange of the key catalytic glutamic acid residue identified as the general acid/base.[11d] Reaction of **11** and **12** catalysed by WT Endo A proved to be very slow; the yield of product **13** rose gradually to a maximum value of 11% after 3 h. However interestingly the product **13** was not a substrate for hydrolysis by Endo A; the yield remained constant even over prolonged reaction times, confirming one aspect of the original hypothesis. Reactions catalysed by the mutants E173H and E173Q were then investigated. However, even after 48 h neither of these mutants was able to effect any appreciable amount of glycosylation of acceptor **12** using donor **11**.

Clearly oxazoline **11** is a poorer substrate for WT Endo A than it is for WT Endo M. Moreover the Endo A E173Q and E173H mutants were completely incapable of affecting glycosylation using donor **11**. These results are perhaps not that surprising given that Endo A selectively cleaves high mannose glycans; the use of an oxazoline as an activated donor is unable to outweigh the natural enzyme specificity. Indeed, during preparation of this manuscript, Wang and co-workers published[12l] a further report in which a bi-antennary complex-type oxazoline, produced by semi-synthesis after isolation of a sialylglycopeptide from natural sources, was investigated as a donor substrate for both Endo A and Endo M-catalysed glycosylation reactions. The donor used in their paper corresponds to a more extended version of oxazoline **11**, with two galactose units β-linked to the 4-position of the two GlcNAc residues at the non-reducing termini. Wang and co-workers reported that while WT Endo M was able to process their more extended complex oxazoline donor, the maximum yield obtainable was in the region of 30%; product hydrolysis was competitive and after approximately 2 h all of the glycopeptide product was consumed. Moreover they also reported that Endo A was completely unable to accept their more extended complex *N*-glycan oxazoline.

The results reported in this paper are therefore corroboratory and complementary to those recently reported by Wang. They confirm that complex glycan oxazolines are poor substrates for Endo A, but they do indicate that the more truncated oxazoline **11** studied here can be processed by WT Endo A. However the synthetic glycosylation process is inefficient and only a poor yield of product may be obtained. Since the product **13** was not hydrolysed by WT Endo A the question arises as to why this yield is in fact so poor. In the absence of further information we can only speculate that this low synthetic efficiency may most probably be due to competing hydrolysis of the oxazoline **11** by water, either in an enzyme catalysed or non-catalysed manner, or, perhaps less possibly because of inhibition of the enzyme by **11**, **12** or **13**. Furthermore the maximum yield of product obtainable in our study using Endo M was in the region of 22%, a similar result to that obtained by Wang. As product hydrolysis is the limiting factor in this respect it can be concluded therefore that the synthesis of neoglycoproteins and glycopeptides containing complex type *N*-glycans is possible using Endo M, but that mutant enzymes which are themselves incapable of product hydrolysis show the greatest potential in this respect. The recent publications[12f,l] of Wang and co-workers are therefore particularly noteworthy as the N175A Endo M mutant engineered by them appears to show exceptional promise as a glycosynthase catalyst for the synthesis of glycoconjugates bearing complex *N*-glycans. However, as yet the ability of such glycosynthases to transfer fully intact complex *N*-glycans using oxazolines which bear sialic acid residues at the non-reducing termini remains to be demonstrated, but will certainly be an area of interest. Moreover application to tri-, tetra-, and penta-antennary complex *N*-glycans remains as a future challenge that will require significant synthetic endeavour. Further investigations into the use of endohexosaminidase catalysed glycosylation processes for the construction of a variety of neo-glycoconjugates and glycoproteins are currently in progress in this laboratory, and the results will be reported in due course.

Experimental

General

Melting points were recorded on a Kofler hot block and are uncorrected. Proton and carbon nuclear magnetic resonance (δ_H, δ_C) spectra were recorded on Bruker DPX 400 (400y), Bruker DQX 400 (400 MHz), Bruker AVC 500 (500 MHz) or Bruker AMX 500 (500 MHz) spectrometers. All chemical shifts are quoted on the δ-scale in ppm using residual solvent as an internal standard. Low-resolution and high-resolution mass spectra were recorded on a Waters 31 LCT Premier XE spectrometer with electrospray ionisation (ESI+ or ESI−) and on a Bruker micrOTOF (ESI) spectrometer, respectively. *m*/*z* values are reported in Daltons and

 This journal is © The Royal Society of Chemistry 2009

are followed by their percentage abundance in parentheses. 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 Inorganic Chemistry Laboratory Elemental Analysis service, Oxford University, UK. Thin Layer Chromatography (t.l.c.) was carried out on Merck Kieselgel $60F_{254}$ pre-coated glass-backed plates. Visualisation of the plates was achieved using a u.v. lamp (l_{max} = 254 or 365 nm), and/or ammonium molybdate (5% in 2 M sulfuric acid), or sulfuric acid (5% in ethanol). Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Dichloromethane and THF were dried on an alumina column. Anhydrous DMF, pyridine, methanol and toluene were purchased from Fluka over molecular sieves. 'Petrol' refers to the fraction of light petrol ether boiling in the range of 40–60 °C.

General procedure for enzymatic glycosylation

Endohexosaminidase-catalysed glycosylations were monitored by HPLC using a Hewlett-Packard 1050 HPLC instrument using Clarity software (version 2.4.1.43) connected to an Agilent 1100 variable wavelength detector at 257 nm wavelength. An analytical HPLC (Phenomenex Gemini 5 µ C-18 column, 250 × 4.6y) was used to monitor the reactions, with 2 µL aliquots taken at appropriate time intervals. The column was eluted with 22% $MeCN/H_2O$. The yield was determined by integration of the product and acceptor peaks. Unit definitions of enzyme activity used in this report are as follows. Endo A activity was assayed with RNase B as the substrate, applying the unit definition of Endo H activity provided by New England Biolabs. One unit of Endo A is defined as the amount of enzyme required to remove >95% of the carbohydrate from 10 micrograms of denatured RNase B in a total reaction volume of 10 µL in 1 h at 37 °C. Endo M activity was assigned as supplied by Prof. Yamamoto; 1 unit of Endo M is defined as the amount of enzyme yielding 1 µmol of GlcNAc-Asn-DNS from $Man_6GlcNAc_2$-Asn-DNS in one minute at 37 °C.

Ethyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-levulinoyl-1-thio-β-D-glucopyranoside (1c). Alcohol **1b**[19] (2.40 g, 6.81 mmol) was dissolved in DCM (100y). Levulinic acid (1.39 mL, 13.6 mmol), dicyclohexylcarbodiimide (2.81 g, 13.6 mmol) and DMAP (0.0832 g, 0.681 mmol) were added and the mixture was stirred at rt under an atmosphere of argon. After 14 h, t.l.c. (petrol : ethyl acetate, 2 : 1) indicated formation of a single product (R_f 0.60) and complete consumption of **1b** (R_f 0.55). The mixture was diluted with DCM (100 mL), washed with water (2 × 50 mL), sodium hydrogen carbonate (2 × 50 mL of a saturated aqueous solution) and brine (50 mL), dried ($MgSO_4$), filtered, concentrated *in vacuo* and the residue purified by flash column chromatography (petrol : ethyl acetate, 2 : 1) to give ester **1c** (2.95 g, 96%) as an amorphous white solid, m.p. 98–100 °C; $[\alpha]_D^{18}$–51.3 (*c*, 0.5 in $CHCl_3$); ν_{max} (KBr disk) 1750, 1716 (s, C=O), 1455, 1388 (s, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.26 (3H, t, *J* 7.5 Hz, CH_2CH_3), 2.21 (3H, s, CH_3), 2.61–2.86 (6H, m, 3 × CH_2), 3.47–3.51 (1H, m, H-5), 3.67–3.71 (2H, m, H-3, H-4), 3.78 (1H, at, *J* 10.3 Hz, H-6), 4.17 (1H, dd, J_{vic} 6.0 Hz, J_{gem} 13.0 Hz, OC*H*H′CH=CH₂), 4.34–4.48 (2H, m, H-6′, OCH*H*′CH=CH₂), 4.50 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 5.02 (1H, at, *J* 9.9 Hz, H-2), 5.16 (1H, d, J_{vic} 10.4 Hz, CH=CHH_Z), 5.25 (1H, dd, J_{gem} 1.7 Hz, J_{vic} 17.2 Hz, CH=CHH_E), 5.56 (1H, s, PhCH(O)), 5.88 (1H, ddd, C*H*=CH_2), 7.36–7.40 (3H, m, 3 × Ar-H), 7.47–7.49 (2H, m, 2 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 15.0 (q, CH_3), 24.1, 28.1, 38.0 (3 × t, 3 × CH_2), 30.0 (q, C(O)CH_3), 68.7 (t, C-6), 70.8 (d, C-5), 71.7 (d, C-2), 73.6 (t, OCH_2CH=CH_2), 79.8 (d, C-3), 81.4 (d, C-4), 84.3 (d, C-1), 101.3 (d, PhCH(O)), 117.1 (t, OCH_2CH=*C*H_2), 126.1, 128.4, 129.1 (3 × s, 5 × Ar-H), 134.9 (d, OCH_2*C*H=CH_2), 137.3 (s, Ar-C), 171.5, 206.3 (2 × s, 2 × C=O); *m/z* (ES^+) 509 ($MMeCN.NH_4^+$, 100), 473 (MNa^+, 79%). HRMS (ES^+) Calcd. For $C_{23}H_{30}NaO_7S$ (MNa^+) 473.1604. Found 473.1606. Found: C, 61.38; H, 6.70. $C_{23}H_{30}O_7S$ requires C, 61.31; H, 6.71%.

***p*-Methoxyphenyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3a).** Glycosyl acceptor **2**[20] (0.100 g, 0.168 mmol), thioglycoside donor **1a**[19] (0.073 g, 0.185 mmol) and tri-*tert*-butylpyrimidine (0.207 g, 0.833 mmol) were dissolved in anhydrous DCM (7.0 mL). The solution was transferred *via* canula to a flame-dried round-bottom flask containing activated 3 Å molecular sieves (0.50 g). The mixture was cooled to 0 °C, methyl trifluoromethanesulfonate (0.0940 mL, 0.833 mmol) added and the mixture allowed to warm to rt while being stirred under an atmosphere of argon. After 15 h, t.l.c. (2 : 1 petrol : ethyl acetate) indicated complete consumption of donor **1a** (R_f 0.75) and formation of a single product (R_f 0.25). Triethylamine (0.116 mL, 0.833 mmol) was added, and the reaction mixture stirred for 10 min, filtered through Celite® and concentrated *in vacuo.* The residue was purified by flash column chromatography (4 : 1 toluene : ethyl acetate) to afford disaccharide **3a** (0.620 g, 80%) as a white foam, m.p. 85–87 °C; $[\alpha]_D^{22}$ + 39.7 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr disk) 1777, 1753, 1716 (s, C=O), 1455, 1388 (s, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.10 (3H, s, CH_3), 3.27 (1H, dat, *J* 5.0 Hz, *J* 9.8 Hz, *J* 9.8 Hz, H-5b), 3.49–3.54 (2H, m, H-3b, H-6b), 3.59–3.66 (2H, m, H-4b, H-5a), 3.72 (3H, s, OCH_3), 3.81–3.86 (2H, m, H-6a, H-6′a), 4.10 (1H, dd, J_{vic} 5.7 Hz, J_{gem} 13.2 Hz, OC*H*H′CH=CH₂), 4.15 (1H, at, *J* 9.1 Hz, H-4a), 4.29 (1H, dd, $J_{5,6'}$ 5.0 Hz, $J_{6,6'}$ 10.4 Hz, H-6′b), 4.32 (1H, dd, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 8.2 Hz, H-3a), 4.36 (1H, dd, J_{vic} 5.0 Hz, OCH*H*′CH=CH₂), 4.41 (1H, dd, $J_{1,2}$ 8.5 Hz, H-2a), 4.47 (1H, d, *J* 12.6 Hz, PhCH_2), 4.54 (1H, d, *J* 12.0 Hz, PhCH_2), 4.67 (1H, d, $J_{1,2}$ 8.2 Hz, H-1b), 4.80 (2H, d, *J* 12.0 Hz, PhCH_2), 4.99 (1H, at, *J* 8.7 Hz, H-2b), 5.17 (1H, dd, J_{vic} 10.4 Hz, J_{gem} 1.7 Hz, CH=CHH_Z), 5.26 (1H, dd, J_{vic} 17.1 Hz, CH=CHH_E), 5.48 (1H, s, PhCH(O)), 5.85 (1H, ddd, C*H*=CH_2), 6.69–6.72 (2H, m, 2 × Ar-H), 6.81–6.84 (2H, m, 2 × Ar-H), 6.87–6.94 (3H, m, 3 × Ar-H), 7.04–7.05 (2H, m, 2 × Ar-H), 7.16–7.20 (4H, m, 4 × Ar-H), 7.26–7.29 (3H, m, 3 × Ar-H), 7.38–7.40 (1H, m, Ar-H), 7.47–7.49 (2H, m, 2 × Ar-H), 7.68–7.73 (4H, m, 4 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 21.0 (q, CH_3), 55.7 (m, C-2a, OCH_3), 66.2 (d, C-5b), 67.7 (t, C-6a), 68.7 (t, C-6b), 73.3 (t, OCH_2CH=CH_2), 73.4 (d, C-2b), 73.8, 74.8 (2 × t, 2 × PhCH_2), 75.2 (d, C-5a), 76.8 (d, C-3a), 78.2 (d, C-4a), 78.8 (d, C-3b), 81.6 (d, C-4b), 97.7 (d, C-1a), 101.0 (d, C-1b), 101.3 (d, PhCH(O)), 114.5, 118.8, 123.5, 125.4, 126.1, 127.3, 128.0, 128.1, 128.2, 128.4, 128.7, 129.1, 129.2, 133.9 (14 × d, 23 × Ar-C), 116.7 (t, OCH_2CH=*C*H_2), 134.8 (d, OCH_2*C*H=CH_2), 131.7, 137.3, 138.0, 138.1, 138.5, 150.9, 155.5 (7 × s, 7 × Ar-C), 167.9, 169.2 (2 × s, 3 × C=O); *m/z* (ES^+) 986 ($MMeCN.NH_4^+$, 100%). HRMS (ES^+) Calcd. For $C_{53}H_{53}NNaO_{14}$ (MNa^+) 950.3358. Found 950.3360.

***p*-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-levulinoyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3b).** Glycosyl acceptor **2**[20] (1.75 g, 2.93 mmol), thioglycoside donor **1c** (1.20 g, 2.66 mmol) and tri-*tert*-butylpyrimidine (2.98 g, 12.0 mmol) were dissolved in anhydrous DCM (100 mL) and transferred to a flame-dried round-bottom flask containing activated 3 Å molecular sieves (8.0 g). The mixture was cooled to 0 °C, stirred under an atmosphere of argon and methyl trifluoromethanesulfonate (1.36 mL, 12.0 mmol) was then added. After 14 h, t.l.c. (2 : 1 petrol : ethyl acetate) indicated complete consumption of **1c** (R_f 0.40) and formation of a single product (R_f 0.10). Triethylamine (1.67 mL, 12.0 mmol) was added and the reaction mixture stirred for 10 min before being filtered through Celite® and concentrated *in vacuo*. The residue was purified by flash column chromatography (2 : 1 petrol : ethyl acetate) to afford disaccharide **3b** (2.38 g, 91%) as a white foam, m.p. 76–78 °C; $[\alpha]_D^{18}$ + 31.2 (*c*, 0.6 in $CHCl_3$); ν_{max} (KBr disk) 1777, 1751, 1716 (s, C=O), 1455, 1388 (s, C=C) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.23 (3H, s, CH_3), 2.55–2.88 (4H, m, 2 × CH_2), 3.25 (1H, dat, *J* 4.8 Hz, *J* 9.5 Hz, *J* 9.5 Hz, H-5b), 3.45–3.52 (2H, m, H-3b, H-6b), 3.58 (1H, at, *J* 9.6 Hz, H-4b), 3.69 (1H, m, H-5a), 3.71 (3H, s, OCH_3), 3.84 (1H, dd, $J_{5,6}$ 1.3 Hz, $J_{6,6'}$ 11.1 Hz, H-6a), 3.93 (1H, dd, $J_{5,6'}$ 3.3 Hz, H-6′a), 4.10 (1H, dd, J_{vic} 5.7 Hz, J_{gem} 13.0 Hz, OC*H*H′CH=CH_2), 4.16 (1H, at, *J* 9.2 Hz, H-4a), 4.28 (1H, dd, $J_{5,6'}$ 4.9 Hz, $J_{6,6'}$ 10.4 Hz, H-6′b), 4.31–4.36 (2H, m, H-3a, OCH*H*′CH=CH_2), 4.40 (1H, dd, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.7 Hz, H-2a), 4.44, 4.81 (2H, ABq, *J* 12.7 Hz, PhCH_2), 4.52, 4.79 (2H, ABq, *J* 12.0 Hz, PhCH_2), 4.65 (1H, d, $J_{1,2}$ 8.0 Hz, H-1b), 4.96 (1H, dd, $J_{2,3}$ 8.8 Hz, H-2b), 5.16 (1H, dd, J_{vic} 10.4 Hz, J_{gem} 1.5 Hz, CH=CHH_Z), 5.24 (1H, dd, J_{vic} 17.3 Hz, CH=CHH_E), 5.46 (1H, s, PhCH(O)), 5.63 (1H, d, H-1a), 5.86 (1H, ddd, C*H*=CH_2), 6.69–6.72 (2H, m, 2 × Ar-H), 6.81–6.83 (2H, m, 2 × Ar-H), 6.87–6.93 (3H, m, 3 × Ar-H), 7.02–7.04 (2H, m, 2 × Ar-H), 7.32–7.41 (8H, m, 8 × Ar-H), 7.46–7.48 (2H, m, 2 × Ar-H), 7.68–7.73 (4H, m, 4 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 27.9 (t, C$H_2$$CH_2$C(O)$CH_3$), 30.0 (q, C(O)C$H_3$), 37.9 (t, CH_2CH_2C(O)CH_3), 55.7 (m, C-2a, OCH_3), 66.1 (d, C-5b), 67.7 (t, C-6a), 68.7 (t, C-6b), 73.3 (t, OCH_2CH=CH_2), 73.6 (d, C-2b), 73.7, 74.8 (2 × t, 2 × PhCH_2), 75.1 (d, C-5a), 76.8 (d, C-3a), 78.1 (d, C-4a), 78.6 (d, C-3b), 81.6 (d, C-4b), 97.6 (d, C-1a), 100.8 (d, C-1b), 101.2 (d, PhCH(O)), 114.4, 118.8, 123.4, 126.1, 127.2, 127.9, 128.0, 128.2, 128.3, 128.6, 129.1, 133.8 (12 × d, 23 × Ar-C), 116.7 (t, OCH_2CH=CH_2), 134.9 (d, OCH_2*C*H=CH_2), 131.6, 137.3, 138.1, 138.6, 150.9, 155.4 (6 × s, 7 × Ar-C), 167.7, 168.2, 171.3, 206.3 (4 × s, 4 × C=O); *m/z* (ES^+) 1043 ($MMeCN.NH_4^+$, 81), 1006 (MNa^+, 82), 1001 (MNH_4^+, 100%). HRMS (ES^+) Calcd. For $C_{56}H_{57}NNaO_{15}$ (MNa^+) 1006.3620. Found 1006.3593. Found: C, 68.38; H, 5.80; N, 1.42. $C_{56}H_{57}NO_{15}$ requires C, 68.35; H, 5.84; N, 1.42%.

***p*-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3c).** Disaccharide **3b** (1.23 g, 1.25 mmol) was dissolved in DCM (5.0 mL) and methanol (12 mL). Hydrazine acetate (0.230 g, 2.50 mmol) was added and the mixture stirred under an atmosphere of argon. After 14 h, t.l.c. (3 : 2 petrol : ethyl acetate) indicated complete consumption of starting material (R_f 0.70) and formation of a single product (R_f 0.50). The reaction mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (2 : 1 petrol : ethyl acetate) to afford alcohol **3c** (1.07 g, 100%) as a white foam, m.p. 80–83 °C; $[\alpha]_D^{19}$ + 33.1 (*c*, 0.3 in $CHCl_3$); ν_{max} (KBr disk) 3475 (m, O-H), 1777, 1715 (s, C=O), 1455, 1389 (s, C=C) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 3.24 (1H, dat, *J* 4.9 Hz, *J* 9.7 Hz, *J* 9.7 Hz, H-5b), 3.45–3.57 (4H, m, H-2b, H-3b, H-4b, H-6b), 3.72 (3H, s, OCH_3), 3.74–3.77 (1H, m, H-5a), 3.88 (1H, dd, $J_{5,6}$ 1.7 Hz, $J_{6,6'}$ 11.3 Hz, H-6a), 4.06 (1H, dd, $J_{5,6'}$ 3.6 Hz, H-6′a), 4.17–4.22 (2H, m, H-4a, H-6′b), 4.26 (1H, dd, J_{vic} 6.1 Hz, J_{gem} 12.8 Hz, OC*H*H′CH=CH_2), 4.42–4.46 (3H, m, H-2a, H-3a, OCH*H*′CH=CH_2), 4.45, 4.82 (2H, ABq, *J* 12.3 Hz, PhCH_2), 4.61, 4.75 (2H, ABq, *J* 12.0 Hz, PhCH_2), 4.69 (1H, d, $J_{1,2}$ 7.0 Hz, H-1b), 5.21 (1H, dd, J_{vic} 10.4 Hz, J_{gem} 1.5 Hz, CH=CHH_Z), 5.32 (1H, dd, J_{vic} 17.3 Hz, CH=CHH_E), 5.47 (1H, s, PhCH(O)), 5.61 (1H, d, H-1a), 5.96 (1H, ddd, C*H*=CH_2), 6.69–6.72 (2H, m, 2 × Ar-H), 6.80–6.83 (2H, m, 2 × Ar-H), 6.88–6.95 (3H, m, 3 × Ar-H), 7.03–7.05 (2H, m, 2 × Ar-H), 7.36–7.40 (8H, m, 8 × Ar-H), 7.46–7.47 (2H, m, 2 × Ar-H), 7.68 (4H, br s, 4 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 55.7 (q, OCH_3), 55.8 (d, C-2a), 66.4 (d, C-5b), 68.1 (t, C-6a), 68.8 (t, C-6b), 73.7 (m, PhCH_2, OCH_2CH=CH_2), 74.9 (t, PhCH_2), 75.0 (d, C-5a), 75.1 (d, C-2b), 78.0 (d, C-3a), 78.9 (d, C-4a), 80.3 (d, C-3b), 81.5 (d, C-4b), 97.9 (d, C-1a), 101.3 (d, PhCH(O)), 103.5 (d, C-1b), 114.5, 118.9, 123.5, 126.1, 127.3, 127.7, 128.1, 128.2, 128.4, 128.6, 129.1, 133.9 (12 × d, 23 × Ar-C), 117.5 (t, OCH_2CH=CH_2), 135.0 (d, OCH_2*C*H=CH_2), 131.7, 137.4, 137.9, 138.5, 151.0, 155.5 (6 × s, 7 × Ar-C), 167.9 (s, 2 × C=O); *m/z* (ES^+) 943 ($MMeCN.NH_4^+$, 45), 908 (MNa^+, 100%). HRMS (ES^+) Calcd. For $C_{51}H_{51}NNaO_{13}$ (MNa^+) 908.3253. Found 908.3249. Found: C, 68.93; H, 5.81; N, 1.52. $C_{51}H_{51}NO_{13}$ requires C, 69.14; H, 5.80; N, 1.58%.

***p*-Methoxyphenyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4a).** *Gluco* alcohol **3c** (2.52 g, 2.84 mmol) was dissolved in DCM (100 mL) and the solution cooled to 0 °C. Pyridine (4.14 mL, 51.2 mmol) was added and the mixture stirred for 5 min under an atmosphere of argon; after this time trifluoromethane sulfonic anhydride (3.83 mL, 22.8 mmol) was added and the mixture allowed to warm to room temperature. After 3 h, t.l.c. (petrol : ethyl acetate, 2 : 1) indicated formation of a single product (R_f 0.35) and complete consumption of starting material (R_f 0.10). The reaction mixture was diluted with DCM (50 mL), washed with sodium hydrogen carbonate (2 × 30 mL of a saturated aqueous solution), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was dissolved in toluene (100 mL) and tetrabutylammonium acetate (6.00 g, 19.9 mmol) added. The reaction mixture was subjected to sonication under an atmosphere of argon. After 16 h, t.l.c. (petrol : ethyl acetate, 3 : 2) indicated formation of a single product (R_f 0.45) and complete consumption of the intermediate triflate (R_f 0.55). The mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (3 : 2 petrol : ethyl acetate) to afford *manno* acetate **4a** (2.39 g, 91% over two steps) as a pale yellow solid, m.p. 76–78 °C; $[\alpha]_D^{19}$ + 28.1 (*c*, 0.7 in $CHCl_3$); ν_{max} (KBr disk) 1776, 1716, 1613 (s, C=O), 1455, 1389 (s, C=C) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.17 (3H, s, CH_3), 3.21 (1H, dat, *J* 4.9 Hz, *J* 9.7 Hz, *J* 9.7 Hz, H-5b), 3.43 (1H, dd, $J_{2,3}$ 2.4 Hz, $J_{3,4}$ 8.9 Hz, H-3b), 3.59 (1H, at, *J* 10.3 Hz, H-6b), 3.67 (1H, br d, *J* 9.9 Hz, H-5a), 3.72 (3H, s, OCH_3), 3.77–3.88 (3H, m, H-4b, H-6a, H-6′a), 4.04-4.07 (2H, m, OC*H*H′CH=CH_2, OCH*H*′CH=CH_2), 4.18-4.23 (2H, m, H-4a, H-6′b), 4.32 (1H, at, *J* 9.0 Hz, H-3a), 4.39–4.45 (2H, m, H-2a,

 This journal is © The Royal Society of Chemistry 2009

PhCH_2), 4.54, 4.80 (2H, ABq, *J* 12.0 Hz, PhCH_2), 4.74 (1H, br s, H-1b), 4.86 (1H, d, *J* 12.3 Hz, PhCH_2), 5.18 (1H, dd, J_{vic} 10.3 Hz, J_{gem} 1.3 Hz, CH=CHH_Z), 5.30 (1H, dd, J_{vic} 17.4 Hz, CH=CHH_E), 5.41 (1H, d, *J* 3.4 Hz, H-2b), 5.51 (1H, s, PhCH(O)), 5.61 (1H, d, $J_{1,2}$ 8.2 Hz, H-1a), 5.85 (1H, ddd, C*H*=CH_2), 6.69–6.72 (2H, m, 2 × Ar-H), 6.80–6.82 (2H, m, 2 × Ar-H), 6.89–6.95 (3H, m, 3 × Ar-H), 7.01–7.04 (2H, m, 2 × Ar-H), 7.32–7.39 (8H, m, 8 × Ar-H), 7.46–7.48 (2H, m, 2 × Ar-H), 7.68 (4H, br s, 4 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 21.2 (q, CH_3), 55.7 (q, OCH_3), 55.8 (d, C-2a), 67.1 (d, C-5b), 68.4 (t, C-6a), 68.6 (t, C-6b), 69.4 (d, C-2b), 71.0 (t, O*C*H_2CH=CH_2), 73.7, 74.8 (2 × t, 2 × $PhCH_2$), 74.7 (d, C-5a), 75.9 (d, C-3b), 77.4 (d, C-3a), 78.0 (d, C-4b), 79.2 (d, C-4a), 97.8 (d, C-1a), 99.7 (d, C-1b), 101.7 (d, PhCH(O)), 114.5, 118.7, 123.5, 126.2, 127.4, 127.9, 128.0, 128.1, 128.2, 128.3, 128.7, 129.1, 133.9 (13 × d, 23 × Ar-C), 117.3 (t, OCH_2CH=*C*H_2), 134.4 (d, OCH_2*C*H=CH_2), 131.7, 137.5, 137.9, 138.0, 138.6, 150.9, 155.5 (7 × s, 7 × Ar-C), 169.8, 170.3 (2 × s, 3 × C=O); *m/z* (ES^+) 950 (MNa^+, 22), 945 (MNH_4^+, 100%). HRMS (ES^+) Calcd. For $C_{53}H_{53}NNaO_{14}$ (MNa^+) 950.3354. Found 950.3358.

***p*-Methoxyphenyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4b).** Freshly distilled THF (15 mL) was added to a flame-dried flask containing (1,5-cyclooctadiene)-bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (72.9 mg, 86.2 μmol). The mixture was degassed and subjected to an atmosphere of hydrogen for 15 min until the catalyst lost its pink colour and had fully dissolved. Allyl ether **4a** (1.00 g, 1.08 mmol) was dissolved in freshly distilled THF (25 mL) in a second flame-dried flask. The solution of the activated iridium catalyst was added and the mixture was stirred for 14 h under an atmosphere of argon. After this time water (5.4 mL, 300 mmol) and *N*-iodosuccinimide (1.21 g, 5.39 mmol) were added. After 18 h, t.l.c. (petrol : ethyl acetate, 1 : 1) indicated formation of a major product (R_f 0.20) and consumption of starting material (R_f 0.55). The reaction mixture was diluted with DCM (100 mL), washed with sodium thiosulfate (2 × 50 mL of a 10% w/w solution), sodium hydrogen carbonate (2 × 50 mL of a saturated solution), dried ($MgSO_4$), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (1 : 1 petrol : ethyl acetate) to afford alcohol **4b** (0.820 g, 86%) as a white solid, m.p. 111–113 °C; $[\alpha]_D^{20}$ + 34.3 (*c*, 0.75 in $CHCl_3$); ν_{max} (KBr disk) 1776, 1747, 1715 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.19 (3H, s, CH_3), 2.23 (1H, d, $J_{OH,3}$ 3.6 Hz, OH), 3.20 (1H, dat, *J* 5.0 Hz, *J* 9.7 Hz, *J* 9.7 Hz, H-5b), 3.59 (1H, at, *J* 9.8 Hz, H-6b), 3.65–3.71 (2H, m, H-3b, H-5a), 3.72 (3H, s, OCH_3), 3.74–3.76 (1H, m, H-4b), 3.78 (1H, dd, $J_{5,6}$ 1.7 Hz, $J_{6,6'}$ 11.3 Hz, H-6a), 3.87 (1H, dd, $J_{5,6'}$ 3.1 Hz, H-6′a), 4.18–4.22 (2H, m, H-4a, H-6′b), 4.30–4.34 (1H, m, H-3a), 4.40–4.45 (2H, m, H-2a, PhCH_2), 4.51, 4.79 (2H, ABq, *J* 12.0 Hz, PhCH_2), 4.76 (1H, d, $J_{1,2}$ 0.6 Hz, H-1b), 4.85 (1H, d, *J* 12.2 Hz, PhCH_2), 5.30 (1H, dd, $J_{2,3}$ 3.5 Hz, H-2b), 5.50 (1H, s, PhCH(O)), 5.61 (1H, d, $J_{1,2}$ 8.4 Hz, H-1a), 6.69–6.72 (2H, m, 2 × Ar-H), 6.79–6.83 (2H, m, 2 × Ar-H), 6.88–6.95 (3H, m, 3 × Ar-H), 7.02–7.04 (2H, m, 2 × Ar-H), 7.33–7.40 (8H, m, 8 × Ar-H), 7.46–7.48 (2H, m, 2 × Ar-H), 7.68–7.80 (4H, m, 4 × Ar-H); δ_D (125.8 MHz, $CDCl_3$) 21.1 (q, CH_3), 55.7 (m, C-2a, OCH_3), 66.8 (d, C-5b), 68.3 (t, C-6a), 68.6 (t, C-6b), 69.9 (d, C-3b), 71.3 (d, C-2b), 73.7, 74.8 (2 × t, 2 × $PhCH_2$), 74.7 (d, C-5a), 77.1 (d, C-3a), 78.6 (d, C-4b), 79.5 (d, C-4a), 97.8 (d, C-1a), 99.7 (d, C-1b), 102.2 (d, PhCH(O)), 114.5, 118.7, 123.5, 126.4, 127.4, 127.9, 128.1, 128.2, 128.5, 128.7, 129.5, 133.9 (12 × d, 23 × Ar-C), 131.7, 137.2, 138.0, 138.6, 151.0, 155.5 (6 × s, 7 × Ar-C), 167.8 (br s, 2 × C=O), 170.7 (s, C=O); *m/z* (ES^+) 910 (MNa^+, 100), 905 (MNH_4^+, 92%). HRMS (ES^+) Calcd. For $C_{50}H_{49}NNaO_{14}$ (MNa^+) 910.3045. Found 910.3048.

Ethyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (6b). Disaccharide **5**[28] (3.00 g, 3.92 mmol) and ethanethiol (0.435 mL, 5.88 mmol) were dissolved in anhydrous $CHCl_3$ (30 mL). To this solution was added boron trifluoride diethyl etherate (0.745 mL, 5.88 mmol), and the mixture stirred and heated to reflux under an atmosphere of argon. After 1 h, t.l.c. (petrol : ethyl acetate 2 : 3) indicated formation of a single product (R_f 0.45) and complete consumption of starting material (R_f 0.25). The reaction mixture was cooled, quenched with triethylamine (3.0 mL), diluted with DCM (50 mL) and washed with water (20 mL) and sodium hydrogen carbonate (2 × 20 mL of a saturated solution). The organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo.* The residue was purified by flash column chromatography (petrol : ethyl acetate, 2 : 3) to afford thioglycoside **6b** (2.12 g, 70%) as a white foam, $[\alpha]_D^{25}$ + 18.7 (*c*, 1.2 in $CHCl_3$); ν_{max} (KBr disk) 1749 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.18 (3H, t, *J* 7.4 Hz, SCH_2CH_3) 1.87, 1.98, 1.98, 2.02, 2.03, 2.11 (18H, 6 × s, 6 × CH_3), 2.40–2.51 (3H, m, SC$H_2$$CH_3$), 3.63 (1H, dd, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.2 Hz, H-6a), 3.75 (1H, dd, $J_{5,6'}$ 5.4 Hz, H-6′a), 3.83 (1H, ddd, $J_{5,6}$ 2.2 Hz, $J_{5,6'}$ 5.1 Hz, $J_{4,5}$ 10.1 Hz, H-5b), 4.04 (1H, ddd, $J_{4,5}$ 9.9 Hz, H-5a), 4.08 (1H, dd, $J_{6,6'}$ 12.3 Hz, H-6b), 4.26 (1H, dd, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2a), 4.30 (1H, dd, H-6′b), 4.42 (1H, dd, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.8 Hz, H-2b), 4.88 (1H, dd, $J_{3,4}$ 9.9 Hz, H-3a), 4.94 (1H, d, H-1a), 5.11 (1H, at, *J* 9.9 Hz, H-4a), 5.14 (1H, dd, $J_{3,4}$ 9.2 Hz, H-4b), 5.39 (1H, d, H-1b), 5.79 (1H, dd, H-3b), 7.70–7.82 (4H, m, 4 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 14.6 (q, SCH_2*C*H_3), 20.6, 20.7, 20.8, 20.8, 20.9 (5 × q, 6 × CH_3), 25.6 (t, S*C*$H_3$$CH_3$), 54.5 (d, C-2b), 62.1 (t, C-6b), 62.6 (t, C-6a), 66.0 (d, C-4a), 68.7 (d, C-5a), 69.0 (d, C-4b), 70.6 (d, C-3a), 70.7 (d, C-3b), 72.2 (d, C-5b), 76.1 (d, C-2a), 81.6 (d, C-1a), 96.7 (d, C-1b), 123.5, 134.3 (2 × d, 4 × Ar-C), 131.6 (s, 2 × Ar-C), 169.4, 169.6, 170.3, 170.5, 170.8, 170.8 (6 × s, 8 × C=O); *m/z* (ES^+) 1557 (M_2Na^+, 88), 1552 ($M_2NH_4^+$, 85), 790 (MNa^+, 100), 785 (MNH_4^+, 98%). HRMS (ES^+) Calcd. For $C_{34}H_{41}NNaO_{17}S$ (MNa^+) 790.1993. Found 790.1993.

***p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7).**

Method 1. Disaccharide acceptor **4b** (0.100 g, 0.113 mmol) and trichloroacetimidate donor **6a**[28] (0.117 g, 0.135 mmol) were dissolved in anhydrous DCM (5.0 mL). The solution was transferred *via* canula to a flame-dried round-bottom flask containing activated 3 Å molecular sieves (0.60 g), cooled to −20 °C and stirred under an atmosphere of argon for 10 min. After this time, boron trifluoride diethyl etherate (7.14 μL, 0.0563 mmol) was added portionwise and the reaction mixture stirred at −20 °C. After 2 h, t.l.c. (petrol : ethyl acetate, 1 : 2) indicated formation of a major product (R_f 0.40) and complete consumption of the donor **6a** (R_f 0.45). The reaction mixture was diluted with DCM (15 mL), filtered

through Celite®, washed with sodium hydrogen carbonate (5 mL of a saturated solution), dried ($MgSO_4$), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (petrol : ethyl acetate, 1 : 1) to afford tetrasaccharide **7** (0.090 g, 50%) as a white foam, together with some recovered acceptor **4b** (0.034 g, 34%). $[\alpha]_D^{25}$ + 18.8 (*c*, 0.25 in $CHCl_3$); ν_{max} (KBr disk) 1750, 1718 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.86, 1.86, 1.98, 1.99, 2.04, 2.05, 2.13 (21H, 7 × s, 7 × CH_3), 3.02–3.07 (1H, m, H-5b), 3.52–3.56 (2H, m, H-3b, H-6b), 3.59–3.61 (1H, m, H-5a), 3.67–3.83 (8H, m, H-4b, H-5c, H-5d, H-6a, H-6′a, H-6c, H-6′c, H-6d), 3.70 (3H, s, OCH_3), 3.95 (1H, dd, $J_{5,6'}$ 3.6 Hz, $J_{6,6'}$ 12.4 Hz, H-6′d), 4.02 (1H, dd, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.3 Hz, H-2c), 4.17–4.21 (2H, m, H-4a, H-6′b), 4.24–4.29 (2H, m, H-2d, H-3a), 4.37–4.42 (3H, m, H-2a, 2 × $PhCH_2$), 4.50 (1H, s, H-1b), 4.80 (1H, d, *J* 12.1 Hz, $PhCH_2$), 4.84 (1H, dd, $J_{3,4}$ 10.3 Hz, H-3c), 4.87 (1H, d, *J* 12.1 Hz, $PhCH_2$), 4.90 (1H, d, $J_{1,2}$ 8.5 Hz, H-1d), 4.95 (1H, d, H-1c), 4.98 (1H, at, *J* 9.6 Hz, H-4d), 5.03 (1H, at, *J* 10.1 Hz, H-4c), 5.18 (1H, d, *J* 3.4 Hz, H-2b), 5.46 (1H, dd, $J_{3,4}$ 9.2 Hz, $J_{2,3}$ 10.3 Hz, H-3d), 5.48 (1H, s, PhCH(O)), 5.60 (1H, d, $J_{1,2}$ 8.5 Hz, H-1a), 6.69–6.71 (2H, m, 2 × Ar-H), 6.79–6.80 (2H, m, 2 × Ar-H), 6.92–6.94 (3H, m, 3 × Ar-H), 6.99–7.01 (2H, m, 2 × Ar-H), 7.39 7.49 (6H, m, 6 × Ar-H), 7.55–7.88 (12H, m, 12 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 20.6, 20.6, 20.8, 20.8, 20.9, 20.9, 20.9 (7 × q, 7 × CH_3), 54.2 (d, C-2d), 55.7 (d, C-2a), 55.7 (q, OCH_3), 61.1 (t, C-6d), 62.9 (t, C-6c), 65.5 (d, C-4c), 66.3 (d, C-5b), 67.9 (t, C-6a), 68.5 (d, C-4d), 68.7 (t, C-6b), 68.9 (d, C-5c), 69.4 (d, C-3c), 70.6 (d, C-3d), 70.7 (d, C-2b), 71.2 (d, C-5d), 72.9 (d, C-2c), 73.9, 74.6 (2 × t, 2 × $PhCH_2$), 74.7 (d, C-5a), 75.3 (d, C-3b), 76.6 (d, C-3a), 78.2 (d, C-4a), 78.8 (d, C-4b), 95.9 (d, C-1d), 97.8 (d, C-1a), 98.1 (d, C-1c), 98.6 (d, C-1b), 102.5 (d, PhCH(O)), 114.5, 118.7, 123.7, 123.7, 127.0, 127.4, 128.0, 128.2, 128.9, 129.0, 129.1, 130.3, 134.0, 134.4 (14 × d, 27 × Ar-C), 131.7, 137.4, 137.9, 138.5, 151.0, 155.5 (6 × s, 9 × Ar-C), 169.3, 169.5, 170.3, 170.3, 170.6, 170.7, 170.7 (7 × s, 11 × C=O); *m/z* (ESI^+) species observed (MNa^+), (MNH_4^+); (MNa^+) peaks observed: 1615.41 (100), 1616.42 (92), 1617.42 (37), 1618.42 (10), 1619.42 (3), peaks calculated: 1615.50 (100), 1616.50 (92), 1617.50 (48), 1618.50 (18), 1619.51 (5), 1620.51 (1%). The stereochemistry of the anomeric linkages was confirmed by measurement of the J_{H1-C1} coupling constants from C-coupled HSQC experiments: $J_{C(1a)-H(1a)}$ 166 Hz; $J_{C(1b)-H(1b)}$ 162 Hz; $J_{C(1c)-H(1c)}$ 174 Hz; $J_{C(1d)-H(1d)}$ 166 Hz.

Method 2. Disaccharide acceptor **4b** (0.250 g, 0.282 mmol) and thioglycoside donor **6b** (0.281 g, 0.366 mmol) were dissolved in anhydrous DCM (40 mL). The solution was transferred *via* canula to a flame-dried round-bottom flask containing activated 3 Å molecular sieves (1.0 g) and NIS (0.095 g, 0.422 mmol) was then added. The solution was cooled to 0 °C and stirred under an atmosphere of argon for 10 min. After this time silver trifluoromethanesulfonate (0.094 g, 0.365 mmol) was added and the reaction mixture stirred at 0 °C. After 1 h, t.l.c. (petrol : ethyl acetate, 1 : 2) indicated formation of a major product (R_f 0.40) and complete consumption of the donor **6b** (R_f 0.55). The reaction mixture was diluted with DCM (15 mL), filtered through Celite®, washed with sodium thiosulfate (2 × 5 mL of a 10% w/v solution) and sodium hydrogen carbonate (5 mL of a saturated solution), dried ($MgSO_4$), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (petrol : ethyl acetate, 1 : 1) to afford tetrasaccharide **7** (0.342 g, 79%, 93% based on recovered **4b**) as a white foam, identical to the material described above.

***p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8).** Tetrasaccharide **7** (0.150 g, 0.0941 mmol) was dissolved in anhydrous DCM (5.0 mL). The solution was transferred *via* canula to a flame-dried round-bottom flask containing activated 3 Å molecular sieves (0.30 g), stirred at rt under an atmosphere of argon for 30 min then cooled to −78 °C. Triethylsilane (45 µL, 0.282 mmol) and dichlorophenyl borane (41 µL, 0.320 mmol) were added and the reaction mixture stirred at −78 °C. After 45 min, t.l.c. (petrol : ethyl acetate, 2 : 3) indicated formation of a major product (R_f 0.25) and complete consumption of starting material (R_f 0.35). Triethylamine (0.50 mL) and methanol (0.50 mL) were added, the reaction mixture diluted with DCM (5.0 mL), washed with sodium hydrogen carbonate (2 × 5 mL of a saturated solution), dried ($MgSO_4$), filtered and concentrated *in vacuo.* The residue was co-distilled with methanol fives times at 50 °C before being purified by flash column chromatography (petrol:ethyl acetate, 2:3) to give alcohol **8** (0.137 g, 91%) as a white foam, $[\alpha]_D^{25}$–4.3 (*c*, 0.4 in $CHCl_3$); ν_{max} (KBr disk) 3458 (s, O-H), 1748, 1717 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.84, 1.89, 1.98, 2.01, 2.02, 2.04, 2.13 (21H, 7 × s, 7 × CH_3), 2.35–2.38 (1H, m, O-H), 2.98 (1H, ddd, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$ 4.0 Hz, H-5b), 3.93-3.43 (1H, m, H-6b), 3.45 (1H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.7 Hz, H-3b), 3.58–3.70 (4H, m, H-4b, H-5a, H-6d, H-6′b), 3.71 (3H, s, OCH_3), 3.73–3.77 (5H, m, H-5d, H-6a, H-6c, H-6′a, H-6′c), 3.82–3.85 (1H, m, H-5c), 3.99–4.03 (2H, m, H-2c, H-6′d), 4.16 (1H, at, *J* 9.3 Hz, H-4a), 4.24–4.30 (2H, m, H-2d, H-3a), 4.36–4.44 (4H, m, H-1b, H-2a, 2 × $PhCH_2$), 4.66 (1H, d, *J* 12.9 Hz, $PhCH_2$), 4.79–4.89 (4H, m, H-3c, 3 × $PhCH_2$), 4.95–4.99 (2H, m, H-1c, H-4d), 5.01 (1H, d, $J_{1,2}$ 8.4 Hz, H-1d), 5.07–5.11 (2H, m, H-2b, H-4c), 5.47 (1H, dd, $J_{3,4}$ 9.1 Hz, $J_{2,3}$ 10.7 Hz, H-3d), 5.61 (1H, d, $J_{1,2}$ 8.5 Hz, H-1a), 6.69-6.71 (2H, m, 2 × Ar-H), 6.79–6.81 (2H, m, 2 × Ar-H), 6.98–7.04 (5H, m, 5 × Ar-H), 7.31–7.53 (10H, m, 10 × Ar-H), 7.68–7.86 (8H, m, 8 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 20.6, 20.6, 20.7, 20.8, 20.9, 20.9, 21.1 (7 × q, 7 × CH_3), 54.3 (d, C-2d), 55.6 (d, C-2a), 55.7 (q, OCH_3), 61.5 (m, C-6b, C-6d), 62.6 (t, C-6c), 65.5 (d, C-4c), 67.7 (t, C-6a), 68.5 (t, C-4d), 69.1 (d, C-5c), 69.7 (d, C-3c), 70.7 (d, C-3d), 71.0 (d, C-2b), 71.3 (d, C-5d), 73.3 (d, C-2c), 73.9, 73.9, 74.4 (3 × t, 3 × $PhCH_2$), 74.6 (d, C-4b), 74.8 (d, C-5a), 75.3 (d, C-5b), 76.7 (d, C-3a), 77.2 (d, C-4a), 79.1 (d, C-3b), 96.3 (d, C-1d), 97.7 (d, C-1b), 97.8 (d, C-1a), 99.5 (d, C-1c), 114.5, 118.8, 123.8, 126.1, 127.3, 127.6, 127.9, 128.1, 128.2, 128.4, 128.8, 128.9, 129.1, 133.7, 134.1, 134.4, 135.7 (17 × d, 27 × Ar-C), 131.7, 131.6, 137.7, 138.4, 138.5, 150.9, 155.5 (7 × s, 9 × Ar-C), 169.4, 169.6, 170.2, 170.3, 170.7, 170.7 (6 × s, 11 × C=O); *m/z* (ESI^+) species observed (MNa^+), (MNH_4^+); (MNa^+) peaks observed: 1617.43 (100), 1618.43 (100), 1619.43 (37), 1620.44 (10), 1621.43 (2), peaks calculated: 1617.51 (100), 1618.51 (92), 1619.52 (48), 1620.52 (18), 1621.52 (5), 1622.53 (1%).

***p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-4-*O*-benzyl-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9a).** Tetrasaccharide acceptor **8** (0.159 g, 0.0996 mmol) and disaccharide donor **6b**

 This journal is © The Royal Society of Chemistry 2009

(0.0994 g, 0.130 mmol) were dissolved in anhydrous DCM (15 mL). The solution was transferred *via* syringe to a flame-dried round-bottom flask containing activated 3 Å molecular sieves (0.5 g) and NIS (0.0336 g, 0.149 mmol) added. The solution was cooled to 0 °C and stirred under an atmosphere of argon for 10 min. After this time silver trifluoromethanesulfonate (0.0333 g, 0.130 mmol) was added and the reaction mixture stirred at 0 °C. After 1 h, t.l.c. (petrol : ethyl acetate, 1 : 2) indicated formation of a major product (R_f 0.20) and complete consumption of donor **6b** (R_f 0.55). The reaction mixture was diluted with DCM (15 mL), filtered through Celite®, washed with sodium thiosulfate (2 × 5 mL of a 10% w/v solution) and sodium hydrogen carbonate (5 mL of a saturated solution), dried ($MgSO_4$), filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography (petrol : ethyl acetate, 1 : 2 then toluene : ethyl acetate, 2 : 3) to afford hexasaccharide **9a** (0.182 g, 79%, 89% based on recovered acceptor **8**) as a white, glassy solid, $[\alpha]_D^{25}$ + 2.6 (*c*, 0.9 in $CHCl_3$); ν_{max} (KBr disk) 1748, 1717 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.84, 1.85, 1.87, 1.90, 1.96, 1.98, 2.01, 2.01, 2.03, 2.03, 2.07, 2.10, 2.13 (39H, 13 × s, 13 × CH_3), 3.14–3.17 (2H, m, H-5b, H-5d′), 3.46–3.52 (2H, m, H-3b, H-6b), 3.54–3.59 (3H, m, H-4b, H-5a, H-5d, H-6c′), 3.61–3.66 (2H, m, H-6′b, H-6d), 3.67–3.71 (1H, m, H-5c′), 3.68 (3H, s, OCH_3), 3.74–3.81 (5H, m, H-6a, H-6′a, H-6c, H-6′c, H-6′c′), 3.84–3.87 (2H, m, H-5c, H-6d′), 3.99–4.02 (2H, m, H-2c, H-6′d), 4.11 (1H, dd, $J_{3,4}$ 9.4 Hz, $J_{4,5}$ 7.4 Hz, H-4a), 4.16–4.20 (3H, m, H-2c′, H-3a, H-6′d′), 4.23 (1H, dd, $J_{1,2}$ 8.3 Hz, $J_{2,3}$ 10.4 Hz, H-2a), 4.28 (1H, dd, $J_{1,2}$ 8.4 Hz, $J_{2,3}$ 10.8 Hz, H-2d), 4.35 (1H, dd, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.8 Hz, H-2d′), 4.39, 4.84 (2H, ABq, *J* 12.0 Hz, PhCH_2), 4.44 (1H, s, H-1c′), 4.45, 4.67 (2H, ABq, *J* 12.8 Hz, PhCH_2), 4.50 (1H, s, H-1b), 4.58, 4.91 (2H, ABq, *J* 12.9 Hz, PhCH_2), 4.83-4.86 (1H, m, H-3c), 4.95 (1H, s, H-1c), 4.97-5.00 (2H, m, H-1d, H-4d), 5.03 (1H, dd, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 10.0 Hz, H-3c′), 5.05-5.09 (2H, m, H-4c, H-4d′), 5.11-5.13 (1H, m, H-2b), 5.13 (1H, s, H-1d′), 5.28 (1H, at, *J* 10.0 Hz, H-4c′), 5.47 (1H, dd, $J_{3,4}$ 9.1 Hz, H-3d), 5.54 (1H, d, $J_{1,2}$ 8.0 Hz, H-1a), 5.56 (1H, dd, $J_{3,4}$ 9.1 Hz, H-3d′), 6.65–6.79 (5H, m, 5 × Ar-H), 7.03–7.05 (2H, m, 2 × Ar-H), 7.33–7.54 (12H, m, 12 × Ar-H), 7.63–7.87 (12H, m, 12 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 20.5, 20.6, 20.6, 20.6, 20.7, 20.8, 20.8, 20.9, 20.9, 21.1, 21.2 (11 × q, 13 × CH_3), 54.3 (d, C-2d), 54.5 (d, C-2d′), 55.6 (d, C-2a), 55.7 (q, OCH_3), 61.5 (t, C-6d), 61.7 (t, C-6d′), 62.4 (t, C-6c′), 62.6 (t, C-6c), 65.3 (d, C-4c′), 65.5 (d, C-4c), 66.8 (t, C-6b), 67.9 (t, C-6a), 68.4 (d, C-4d), 68.6 (d, C-4d′), 68.7 (d, C-5c′), 69.2 (d, C-5c), 69.7 (d, C-3c), 70.4 (d, C-3c′), 70.7 (d, C-3d), 70.8 (d, C-3d′), 71.1 (d, C-2b), 71.4 (d, C-5d), 71.6 (d, C-5d′), 73.4 (d, C-2c), 73.6 (d, C-5b), 73.7, 74.2, 74.6 (3 × t, 3 × PhCH_2), 74.5 (d, C-2c′), 74.7 (d, C-5a), 75.0 (d, C-4b), 76.2 (d, C-3a), 78.2 (d, C-4a), 79.9 (d, C-3b), 96.4 (d, C-1d), 97.3 (d, C-1c′), 97.3 (d, C-1d′), 97.5 (d, C-1a), 97.8 (d, C-1b), 99.8 (d, C-1c), 114.5, 118.4, 123.6, 125.9, 127.6, 128.1, 128.2, 128.5, 128.9, 129.0, 129.0, 133.5, 134.0, 134.4, 134.5, 134.7 (16 × d, 31 × Ar-C), 131.6, 138.1, 138.3, 138.4, 150.9, 155.4 (6 × s, 11 × Ar-C), 169.4, 169.4, 169.6, 169.8, 170.3, 170.3, 170.4, 170.7, 170.7, 170.9 (10 × s, 19 × C=O); *m/z* (ESI$^+$) species observed (MNa$^+$), (MNH$_4^+$); (MNa$^+$) peaks observed: 2322.49 (61), 2323.49 (81), 2324.49 (49), 2325.50 (18), 2326.50 (6), peaks calculated: 2322.70 (78), 2323.70 (100), 2324.71 (71), 2325.71 (36), 2326.71 (14), 2327.72 (5), 2328.72 (1.0); (MNH$_4^+$) peaks observed: 2317.53 (70), 2318.54 (100), 2319.54 (62), 2320.54 (24), 2321.54 (8), peaks calculated: 2317.75 (78), 2318.75 (100), 2319.75 (71), 2320.76 (36), 2321.76 (14), 2322.76 (5), 2323.76 (1%).

The stereochemistry of the anomeric linkages was confirmed by measurement of the $J_{H1\text{-}C1}$ coupling constants from C-coupled HSQC experiments: $J_{C(1a)\text{-}H(1a)}$ 166 Hz; $J_{C(1b)\text{-}H(1b)}$ 162 Hz; $J_{C(1c)\text{-}H(1c)}$ 173 Hz; $J_{C(1c')\text{-}H(1c')}$ 171 Hz; $J_{C(1d')\text{-}H(1d')}$ 165 Hz; $J_{C(1d)\text{-}H(1d)}$ 166 Hz.

***p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9b).** Benzyl ether **9a** (0.136 g, 0.0591 mmol) was dissolved in methanol (2 mL) and THF (2 mL). The solution was degassed and put under an atmosphere of nitrogen. Palladium hydroxide (0.040 g) was added and the mixture stirred at rt under an atmosphere of hydrogen. After 10 days, t.l.c. (petrol : ethyl acetate, 1 : 4) indicated complete consumption of starting material (R_f 0.35) and formation of a single product (R_f 0.25). The reaction mixture was filtered through Celite®, the Celite® washed with methanol (2 × 10 mL) and ethyl acetate (2 × 10 mL) and the combined organic washings were concentrated *in vacuo.* The residue was dissolved in pyridine (5 mL), acetic anhydride (2 mL) was added, and the reaction mixture was stirred under an atmosphere of argon. After 16 h, t.l.c. (1 : 4 petrol : ethyl acetate) indicated formation of a single product (R_f 0.30) and complete consumption of triol intermediate (R_f 0.25). The reaction mixture was concentrated *in vacuo* and the residue was co-distilled with toluene 4 times. The residue was purified by flash column chromatography (1 : 4 petrol : ethyl acetate) to afford peracetate **9b** (0.110 g, 86% over two steps) as a white foam, $[\alpha]_D^{20}$–7.2 (*c*, 0.25 in $CHCl_3$); ν_{max} (KBr disk) 1749, 1718, 1636 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.86, 1.88, 1.96, 1.98, 2.00, 2.02, 2.03, 2.04, 2.05, 2.07, 2.09, 2.11, 2.12, 2.13 (48H, 14 × s, 16 × CH_3), 3.37 (1H, dd, *J* 3.6 Hz, *J* 10.1 Hz, H-6b), 3.44–3.49 (1H, m, H-5b), 3.64 (1H, dd, *J* 6.3 Hz, *J* 10.2 Hz, H-6′b), 3.70 (3H, s, OCH_3), 3.74–3.88 (10H, m, H-3b, H-5a, H-5c, H-5c′, H-5d, H-6a, H-6c, H-6c′, H-6′c, H-6‘c’), 3.89–3.91 (1H, m, H-5d′), 3.93–3.97 (2H, m, H-4a, H-6′a), 4.05 (1H, d, *J* 2.4 Hz, H-2c), 4.06–4.10 (2H, m, H-6d, H-6d′), 4.24 (1H, d, *J* 2.4 Hz, H-2c′), 4.31–4.47 (6H, m, H-1c′, H-2a, H-2d, H-2d′, H-6′d, H-6′d′), 4.58 (1H, s, H-1b), 4.62 (1h, s, H-1c), 4.77 (1H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.3 Hz, H-3c), 4.95–4.99 (2H, m, H-3c′, H-4b), 5.12–5.19 (3H, m, H-4c, H-4d, H-4d′), 5.24 (1H, d, *J* 3.4 Hz, H-2b), 5.27 (1H, at, *J* 10.1 Hz, H-4c′), 5.28 (1H, d, $J_{1,2}$ 8.9 Hz, H-1d), 5.44 (1H, d, $J_{1,2}$ 8.5 Hz, H-1d′), 5.67 (1H, dd, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 9.2 Hz, H-3d), 5.76 (1H, d, $J_{1,2}$ 8.4 Hz, H-1a), 5.77 (1H, dd, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 9.1 Hz, H-3d′), 5.81 (1H, dd, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 9.1 Hz, H-3a), 6.70–6.73 (2H, m, 2 × Ar-H), 6.76–6.81 (2H, m, 2 × Ar-H), 7.70–7.86 (12H, m, 12 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 20.6, 20.6, 20.7, 20.7, 20.8, 20.9, 20.9, 21.0, 21.0, 21.0, 21.2, 21.3, 22.8 (13 × q, 16 × CH_3), 54.5 (d, C-2d′), 54.5 (d, C-2a), 54.6 (d, C-2d), 55.7 (q, OCH_3), 62.0 (t, C-6d), 62.2 (t, C-6d′), 62.3 (t, C-6a), 62.4 (m, C-6c, C-6c′), 65.2 (d, C-4c), 65.3 (d, C-4c′), 67.9 (t, C-6b), 68.7 (d, C-4d), 68.9 (d, C-4d′), 68.9 (m, C-5c, C-5c′), 69.4 (d, C-2b), 69.4 (d, C-3c), 69.6 (d, C-4b), 70.0 (d, C-3a), 70.3 (d, C-3c′), 70.8 (m, C-3d, C-3d′), 72.1 (d, C-5d′), 72.4 (d, C-5d), 72.7 (d, C-5a), 73.0 (d, C-5b), 74.0 (d, C-2c′), 74.2 (d, C-3b), 74.3 (d, C-2c), 74.5 (d, C-4a), 96.9 (d, C-1b), 97.3 (m, C-1d, C-1d′), 97.4 (d, C-1a), 97.7 (d, C-1c′), 98.6 (d, C-1c), 114.6, 118.6, 123.7, 123.9, 128.9, 131.4 (6 × d, 16 × Ar-C), 134.5, 134.7, 151.0, 155.8 (4 × s, 8 × Ar-C), 169.5, 169.5, 169.7, 170.0, 170.3, 170.4, 170.4, 170.5, 170.6,

170.8, 170.9 (11 × s, 22 × C=O); *m/z* (ESI$^+$) species observed (MMeCN.NH_4^+), (MNa$^+$), (M[MeCN.$NH_4]_2^{2+}$); (MNa$^+$) peaks observed: 2178.54 (79), 2179.54 (100), 2180.55 (66), 2181.55 (33), 2182.55 (11), 2183.55 (4), peaks calculated: 2178.59 (90), 2179.60 (100), 2180.60 (65), 2181.60 (31), 2182.60 (12), 2183.61 (4), 2184.61 (1%).

***p*-Methoxyphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (9c).** Phthalamide protected hexasaccharide **9b** (0.098 g, 0.0454 mmol) was dissolved in a mixture of methanol (4 mL) and ethylene diamine (2 mL). The reaction mixture was stirred at 70 °C under an atmosphere of argon. After 16 h, t.l.c. (*iso*-propanol : ammonium acetate [1M aqueous solution], 2 : 1) indicated complete consumption of starting material (R_f 0.95) and formation of a single product (R_f 0.10). The reaction mixture was concentrated *in vacuo* and co-distilled 5 times with toluene at 60 °C. The residue was dissolved in pyridine (3 mL), acetic anhydride (1 mL) was added, and the reaction mixture was then stirred at rt under an atmosphere of argon. After 24 h, t.l.c. (DCM : MeOH, 94 : 6) indicated formation of a single product (R_f 0.25) and complete consumption of the intermediate tri-amine (R_f 0.0). The reaction mixture was concentrated *in vacuo*, co-distilled three times with toluene and the residue purified by flash column chromatography (DCM : MeOH, 94 : 6) to afford acetamide **9c** (0.065 g, 76%) as a white foam, $[\alpha]_D^{20}$ – 8.7 (*c*, 0.15 in $CHCl_3$); ν_{max} (KBr disk) 3396 (br, N-H stretch), 1750 (s, C=O), 1672 (br, amide) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.97, 1.98, 1.98, 1.99, 2.00, 2.01, 2.01, 2.03, 2.05, 2.07, 2.08, 2.08, 2.09, 2.10, 2.15, 2.16, 2.18, 2.22 (57H, 18 × s, 19 × CH_3), 3.23 (1H, d, *J* 9.2 Hz, H-2d′), 3.44–3.46 (1H, m, H-5b), 3.44–3.46 (1H, m, H-5d), 3.65–3.72 (3H, m, H-5a, H-5d′, H-6b), 3.74 (3H, s, OCH_3), 3.75–3.80 (2H, m, H-4a, H-6′b), 3.93–4.03 (5H, m, H-2c, H-3b, H-5c, H-5c′, H-6d′), 4.13–4.20 (5H, m, H-6a, H-6′a, H-6c′, H-6′c′, H-6′d′), 4.21–4.28 (4H, m, H-2c′, H-2d, H-6c, H-6′c), 4.34–4.46 (4H, m, H-1b, H-2a, H-6d, H-6′d), 4.72–4.78 (3H, m, H-1a, H-1c′, H-1d), 4.85 (1H, s, H-1c), 4.92–4.96 (2H, m, H-3c, H-4d′), 5.02 (1H, at, *J* 9.7 Hz, H-4d), 5.13–5.17 (3H, m, H-1d′, H-3c′, H-3d), 5.20–5.24 (2H, m, H-3a, H-4c), 5.30 (1H, at, *J* 8.0 Hz, H-4c′), 5.40 (1H, at, *J* 9.4 Hz, H-4b), 5.58 (1H, s, H-2b), 5.66 (1H, at, *J* 9.6 Hz, H-3d′), 6.03 (1H, br s, NH), 6.73–6.75 (3H, m, NH, 2 × Ar-H), 6.82 (1H, d, $J_{2,NH}$ 9.8 Hz, NH), 6.91–6.94 (2H, m, 2 × Ar-H); δ_C (125.8 MHz, $CDCl_3$) 20.1, 20.5, 20.5, 20.7, 20.8, 20.9, 21.0, 21.1, 21.3, 21.7, 23.0, 23.2, 23.2, 23.4 (14 × q, 19 × CH_3), 52.6 (d, C-2d), 53.4 (d, C-2a), 55.8 (q, CH_3), 56.8 (d, C-2d′), 61.6 (t, C-6a), 62.1 (t, C-6d′), 62.2 (t, C-6d), 62.5 (t, C-6c′), 62.7 (t, C-6c), 65.8 (d, C-4c′), 66.6 (m, C-4c, C-6b), 66.8 (d, C-4b), 69.0 (d, C-4d), 69.1 (d, C-5c′), 69.2 (m, C-4d′, C-5c), 69.6 (d, C-3c), 69.9 (d, C-3c′), 70.4 (d, C-2b), 71.2 (d, C-3d′), 72.0 (d, C-5a), 72.0 (d, C-5d′), 72.5 (d, C-5d), 73.4 (d, C-3d), 74.6 (m, C-3b, C-5b), 74.8 (d, C-2c), 75.5 (d, C-2c′), 76.1 (d, C-3a), 79.1 (d, C-4a), 98.3 (d, C-1d′), 98.5 (d, C-1c), 100.6 (d, C-1d), 101.0 (d, C-1b), 101.3 (d, C-1c′), 101.6 (d, C-1a), 114.5, 119.9, 129.1, 129.9 (4 × d, 4 × Ar-C), 134.6, 136.5 (2 × s, 2 × Ar-C), 169.0, 169.3, 169.8, 169.9, 170.3, 170.6, 170.7, 170.8, 170.9, 170.9, 171.0, 171.5, 171.7, 172.4, 173.4, 174.0 (16 × s, 19 × C=O); *m/z* (ESI$^+$) species observed (MMeCN.NH_4^+), (MNa$^+$), (M[MeCN.$NH_4]_2^{2+}$); (MNa$^+$) peaks observed: 1914.54 (100), 1915.54 (70), 1916.54 (20), 1917.55 (2), peaks calculated: 1914.61 (100), 1915.61 (92), 1916.61 (52), 1917.62 (22), 1918.62 (7), 1919.62 (2%).

Acetyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (9d). PMP glycoside **9c** (0.033 g, 0.0174 mmol) was suspended in a mixture of acetonitrile (1.0 mL) and water (0.25 mL). To this suspension was added ceric ammonium nitrate (0.0304 g, 0.0555 mmol) and the reaction mixture stirred at rt. After 18 h, t.l.c. (DCM : MeOH, 94 : 6) indicated complete consumption of starting material (R_f 0.25) and formation of a single product (R_f 0.20). The reaction mixture was diluted with DCM (5 mL) and washed with sodium hydrogen carbonate (2 × 2 mL of a saturated solution), sodium thiosulfate (2 × 2 mL of a 5% w/v solution), EDTA (2 × 2 mL of a 0.1 M solution) and water (2 mL). The organic fraction was dried ($MgSO_4$), filtered, concentrated *in vacuo* and the residue dissolved in pyridine (2 mL). Acetic anhydride (0.5 mL) was added and the reaction stirred at rt under an atmosphere of argon. After 16 h, t.l.c. (DCM : MeOH, 94 : 6) indicated complete consumption of the alcohol intermediate (R_f 0.20) and formation of a single product (R_f 0.25). The reaction mixture was concentrated *in vacuo*, co-distilled 3 times with toluene and the residue purified by flash column chromatography (DCM : MeOH, 94 : 6) to afford peracetate **9d** (0.022 g, 69% over two steps) as a pale yellow foam, $[\alpha]_D^{25}$ + 3.2 (*c*, 0.25 in $CHCl_3$); ν_{max} (KBr disk) 3426 (br, N-H stretch), 1750 (s, C=O), 1638 (br, amide) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.92, 1.94, 1.95, 1.99, 2.00, 2.01, 2.01, 2.03, 2.04, 2.05, 2.07, 2.09, 2.11, 2.13, 2.14, 2.15, 2.22 (60H, 17 × s, 20 × CH_3), 3.29–3.30 (1H, m, H-2d′), 3.53–3.55 (1H, m, H-5b), 3.64–3.69 (3H, m, H-5d, H-5d′, H-6b), 3.75–3.77 (1H, m, H-6′b), 3.80 (1H, at, *J* 9.1 Hz, H-4a), 3.87–3.89 (3H, m, H-2d, H-5a, H-5c′), 3.96–3.98 (2H, m, H-3b, H-5c), 4.01–4.03 (3H, m, H-2c, H-6d, H-6d′), 4.08 (2H, dat, *J* 2.2 Hz, *J* 12.0 Hz, H-6a, H-6c), 4.16–4.28 (6H, m, H-2c′, H-6c′, H-6′a, H-6′c, H-6′d, H-6′d′), 4.41 (1H, dat, *J* 3.6 Hz, *J* 10.4 Hz, H-2a), 4.59 (1H, d, *J* 12.0 Hz, H-6′c′), 4.67 (1H, s, H-1b), 4.73 (2H, br s, H-1c′, H-1d), 4.88–4.92 (2H, m, H-1c, H-3c), 4.95 (1H, at, *J* 9.4 Hz, H-4d′), 5.02 (1H, dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.2 Hz, H-3c′), 5.08-5.12 (2H, m, H-1d′, H-4d), 5.22–5.33 (5H, m, H-3a, H-3d, H-4b, H-4c, H-4c′), 5.51 (1H, s, H-2b), 5.62 (1H, at, *J* 9.8 Hz, H-3d′), 5.91 (1H, br s, NH), 6.07 (1H, d, *J* 9.6 Hz, NH), 6.09 (1H, d, $J_{1,2}$ 3.7 Hz, H-1a), 6.37 (1H, br s, NH); δ_C (125.8 MHz, $CDCl_3$) 20.8, 20.9, 20.9, 21.0, 21.0, 21.1, 21.3, 21.4, 22.8, 23.0, 23.2, 23.4 (12 × q, 20 × CH_3), 50.6 (d, C-2a), 54.6 (d, C-2d), 56.6 (d, C-2d′), 61.8 (t, C-6c′), 62.1 (t, C-6d′), 62.2 (t, C-6d), 62.7 (m, C-6a, C-6c), 65.9 (d, C-4c′), 66.0 (d, C-4c), 66.4 (t, C-6b), 68.0 (d, C-4b), 68.7 (d, C-4d), 68.9 (d, C-5c′), 69.2 (d, C-4d′), 69.4 (d, C-5c), 69.8 (d, C-3c), 69.9 (d, C-2b), 70.3 (d, C-3c′), 70.4 (d, C-5a), 71.2 (d, C-3d′), 72.0 (d, C-5d′), 72.1 (d, C-3d), 72.3 (d, C-5d), 72.5 (d, C-3a), 73.5 (d, C-5b), 74.3 (d, C-2c′), 74.7 (m, C-2c, C-3b), 76.2 (d, C-4a), 90.7 (d, C-1a), 98.6 (m, C-1c, C-1d), 98.7 (d, C-1d′), 99.5 (d, C-1b), 99.9 (d, C-1c′), 168.8, 169.3, 169.5, 169.8, 169.8, 170.2, 170.3, 170.4, 170.5, 170.7, 170.8, 170.8, 170.9, 171.0, 171.2, 171.3, 171.7 (17 × s, 20 × C=O); *m/z* (ESI$^+$) species observed

This journal is © The Royal Society of Chemistry 2009

(MMeCN.NH_4^+), (MNa^+), (M[MeCN.$NH_4]_2^{2+}$); (MNa^+) peaks observed: 1850.52 (100), 1851.53 (81), 1852.53 (43), 1853.53 (16), 1854.52 (5), 1855.53 (2), peaks calculated: 1850.58 (100), 1851.58 (86), 1852.58 (47), 1853.59 (19), 1854.59 (6), 1855.59 (2%).

2-Methyl-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-*d*]-oxazoline (10). Hexasaccharide **9d** (0.015 g, 8.20 μmol) was dissolved in anhydrous DCE (1.0 mL) in a flame-dried flask. Trimethylsilyl bromide (13 μL, 0.0984 mmol), boron trifluoride diethyl etherate (12.5 μL, 0.0984 mmol) and tri-*tert*-butyl pyrimidine (0.0285 g, 0.115 mmol) were added sequentially and the reaction mixture stirred at 40 °C under an atmosphere of argon. After 6 h, t.l.c. (DCM : MeOH, 94 : 6) indicated complete consumption of starting material (R_f 0.25) and formation of a single product (R_f 0.30). The reaction was diluted with DCM (3 mL), washed with sodium hydrogen carbonate (2 mL of a saturated aqueous solution), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (DCM : MeOH : Et_3N, 94 : 6 : 3) to afford protected oxazoline **10** (12.7 mg, 88%) as a pale yellow amorphous solid, $[\alpha]_D^{25}$–7.0 (*c*, 0.20 in $CHCl_3$); ν_{max} (KBr disk) 3441 (br, N-H stretch), 1749 (s, C=O), 1671 (s, C=N), 1636 (br, amide) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.82, 1.89, 1.96, 1.96, 1.97, 1.99, 2.01, 2.03, 2.05, 2.05, 2.12, 2.17 (54H, 12 × s, 18 × CH_3), 1.98 (3H, d, $J_{2a,CH3}$ 1.8 Hz, N=C(CH_3)), 3.35–3.38 (1H, m, H-5a), 3.51–3.55 (2H, m, H-5b, H-6b), 3.62–3.72 (3H, m, H-2d′, H-4a, H-5d′), 3.79 (1H, dd, *J* 2.1 Hz, *J* 11.8 Hz, H-6′b), 3.86-3.92 (2H, m, H-5c, H-5c′), 3.93-4.05 (8H, m, H-2c, H-3b, H-5d, H-6b, H-6c′, H-6d, H-6d′), 4.07 (2H, d, *J* 3.4 Hz, H-6c, H-6′c), 4.13–4.20 (5H, m, H-6a, H-6′a, H-6′c′, H-6′d, H-6′d′), 4.21–4.24 (2H, m, H-2a, H-2c′), 4.63 (1H, d, $J_{1,2}$ 8.5 Hz, H-1d), 4.70 (1H, d, $J_{1,2}$ 8.4 Hz, H-1d′), 4.81 (1H, dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 10.4 Hz, H-3c), 4.83 (1H, s, H-1b), 4.89 (1H, d, $J_{1,2}$ 1.3 Hz, H-1c′), 4.90-5.01 (4H, m, H-1c, H-3c′, H-4d, H-4d′), 5.07 (1H, dd, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 10.8 Hz, H-3d), 5.11 (1H, at, *J* 10.1 Hz, H-4c′), 5.12 (1H, at, *J* 10.2 Hz, H-4c), 5.25 (1H, dd, $J_{2,3}$ 9.4 Hz, $J_{3,4}$ 10.7 Hz, H-3d′), 5.33 (1H, at, *J* 10.0 Hz, H-4b), 5.38 (1H, d, $J_{1,2}$ 3.1 Hz, H-2b), 5.45 (1H, d, *J* 2.3 Hz, H-3a), 5.90 (1H, d, $J_{1,2}$ 7.4 Hz, H-1a), 6.55 (1H, d, $J_{2d\text{-}NH}$ 9.9 Hz, NH(d)), 6.58 (1H, d, $J_{2d'\text{-}NH}$ 8.9 Hz, NH); δ_C (125.8 MHz, CD_3CN) 13.8 (q, C=N(CH_3)), 20.8, 20.8, 20.9, 20.9, 21.0, 21.2, 21.4, 21.5, 23.1, 23.1 (10 × q, 18 × CH_3), 53.8 (d, C-2d), 55.1 (d, C-2d′), 62.7 (t, C-6c′), 62.9 (t, C-6c), 63.0 (t, C-6d′), 63.2 (t, C-6d), 64.5 (t, C-6a), 64.7 (d, C-2a), 65. 9(d, C-4c), 66.1 (d, C-4c′), 67.6 (t, C-6b), 67.7 (d, C-4b), 68.6 (d, C-5a), 69.3 (d, C-5c′), 69.8 (d, C-4a), 69.9 (d, C-4d′), 70.2 (d, C-5c), 70.6 (d, C-3c), 71.3 (d, C-3c′), 71.5 (d, C-2b), 71.8 (d, C-3a), 72.0 (d, C-5d′), 72.1 (d, C-3d), 72.3 (d, C-5d), 72.5 (d, C-3a), 73.5 (d, C-5b), 74.3 (d, C-2c′), 72.3 (d, C-5d), 72.6 (d, C-3d′), 72.7 (C-5d′), 72.7 (d, C-3d), 73.6 (d, C-5b), 75.1 (d, C-2c), 75.8 (d, C-2c′), 77.3 (d, C-4a), 78.0 (d, C-3b), 99.8 (d, C-1a), 100.0 (d, C-1c′), 100.4 (d, C-1d′), 100.5 (d, C-1c), 101.0 (d, C-1b), 101.5 (d, C-1d), 167.0 (s, C=N), 170.4, 170.5, 170.5, 170.5, 170.6, 170.9, 171.0, 171.0, 171.1, 171.3, 171.3, 171.4, 171.4, 171.4, 171.5, 171.9 (16 × s, 18 × C=O); *m*/*z* (ESI^+) species observed (MMeCN.NH_4^+), (MNa^+), (M[MeCN.$NH_4]_2^{2+}$); (MNa^+) peaks observed: 1790.55 (100), 1791.56 (91), 1792.55 (41), 1793.54 (13), 1794.57 (5), peaks calculated: 1790.55 (100), 1791.56 (84), 1792.56 (44), 1793.56 (18), 1794.57 (6), 1795.57 (2%).

2-Methyl-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-*d*]-oxazoline 11. Protected oxazoline **10** (12.7 mg, 7.18 μmol) was dissolved in dry methanol (1 mL) and sodium methoxide (2 μL of a 25% w/v solution) added. The reaction mixture was stirred at rt under an atmosphere of argon. After 40 h, mass spectrometry indicated the presence of a single product. The solution was concentrated in *vacuo*, dissolved in deionised water and lyophilised to afford deprotected oxazoline **11** (7.9 mg, quantitative yield) as a pale yellow foam, ν_{max} (KBr disk) 3441 (br, O-H stretch), 1632 (br, C=N) cm^{-1}; δ_H (500 MHz, D_2O) 1.87 (3H, d, $J_{2a,CH3}$ 1.0 Hz, N=C(CH_3)), 2.02, 2.03 (6H, 2 × s, 2 × CH_3), 4.51–4.54 (2H, m, H-1d, H1d′), 4.84–4.85 (2H, m, H-1c, H-1c′), 4.89 (1H, d, $J_{1,2}$ 2.4 Hz, H-1b), 6.05 (1H, d, $J_{1,2}$ 7.2 Hz, H-1a); *m*/*z* (ESI^-) species observed ($[M\text{-}H]^-$), ($[M\text{-}2H]^{2-}$); ($[M\text{-}H]^-$) peaks observed: 1094.38 (100), 1095.38 (49), 1096.39 (18), 1097.39 (5), peaks calculated: 1094.39 (100), 1095.39 (49), 1096.40 (18), 1097.40 (5) 1098.40 (1%).

***N*⁴-β-D-glucopyranosyl-D-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-methyl-*N*²-(benzyloxycarbonyl)-L-asparagine 13.**

Method 1: Using Endo M. Glycosyl amino acid acceptor **12**[11a] (100 μg, 207 nmol) and the oxazoline donor **11** (681 μg, 621 nmol, 3 eq.) were dissolved in sodium phosphate buffer (0.1 M solution, pH 6.5, total reaction volume: 50 μL). Endo M (10 mU) was added and the temperature maintained at 23 °C. The reaction was analysed by HPLC and UV integration indicated 22% consumption of **12** to give **13** after 70 min. The product was isolated and characterised by HRMS. *m*/*z* (ESI^+) species observed (MMeCN.NH_4^+), (MNa^+), (M[MeCN.$NH_4]_2^{2+}$); (MNa^+) peaks observed: 1601.53 (100), 1602.53 (69), 1603.53 (33), 1604.53 (11), 1605.54 (4), peaks calculated: 1601.57 (100), 1602.57 (73), 1603.58 (35), 1604.58 (12), 1605.58 (4%).

Method 2: Using Endo A. Similarly **12**[11a] (100 μg, 207 nmol) and **11** (681 μg, 621 nmol, 3 eq.) were dissolved in sodium phosphate buffer (0.1 M solution, pH 6.5, total reaction volume: 50 μL). Endo A (50 mU) was added and the temperature maintained at 23 °C. HPLC indicated 11% consumption of **12** to give **13** after 166 min.

Acknowledgements

We thank Professor K. Yamamoto (University of Kyoto) for kindly providing the Endo M enzyme, Professor K. Takegawa (University of Kagawa) for providing the Endo A plasmid, Dr Zhenlain Ling (University of York) for production of WT Endo A and the E173Q and E173H mutants and Mr Robin Proctor for assistance with mass spectrometric analysis. We also thank the EPSRC (DTA studentship to TBP) for funding.

References

1 A. Helenius and M. Aebi, *Science*, 2001, **291**, 2364–2369.
2 (*a*) D. F. Wyss, J. S. Choi, J. Li, M. H. Knoppers and K. J. Willis, *Science*, 1995, **269**, 1273–1278; (*b*) J. D. Aplin and J. C. Wriston, *CRC Crit. Rev. Biochem.*, 1981, **10**, 259–306; (*c*) T. Misaizu, S. Matsuki, T. W. Strickland, M. Takeuchi, A. Kobata and S. Takasaki, *Blood*, 1995, **86**, 4097–4104; (*d*) S. Elliot, A. Lorenzini, S. Asher, K. Aoki, D. Brankow, L. Buck, L. Busse, D. Chang, J. Fuller, J. Gant, N. Hernday, M. Hokum, S. Hu, A. Knudten, N. Levin, R. Komorrowski, F. Martin, R. Navarro, T. Osslund, G. Rogers, N. Orgers, G. Trail and J. Egrie, *Nat. Biotechnol.*, 2003, **21**, 414–421; (*e*) A. Varki, *Glycobiology*, 1993, **3**, 97–130.
3 R. Kornfield and S. Kornfield, *Annu. Rev. Biochem.*, 1985, **54**, 631–664.
4 C. S. Bennett and C.-H. Wong, *Chem. Soc. Rev.*, 2007, **36**, 1227–1238.
5 K. Witte, P. Sears and C.-H. Wong, *J. Am. Chem. Soc.*, 1997, **119**, 2114–2118.
6 K. Haneda, M. Takeuchi, M. Tagashira, T. Inazu, K. Toma, Y. Isogai, M. Hori, K. Kobayashi, M. Takeuchi, K. Takegawa and K. Yamamoto, *Carbohydr. Res.*, 2006, **341**, 181–190.
7 (*a*) K. Yamamoto, S. Kadowaki, J. Watanabe and H. Kumagai, *Biochem. Biophys. Res. Commun.*, 1994, **203**, 244–252; (*b*) K. Haneda, T. Inazu, K. Yamamoto, H. Kumagai, Y. Nakahara and A. Kobata, *Carbohydr. Res.*, 1996, **292**, 61–70; (*c*) K. Yamamoto, K. Fujimori, K. Haneda, M. Mizuno, T. Inazu and H. Kumagai, *Carbohydr. Res.*, 1998, **305**, 415–422; (*d*) M. Mizuno, K. Haneda, R. Iguchi, I. Muramoto, T. Kawakami, S. Aimoto, K. Yamamoto and T. Inazu, *J. Am. Chem. Soc.*, 1999, **121**, 284–290.
8 (*a*) K. Takegawa, M. Tabuchi, S. Yamaguchi, A. Kondo, I. Kato and S. Iwahara, *J. Biol. Chem.*, 1995, **270**, 3094–3099; (*b*) J.-Q. Fan, L. H. Huynh, B. B. Reinhold, V. N. Reinhold, K. Takegawa, S. Iwahara, A. Kondo, I. Kato and Y. C. Lee, *Glycoconjugate J.*, 1996, **13**, 643–652.
9 http://www.cazy.org/fam/GH85.html.
10 M. Fujita, S.-i. Shoda, K. Haneda, T. Inazu, K. Takegawa and K. Yamamoto, *Biochim. Biophys. Acta*, 2001, **1528**, 9–14.
11 (*a*) T. W. D. F. Rising, T. D. W. Claridge, N. Davies, D. P. Gamblin, J. W. B. Moir and A. J. Fairbanks, *Carbohydr. Res.*, 2006, **341**, 1574–1596; (*b*) T. W. D. F. Rising, T. D. W. Claridge, J. W. B. Moir and A. J. Fairbanks, *ChemBioChem*, 2006, **7**, 1177–1180; (*c*) T. W. D. F. Rising, C. D. Heidecke, J. W. B. Moir, Z. Ling and A. J. Fairbanks, *Chem. Eur. J.*, 2008, **14**, 6444–6464; (*d*) C. D. Heidecke, Z. Ling, N. C. Bruce, J. W. B. Moir, T. B. Parsons and A. J. Fairbanks, *ChemBioChem*, 2008, **9**, 2045–2051.
12 (*a*) B. Li, Y. Zeng, S. Hauser, H. J. Song and L.-X. Wang, *J. Am. Chem. Soc.*, 2005, **127**, 9692–9693; (*b*) H. Li, B. Li, H. Song, L. Breydo, I. V. Baskakov and L.-X. Wang, *J. Org. Chem.*, 2005, **70**, 9990–9996; (*c*) L.-X. Wang, H. J. Song, S. W. Liu, H. Lu, S. B. Jiang, J. H. Ni and H. G. Li, *ChemBioChem*, 2005, **6**, 1068–1074; (*d*) Y. Zeng, J. S. Wang, B. Li, S. Hauser, H. G. Li and L.-X. Wang, *Chem. Eur. J.*, 2006, **12**, 3355–3364; (*e*) B. Li, H. Song, S. Hauser and L.-X. Wang, *Org. Lett.*, 2006, **8**, 3081–3084; (*f*) M. Umekawa, W. Huang, B. Li, K. Fujita, H. Ashida, L.-X. Wang and K. Yamamoto, *J. Biol. Chem.*, 2008, **283**, 4469–4479; (*g*) L.-X. Wang, *Carbohydr. Res.*, 2008, **343**, 1509–1522; (*h*) W. Huang, H. Ochiai, X. Zhang and L.-X. Wang, *Carbohydr. Res.*, 2008, **343**, 2903–2913; (*i*) Y. Wei, C. Li, W. Huang, B. Li, S. Strome and L.-X. Wang, *Biochemistry*, 2008, **47**, 10294–10304; (*j*) H. Ochiai, W. Huang and L.-X. Wang, *J. Am. Chem. Soc.*, 2008, **130**, 13790–137803; (*k*) H. Ochiai, W. Huang and L.-X. Wang, *Carbohydr. Res.*, 2009, **344**, 592–598; (*l*) W. Huang, C. Li, B. Li, M. Umekawa, K. Yamamoto, X. Zhang and L.-X. Wang, *J. Am. Chem. Soc.*, 2009, **131**, 2214–2223.
13 (*a*) L. F. Mackenzie, Q. R. Wang, R. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 1998, **120**, 5583–5584; (*b*) C. Malet and A. Planas, *FEBS Lett.*, 1998, **440**, 208–212.
14 PDB code 2vtf. See: Z. Ling, M. D. L. Suits, R. J. Bingham, N. C. Bruce, G. J. Davies, A. J. Fairbanks, J. W. B. Moir and E. J. Taylor, *J. Mol. Biol.*, 2009, **389**, 1–9.
15 J. Yin, L. Li, N. Shaw, Y. Li, J. K. Song, W. Zhang, C. Xia, R. Zhang, A. Joachimiak, H.-C. Zhang, L.-X. Wang, Z.-J. Liu and P. Wang, *PLoS ONE*, 2009, **4**, e4658.
16 D. W. Abbott, M. S. Macauley, D. J. Vocadlo and A. B. Boraston, *J. Biol. Chem.*, 2009, **284**, 11676–11689.
17 (*a*) K. Yamaguchi, K. Akai, G. Kawanishi, M. Ueda, S. Masuda and R. Sasaki, *J. Biol. Chem.*, 1991, **266**, 20434–20439; (*b*) L. C. Wasley, G. Timony, P. Murtha, J. Stoudemire, A. J. Dorner, J. Caro, M. Krieger and R. J. Kaufman, *Blood*, 1991, **77**, 2624–2632; (*c*) T. Misaizu, S. Matsuki, T. W. Strickland, M. Takeuchi, A. Kobata and S. Takasaki, *Blood*, 1995, **86**, 4097–4104; (*d*) J. C. Egrie and J. K. Browne, *Nephrol. Dial. Transplant.*, 2001, **16**(Suppl. 3), 3–13; (*e*) A. W. Gross and H. F. Lodish, *J. Biol. Chem.*, 2006, **281**, 2024–2032.
18 S. Elliott, T. Lorenzini, S. Asher, K. Aoki, D. Brankow, L. Buck, L. Busse, D. Chang, J. Fuller, J. Grant, N. Hernday, M. Hokum, S. Hu, A. Knudten, N. Levin, R. Komorowski, F. Martin, R. Navarro, T. Osslund, G. Rogers, N. Rogers, G. Trail and J. Egrie, *Nat. Biotechnol.*, 2003, **21**, 414–421.
19 M. Alpe and S. Oscarson, *Carbohydr. Res.*, 2002, **337**, 1715–1722.
20 T. Nakano, Y. Ito and T. Ogawa, *Carbohydr. Res.*, 1993, **243**, 43–69.
21 G. M. Watt and G.-J. Boons, *Carbohydr. Res.*, 2004, **339**, 181–193.
22 R. Boss and R. Scheffold, *Angew. Chem., Int. Ed. Engl.*, 1976, **15**, 558–559.
23 T. Ogawa and S. Nakabayashi, *Carbohydr. Res.*, 1981, **93**, C1–C5.
24 J. Cunningham, R. Gigg and C. D. Warren, *Tetrahedron Lett.*, 1964, **5**, 1191–1196.
25 Y.-J. Hu, R. Dominique, S. K. Das and R. Roy, *Can. J. Chem.*, 2000, **78**, 838–845.
26 (*a*) E. J. Corey and J. W. Suggs, *J. Org. Chem.*, 1973, **38**, 3224; (*b*) P. A. Gent and R. Gigg, *J. Chem. Soc. Chem. Commun.*, 1974, 277–278.
27 J.-i. Tamura and J. Nishihara, *J. Org. Chem.*, 2001, **66**, 3074–3083.
28 C. Unverzagt, S. Eller, S. Mezzato and R. Schuberth, *Chem. Eur. J.*, 2008, **14**, 1304–1311.

This journal is © The Royal Society of Chemistry 2009