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Carbohydrate Research 339 (2004) 2487–2497
Carbohydrate RESEARCH

The synthesis of a series of modified mannotrisaccharides as probes of the enzymes involved in the early stages of mammalian complex *N*-glycan formation

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Received 12 July 2004; accepted 17 August 2004
Available online 21 September 2004

Abstract—A series of mannotrisaccharides were synthesized by two distinct chemical pathways as probes of the enzymes involved in the early stages of mammalian complex *N*-glycan formation. Methyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (**6**) and methyl (2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-[(2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (**8**) were rapidly synthesized from unprotected methyl β-D-mannopyranoside (**12**). Methyl (2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (**7**) and methyl (α-D-mannopyranosyl)-(1→3)-[(2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (**9**) were synthesized from the common orthogonally protected precursor methyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**15**). The 2-deoxy-2-fluoro substitution common to trisaccharides **7**–**9** renders these analogues resistant to enzyme action in two distinct ways. Firstly the fluorine serves as a non-nucleophilic isostere for the acceptor hydroxyl in studies with glycosyl transferases GnT-I and GnT-II (**7** and **9**, respectively). Secondly it should render trisaccharide **8** stable to hydrolysis by the mannosidases Man-II and Man-III by inductive destabilization of their oxocarbenium ion-like transition states. These analogues should be useful for structural studies on these enzymes.

Keywords: *N*-Acetylglucosaminyl transferase; Mannosyl hydrolase; Mannotrisaccharide; Fluorine; Inhibitors

1. Introduction

Glycoprotein glycans are protein bound oligosaccharides arising from post-translational modifications of proteins. These glycans, which can be either *O*- (Ser/Thr) or *N*-linked (Asn), are vital for many cellular or intercellular processes. Among the roles implicated for these glycoconjugates are cell differentiation, cancer metastasis, and bacterial and viral infection.[1] The assembly of complex *N*-glycans follows a series of well-defined steps involving many sugar-processing enzymes (Chart 1).[2] Entry into the biosynthetic pathway of complex *N*-glycans is gained solely through the addition of an *N*-acetyl-glucosamine residue to an oligomannose core, a process performed by the glycosyl transferase GnT-I.[3–5] No downstream enzymes can become involved without prior action of GnT-1. Absence of this enzyme, and therefore a complete deficiency of complex *N*-glycans, has been shown to be embryonic lethal in mice.[6,7]

Subsequent to the action of GnT-I, the next enzyme in the pathway is Golgi α-mannosidase II (Man-II).[8] This enzyme is responsible for the hydrolysis of two terminal mannose units to give the oligosaccharide **3** (Chart 1). In the absence of Man-II a rescue pathway involving a different mannosidase (Golgi α-mannosidase III, Man-III) prior to the action of GnT-I has been observed.[9] In this alternate route, the Man5 core **1** is first trimmed of its two terminal mannose units to give **5**, followed by GnT-I mediated glycosylation to arrive at the same oligosaccharide **3**. It is upon this substrate that another *N*-acetyl-glucosamine transferase, GnT-II, is responsible for the introduction of a second branching GlcNAc residue.[10] Upon formation of this doubly *N*-acetyl-glucosaminylated oligosaccharide **4**, the pathway

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doi:10.1016/j.carres.2004.08.007

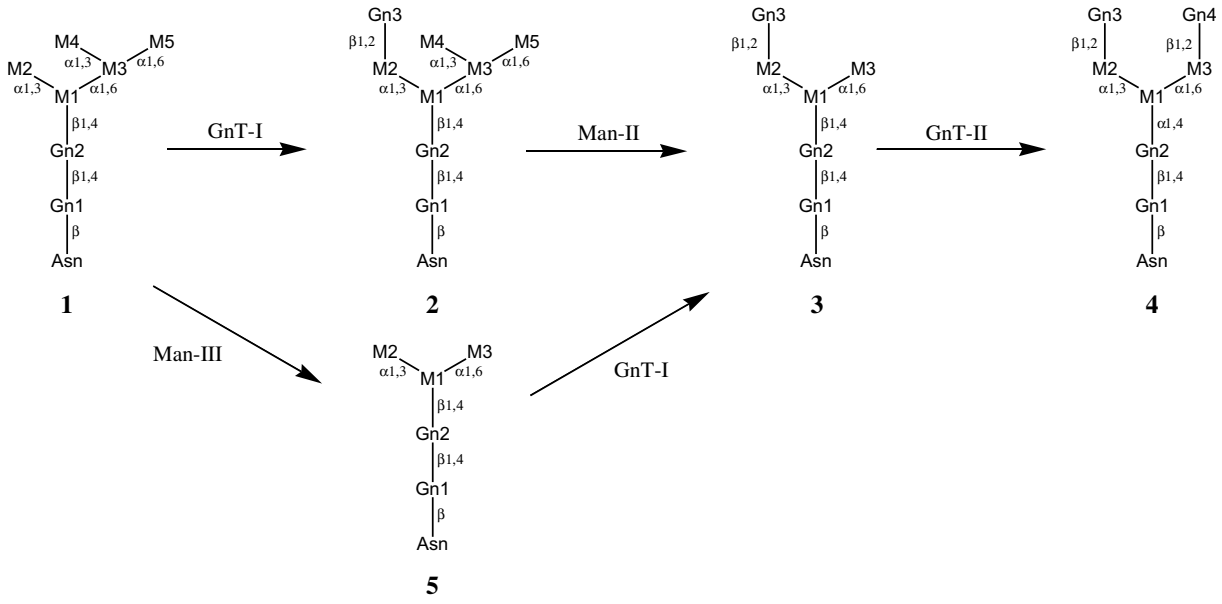


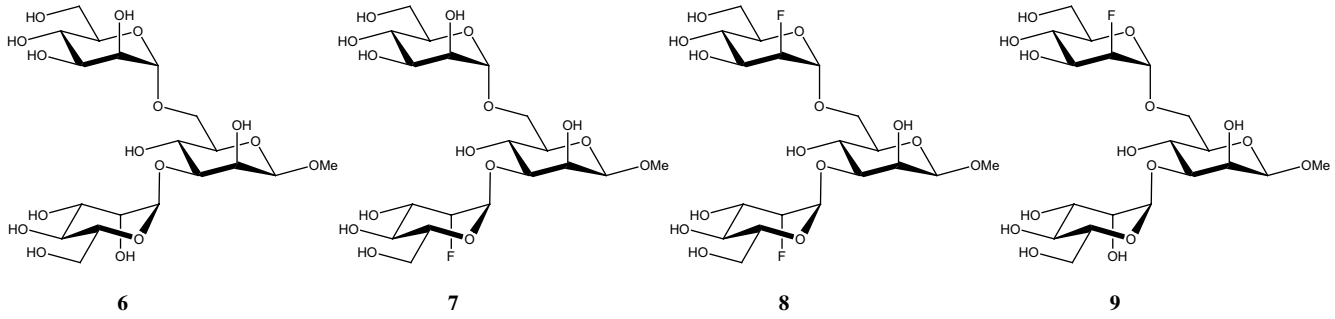
Chart 1. The early stages of the mammalian complex *N*-glycan pathway. Gn = *N*-acetyl-glucosamine; M = mannose.

diverges, with many different enzymes being responsible for the complex structures that eventually arise from this common precursor.

The aim of this work is to produce a series of modified mannotrisaccharides, utilizing a divergent strategy, as mechanistic or structural probes for the investigation of the first four enzymes involved in the elaboration of oligomannose **1** into complex *N*-glycans. Three dimensional structures of both GnT-1 and Man-II have recently been solved by X-ray crystallography.[11,12] However, in neither case has a structure with all relevant substrates present been determined. Missing components of complexes are the mannotrisaccharide acceptor for GnT-I and the trisaccharide substrate for Man-II. The absence of structures including these key oligosaccharides leaves much unknown regarding the details of the binding pocket for these residues. One of the key problems in attaining such binary (Man-II) or ternary (GnT-I) complexes for crystallographic studies is prevention of the natural enzyme-catalyzed process so that a substrate complex, rather than a product complex, may be observed. A commonly used strategy to combat this problem involves the use of incompetent substrates; compounds that structurally resemble the natural substrate but which have been modified in such a way that they are unable to participate in the enzyme catalyzed reaction. In this study we describe the synthesis of fluorinated substrate analogues that should block the action of several enzymes in this pathway by two quite distinct processes. In the first instance, fluorine is utilized as an isostere for the hydroxyl group onto which the *N*-acetylglucosamine residue is transferred by GnT-I or GnT-II (**7** and **9**, respectively). It is expected, although not known, that truncated mannotrisaccharides of the type **6** would be processed by the α-mannosyl hydrolases Man-II and Man-III. Therefore, in the second approach, the 2-deoxy-2-fluoro substitution is used to inductively destabilize the transition states involved in the enzyme catalyzed reaction and thereby render the trisaccharide **8** stable to these enzymes (Chart 2).

Previous work has shown that trisaccharide **6** is the minimal optimal acceptor for GnT-I.[13–15] The three mannose units present are equivalent to the M1–M2–M3 trisaccharide moiety present in oligosaccharide **1**. In a previous study of acceptor specificity, the branched mannotrisaccharides 2-deoxy-Man(α1→3)[Man(α1→6)]-Manβ-OMe and 2-*O*-methyl-Man(α1→3)[Man-(α1→6)]-Manβ-OMe were shown to be neither substrates nor inhibitors of GnT-I.[14] The removal of the key 2-hydroxyl group presumably eliminates one or more important hydrogen bonding interactions. That the 2-*O*-methyl analogue did not bind in the GnT-I active site is likely due to the increased steric bulk at this crucial position (an event also observed upon an equivalent modification to the acceptor for GnT-II).[16] A more conservative modification is to replace the 2′-hydroxyl with a fluorine, and indeed fluorinated analogues have been shown to bind with similar affinities to those of the parent sugar.[16] Removal of the 2′-hydroxyl group will render the trisaccharide **7** unable to accept the *N*-acetylglucosamine residue offered by GnT-I. Such an incompetent substrate analogue may well bind tightly and allow for structural studies to be performed.

The bis(2-deoxy-2-fluoro)-compound **8**, may prove to be an interesting mechanistic probe for both Golgi α-mannosidase II and III since this trisaccharide strongly resembles the terminal M3–M4–M5 portion of the natural substrate for these enzymes. The presence of the



6 **7** **8** **9**

Chart 2. Modified mannotrisaccharides for probing enzymes involved in complex *N*-glycan biosynthesis.

2-fluorine substitution without a chemically activated leaving group should render these sugars stable to hydrolysis by a glycosidase, a strategy, which has been employed with much success in the investigation of β-glycosidases.[17]

The final enzyme in this pathway, GnT-II, is responsible for the addition of an *N*-acetyl glucosamine residue to the 2-position of M3 in the M1–M2(Gn3)–M3 tetrasaccharide. We envisage that a non-competent analogue, with a 2-deoxy-2-fluoro sugar in place of M3, should be readily accessible from GnT-I enzyme-mediated glycosylation of trisaccharide **9**.[15]

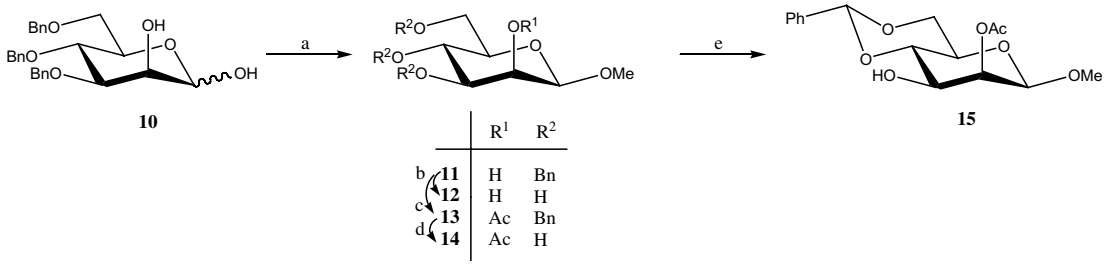
These compounds should prove to be useful tools for detailed kinetic and structural studies of this important group of enzymes.

2. Results and discussion

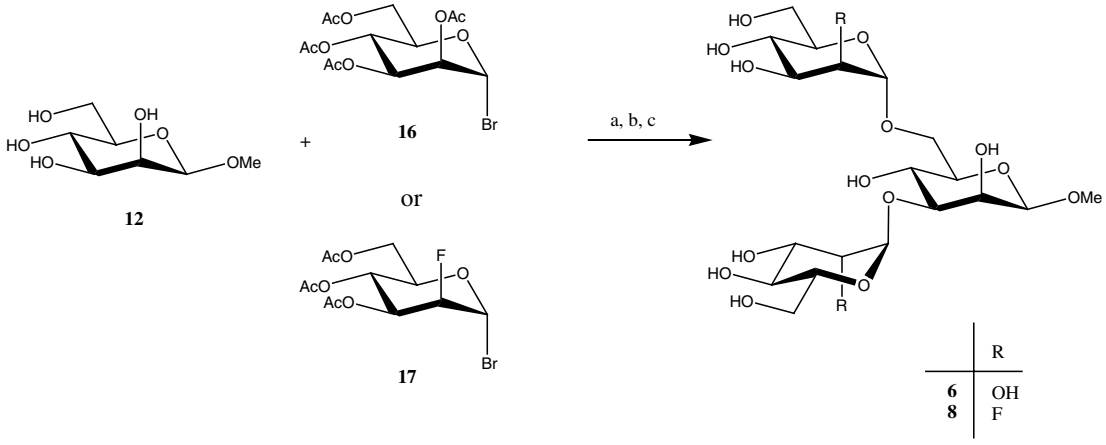
Whilst several synthetic strategies to 2,4-di-*O*-protected mannosides are reported in the literature,[18–20] Kaur and Hindsgaul have developed a rapid synthesis of a structurally related analogue of the branched mannotrisaccharide **6** requiring no prior protection steps.[21] This strategy involved mannosylation of unprotected octyl β-D-mannopyranoside with 2.5 equiv of donor sugar, giving a complex mixture of products. However, by making ingenious use of the fact that the desired 3,6-disubstituted mannoside contain no vicinal diols about the core bisecting sugar residue, the unwanted regioisomers could be selectively oxidized by treatment with sodium periodate. Subsequent to Zemplen deprotection of the acetyl protecting groups on the terminal sugar residues, the desired product was readily isolated by column chromatography. Although the reported yield is low (17% over these three key steps), this synthesis represents an extremely efficient route into this class of compounds due to the significant number of protecting group manipulation steps that are avoided. A limitation of this approach for our purposes is that it is applicable only to trisaccharide targets arising from the same donor sugar. This method is therefore suitable for the synthesis of target compounds **6** and **8**, however, a more traditional approach must be employed for targets **7** and **9**.

The differentially protected mannose unit **15** containing a synthetically challenging β-mannosyl linkage was required as the key building block for access to target trisaccharides **7** and **9**. The installation of this β-methyl glycosidic linkage was previously reported via a stannylene acetal mediated alkylation of 3,4,6-tri-*O*-benzyl mannopyranose.[22] This approach was particularly appealing to our synthetic route since after methylation the 2-hydroxyl group remains free and can be orthogonally protected. Briefly, regio- and stereoselective glycosylation of **10** is accomplished via formation of a cyclic 1,2-stannylene acetal with dibutyltin oxide under Dean–Stark conditions, followed by treatment with methyl iodide to give the required β-methyl glycoside **11**.[22] For the synthesis of targets **6** and **8** via the Hindsgaul route, fully deprotected β-methyl mannoside **12** was required. Catalytic hydrogenolysis of the benzyl ethers gave the tetrol starting material **12** for this route. Target compounds **7** and **9** require a more complex precursor, so mannoside **11** was further elaborated. After protection of the remaining free hydroxyl at the 2-position as its acetyl derivative, the benzyl ethers were removed by catalytic hydrogenolysis to give the triol **14**. Regioselective protection of the 4- and 6-hydroxyls with benzaldehyde dimethyl acetal afforded the required key building block methyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranoside **15** (Scheme 1).

Target mannosides **6** and **8** were readily amenable to synthesis via the Hindsgaul methodology (Scheme 2).[21] Glycosylation of the unprotected β-methyl mannoside **12** with 2.5 equiv of either mannosyl bromide **16** or 2-deoxy-2-fluoro-mannosyl bromide **17** donor gave a complex mixture of products from which a mixture of isomeric trisaccharides was isolated by column chromatography. Reaction of these two mixtures with sodium periodate followed by global deprotection of the acetate groups with sodium methoxide allowed, in both cases, isolation of the desired mannotrisaccharide **6** or **8**. Although the obtained yields were low (14% for **6** and



Scheme 1. Reagents and conditions: (a) Bu_2SnO, MeI, toluene/benzene (3:1), 73%; (b) H_2, Pd/C, MeOH, 88%; (c) Ac_2O, pyridine, 96%; (d) H_2, Pd/C, MeOH, 88%; (e) $PhCH(OMe)_2$, TsOH, DMF, 56%.



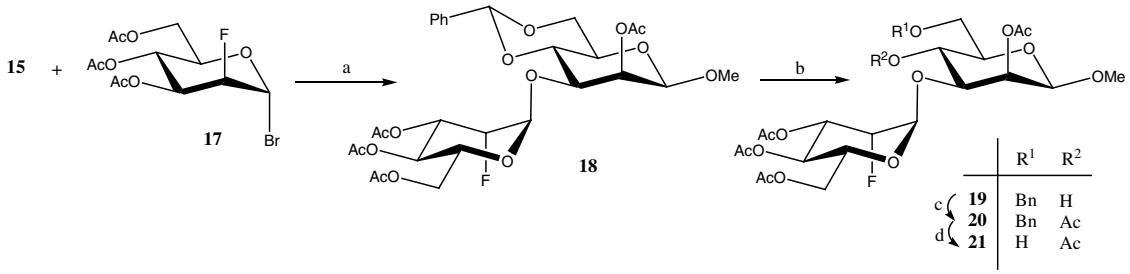
Scheme 2. Reagents and conditions: (a) $HgBr_2$, $Hg(CN)_2$, MeCN, **16** or **17**; (b) $NaIO_4$, MeOH, H_2O; (c) MeONa, MeOH, 14% for **6**, 5% for **8**.

5% for **8**), they still represent a more efficient route into this class of compounds than traditional synthetic routes involving multiple protection and deprotection steps.

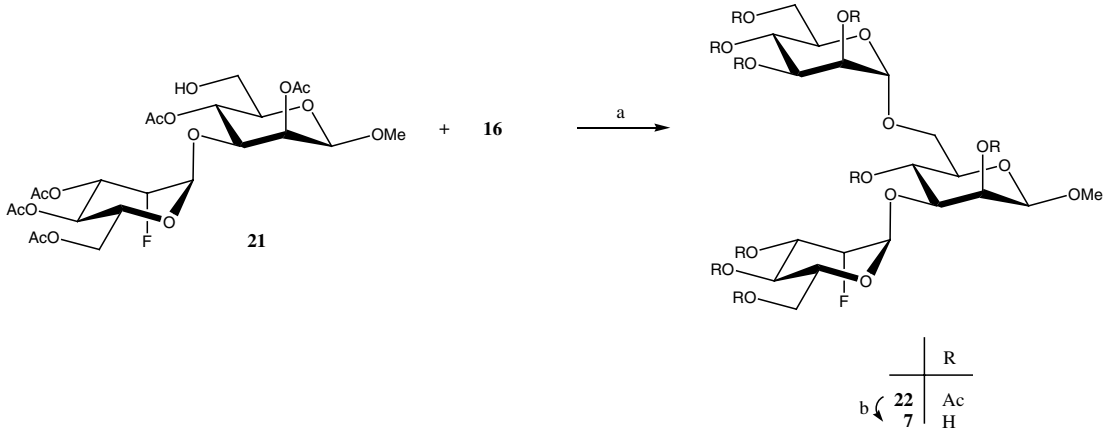
With the two symmetric mannoside targets **6** and **8** in hand, attention turned to the synthetically more complex targets **7** and **9**. The routes to these two trisaccharides both utilize the same orthogonally protected precursor **15**. Elaboration of this core structure to target **7** began with glycosylation of the 3-hydroxyl with 2-deoxy-2-fluoro-mannose (Scheme 3). Glycosylation of **15** was achieved in good yield using 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannosyl bromide **17** as the donor. The disaccharide product **18** was difficult to purify from unreacted monosaccharide acceptor **15**. Therefore, the reaction mixture was subjected to acetylation conditions in order to facilitate purification of **18**. Although the glycosyl donor **17** has no C-2 acetoxy group to provide neighboring group participation, only formation of the α-linked anomer **18** was observed, as determined by ^1H and ^{19}F NMR.[23] This stereochemical outcome is likely due to a combination of the anomeric effect exerted by the endocyclic oxygen and the dipolar interaction from the axially oriented C-2 fluorine. Regioselective reductive ring opening of the benzylidene acetal to the benzyl ether **19** was accomplished with trimethylamine–borane complex. In contrast to previous reports using this reagent,[24,25] only the 6-*O*-benzyl ether regioisomer was observed. Confirmation of this assignment was established by the observation (^1H NMR) of an induced downfield chemical shift (1.3 ppm) of the H-4 proton upon acetylation to provide **20**. Deprotection of the benzyl ether of **20** by catalytic hydrogenolysis gave the 6-hydroxy disaccharide **21** ready for the final glycosylation.

The target trisaccharide **7** was realized by addition of the final mannosyl moiety using the bromide donor **16** (Scheme 4). Global deprotection was accomplished employing Zemplen conditions. It is worth noting that this deprotection was slow to proceed to completion, a phenomenon previously reported for the Zemplen deprotection of structurally similar acylated branched mannotrisaccharides.[26]

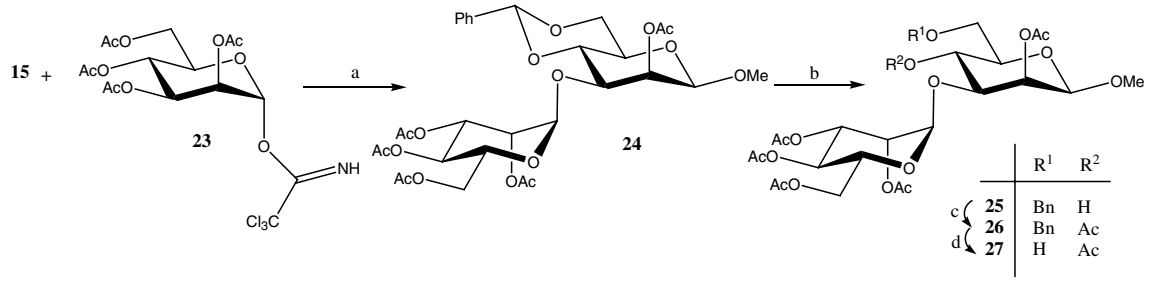
The regioisomeric trisaccharide **9** was synthesized from the common, selectively protected mannoside **15**. Glycosylation of **15** with the mannosyl bromide donor **16** was observed to proceed only in low yield. A more successful glycosylation was accomplished when the corresponding trichloroacetimidate donor **23** was used (Scheme 5). Regioselective ring opening of the benzylidene moiety again afforded only the 6-*O*-benzyl regioisomer **25**. Simple protecting group manipulations



Scheme 3. Reagents and conditions: (a) i. $HgBr_2$, $Hg(CN)_2$, MeCN; ii. Ac_2O, pyridine, 64%; (b) $Me_3N{\cdot}BH_3$, CH_2Cl_2, 52%; (c) Ac_2O, pyridine, 90%; (d) H_2, Pd/C, MeOH, 78%.



Scheme 4. Reagents and conditions: (a) $HgBr_2$, $Hg(CN)_2$, MeCN, 85%; (b) NaOMe, MeOH, 71%.



Scheme 5. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 73%; (b) $Me_3N{\cdot}BH_3$, CH_2Cl_2, 57%; (c) Ac_2O, pyridine, 88%; (d) H_2, Pd/C, MeOH, 87%.

gave disaccharide **27** ready for addition of the final sugar moiety.

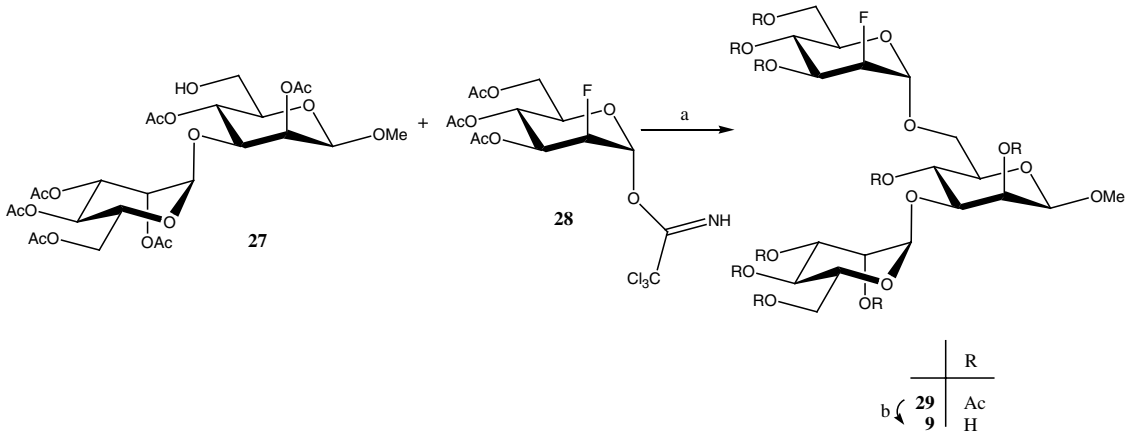
An appropriate 2-deoxy-2-fluoro-mannosyl donor was readily synthesized from 3,4,6-tri-*O*-acetyl glucal using the Selectfluor procedure described by Dax and co-workers.[27] The hemi-acetal was then converted to the required trichloroacetimidate donor **28** upon treatment with trichloroacetonitrile. Glycosylation of the 6-position of disaccharide **27** with donor **28** gave the protected mannotrisaccharide **29** (Scheme 6). As was previously observed in the synthesis of **24**, the trichloroacetimidate donor **28** was found to give superior yields over the equivalent glycosyl bromide. Global deprotection of the acetate groups was again accomplished with a prolonged reaction time under Zemplen conditions to give the target compound **9**.

Structural studies and kinetic analysis of these four key compounds (**6**–**9**) with GnT-I and Man-II are currently in progress.

3. Experimental

3.1. General methods

All reagents were obtained from commercial suppliers and were used without further purification. Column chromatography was performed with silica gel



Scheme 6. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 57%; (b) NaOMe, MeOH, 73%.

(230–400 mesh). TLC analysis was performed on precoated E. Merck 60 F-254 silica plates and visualized by staining with 10% ammonium molybdate in 2 M H_2SO_4 followed by heating. Methylene chloride was distilled over calcium hydride. Methanol was distilled over magnesium and iodine. DMF was dried over 4 Å molecular sieves. All ^{1}H NMR assignments were based on COSY experiments.

3.1.1. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (13). Known methyl 3,4,6-tri-*O*-benzyl-β-D-mannoside **11**[19] (2 g, 4.3 mmol) in 2:1 pyridine–Ac_2O (30 mL) was stirred overnight at room temperature. MeOH (5 mL) was added and the mixture stirred for a further 0.5 h. The reaction mixture was then concentrated under diminished pressure and poured onto EtOAc. The organic layer was washed with satd $NaHCO_3$ (× 2) and water and dried over $MgSO_4$. The solvent was evaporated under diminished pressure and the crude material purified by flash column chromatography over silica gel (3:1 hexane–EtOAc) to give the title compound **13** as a colorless oil (2.1 g, 4.1 mmol, 96%). ^{1}H and ^{13}C NMR data were found to be in accordance with the limited data previously reported in the literature.[28] ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.33–7.24 (13H, m, Ar), 7.18–7.15 (2H, m, Ar), 5.61 (1H, d, *J* 3.3 Hz, H-2), 4.86 (1H, d, *J* 10.8 Hz, OCH_2Ar$_a$), 4.74 (1H, d, *J* 11.2 Hz, OCH_2Ar$_b$), 4.65 (1H, d, *J* 12.0 Hz, OCH_2Ar$_c$), 4.55 (1H, d, *J* 12.0 Hz, OCH_2Ar$_c$), 4.50 (1H, d, *J* 10.8 Hz, OCH_2Ar$_a$), 4.48 (1H, d, *J* 11.2 Hz, OCH_2Ar$_b$), 4.90 (1H, s, H-1), 3.80–3.74 (3H, m, H-4, H-6$_a$ and H-6$_b$), 3.65 (1H, dd, *J* 3.3 and 9.3 Hz, H-3), 3.51 (3H, s, OCH_3), 3.47 (1H, ddd, *J* 2.3, 4.8, and 9.7 Hz, H-5), 2.19 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 138.3, 138.3, 137.6, 128.5, 128.4, 128.4, 128.2, 128.0, 127.9, 127.9, 127.7, 127.6, 100.0, 80.4, 75.6, 75.2, 74.4, 73.6, 71.5, 69.3, 67.9, 57.2, and 21.1.

3.1.2. Methyl 2-*O*-acetyl-β-D-mannopyranoside (14). To a solution of methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannoside **12** (1.0 g, 1.97 mmol) in MeOH (20 mL) was added 10% Pd/C (100 mg) and catalytic AcOH and the mixture stirred under an atmosphere of hydrogen for 12 h. The reaction mixture was then filtered through Celite and the filtrate concentrated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (5 → 20% MeOH–$CHCl_3$) to yield the title compound **14** a colorless oil (470 mg, 1.97 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.27 (1H, d, *J* 3.4 Hz, H-2), 4.65 (1H, s, H-1), 3.86 (1H, dd, *J* 1.7 and 12.4 Hz, H-6$_a$), 3.74 (1H, dd, *J* 3.4 and 9.7 Hz, H-3), 3.65 (1H, dd, *J* 6.6 and 12.4 Hz, H-6$_b$), 3.48 (1H, t, *J* 9.7 Hz, H-4), 3.42 (3H, s, OCH_3), 3.36 (1H, ddd, *J* 1.7, 6.6 and 9.7 Hz, H-5), 2.07 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.6, 99.6, 76.5, 72.0, 71.4, 67.1, 61.0, 57.0 and 20.3.

3.1.3. Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranoside (15). To a soln of methyl 2-*O*-acetyl-β-D-mannoside **14** (460 mg, 1.95 mmol) in DMF (3 mL) containing benzaldehyde dimethyl acetal (3.0 g, 19.5 mmol) was added catalytic *p*-TsOH (10 mg). The reaction mixture was stirred at room temperature for 12 h then poured onto EtOAc. The organic layer was washed with satd $NaHCO_3$, water (× 5), and brine. The organic layer was then dried over $MgSO_4$ and the solvent evaporated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (3:2 EtOAc–hexane) to give the product **15** as a colorless crystalline solid (357 mg, 1.10 mmol, 56%). Mp 149–150 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.49–7.45 (2H, m, Ar), 7.38–7.34 (3H, m, Ar), 5.57 (1H, s, ArC*H*), 5.46 (1H, dd, *J* 1.2 and 3.6 Hz, H-2), 4.50 (1H, d, *J* 1.2 Hz, H-1), 4.34 (1H, dd, *J* 5.0 and 10.5 Hz, H-6$_a$), 3.95 (1H, dd, *J* 3.6 and 9.8 Hz, H-3), 3.87 (1H, t, *J* 10.5 Hz, H-6$_b$), 3.84 (1H, t, *J* 9.8 Hz, H-4), 3.51 (3H, s, OCH_3), 3.39 (1H, ddd, *J* 5.0, 9.8, and 10.5 Hz, H-5), 2.18 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.8, 137.0, 129.3,

128.4, 126.3, 102.2, 100.6, 78.7, 70.9, 69.8, 68.5, 66.9, 57.5, and 20.9. HRMS-ESI: calcd for $C_{16}H_{20}O_7Na$: 347.1107; found: 347.1094. Anal. Calcd for $C_{16}H_{20}O_7$: C, 59.25; H, 6.22. Found: C, 58.89; H, 6.04.

3.1.4. Methyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (6). To a soln of methyl β-D-mannoside **12** (165 mg, 0.85 mmol) in MeCN (100 mL) containing 4 Å molecular sieves, mercuric bromide (760 mg, 2.12 mmol) and mercuric cyanide (540 mg, 2.12 mmol) was added donor **16** (870 mg, 2.12 mmol). The mixture was stirred at 35 °C for 16 h then filtered through Celite. The filtrate was concentrated under diminished pressure then redissolved in CH_2Cl_2 and extracted with water. The organic layer was washed sequentially with water, $NaHCO_3$ soln, and brine then dried over $MgSO_4$ and the solvent evaporated under diminished pressure. The crude material was fractionated by flash column chromatography over silica (2:1 EtOAc–hexane → EtOAc) and the trisaccharide-containing fractions (R_f 0.19–0.38, 2:1 EtOAc–hexane) pooled and used in the next step without further purification. The crude trisaccharide mixture (280 mg) was dissolved in MeOH–water (1:2, 15 mL) containing sodium periodate (300 mg) and stirred at room temperature overnight. The reaction mixture was poured onto water, extracted with CH_2Cl_2, the organic layer was dried over $MgSO_4$, and the solvent was evaporated under diminished pressure. Without further purification the crude material was dissolved in MeOH (10 mL) and catalytic sodium methoxide was added. The mixture was stirred at room temperature for 4 h. The sodium methoxide was neutralized with Amberlite IR-120 H^+ resin and the solvent evaporated under diminished pressure. The crude mixture was purified by flash column chromatography over silica (7:4:1 CH_2Cl_2–MeOH–H_2O) to give the title compound **6** as a colorless powder (60 mg, 0.12 mmol, 14%). ^{1}H NMR (400 MHz, D_2O): δ_H 4.95 (1H, d, J 1.6 Hz, H-1′), 4.76 (1H, d, J 1.5 Hz, H-1″), 4.44 (1H, s, H-1), 3.99 (1H, d, J 3.0 Hz, H-2), 3.92 (1H, dd, J 1.6 and 3.2 Hz, H-2′), 3.85 (1H, dd, J 1.5 and 3.3 Hz, H-2″), 3.84–3.48 (14H, m), 3.43–3.35 (1H, m), 3.37 (3H, s, OAc). ^{13}C NMR (100 MHz, D_2O): δ_C 102.2, 100.9, 80.5, 74.6, 73.9, 73.1, 70.4, 70.2, 70.0, 69.9, 69.8, 69.7, 66.6, 66.6, 65.6, 65.2, 65.2, 60.8, 56.7. HRMS-ESI: calcd for $C_{19}H_{34}O_{16}Na$: 541.1745; found: 541.1736.

3.1.5. Methyl (2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-[(2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (8). To a soln of methyl β-D-mannoside **12** (120 mg, 0.62 mmol) in acetonitrile (25 mL) containing 4 Å molecular sieves, mercuric bromide (490 mg, 1.36 mmol) and mercuric cyanide (340 mg, 1.36 mmol) was added donor **17** (470 mg, 1.27 mmol). The reaction conditions were similar to those used for compound **6**. The crude mixture was purified by flash column chromatography over silica (7:3:1 CH_2Cl_2–MeOH–H_2O) to give the title compound **8** as a colorless powder (15 mg, 0.03 mmol, 5%). ^{1}H NMR (400 MHz, D_2O): δ_H 5.17 (1H, d, J 7 Hz, H-1′), 4.97 (1H, d, J 7 Hz, H-1″), 4.79–4.60 (2H, m partially obscured by water peak, H-2′ and H-2″), 4.43 (1H, s, H-1), 4.00 (1H, d, J 2.6 Hz, H-2), 3.89–3.53 (14H, m), 3.42–3.34 (1H, m), 3.36 (3H, s, OCH_3). ^{13}C NMR (100 MHz, D_2O): δ_C 100.8, 99.2 (d, J 30 Hz), 96.5 (d, J 30 Hz), 89.4 (d, J 173 Hz), 89.3 (d, J 172 Hz), 81.0, 73.8, 73.1, 72.5, 69.8, 69.6 (d, J 17 Hz), 69.4 (d, J 17 Hz), 66.5, 66.5, 65.5, 65.4, 60.3, 60.3, 56.7. ^{19}F NMR (376 MHz, D_2O): δ_F −128.1 (ddd, J 7, 31, and 50 Hz, F-2′), −129.5 (ddd, J 7, 31, and 49 Hz, F-2″). HRMS-ESI: calcd for $C_{19}H_{32}F_2O_{16}Na$: 545.1658; found: 545.1655.

3.1.6. Methyl 2-*O*-acetyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-β-D-mannopyranoside (18). To a soln of acceptor **15** (590 mg, 1.82 mmol) and donor **17** (1.2 g, 3.23 mmol) in MeCN (20 mL) containing 4 Å molecular sieves was added mercuric cyanide (816 mg, 3.23 mmol) and mercuric bromide (1.16 g, 3.23 mmol). The reaction mixture was allowed to stir at room temperature for 12 h. TLC analysis showed the formation of product, but the product and starting material were observed to have very similar mobility under all solvent systems investigated. The reaction mixture was filtered through Celite, concentrated under diminished pressure then concentrate was redissolved in EtOAc and the organic layer washed with satd $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated under diminished pressure. To resolve the separation problem, the crude reaction mixture was acetylated with 2:1 pyridine–Ac_2O (30 mL) overnight at room temperature. MeOH (5 mL) was added and the mixture stirred for a further 0.5 h. The reaction mixture was then concentrated under diminished pressure and poured onto EtOAc. The organic layer was washed with 0.1 M HCl, satd $NaHCO_3$ (×2) and brine, dried over $MgSO_4$, and the solvent was evaporated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (2:1→3:1 EtOAc–hexane) to give the title compound **18** as a colorless oil (720 mg, 1.17 mmol, 64%). Mp 88–90 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.38–7.32 (5H, m, Ar), 5.56 (1H, s, PhCH), 5.51 (1H, dd, J 1.1 and 3.6 Hz, H-2), 5.34 (1H, dd, J 10.3 and 9.3 Hz, H-4′), 5.32 (1H, d, J 6.8 Hz, H-1′), 5.05 (1H, ddd, J 2.3, 10.3, and 28.6 Hz, H-3′), 4.76 (1H, dt, J 2.3 and 49.8 Hz, H-2′), 4.56 (1H, d, J 1.1 Hz, H-1), 4.34 (1H, dd, J 4.9 and 10.5 Hz, H-6$_a$), 4.26 (1H, dd, J 4.2 and 12.6 Hz, H-6′$_a$), 4.15–4.10 (2H, m, H-5′ and H-6′$_b$), 4.08 (1H, dd, J 3.6 and 9.6 Hz, H-3), 3.98 (1H, t, J 9.6 Hz, H-4), 3.88 (1H, t, J 10.5 Hz, H-6$_b$), 3.53

(3H, s, OCH_3), 3.41 (1H, ddd, *J* 4.9, 9.6, and 10.5 Hz, H-5), 2.22 (3H, s, O*Ac*), 2.07 (3H, s, O*Ac*), 2.04 (3H, s, O*Ac*), 2.04 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.7, 170.1, 169.6, 136.8, 129.2, 128.4, 125.9, 101.7, 100.5, 98.0 (d, *J* 30 Hz), 88.6 (d, *J* 179 Hz), 78.5, 77.4, 73.6, 70.2, 69.7 (d, *J* 17 Hz), 69.4, 68.4, 66.8, 65.1, 62.0, 57.7, and 20.8 (4 × OAc). ^{19}F NMR (376 MHz, $CDCl_3$): δ_F −127.7 (ddd, *J* 7, 29, and 50 Hz). HRMS-ESI: calcd for $C_{28}H_{35}FO_{14}Na$: 637.1909; found: 637.1919. Anal. Calcd for $C_{28}H_{35}FO_{14}$: C, 54.72; H, 5.74. Found: C, 54.87; H, 5.77; and *methyl 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-mannopyranoside* (100 mg, 0.27 mmol, 15%). Mp 105–106 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.44–7.43 (2H, m, Ar), 7.34–7.33 (3H, m, Ar), 5.56 (1H, dd, *J* 1.3 and 3.4 Hz, H-2), 5.55 (1H, d, *J* 1.2 Hz, PhC*H*), 5.12 (1H, ddd, *J* 1.3, 3.4, and 10.4 Hz, H-3), 4.60 (1H, t, *J* 1.3 Hz, H-1), 4.35 (1H, ddd, *J* 1.2, 4.9, and 10.5 Hz, H-6$_a$), 4.01 (1H, td, *J* 1.2 and 10.5 Hz, H-4), 3.90 (1H, td, *J* 1.2 and 10.5 Hz, H-6$_b$), 3.52 (3H, d, *J* 1.3 Hz, OCH_3), 3.52–3.47 (1H, m, H-5), 2.18 (3H, d, *J* 1.3 Hz, O*Ac*), 2.00 (3H, d, *J* 1.3 Hz, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.3, 170.0, 137.0, 129.2, 128.3, 126.2, 101.9, 100.3, 75.7, 70.3, 69.3, 68.5, 67.3, 57.7, 20.8, and 20.8. HRMS-ESI: calcd for $C_{18}H_{22}O_8Na$: 389.1212; found: 389.1202. Anal. Calcd for $C_{18}H_{22}O_8$: C, 59.01; H, 6.05. Found: C, 59.17; H, 6.24.

3.1.7. Methyl 2-*O*-acetyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-6-*O*-benzyl-β-D-mannopyranoside (19). To a soln of acetal **18** (100 mg, 0.16 mmol), 4 Å molecular sieves (100 mg), and trimethylamine borane (60 mg, 0.81 mmol) in CH_2Cl_2 (6 mL) at 0 °C was added dropwise a suspension of aluminum trichloride (87 mg, 0.65 mmol) in Et_2O (3 mL). The reaction mixture was stirred at 0 °C for 1 h then filtered through Celite. CH_2Cl_2 (10 mL) and 0.75 M H_2SO_4 (1 mL) were added and the mixture stirred for 1 h at room temperature. The mixture was transferred to a separating funnel and the organic layer was washed with water, satd $NaHCO_3$ solution, and brine. The organic layer was dried over $MgSO_4$, the solvent evaporated under diminished pressure, and the crude material then purified by flash column chromatography over silica gel (2:1 EtOAc–hexane) to give the title compound **19** as a colorless solid (52 mg, 0.084 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.37–7.28 (5H, m, Ar), 5.39 (1H, d, *J* 7.3 Hz, H-1′), 5.39 (1H, dd, *J* 1.0 and 3.4 Hz, H-2), 5.09 (1H, t, *J* 10.3 Hz, H-4′), 5.07 (1H, ddd, *J* 2.4, 10.3, and 28 Hz, H-3′), 4.79 (1H, dt, *J* 2.4 and 50 Hz, H-2′), 4.61 (1H, d, *J* 11.7 Hz, PhCH_2), 4.53 (1H, d, *J* 11.7 Hz, PhCH_2), 4.44 (1H, d, *J* 1.0 Hz, H-1), 4.24 (1H, dd, *J* 4.3 and 12.6 Hz, H-6′$_a$), 4.14 (1H, dd, *J* 2.3 and 12.6 Hz, H-6′$_b$), 4.13–4.10 (1H, m, H-5′), 3.92 (1H, t, *J* 9.4 Hz, H-4), 3.83 (1H, dd, *J* 4.7 and 9.7 Hz, H-6$_a$), 3.78 (1H, dd, *J* 3.4 and 9.4 Hz, H-3), 3.74 (1H, dd, *J* 6.6 and 9.7 Hz, H-6$_b$), 3.47 (3H, s, OCH_3), 3.42 (1H, ddd, *J* 4.7, 6.6, and 9.4 Hz, H-5), 2.16 (3H, s, O*Ac*), 2.06 (3H, s, O*Ac*), 2.05 (3H, s, O*Ac*), 2.03 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.7, 170.2, 169.7, 137.2, 128.7, 128.2, 128.0, 99.9, 98.6 (d, *J* 30 Hz), 86.8 (d, *J* 179 Hz), 77.5, 74.0, 73.1, 71.2, 71.0, 70.0, 69.8 (d, *J* 17 Hz), 69.2, 65.3, 62.1, 57.3, and 20.8 (4 × OAc). ^{19}F NMR (376 MHz, $CDCl_3$): δ_F −127.7 (ddd, *J* 7, 28 and 50 Hz). HRMS-ESI: calcd for $C_{28}H_{37}FO_{14}Na$: 639.2065; found: 639.2034.

3.1.8. Methyl 2,4-di-*O*-acetyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-6-*O*-benzyl-β-D-mannopyranoside (20). Disaccharide **19** (230 mg, 0.37 mmol) in 2:1 pyridine–Ac_2O (15 mL) was stirred overnight at room temperature. MeOH (2 mL) was added and the mixture stirred for a further 0.5 h. The reaction mixture was then concentrated under diminished pressure, redissolved in EtOAc, then washed with 1 M HCl, satd $NaHCO_3$ (× 2) and water, and dried over $MgSO_4$. The solvent was evaporated under diminished pressure and the crude material purified by flash column chromatography over silica gel (1:1 hexane–EtOAc) to give the title compound **20** as a colorless oil (220 mg, 0.33 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.33–7.24 (5H, m, Ar), 5.43 (1H, d, *J* 3.4 Hz, H-2), 5.32 (1H, t, *J* 10.1 Hz, H-4′), 5.18 (1H, t, *J* 9.7 Hz, H-4), 5.05 (1H, d, *J* 7 Hz, H-1′), 5.03 (1H, ddd, *J* 2.3, 10.1, and 29 Hz, H-3′), 4.59 (1H, dt, *J* 2.3 and 50 Hz, H-2′), 4.50 (2H, s, PhCH_2), 4.47 (1H, s, H-1), 4.23 (1H, dd, *J* 4.3 and 12.7 Hz, H-6′$_a$), 4.17–4.10 (2H, m, H-5′ and H-6′$_b$), 3.92 (1H, dd, *J* 3.4 and 9.7 Hz, H-3), 3.64–3.51 (3H, m, H-5, H-6$_a$, and H-6$_b$), 3.50 (3H, s, OCH_3), 2.19 (3H, s, O*Ac*), 2.06 (3H, s, O*Ac*), 2.03 (3H, s, O*Ac*), 2.02 (3H, s, O*Ac*), 1.91 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.6, 169.9, 169.6, 169.6, 137.7, 128.4, 127.9, 127.8, 99.8, 98.7 (d, *J* 31 Hz), 86.9 (d, *J* 180 Hz), 76.2, 73.7, 73.6, 70.0, 69.7, 69.6, 69.6, 69.3 (d, *J* 17 Hz), 65.2, 61.9, 57.4, 20.9, and 20.7 (4 × OAc). ^{19}F NMR (376 MHz, $CDCl_3$): δ_F −126.8 (ddd, *J* 7, 29, and 50 Hz). HRMS-ESI: calcd for $C_{30}H_{39}FO_{15}Na$: 681.2171; found: 681.2148.

3.1.9. Methyl 2,4-di-*O*-acetyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-β-D-mannopyranoside (21). Benzyl ether **20** (34 mg, 0.052 mmol) was dissolved in 2:1 MeOH–CH_2Cl_2 (1.5 mL) containing catalytic AcOH, and 10% Pd/C was added (5 mg). The reaction mixture was stirred under an atmosphere of hydrogen for 12 h then filtered through Celite. The solvent was evaporated under diminished pressure and the crude material purified by flash column chromatography over silica gel (2:1 EtOAc–hexane) to give the title compound **21** as a colorless solid (23 mg, 0.04 mmol, 78%). Mp 156–157 °C (EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.45 (1H, dd, *J* 0.9 and 3.4 Hz,

H-2), 5.31 (1H, t, *J* 10.1 Hz, H-4′), 5.14 (1H, t, *J* 9.8 Hz, H-4), 5.08 (1H, dd, *J* 1.8 and 8 Hz, H-1′), 5.02 (1H, ddd, *J* 2.6, 10.1, and 28 Hz, H-3′), 4.61 (1H, dt, *J* 2.2 and 50 Hz, H-2′), 4.50 (1H, d, *J* 0.9 Hz, H-1), 4.22 (1H, dd, *J* 4.1 and 12.5 Hz, H-$6'_a$), 4.17–4.06 (2H, m, H-5′ and H-$6'_b$), 3.97 (1H, dd, *J* 3.4 and 9.8 Hz, H-3), 3.71 (1H, dd, *J* 2.9 and 12.5 Hz, H-6_a), 3.63 (1H, dd, *J* 5.1 and 12.5 Hz, H-6_b), 3.50 (3H, s, OCH_3), 3.38 (1H, ddd, *J* 2.9, 5.1, and 9.8 Hz, H-5), 2.18 (3H, s, O*Ac*), 2.07 (3H, s, O*Ac*), 2.06 (3H, s, O*Ac*), 2.04 (3H, s, O*Ac*), 2.03 (3H, s, O*Ac*). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 170.6, 170.6, 170.1, 169.8, 169.6, 99.8, 98.7 (d, *J* 30 Hz), 86.8 (d, *J* 180 Hz), 76.0, 74.5, 69.9, 69.6, 69.3 (d, *J* 17 Hz), 68.8, 65.0, 61.8, 61.4, 57.4, 20.8, and 20.6 (4 × OAc). ^{19}F NMR (282 MHz, $CDCl_3$): δ_F −126.9 (ddd, *J* 8, 28, and 50 Hz). HRMS-ESI: calcd for $C_{23}H_{33}FO_{15}Na$: 591.1701; found: 591.1705. Anal. Calcd for $C_{23}H_{33}FO_{15}$: C, 48.59; H, 5.85. Found: C, 48.98; H, 5.74.

3.1.10. Methyl (2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (7). To a soln of acceptor **21** (90 mg, 0.158 mmol) and donor **16** (130 mg, 0.316 mmol) in MeCN (10 mL) containing 4 Å molecular sieves was added mercuric cyanide (75 mg, 0.284 mmol) and mercuric bromide (108 g, 0.284 mmol). The reaction mixture was allowed to stir at room temperature for 12 h, then filtered through Celite, and concentrated under diminished pressure. The concentrate was redissolved in EtOAc and the organic layer was washed with satd $NaHCO_3$ soln and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (2:1 EtOAc–hexane) to give the protected compound **22** as a colorless oil (120 mg, 0.134 mmol, 85%). After column chromatography the product was observed to contain a co-eluting minor impurity. This material was therefore deprotected and characterized as the final product described below. To a soln of **22** (110 mg, 0.122 mmol) in MeOH (10 mL) was added catalytic sodium methoxide, the mixture stirred at room temperature for 2 days. The sodium methoxide was then neutralized with Amberlite IR-120 H^+ resin and the solvent was evaporated under diminished pressure. The crude material was purified by flash column chromatography over silica (7:4:1 CH_2Cl_2–MeOH–H_2O) to give the title compound **7** as a colorless powder (45 mg, 0.086 mmol, 71%). ^{1}H NMR (400 MHz, D_2O): δ_H 5.23 (1H, d, *J* 7.9 Hz, H-1′), 4.81 (1H, s, H-1″), 4.78 (1H, d, *J* 49 Hz, H-2′), 4.49 (1H, s, H-1), 4.05 (1H, br s, H-2), 3.90–3.89 (1H, m, H-2″), 4.05–3.86 (1H, m, H-3′), 3.81–3.56 (13 H, m), 3.47–3.42 (1H, m), 3.42 (3H, s, OCH_3). ^{13}C NMR (75 MHz, D_2O): δ_C 100.9, 99.4, 99.2 (d, *J* 30 Hz), 89.5 (d, *J* 172 Hz), 81.1, 74.0, 73.2, 72.6, 70.5, 69.9, 69.9, 69.5 (d, *J* 17 Hz), 66.7, 66.7, 65.6, 65.3, 60.9, 60.4, 56.8. ^{19}F NMR (376 MHz, D_2O): δ_F −128.1 (ddd, *J* 8, 32, and 49 Hz). HRMS-ESI: calcd for $C_{19}H_{33}FO_{15}Na$: 543.1701; found: 543.1705.

3.1.11. Methyl 2-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-β-D-mannopyranoside (24). To a cooled (−40 °C) soln of acceptor **15** (130 mg, 0.40 mmol) in CH_2Cl_2 (10 mL) containing the trichloroacetimidate donor **23** (490 mg, 1.0 mmol) was added catalytic boron trifluoride diethyl etherate. The reaction was allowed to warm to 0 °C over 4 h then Et_3N (0.1 mL) was added. The organic layer was washed with satd $NaHCO_3$ soln and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (2:1 EtOAc–hexane) to give the title compound **24** as a colorless crystalline solid (191 mg, 0.29 mmol, 73%). Mp 177–178 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.40–7.37 (2H, m, Ar), 7.31–7.29 (3H, m, Ar), 5.78 (1H, s, PhC*H*), 5.52 (1H, br s, H-2), 5.34 (1H, br s, H-2′), 5.29 (1H, t, *J* 10.3 Hz, H-4′), 5.19 (1H, s, H-1′), 5.15 (1H, dd, *J* 3.4 and 10.3 Hz, H-3′), 4.56 (1H, s, H-1), 4.35 (1H, dd, *J* 4.9 and 10.4 Hz, H-6_a), 4.28 (1H, dd, *J* 4.8 and 13.0 Hz, H-$6'_a$), 4.11–4.08 (2H, m, H-5′ and H-$6'_b$), 4.03–4.01 (2H, m, H-3 and H-4), 3.89 (1H, t, *J* 10.4 Hz, H-6_b), 3.53 (3H, s, OCH_3), 3.42–3.36 (1H, m, H-5), 2.25 (3H, s, O*Ac*), 2.08 (3H, s, O*Ac*), 2.06 (3H, s, O*Ac*), 2.04 (3H, s, O*Ac*), 1.94 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.8, 170.6, 169.9, 169.9, 169.7, 136.9, 129.0, 128.2, 125.9, 101.4, 100.5, 98.3, 78.7, 73.1, 70.1, 69.3, 69.1, 68.8, 68.3, 66.8, 65.5, 62.3, 57.6, and 20.8 (4 × OAc), 20.7 (OAc). HRMS-ESI: calcd for $C_{30}H_{38}O_{16}Na$: 677.2058; found: 677.2036. Anal. Calcd for $C_{30}H_{38}O_{16}$: C, 55.05; H, 5.85. Found: C, 55.04; H, 5.88.

3.1.12. Methyl 2-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-6-*O*-benzyl-β-D-mannopyranoside (25). To a soln of acetal **24** (315 mg, 0.48 mmol), 4 Å molecular sieves (100 mg), and trimethylamine borane (176 mg, 2.4 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added dropwise a suspension of aluminum trichloride (257 mg, 1.9 mmol) in Et_2O (10 mL). The reaction mixture was stirred at 0 °C for 1.5 h then filtered through Celite. CH_2Cl_2 (20 mL) and 0.75 M H_2SO_4 (5 mL) was added and the mixture was stirred for 1.5 h at room temperature. The mixture was transferred to a separating funnel and the organic layer was washed with water, satd $NaHCO_3$ soln, and brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (2:1 EtOAc–hexane) to give the title compound **25** as a colorless oil (180 mg, 0.27 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.37–7.27 (5H, m, Ar), 5.40 (1H, dd, *J* 0.9 and 3.5 Hz, H-2), 5.31 (1H, dd, *J* 1.7

and 3.4 Hz, H-2′), 5.28 (1H, t, *J* 10.2 Hz, H-4′), 5.23 (1H, d, *J* 1.7 Hz, H-1′), 5.15 (1H, dd, *J* 3.4 and 10.2 Hz, H-3′), 4.61 (1H, d, *J* 11.8 Hz, PhC*H*$_2$), 4.53 (1H, d, *J* 11.8 Hz, PhC*H*$_2$), 4.44 (1H, d, *J* 0.9 Hz, H-1), 4.26 (1H, dd, *J* 4.6 and 12.3 Hz, H-6′$_a$), 4.16–4.09 (2H, m, H-5′ and H-6′$_b$), 3.95 (1H, dt, *J* 2.5 and 9.4 Hz, H-4), 3.82 (1H, dd, *J* 4.9 and 9.8 Hz, H-6$_a$), 3.77–3.73 (2H, m, H-3 and H-6$_b$), 3.48 (3H, s, OC*H*$_3$), 3.44–3.40 (1H, m, H-5), 3.08 (1H, d, *J* 2.5 Hz, C^4-O*H*), 2.18 (3H, s, O*Ac*), 2.11 (3H, s, O*Ac*), 2.08 (3H, s, O*Ac*), 2.04 (3H, s, O*Ac*), 1.96 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.5, 170.0, 169.8, 169.8, 137.2, 128.5, 128.0, 127.8, 99.8, 98.8, 77.1, 73.8, 73.4, 70.8, 70.5, 69.9, 69.3, 69.1, 68.9, 65.5, 62.3, 57.2, 20.8, 20.7, 20.7, 20.6, 20.6. HRMS-ESI: calcd for $C_{30}H_{40}O_{16}Na$: 679.2214; found: 679.2192.

3.1.13. Methyl 2,4-di-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-6-*O*-benzyl-β-D-mannopyranoside (26). Disaccharide **25** (150 mg, 0.23 mmol) in 2:1 pyridine–Ac_2O (15 mL) was stirred overnight at room temperature. MeOH (5 mL) was added and the mixture stirred for a further 0.5 h. The reaction mixture was then concentrated under diminished pressure, redissolved in EtOAc, then washed with 1 M HCl, satd $NaHCO_3$ (× 2) and water, and dried over $MgSO_4$. The solvent was evaporated under diminished pressure and the crude material purified by flash column chromatography over silica gel (3:2 EtOAc–hexane) to give the title compound **26** as a colorless solid (140 mg, 0.20 mmol, 88%). Mp 149–150 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.34–7.26 (5H, m, Ar), 5.44 (1H, dd, *J* 1.0 and 3.4 Hz, H-2), 5.27 (1H, t, *J* 10.0 Hz, H-4′), 5.18 (1H, t, *J* 10.0 Hz, H-4), 5.17 (1H, dd, *J* 3.4 and 10.0 Hz, H-3′), 4.98 (1H, dd, *J* 1.8 and 3.4 Hz, H-2′), 4.96 (1H, d, *J* 1.8 Hz, H-1′), 4.52 (2H, s, PhC*H*$_2$), 4.47 (1H, d, *J* 1.0 Hz, H-1), 4.24 (1H, dd, *J* 4.8 and 12.6 Hz, H-6′$_a$), 4.16–4.11 (2H, m, H-5′ and H-6′$_b$), 3.85 (1H, dd, *J* 3.4 and 10.0 Hz, H-3), 3.66–3.52 (3H, m, H-5, H-6$_a$, and H-6$_b$), 3.51 (3H, s, OC*H*$_3$), 2.21 (3H, s, O*Ac*), 2.11 (3H, s, O*Ac*), 2.08 (3H, s, O*Ac*), 2.03 (3H, s, O*Ac*), 1.99 (3H, s, O*Ac*), 1.97 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.4, 169.9, 169.9, 169.8, 169.4, 137.7, 128.3, 127.8, 127.6, 99.6, 98.9, 76.5, 73.8, 73.6, 70.0, 69.9, 69.7, 69.3, 69.0, 68.1, 65.6, 62.1, 57.2, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5. HRMS-ESI: calcd for $C_{32}H_{42}O_{17}Na$: 721.2320; found: 721.2332. Anal. Calcd for $C_{32}H_{42}O_{17}$: C, 55.01; H, 6.06. Found: C, 55.40; H, 6.11.

3.1.14. Methyl 2,4-di-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-β-D-mannopyranoside (27). Benzyl ether **26** (115 mg, 0.17 mmol) was dissolved in MeOH (20 mL) containing catalytic AcOH and 10% Pd/C was added (15 mg). The reaction mixture was stirred under an atmosphere of hydrogen for 12 h then filtered through Celite. The solvent was evaporated under diminished pressure and the crude material purified by flash column chromatography over silica gel (EtOAc) to give the title compound **27** as a colorless solid (87 mg, 0.14 mmol, 87%). Mp 191–193 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.45 (1H, d, *J* 3.4 Hz, H-2), 5.29 (1H, t, *J* 9.9 Hz, H-4′), 5.16–5.11 (2H, m, H-3′ and H-4), 4.99 (2H, br s, H-1′ and H-2′), 4.50 (1H, d, *J* 0.8 Hz, H-1), 4.24 (1H, dd, *J* 4.6 and 12.5 Hz, H-6′$_a$), 4.17–4.12 (2H, m, H-5′ and H-6′$_b$), 3.91 (1H, dd, *J* 3.4 and 9.9 Hz, H-3), 3.69–3.65 (2H, m, H-6$_a$ and H-6$_b$), 3.51 (3H, s, OC*H*$_3$), 3.37–3.33 (1H, m, H-5), 2.21 (3H, s, O*Ac*), 2.12 (3H, s, O*Ac*), 2.12 (3H, s, O*Ac*), 2.08 (3H, s, O*Ac*), 2.03 (3H, s, O*Ac*), 1.97 (3H, s, O*Ac*). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.7, 170.6, 170.5, 170.0, 169.8, 169.5, 99.8, 98.9, 76.3, 74.6, 70.6, 69.9, 69.4, 68.4, 68.1, 65.6, 62.1, 61.5, 57.3, 20.8, 20.8, 20.7, 20.6, 20.5, 20.5. HRMS-ESI: calcd for $C_{25}H_{36}O_{17}Na$: 631.1850; found: 631.1851. Anal. Calcd for $C_{25}H_{36}O_{17}$: C, 49.34; H, 5.96. Found: C, 49.72; H, 6.03.

3.1.15. Methyl 2,4-di-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-[(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (29). To a cooled (−40 °C) soln of acceptor **27** (50 mg, 0.08 mmol) in CH_2Cl_2 (10 mL) containing the trichloroacetimidate donor **28**[29] (150 mg, 0.33 mmol) was added catalytic boron trifluoride diethyl etherate. The reaction mixture was allowed to warm to −20 °C over 4 h then Et_3N (0.1 mL) was added. The organic layer was washed with satd $NaHCO_3$ soln and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The crude material was purified by flash column chromatography over silica gel (2:1→4:1 EtOAc–hexane) to give the title compound **29** as a colorless crystalline solid (42 mg, 0.05 mmol, 57%). Mp 194–195 °C (EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.43 (1H, d, *J* 3.2 Hz, H-2), 5.33–5.10 (5H, m, H-3′, H-3″, H-4, H-4′, and H-4″), 5.01–4.96 (3H, m, H-1′, H-1″, and H-2′), 4.74 (1H, br d, *J* 50 Hz, H-2″), 4.47 (1H, s, H-1), 4.26–4.03 (6H, m, H-5′, H-5″, H-6′$_a$, H-6″$_a$, H-6′$_b$, and H-6″$_b$), 3.88–3.81 (2H, m, H-3 and H-6$_a$), 3.57–3.49 (2H, m, H-5 and H-6$_b$), 3.49 (3H, s, OC*H*$_3$), 2.19 (3H, s, OAc), 2.11 (3H, s, OAc), 2.10 (3H, s, OAc), 2.08 (3H, s, OAc), 2.07 (3H, s, OAc), 2.05 (3H, s, OAc), 2.02 (3H, s, OAc), 1.99 (3H, s, OAc), 1.96 (3H, s, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 170.6, 170.5, 170.4, 170.0, 169.9, 169.9, 169.8, 169.4, 169.3, 99.6, 98.9, 99.8 (d, *J* 29 Hz), 86.6 (d, *J* 180 Hz), 76.4, 73.0, 69.9, 69.8 (d, *J* 17 Hz), 69.4, 68.6, 68.5, 68.1, 66.8, 65.6, 65.6, 62.1, 61.9, 60.3, 57.3, 20.7, 20.7, 20.6, 20.6, 20.6, 20.6, 20.6, 20.5, 20.5. ^{19}F NMR (376 MHz, $CDCl_3$): δ_F −128.2 (ddd, *J* 7, 28, and 50 Hz). HRMS-ESI: calcd for $C_{37}H_{51}FO_{24}Na$: 921.2652; found: 921.2630. Anal. Calcd for

$C_{37}H_{51}FO_{24}$: C, 49.44; H, 5.72. Found: C, 49.46; H, 5.69.

3.1.16. Methyl (α-D-mannopyranosyl)-(1→3)-[(2-deoxy-2-fluoro-α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (9). To a soln of the protected sugar **29** (19 mg, 0.02 mmol) in MeOH (5 mL) at room temperature was added catalytic sodium methoxide. The mixture was stirred at room temperature for 24 h. The sodium methoxide was neutralized with Amberlite IR-120 H^+ resin and the solvent evaporated under diminished pressure. The crude material was purified by flash column chromatography over silica (7:4:1 CH_2Cl_2–MeOH–H_2O) to give the title compound **9** as a colorless powder (8 mg, 0.015 mmol, 73%). ^{1}H NMR (400 MHz, D_2O): δ_H 4.99 (1H, d, *J* 8 Hz, H-1″), 4.96 (1H, s, H-1′), 4.74–4.60 (1H, m, partially obscured by water peak), 4.45 (1H, s, H-1), 4.01 (1H, d, *J* 3.1 Hz, H-2), 3.92 (1H, br s, H-2′), 3.88 (1H, dd, *J* 4.5 and 11.3 Hz), 3.83–3.49 (13H, m), 3.43–3.36 (1H, m), 3.38 (3H, s, OCH_3). ^{13}C NMR (100 MHz, D_2O): δ_C 102.2, 100.9, 96.6 (d, *J* 30 Hz), 89.3 (d, *J* 172 Hz), 80.5, 73.9, 73.2, 72.5, 70.1 (d, *J* 17 Hz), 70.2, 69.9, 69.8, 66.6, 66.5, 65.6, 65.6, 60.8, 60.4, 56.7. ^{19}F NMR (376 MHz, D_2O): δ_F −129.4 (ddd, *J* 7, 31, and 49 Hz). HRMS-ESI: calcd for $C_{19}H_{33}FO_{15}Na$: 543.1701; found: 543.1708.

Acknowledgements

We thank the Protein Engineering Network of Centres of Excellence of Canada (PENCE) and GlycoDesign Inc. for financial support. C.A.T. thanks the Royal Society (UK) for a postdoctoral fellowship.

References

1. Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. *Essentials of Glycobiology*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1999.
2. Herscovics, A. *Biochim. Biophys. Acta* **1999**, *1473*, 96–107.
3. Stanley, P.; Narasimhan, S.; Siminovitch, L.; Schachter, H. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3323–3327.
4. Narasimhan, S.; Stanley, P.; Schachter, H. *J. Biol. Chem.* **1977**, *252*, 3926–3933.
5. Harpaz, N.; Schachter, H. *J. Biol. Chem.* **1980**, *255*, 4885–4893.
6. Ioffe, E.; Stanley, P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 728–732.
7. Metzler, M.; Gertz, A.; Sarkar, M.; Schachter, H.; Schrader, J. W.; Marth, J. D. *EMBO J.* **1994**, *13*, 2056–2065.
8. Moremen, K. W. *Biochim. Biophys. Acta* **2002**, *1573*, 225–235.
9. Chui, D.; OhEda, M.; Liao, Y. F.; Panneerselvam, K.; Lal, A.; Marek, K. W.; Freeze, H. H.; Moremen, K. W.; Fukuda, M. N.; Marth, J. D. *Cell* **1997**, *90*, 157–167.
10. Schachter, H.; Chen, S. H.; Zhou, S.; Tan, J.; Yip, B.; Sarkar, M.; Spence, A. *Biochem. Soc. Trans.* **1997**, *25*, 875–880.
11. Unligil, U. M.; Zhou, S. H.; Yuwaraj, S.; Sarkar, M.; Schachter, H.; Rini, J. M. *EMBO J.* **2000**, *19*, 5269–5280.
12. van den Elsen, J. M. H.; Kuntz, D. A.; Rose, D. R. *EMBO J.* **2001**, *20*, 3008–3017.
13. Vella, G. J.; Paulsen, H.; Schachter, H. *Can. J. Biochem. Cell B* **1984**, *62*, 409–417.
14. Moller, G.; Reck, F.; Paulsen, H.; Kaur, K. J.; Sarkar, M.; Schachter, H.; Brockhausen, I. *Glycoconjugate J.* **1992**, *9*, 180–190.
15. Reck, F.; Springer, M.; Meinjohanns, E.; Paulsen, H.; Brockhausen, I.; Schachter, H. *Glycoconjugate J.* **1995**, *12*, 747–754.
16. Reck, F.; Meinjohanns, E.; Springer, M.; Wilkens, R.; Vandorst, J. A. L. M.; Paulsen, H.; Moller, G.; Brockhausen, I.; Schachter, H. *Glycoconjugate J.* **1994**, *11*, 210–216.
17. Wicki, J.; Rose, D. R.; Withers, S. G. *Methods Enzymol.* **2002**, *354*, 84–105.
18. Oscarson, S.; Tiden, A. K. *Carbohydr. Res.* **1993**, *247*, 323–328.
19. Arnarp, J.; Lonngren, J. *Acta Chem. Scand. B* **1978**, *32*, 696–697.
20. Ogawa, T.; Matsui, M. *Carbohydr. Res.* **1978**, *62*, C1–C4.
21. Kaur, K. J.; Hindsgaul, O. *Glycoconjugate J.* **1991**, *8*, 90–94.
22. Srivastava, V. K.; Schuerch, C. *Tetrahedron Lett.* **1979**, *35*, 3269–3272.
23. Csuk, R.; Glanzer, B. I. *Adv. Carbohydr. Chem. Biochem.* **1988**, *46*, 73–177.
24. Ek, M.; Garegg, P. J.; Hultberg, H.; Oscarson, S. *J. Carbohydr. Chem.* **1983**, *2*, 305–311.
25. Karst, N.; Jacquinet, J. C. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2709–2717.
26. Agoston, K.; Dobo, A.; Rako, J.; Kerekgyarto, J.; Szurmai, Z. *Carbohydr. Res.* **2001**, *330*, 183–190.
27. Ortner, J.; Albert, M.; Weber, H.; Dax, K. *J. Carbohydr. Chem.* **1999**, *18*, 297–316.
28. Ogawa, T.; Katano, K.; Sasajima, K.; Matsui, M. *Tetrahedron* **1981**, *37*, 2779–2786.
29. Spijker, N. M.; Slief, J. W.; Vanboeckel, C. A. A. *J. Carbohydr. Chem.* **1993**, *12*, 1017–1041.