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Synthesis of a hexasaccharide, the repeating unit of *O*-deacetylated GXM of *C. neoformans* serotype A

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Abstract

β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→3)-[β-D-Xyl*p*-(1→2)]-α-D-Man*p*-(1→3)[-β-D-Xyl*p*-(1→2)]-α-D-Man*p*, the repeating unit of the exopolysaccharide from *Cryptococcus neoformans* serovar A, was synthesized as its allyl glycoside. Thus, 3-O-selective acetylation of allyl 4,6-*O*-benzylidene-α-D-mannopyranoside afforded **2**, and subsequent glycosylation of **2** with 2,3,4-tri-*O*-benzoyl-D-xylopyranosyl trichloroacetimidate furnished the β-(1→2)-linked disaccharide **4**. Debenzylidenation followed by benzoylation gave allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-3-*O*-acetyl-4,6-di-*O*-benzoyl-α-D-mannopyranoside (**5**), and selective 3-O-deacetylation gave the disaccharide acceptor **6**. Coupling of **6** with 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate yielded the trisaccharide **8**, and subsequent deallylation and trichloroacetimidation gave 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-4,6-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**9**). Condensation of the trisaccharide donor **9** with the disaccharide acceptor **6** gave the pentasaccharide **10** whose 2-O-deacetylation gave the acceptor **11**. Glycosylation of **11** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate and subsequent deprotection gave the target hexasaccharide.

Keywords: Mannose; Xylose; Glucouronic acid

1. Introduction

Cryptococcus neoformans, a primary cause of opportunistic infections associated with AIDS, produces glucuronoxylomannan (GXM) as the major capsule component.[1,2] There are four major serotypes[3] for GXM designated A–D (Fig. 1). All four serotypes are composed of a linear α-(1→3)-linked mannosyl backbone with β-glucopyranosyluronic acid, β-xylopyranosyl, and 6-*O*-acetyl substituents.[4]

GXM is antipathogocytic and poorly immunogenic, and acapsular strains have significantly reduced virulence.[5] In vitro, GXM inhibits leukocyte migration,[6] enhances HIV infection in human lymphocytes[7] and promotes L-selectin shedding from neutrophils.[8] Because of its prominent virulence, synthesis of the GXM repeating unit will be very helpful for the research on the structure–activity relationships of oligosaccharides.

The synthesis of tri- and tetrasaccharide fragments corresponding to structures in the capsular polysaccharides of *C. neoformans* has been reported,[9] and the synthesis of a pentasaccharide, the repeating unit of the polysaccharide in *C. neoformans* serovar D, has appeared.[10] We have reported in a preliminary communication[11] the successful synthesis of the hexasaccharide repeating unit of *O*-deacetylated GXM of *C. neoformans* serotype A with 4,6-*O*-isopropylidenated mannose derivatives as the acceptors based on our previous studies on the syntheses of cell-wall components. We now report an alternative highly efficient and conver-

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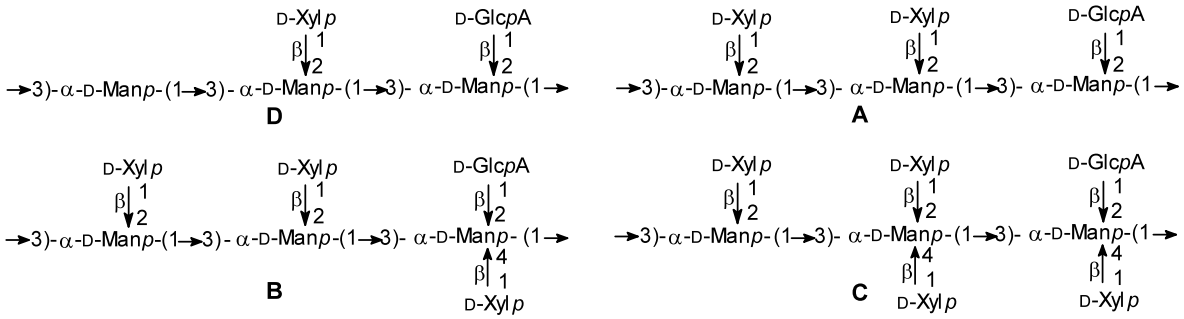


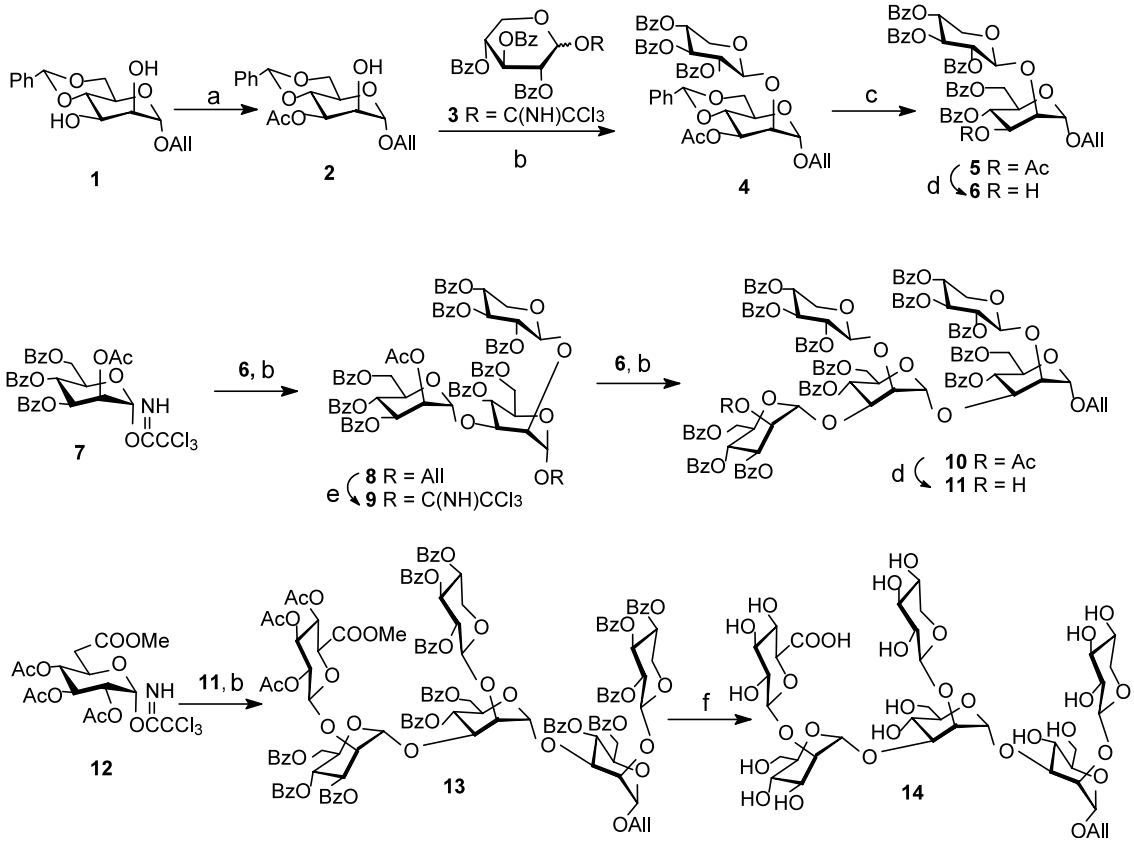
Fig. 1. Model structures of deacetylated GXM of *C. neoformans* serotypes A–D.

gent synthesis of the hexasaccharide repeating unit of *O*-deacetylated GXM of *C. neoformans* serotype A.

2. Results and discussion

As outlined in Scheme 1, selective acetylation of allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**)[12] with acetyl chloride in pyridine went smoothly giving allyl 3-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranoside (**2**) in high yield (91%), and no acetyl migration was found.

The ^{1}H NMR spectrum of **2** showed a characteristic downfield doublet of doublets at δ 5.36 ppm with $J_{2,3} = 3.2$ and $J_{3,4} = 10.0$ Hz for H-3, confirming the 3-OH regioselectivity. This selective 3-O-acetylation was the key step in the synthesis since, on the one hand, **2** contained a free hydroxyl group at the position where the xylosyl residue should be attached, and on the another hand, the coupling of **2** with perbenzoylated xylosyl trichloroacetimidate (**3**)[13] gave a product that was readily transformed to either an acceptor or a donor for further reactions. Thus, condensation of **2** with **3**



Scheme 1. (a) CH_3COCl, CH_2Cl_2, Pyridine; (b) TMSOTf, CH_2Cl_2, −10 °C to room temperature (dry); (c) 90% TFA; then BzCl–Pyridine; (d) 2% CH_3COCl–CH_3OH, 0 °C to room temperature; (e) $PdCl_2$, 90% acetic acid–NaOAc, room temperature, 12 h; then Cl_3CN, DBU, CH_2Cl_2 2 h; (f) saturated NH_3–MeOH, room temperature, 36 h; then H_2O, room temperature, 5 h.

afforded (1→2)-linked disaccharide **4** in good yield (85%). Because of the presence of a benzylidene group, selective deacetylation of **4** with 2% CH_3COCl–MeOH[14] was not successful since a benzylidene group is more sensitive to acidic conditions than either acetyl or benzoyl groups. Thus, debenzylidenation of **4** was carried out with 90% trifluoroacetic acid (TFA), and subsequent benzoylation gave disaccharide **5** (87%). Selective deacetylation of **5** with 2% CH_3COCl–MeOH gave the disaccharide acceptor **6** (73%).

Due to the unstability of methyl ester linkage of a glucuronate residue under either basic or acidic conditions, assembly of the glucuronate unit was arranged at the end of the reaction series. So, 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**7**)[15] was used as the donor to couple **6**, and trisaccharide **8** was obtained in satisfactory yield (88%). Subsequent 1-O-deallylation with $PdCl_2$ and activation with trichloroacetonitrile[16a] in the presence of potassium carbonate gave the trisaccharide donor **9** (84%). Again, glycosylation of **6** with **9** readily afforded the pentasaccharide **10** (83%). Selective deacetylation of **10** with 2% CH_3COCl–MeOH was accompanied by some decomposition, perhaps caused by breaking of the xylosyl linkage, giving the pentasaccharide acceptor **11** in 65% yield. Coupling of **11** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate (**12**)[16] went smoothly affording the protected hexasaccharide in good yield (86%). The ^1H and ^{13}C NMR spectra of **13** showed a methyl signal (δ 3.69 ppm), 13 benzoyl C=O signals (δ 165.9, 165.9, 165.8, 165.4, 165.4, 165.3, 165.3, 165.2, 165.1, 165.0, 164.8, 164.6, 164.6 ppm), four C=O signals (δ 169.0, 168.5, 168.5, 168.3 ppm), and six anomeric C signals (100.9, $J_{C1,H1} = 176$ Hz, Man*p*; 100.1, $J_{C1,H1} = 162$ Hz, GluA*p*; 99.9, $J_{C1,H1} = 164$ Hz, Xyl*p*; 99.5 $J_{C1,H1} = 164$ Hz, Xyl*p*; 96.7, $J_{C1,H1} = 173$ Hz, Man*p*; 96.2, $J_{C1,H1} = 175$ Hz, Man*p*). It is noted that although it was difficult to assign the anomeric configuration of the two Xyl*p* just from the relatively small $J_{H1,H2}$ values (6.1 and 5.2 Hz, respectively), the chemical shifts of H-1 (δ 4.93 and 4.74, respectively) and the $J_{C1,H1}$ values clearly indicate the β linkages. Deprotection of **13** was carried out in a saturated solution of ammonia in methanol for 36 h, then water (2 equiv) was added to cleave the methyl ester. After standing at room temperature for 5 h, the reaction mixture was concentrated and purified on a Bio-Gel P2 column (eluent:-water), affording the target hexasaccharide **14** as a foamy solid.

In summary, an alternative highly efficient and convergent synthesis of the hexasaccharide repeat unit of *O*-deacetylated GXM of *C. neoformans* serotype A was achieved. The strategy presented here also provides a route to the synthesis of more complex repeating units of GXM of *C. neoformans* serotypes B and C.

3. Experimental

3.1. General methods

Melting points were determined with a 'Mel-Temp' apparatus. Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solutions in a 1-dm jacketed cell. ^1H and ^{13}C NMR spectra were recorded with Varian XL-400 and Varian XL-200 spectrometers, for solutions in $CDCl_3$ or in D_2O as indicated. Chemical shifts are expressed in ppm downfield from the Me_4Si absorption. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the electronspray-ionization (ESI) mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) H_2SO_4 in CH_3OH or by UV detection. Column chromatography was conducted by elution of a column (8 × 100, 16 × 240, 18 × 300, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Analytical LC was performed with a Gilson HPLC consisting of a pump (model 306), stainless steel column packed with silica gel (Spherisorb SiO_2, 10 × 300 or 4.6 × 250 mm), a differential refractometer (132-RI Detector), and a UV–Vis detector (model 118). EtOAc–petroleum ether (bp 60–90 °C) was used as the eluent at a flow rate of 1–4 mL/min. Solutions were concentrated at a temperature < 60 °C under diminished pressure.

3.2. Allyl 3-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (2)

Compound **1** (3.08 g, 10 mmol) was dissolved in dry CH_2Cl_2 (40 mL) containing pyridine (8.1 mL, 100 mmol), then under N_2 protection and stirring, a solution of acetyl chloride (0.8 mL, 11 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise within 30 min at 0 °C. The reaction mixture was slowly raised to room temperature (rt) and stirred for 2 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with water, 1 N HCl, and dried over Na_2SO_4. The solution was concentrated, and purification of the residue by column chromatography on a silica gel column (3:1 petroleum ether–EtOAc) gave compound **2** (3.17 g, 90.6%) as a syrup: $[\alpha]_D$ + 42.0° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.46–7.34 (m, 5 H, Ph*H*), 5.88 (m, 1 H, CH_2=C*H*CH_2O), 5.55 (s, 1 H, PhC*H*O_2), 5.36 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 5.20 (m, 1 H, C*H*$_2$=CHCH_2O), 5.23 (m, 1 H, C*H*$_2$=CHCH_2O), 4.89 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.28 (dd, 1 H, *J* 4.8, 10.6 Hz, H-6a), 4.19 (m, 1 H, CH_2=CHC*H*$_2$O), 4.15 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.2 Hz, H-2), 4.09 (dd, 1 H, *J* 10.0, 10.6 Hz, H-6b), 4.02 (m, 1 H, CH_2=CHC*H*$_2$O), 3.99 (ddd, 1 H, *J* 4.8, 10.0, 10.6 Hz,

H-5), 3.84 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 2.12 (s, 3 H, CH_3CO). Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.70; H, 6.33. Found: C, 61.94; H, 6.21.

3.3. Allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-3-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (4)

To a cooled solution (−10 °C) of **2** (3.50 g, 10.0 mmol) and **3** (6.70 g, 11.0 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (18 μL, 0.1 mmol). The mixture was stirred for 2 h and then quenched with Et_3N (four drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give disaccharide **4** (6.76 g, 85.1%) as a foamy solid: $[\alpha]_D$ −38.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13–7.33 (m, 20 H, 4 Ph*H*), 5.85 (m, 1 H, CH_2=C*H*CH_2O), 5.71 (dd, 1 H, $J_{2',3'} = J_{3',4'} = 5.8$ Hz, H-3′), 5.37 (dd, 1 H, $J_{1',2'}$ 4.2, $J_{2',3'}$ 5.8 Hz, H-2′), 5.36 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.5 Hz, H-3), 5.28 (m, 1 H, H-4′), 5.25 (m, 1 H, CH_2=CHCH_2O), 5.24 (s, 1 H, PhCHO_2), 5.20 (m, 1 H, CH_2=CHCH_2O), 4.91 (d, 1 H, $J_{1',2'}$ 4.2 Hz, H-1′), 4.80 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.59 (dd, 1 H, *J* 3.5, 12.4 Hz, H-6a), 4.30 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.5 Hz, H-2), 4.10 (m, 1 H, CH_2=CHCH_2O), 4.06 (dd, 1 H, $J_{4',5a'}$ 4.8, $J_{5'a,5'b}$ 10.3 Hz, H-5′a), 3.98 (dd, 1 H, $J_{4',5b'} = J_{5'a,5'b}$ 10.3 Hz, H-5′b), 3.92 (m, 1 H, CH_2=CHCH_2O), 3.88–3.76 (m, 2 H, H-5, H-6b), 3.49 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.5$ Hz, H-4), 2.14 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2 (CH_3CO), 165.6, 165.1, 165.0 (3 C, 3 Ph*C*O), 118.1 (*C*H_2=CHCH_2O), 101.6 (Ph*C*HO_2), 98.2, 96.9 (2 C, 2 C-1), 75.8, 75.3, 69.5, 69.1, 68.6, 68.5, 68.3, 68.2, 64.0, 60.2 (10 C, C-2–6, C-2′–5′, CH_2=CH*C*H_2O), 21.1 (*C*H_3CO). Anal. Calcd for $C_{44}H_{42}O_{14}$: C, 66.49; H, 5.33. Found: C, 66.20; H, 5.48.

3.4. Allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-3-*O*-acetyl-4,6-di-*O*-benzoyl-α-D-mannopyranoside (5)

Compound **4** (7.94 g, 10 mmol) was dissolved in 80 mL 90% TFA and stirred for 2 h, at the end of which time the reaction mixture was poured into toluene (300 mL) and concentrated. After drying under high vacuum for 2 h, the residue was dissolved in Py (25 mL), and BzCl (4.70 mL, 40 mmol) was added. The mixture was stirred at rt for 12 h, then quenched with MeOH (3 mL). The reaction mixture was evaporated and coevaporated with toluene in vacuo to give a residue. Purification of the residue by silica gel column chromatography (3:1 petroleum ether–EtOAc) gave **5** (7.93 g, 86.8%) as a foamy solid: $[\alpha]_D$ −46.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12–7.29 (m, 25 H, 5 Ph*H*), 5.91 (m, 1 H, CH_2=C*H*CH_2O), 5.71 (dd, 1 H, $J_{2',3'} = J_{3',4'} = 5.0$ Hz, H-3′), 5.67 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.50 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0 Hz, H-3), 5.37 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 5.0 Hz, H-2′), 5.28–5.16 (m, 3 H, H-4, CH_2=CHCH_2O), 4.97 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.94 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.61 (dd, 1 H, *J* 3.2, 12.5 Hz, H-6a), 4.34 (dd, 1 H, $J_{4',5a'}$ 3.0, $J_{5'a,5'b}$ 11.8 Hz, H-5′a), 4.26 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.3 Hz, H-2), 4.22–4.16 (m, 2 H), 4.08–3.97 (m, 2 H), 3.78 (dd, 1 H, *J* 4.8, 12.5 Hz, H-6b), 1.97 (s, 3 H, CH_3CO). Anal. Calcd for $C_{51}H_{46}O_{16}$: C, 66.95; H, 5.07. Found: C, 67.09; H, 5.31.

3.5. Allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-4,6-di-*O*-benzoyl-α-D-mannopyranoside (6)

To a solution of **5** (4.57 g, 5 mmol) in anhyd CH_2Cl_2 (10 mL) was added anhyd MeOH (40 mL). Acetyl chloride (1.0 mL) was then added to the reaction mixture at 0 °C. The solution was stoppered in a flask and stirred at rt until TLC (3:1 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column to give **6** (3.20 g, 73.4%) as a foamy solid: $[\alpha]_D$ −7.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05–7.30 (m, 25 H, 5 Ph*H*), 5.88 (m, 1 H, CH_2=C*H*CH_2O), 5.77 (dd, 1 H, $J_{2',3'} = J_{3',4'} = 6.9$ Hz, H-3′), 5.46 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.37 (dd, 1 H, $J_{1',2'}$ 5.2, $J_{2',3'}$ 6.9 Hz, H-2′), 5.29 (m, 1 H, H-4′), 5.19–5.13 (m, 2 H, CH_2=CHCH_2O), 4.97 (d, 1 H, $J_{1',2'}$ 5.2 Hz, H-1′), 4.94 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.61 (dd, 1 H, *J* 4.0, 12.3 Hz, H-6a), 4.34 (dd, 1 H, $J_{4',5a'}$ 2.5, $J_{5'a,5'b}$ 11.9 Hz, H-5′a), 4.24 (dd, 1 H, $J_{4',5b'}$ 5.8, $J_{5'a,5'b}$ 11.9 Hz, H-5′b), 4.18–4.12 (m, 4 H), 3.95–3.84 (m, 2 H), 1.60 (bs, 1 H, O*H*). Anal. Calcd for $C_{49}H_{44}O_{15}$: C, 67.42; H, 5.08. Found: C, 67.19; H, 5.01.

3.6. Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranoside (8)

Compound **6** (2.62 g, 3.0 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**7**) (2.23 g, 3.3 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (50 mL). TMSOTf (18 μL, 0.1 mmol) was added dropwise at −10 °C with nitrogen protection. The reaction mixture was stirred for 3 h, during which time the mixture was allowed to gradually warm to ambient temperature. Then the mixture was neutralized with Et_3N and concentrated to dryness. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave **8** (3.66 g, 87.9%) as a syrup: $[\alpha]_D$ −45.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12–7.13 (m, 40 H, 8 Ph*H*), 5.89 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0 Hz, H-3 of Man*p*), 5.77 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.76 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.71 (m, 1 H, CH_2=C*H*CH_2O), 5.65 (dd, 1 H, $J_{2,3} = J_{3,4} = 4.6$ Hz, H-3 of Xyl*p*), 5.47 (m, 1 H, H-4 of Xyl*p*), 5.38 (dd, 1 H, $J_{1,2} = J_{2,3} = 4.6$ Hz, H-2 of

Xyl*p*), 5.20 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.3 Hz, H-2 of Man*p*), 5.18 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1 of Man*p*), 5.18–5.10 (m, 2 H, CH_2=CHCH$_2$O), 5.07 (d, 1 H, $J_{1,2}$ 4.6 Hz, H-1 of Xyl*p*), 4.92 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1 of Man*p*), 4.85–4.76 (m, 2 H), 4.58–4.54 (m, 2 H), 4.40 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.9 Hz, H-3 of Man*p*), 4.28 (m, 1 H), 4.24 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.1 Hz, H-2 of Man*p*), 4.09–4.03 (m, 2 H), 3.92–3.86 (m, 2 H), 1.92 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7 (CH$_3$*C*O), 166.1, 165.9, 165.7, 165.3, 165.2, 165.1, 165.0, 164.8 (8 C, 8 Ph*C*O), 118.1 (*C*H$_2$=CHCH$_2$O), 99.8, 98.6, 96.5 (3 C, 3 C-1), 69.7, 69.7, 69.5, 69.3, 69.2, 69.2, 68.9, 68.9, 68.7, 68.5, 68.5, 68.0, 67.7, 63.8, 63.6, 60.3 (15 C, C-2–6 of Man*p*; C-2–5 of Xyl*p*; CH$_2$=CH*C*H$_2$O), 20.3 (*C*H$_3$CO); Anal. Calcd for $C_{78}H_{68}O_{24}$: C, 67.43; H, 4.93. Found: C, 67.26; H, 5.10.

3.7. 2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (9)

To a solution of **8** (2.08 g, 1.5 mmol) in 90% AcOH (15 mL) containing AcONa (0.44 g, 4.5 mmol) was added $PdCl_2$ (81 mg, 0.75 mmol), and the mixture was stirred for 12 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 (150 mL), washed with water and satd aq sodium bicarbonate. The organic layer was concentrated, and the residue was passed through a short silica gel column with 1:1 petroleum ether–EtOAc as the eluent to give crude 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α,β-D-mannopyranose as a syrup. After drying under high vacuum for 2 h, the solid was dissolved in CH_2Cl_2 (10 mL), and CCl_3CN (0.5 mL, 5 mmol) and DBU (40 μL, 0.3 mmol) were added. The reaction mixture was stirred for 2 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the reaction mixture, followed by purification of the crude product on a silica gel column with 2:1 petroleum ether–EtOAc as the eluent, furnished the trisaccharide donor **9** (1.88 g, 83.9%) as a foamy solid: $[\alpha]_D$ −42.1° (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1 H, CN*H*CCl$_3$), 8.06–7.21 (m, 40 H, 8 *Ph*), 6.43 (s, 1 H, H-1 of Man*p*), 5.89 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.8 Hz, H-3 of Man*p*), 5.86 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4 of Man*p*), 5.76 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4 of Man*p*), 5.74 (dd, 1 H, $J_{1,2} = J_{2,3} = 4.4$ Hz, H-3 of Xyl*p*), 5.48 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.0 Hz, H-2 of Man*p*), 5.45 (dd, 1 H, $J_{1,2}$ 4.9, $J_{2,3}$ 4.4 Hz, H-2 of Xyl*p*), 5.27 (m, 1 H, H-4 of Xyl*p*), 5.26 (d, 1 H, $J_{1,2}$ 4.9 Hz, H-1 of Xyl*p*), 5.20 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1 of Man*p*), 1.94 (s, 3 H, CH_3CO). Anal. Calcd for $C_{77}H_{64}Cl_3NO_{24}$: C 61.91; H 4.32. Found: C 61.65; H 4.58.

3.8. Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranoside (10)

Compound **9** (1.64 g, 1.1 mmol) and **6** (873 mg, 1.0 mmol) were coupled under the same conditions as those used for the preparation of **8** from **7** and **6**, giving **10** (1.82 g, 82.7%) as a foamy solid: $[\alpha]_D$ −20.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10–7.22 (m, 65 H, 13 Ph*H*), 5.95 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.79–5.70 (m, 3 H), 5.62 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.57 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.49–5.43 (m, 3 H), 5.33–5.28 (m, 2 H), 5.20 (m, 1 H, OCH$_2$CH=CH_2), 5.17 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1 of Xyl*p*), 5.17 (m, 1 H, OCH$_2$CH=CH_2), 5.13 (s, 1 H, H-1 of Man*p*), 5.00 (s, 1 H, H-1 of Man*p*), 4.78 (d, 1 H, $J_{1,2}$ 4.9 Hz, H-1 of Xyl*p*), 4.71 (s, 1 H, H-1 of Man*p*), 1.90 (s, 3 H, COCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 168.9 (*C*OCH$_3$), 166.0, 165.9, 165.8, 165.4, 165.4, 165.3, 165.2, 165.1, 165.0, 164.8, 164.7, 164.6, 164.5 (13 C, 13 *C*OPh), 118.2 (OCH$_2$CH=*C*H$_2$), 100.0, 99.7, 99.7, 98.9, 96.7 (5 C, 5 C-1), 20.3 (CO*C*H$_3$). Anal. Calcd for $C_{124}H_{106}O_{38}$: C, 67.56; H, 4.85. Found: C, 67.75; H, 5.03.

3.9. Allyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranoside (11)

To a solution of **10** (1.10 g, 0.05 mmol) in anhyd CH_2Cl_2 (10 mL) was added anhyd MeOH (40 mL). Acetyl chloride (1.0 mL) was added to the reaction mixture at 0 °C. The solution was stoppered in a flask and stirred at rt until TLC (3:1 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column to give **11** (700 mg, 64.7%) as a foamy solid: $[\alpha]_D$ −19.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12–7.22 (m, 65 H, 13 Ph*H*), 6.02 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.77 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.76 (dd, $J_{1,2} = J_{2,3} = 6.9$ Hz, H-3 of Xyl*p*), 5.72 (dd, $J_{1,2} = J_{2,3} = 6.1$ Hz, H-3 of Xyl*p*), 5.66 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.9 Hz, H-3 of Man*p*), 5.61 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4 of Man*p*), 5.57 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.47 (dd, 1 H, $J_{1,2}$ 5.1, $J_{2,3}$ 6.1 Hz, H-2 of Xyl*p*), 5.43–5.37 (m, 2 H, 2 H-4 of Xyl*p*), 5.35–5.29 (m, 2 H, H-3 of Xyl*p*; OCH$_2$CH=CH_2), 5.23 (s, 1 H, H-1 of

Man*p*), 5.16 (s, 1 H, $OCH_2CH{=}CH_2$), 5.12 (s, 1 H, H-1 of Man*p*), 4.82 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1 of Xyl*p*), 4.70 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1 of Man*p*), 4.61 (d, 1 H, $J_{1,2}$ 5.4 Hz, H-1 of Xyl*p*); Anal. Calcd for $C_{122}H_{104}O_{37}$: C, 67.77; H, 4.85. Found: C, 67.93; H, 4.64.

3.10. Allyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranoside (13)

To a cooled solution (0 °C) of **11** (648 mg, 0.3 mmol) and methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate (**12**) (240 mg, 0.5 mmol) in anhyd CH_2Cl_2 (10 mL) was added TMSOTf (8 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (one drop). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give hexasaccharide **13** (638 mg, 85.9%): $[\alpha]_D$ −26.9° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08–7.23 (m, 65 H, 13 Ph*H*), 6.00 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.79 (m, 1 H, $OCH_2CH{=}CH_2$), 5.71 (dd, 1 H, $J_{2,3} = J_{3,4} =$ 6.9 Hz, H-3 of Xyl*p*), 5.15 (s, 1 H, H-1 of Man*p*), 4.97 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1 of Man*p*), 4.93 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1 of Xyl*p*), 4.74 (d, 1 H, $J_{1,2}$ 5.2 Hz, H-1 of Xyl*p*), 4.78 (s, 1 H, H-1 of Man*p*), 4.07 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1 of GluA*p*), 3.69 (s, 3 H, $COOCH_3$), 1.96, 1.93, 1.27 (3 s, 9 H, 3 $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 169.0, 168.5, 168.5, 168.3 (4 C, 3 $COCH_3$, *C*OOMe), 165.9, 165.9, 165.8, 165.4, 165.4, 165.3, 165.3, 165.2, 165.1, 165.0, 164.8, 164.6, 164.6 (13 C, 13 *C*OPh), 118.3 ($OCH_2CH{=}CH_2$), 100.9 (C-1, $J_{C1,H1} = 176$ Hz, Man*p*), 100.1 (C-1, $J_{C1,H1} = 162$ Hz, GluA*p*), 99.9 (C-1, $J_{C1,H1} = 164$ Hz, Xyl*p*), 99.5 (C-1, $J_{C1,H1} = 164$ Hz, Xyl*p*), 96.7 (C-1, $J_{C1,H1} = 173$ Hz, Man*p*), 96.2 (C-1, $J_{C1,H1} = 175$ Hz, Man*p*), 51.9 ($COOCH_3$), 20.5, 20.4, 20.4 (4 C, 4 $COCH_3$). Anal. Calcd for $C_{135}H_{120}O_{46}$: C, 65.42; H, 4.88. Found: C, 65.61; H, 4.79.

3.11. Allyl (β-D-glucopyranosyluronic acid)-(1→2)-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranoside, ammonium salt (14)

Hexasaccharide **13** (490 mg, 0.2 mmol) was dissolved in a satd methanolic ammonia (50 mL). After 36 h at rt, water (1.0 mL) was added to the mixture to cleave the methyl ester. After stirring at rt for 5 h, the reaction mixture was concentrated and purified on a Bio-Gel P2 column (eluent:water), affording the target hexasaccharide **14** (126 mg, 63.0%) as a foamy solid: $[\alpha]_D$ +72.8° (*c* 0.5, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.87 (m, 1 H, $OCH_2CH{=}CH_2$), 5.31–5.17 (m, 2 H, $OCH_2CH{=}CH_2$), 5.13 (s, 1 H, H-1 of Man*p*), 4.92 (s, 1 H, H-1 of Man*p*), 4.70 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1 of GluA*p*), 4.65 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 of Man*p*), 4.42 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1 of Xyl*p*), 4.40 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1 of Xyl*p*); ^{13}C NMR (100 MHz, D_2O): δ 173.6 ($-COONH_4$), 118.5 ($CH_2{=}CHCH_2O$), 103.6, 103.5, 102.7, 100.5, 100.5, 97.5 (6 C-1), 79.0, 78.5, 78.4, 78.3, 78.3, 76.9, 76.2, 75.8, 75.8, 73.9, 73.4, 73.2, 72.9, 72.9, 70.7, 70.3, 69.5, 69.5, 68.5, 67.2, 67.0, 66.7, 66.5, 65.4, 65.3, 61.5, 60.7, 60.6. MALDI-TOF MS Calcd for the ammonium salt of **14**, $C_{37}H_{63}NO_{30}$: 1001.9 [M]. Found: 1001.6 (M); 1006.9 ($M\text{-}NH_4^+ + Na^+$).

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