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Facile synthesis of the heptasaccharide repeating unit of *O*-deacetylated GXM of *C. neoformans* serotype B

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Abstract—A heptasaccharide, β-D-Xyl*p*-(1→2)-α-D-Man*p*-(1→3)-[β-D-Xyl*p*-(1→2)]-α-D-Man*p*-(1→3)-[β-D-Glc*p*A-(1→2)][β-D-Xyl*p*-(1→4)]-α-D-Man*p*, the repeating unit of the exopolysaccharide from *Cryptococcus neoformans* serovar B, was synthesized as its methyl glycoside. Thus 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**7**) and allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-4,6-di-*O*-benzoyl-α-D-mannopyranoside (**8**), readily obtained from the corresponding monosaccharide derivatives via simple transformation, were coupled to give a (1→3)-linked tetrasaccharide **9**. Deallylation of **9** followed by trichloroacetimidate formation produced the tetrasaccharide donor **11**. Condensation of methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzoyl-α-D-mannopyranoside (**18**) with **11** followed by selective deacetylation yielded hexasaccharide acceptor **20**. Coupling of **20** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**21**) and subsequent deprotection furnished the target heptaoside. A hexasaccharide fragment, α-D-Man*p*-(1→3)-[β-D-Xyl*p*-(1→2)]-α-D-Man*p*-(1→3)-[β-D-Glc*p*A-(1→2)][β-D-Xyl*p*-(1→4)]-α-D-Man*p*, was also similarly synthesized as its methyl glycoside.

1. Introduction

C. neoformans, the etiological agent of cryptococcosis, is an encapsulated yeast. The major component of the capsular envelop of *C. neoformans* is composed of an acidic hetero-polysaccharide, glucuronoxylomannan (GXM), consisting of mannose, xylose, glucuronic acid, and *O*-acetyl groups. Initially, 12 strains of *C. neoformans* were classified into three antigenic types (A, B, and C) on the basis of agglutinin titers.[1] A fourth, type D, was introduced later.[2] Of the four major serotypes A–D for GXM, D has the simplest pentaose structure while C has the most complex octaose structure. All the four serotypes are composed of a linear α-1,3-linked mannosyl backbone with β-glucopyranosyluronic acid, β-xylopyranosyl, and 6-*O*-acetyl substituents (Fig. 1).[3]

We have reported the successful syntheses of the hexasaccharide repeating unit of *O*-deacetylated GXM of *C. neoformans* serotype A and its frame-shifted hexaoside,[4] and a hexasaccharide fragment[5] of the GXM of *C. neoformans* serotype B. Earlier, the synthesis of trisaccharide and tetrasaccharide fragments[6] corresponding to structures in capsular polysaccharides of *C. neoformans* and the synthesis of a pentasaccharide[7]—the repeating unit of the polysaccharide in *C. neoformans* serovar D were reported. We now report a convergent synthesis of the heptasaccharide repeating unit of *O*-deacetylated GXM of *C. neoformans* serotype B, and a hexasaccharide fragment of the GXM of serotypes B and C.

2. Results and discussion

In our previous work,[5] a trial for the synthesis of methyl glycoside of the frame-shifted heptaoside, β-D-Glc*p*A-(1→2)-[β-D-Xyl*p*-(1→4)]-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*, was not successful, since the coupling of the hexasaccharide acceptor, methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-3,6-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-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzoyl-α-D-mannopyranoside, with glucuronate donors either

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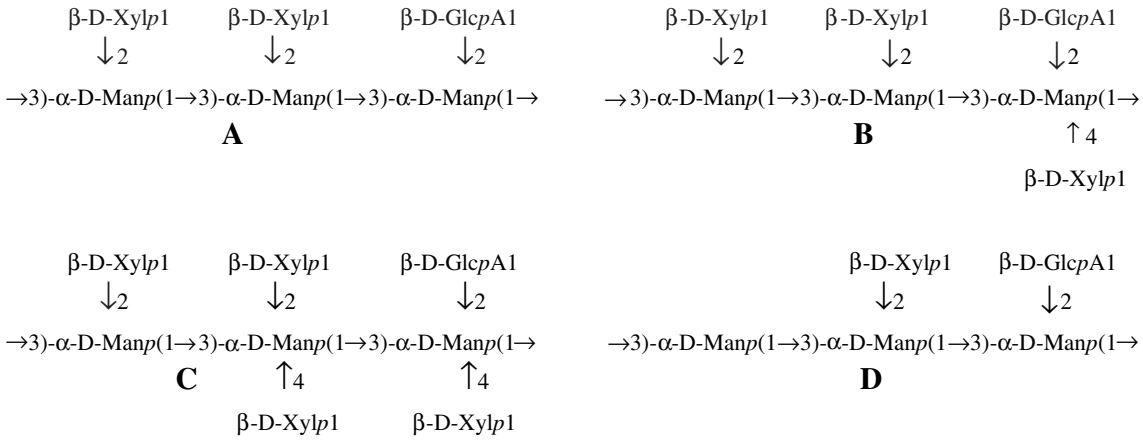


Figure 1. Structures of deacetylated GXM of *C. neoformans* serotypes A–D.

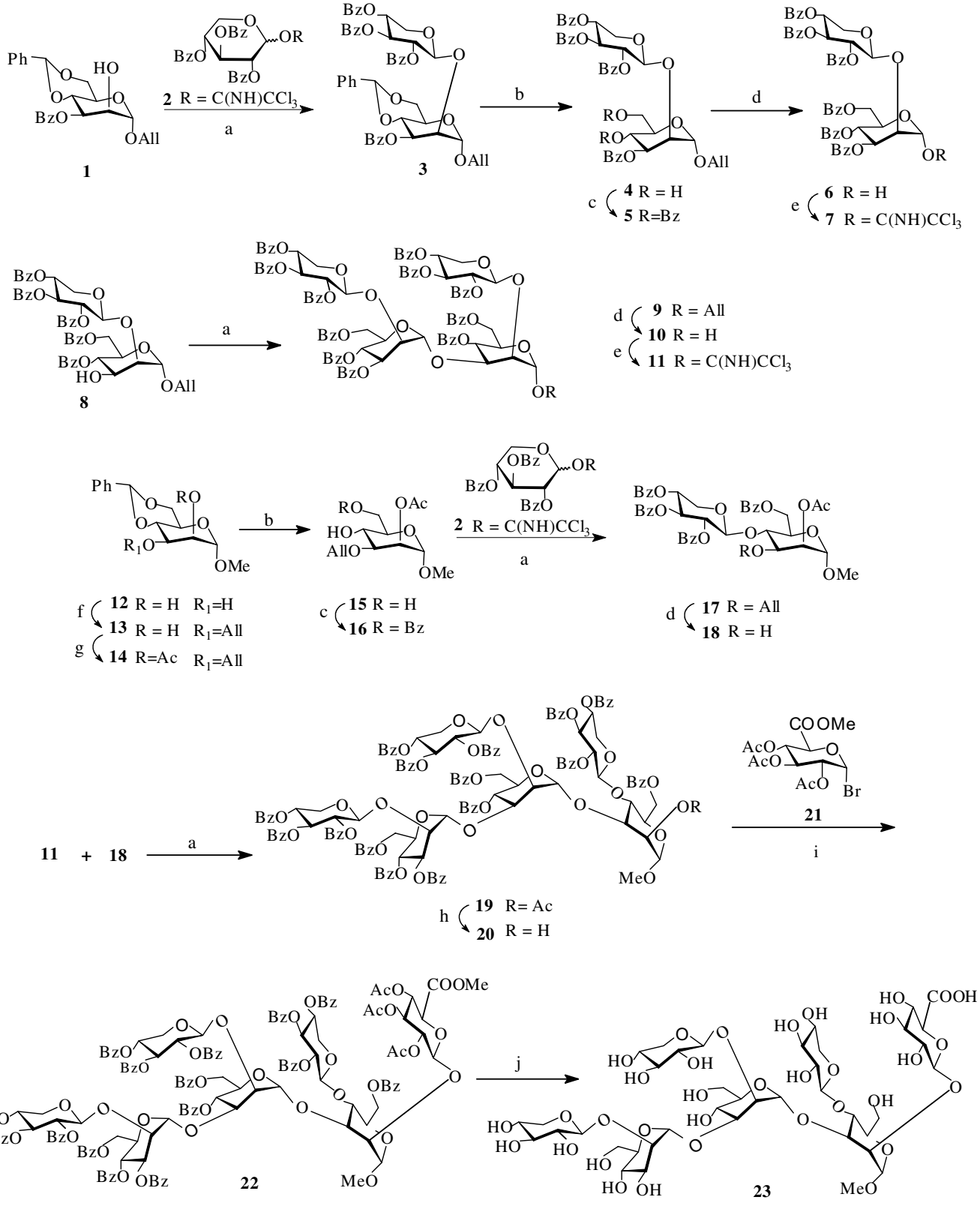
gave transacetylated hexasaccharide product when the acetylated glucuronate trichloroacetimidate was used, or did not occur when the acetylated glucuronate bromide was used. We suppose that, a serious steric hindrance at C-2 of the upstream mannose residue of the hexasaccharide acceptor restricted the coupling. Thus, we expected that a hexasaccharide acceptor with C-2 free hydroxyl group at the downstream mannose residue would be suitable for its coupling with the glucuronate donor due to smaller steric hindrance. The experimental results verified the expectation, and a successful synthesis of the title heptaoside was achieved.

Scheme 1 outlined the synthesis of the target heptaoside. Allyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside[5] (**1**) was chosen as the starting material since it was obtained in nearly quantitative yield from 3-*O*-selective benzoylation of 4,6-*O*-benzylidene-α-D-mannopyranoside.[5] Glycosylation (85%) of allyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside[5] (**1**) with 2,3,4-tri-*O*-benzoyl-D-xylopyranosyl trichloroacetimidate (**2**) followed by debenzylidenation (90%) and benzoylation (80%) gave the disaccharide **5**. Subsequent deallylation (85%) with $PdCl_2$ and trichloroacetimidate formation (89%) with trichloroacetonitrile[8] afforded the upstream disaccharide donor **7**. Condensation (62%) of allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-4,6-di-*O*-benzoyl-α-D-mannopyranoside[5] (**8**) with the disaccharide donor **7** yielded the tetrasaccharide **9** that showed two signals in its ^{13}C NMR spectrum at δ 100.0 and 99.3 ppm with $J_{C1\text{-}H1}$ 163.4 Hz for Xyl*p*-β-C-1, and two signals at δ 99.1 ppm ($J_{C1\text{-}H1}$ 176 Hz) and 96.7 ppm ($J_{C1\text{-}H1}$ 172 Hz) for Man*p*-α-C-1. Deallylation (89%) of **9** and subsequent trichloroacetimidate formation (89%) produced the upstream tetrasaccharide donor **11**. The downstream disaccharide was readily prepared by the following way. Selective 3-*O*-allylation (85%) of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**12**) through dibutyltin complex, followed by acetylation (90%), debenzylidenation (90%), and selective 6-*O*-benzoylation (90%) furnished **16**. The ^1H NMR spectrum of **14** showed H-2 at δ 5.31 ppm with $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 3.5 Hz, and H-3 at δ 3.91 ppm with $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.9 Hz confirming the 3-*O*-allylation of **12**, and the ^1H NMR spectrum of **16** gave H-4 in the range of δ 4.18–3.87 ppm indicating the selective 6-*O*-benzoylation of **15**. Condensation (90%) of **16** with **2** followed by deallylation (85%) produced the downstream disaccharide acceptor **18**. Coupling of **18** with **11** went smoothly giving the hexasaccharide **19** (70%) with the ^{13}C NMR spectrum showing three signals at δ 102.6, 99.7, and 98.6 ppm with $J_{C1\text{-}H1}$ 163.4–164.1 Hz for Xyl*p*-β-C-1, and 98.5, 97.7, and 97.0 ppm with $J_{C1\text{-}H1}$ 173.4–176.1 Hz for Man*p*-α-C-1. Selective removal of 2-*O*-acetyl group of **19** by methanolysis[9] with $MeCOCl/MeOH/CH_2Cl_2$ (3.5/40/10 mL) afforded the hexasaccharide acceptor **20** in acceptable yield (45%). Condensation of **20** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**21**) in the presence of AgOTf furnished the heptaoside **22** in good yield (78%), and the ^{13}C NMR spectrum of **22** gave Glc*p*A-β-C-1 at δ 95.7 ppm with $J_{C1\text{-}H1}$ 166.8 Hz. Deprotection of **22** in ammonia-saturated methanol gave the target free heptaoside **23**.

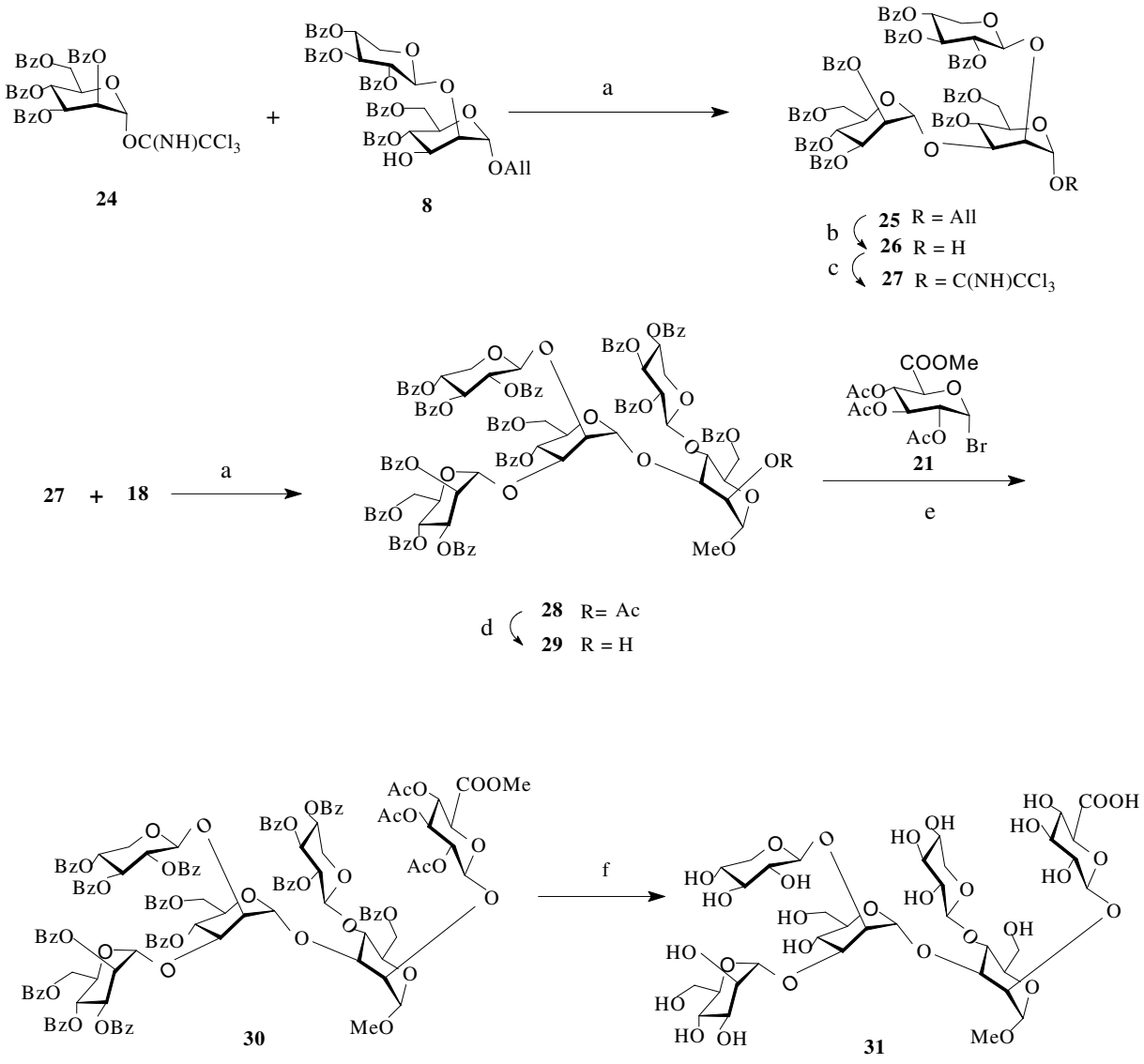
A hexasaccharide fragment of the GXM of serotypes B and C was also synthesized similarly as shown in Scheme 2. Thus, coupling (95%) of the disaccharide acceptor **8** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**24**) followed by deallylation (86%) and trichloroacetimidate formation (89%) afforded the trisaccharide donor **27**. Condensation (80%) of the disaccharide acceptor **18** with **27**, followed by selective deacetylation (42%) gave the pentasaccharide acceptor **29**. Coupling (76%) of **29** with **21** followed by deacylation produced the hexasaccharide fragment **31**.

3. Conclusion

In summary, a convergent synthesis of complex glucurono-xylo-mannosyl oligosaccharide was achieved through a regio- and stereoselective manner with readily accessible materials. The described method is suitable for preparation of other oligosaccharide fragments of the deacetylated GXM of serotypes A, B, and D.



Scheme 1. Reagents and conditions: (a) TMSOTf (0.01–0.05 equiv), CH_2Cl_2, −20 to 0 °C, 2–4 h, 85% for **3**, 62% for **9**, 90% for **17**, 70% for **19**, respectively; (b) 90% HOAc–H_2O, 70 °C, 2 h, 90% for **4**, 90% for **15**; (c) BzCl–pyridine, 80% for **5**; 90% for **16**; (d) $PdCl_2$, CH_3OH, rt, 4 h, 85% for **6**, 89% for **10**, 85% for **18**; (e) CCl_3CN, DBU, CH_2Cl_2, 3 h, 89% for **7**, 89% for **11**; (f) (i) MeOH, Bu_2SnO, reflux, (ii) AllBr, toluene; (g) Ac_2O–pyridine, 90% for **14**; (h) MeCOCl/MeOH/CH_2Cl_2 (3.5/40/10 mL), rt, 48 h, 45% for **20**; (i) 2,4-lutidine, silver triflate, CH_2Cl_2, −20 to 0 °C, 2–4 h, 78% for **22**; (j) satd NH_3–MeOH, rt, 72 h, 65% for **23**.



Scheme 2. Reagents and conditions: (a) TMSOTf (0.01–0.05 equiv), CH_2Cl_2, −20 to 0 °C, 2–4 h, 95% for **25**, 80% for **28**, respectively; (b) $PdCl_2$, CH_3OH, rt, 4 h, 86% for **26**; (c) CCl_3CN, DBU, CH_2Cl_2, 3 h, 89% for **27**; (d) MeCOCl/MeOH/CH_2Cl_2 (3.5/40/10 mL), rt, 48 h, 42% for **29**; (e) 2,4-lutidine, silver triflate, CH_2Cl_2, −20 to 0 °C, 2–4 h, 76% for **30**; (f) satd NH_3–MeOH, rt, 72 h, 64% for **31**.

4. Experimental

4.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^1H, 100 MHz for ^{13}C) for solutions in $CDCl_3$ or D_2O as indicated. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using MALTI-TOF-MS with CCA as matrix or recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (16 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

4.2. General procedure for the glycosylations

The mixture of donor and acceptor was dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2. TMSOTf (0.05 equiv) was added dropwise at −20 °C with nitrogen protection. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. Then the mixture was neutralized with Et_3N. Concentration of the reaction mixture, followed by purification on a silica gel column, gave the desired products.

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

As described in the general procedure, **1** (5.14 g, 12.50 mmol) and **2** (8.00 g, 13.16 mmol) were coupled, and the product was purified by column chromatography with 2.5:1 petroleum ether–EtOAc as the eluent to give **3** (9.02 g, 85%) as a foamy solid. $[\alpha]_D$ −38.9 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.13–7.25 (m, 25H, 5*Ph*), 5.87–5.77 (m, 1H, $-CH_2-CH{=}CH_2$), 5.66 (dd, 1H, $J_{3,4} = J_{2,3} = 4.9$ Hz, H-3 of Xyl*p*), 5.57 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.1$ Hz, H-3 of Man*p*), 5.38 (dd, 1H, $J_{1,2} = 4.0$ Hz, $J_{2,3} = 4.9$ Hz, H-2 of Xyl*p*), 5.31 (s, 1H, PhC*H*), 5.27–5.17 (m, 2H, $-CH_2-CH{=}CH_2$), 5.17 (m, 1H, H-4 of Xyl*p*), 4.89 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1 of Xyl*p*), 4.84 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1 of Man*p*), 4.47 (dd, 1H, $J_{1,2} = 1.3$ Hz, $J_{2,3} = 3.3$ Hz, H-2 of Man*p*), 4.30–3.43 (m, 8H, H-5 of Xyl*p*, H-4, 5, 6 of Man*p*, $-CH_2-CH{=}CH_2$). Anal. Calcd for $C_{49}H_{44}O_{14}$: C, 68.69; H, 5.14. Found: C, 68.77; H, 5.10.

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

A mixture of **3** (8.69 g, 10.15 mmol) and 90% HOAc–H_2O (100 mL) was stirred for 2 h at 70 °C, then concentrated to dryness. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave **4** (7.02 g, 90%) as an amorphous solid. $[\alpha]_D$ −78.6 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.13–7.35 (m, 20H, 4*Ph*), 5.83–5.76 (m, 1H, $-CH_2-CH{=}CH_2$), 5.76 (dd, 1H, $J_{2,3} = J_{3,4} = 5.0$ Hz, H-3 of Xyl*p*), 5.46 (dd, 1H, $J_{1,2} = 4.1$ Hz, $J_{2,3} = 5.0$ Hz, H-2 of Xyl*p*), 5.42 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 10.1$ Hz, H-3 of Man*p*), 5.28 (m, 1H, H-4 of Xyl*p*), 5.27–5.10 (m, 2H, $-CH_2-CH{=}CH_2$), 4.75 (d, 1H, $J_{1,2} = 4.1$ Hz, H-1 of Xyl*p*), 4.69 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1 of Man*p*), 4.27 (dd, 1H, $J_{1,2} = 1.2$ Hz, $J_{2,3} = 3.3$ Hz, H-2 of Man*p*), 4.30–3.33 (m, 8H, H-5 of Xyl*p*, H-4, 5, 6 of Man*p*, $-CH_2-CH{=}CH_2$). Anal. Calcd for $C_{42}H_{40}O_{14}$: C, 65.63; H, 5.21. Found: C, 65.58; H, 5.23.

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

Compound **4** (6.25 g, 8.14 mmol) was dissolved in pyridine (30 mL), and benzoyl chloride (3.5 mL, 30 mmol) was added dropwise within 30 min. The mixture was stirred overnight at rt, and TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Ice water was added, and the mixture was diluted with dichloromethane, washed with 1 M HCl, water, and satd aq sodium bicarbonate subsequently. The organic layer was combined, dried, and concentrated. Purification by column chromatography (2:1 petroleum ether–EtOAc) gave **5** (5.26 g, 80%) as an amorphous solid. $[\alpha]_D$ −23.7 (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.19–7.30 (m, 30H, 6*Ph*), 5.94 (m, 1H, $CH_2{=}CHCH_2O$), 5.92 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4 of Man*p*), 5.66 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 10.1$ Hz, H-3 of Man*p*), 5.63 (dd, 1H, $J_{2,3} = J_{3,4} = 4.9$ Hz, H-3 of Xyl*p*), 5.34 (dd, 1H, $J_{1,2} = 3.6$ Hz, $J_{2,3} = 4.9$ Hz, H-2 of Xyl*p*), 5.33–5.20 (m, 2H, $CH_2{=}CHCH_2O$), 5.12 (m, 1H, H-4 of Xyl*p*), 4.99 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1 of Man*p*), 4.98 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1 of Xyl*p*), 4.50 (dd, 1H, $J_{1,2} = 1.6$ Hz, $J_{2,3} = 3.3$ Hz, H-2 of Man*p*), 4.40–4.33 (m, 2H, H-5 of Xyl*p*), 4.30 (ddd, 1H, $J_{4,5} = 10.1$ Hz, $J_{5,6a} = 6.3$ Hz, $J_{5,6b} = 4.6$ Hz, H-5 of Man*p*), 4.20 (m, 1H, $CH_2{=}CHCH_2O$), 4.06 (dd, 1H, $J_{5,6a} = 6.3$ Hz, $J_{6a,6b} = 12.0$ Hz, H-6a of Man*p*), 4.00 (m, 1H, $CH_2{=}CHCH_2$), 3.33 (dd, 1H, $J_{5,6b} = 4.6$ Hz, $J_{6a,6b} = 12.0$ Hz, H-6b of Man*p*). Anal. Calcd for $C_{56}H_{48}O_{16}$: C, 68.85; H, 4.92. Found: C, 68.78; H, 5.00.

4.6. 2,3,4-Tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranose (6)

To a solution of **5** (4.29 g, 5.31 mmol) in anhyd MeOH (25 mL) was added $PdCl_2$ (20 mg). After stirring the mixture at rt for 2 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the solution was concentrated to dryness, and the resultant residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **6** (4.22 g, 85%) as an amorphous solid. $[\alpha]_D$ −23.7 (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.19–7.30 (m, 30H, 6*Ph*), 5.94 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.70 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 10.0$ Hz, H-3 of Man*p*), 5.63 (dd, 1H, $J_{2,3} = J_{3,4} = 4.9$ Hz, H-3 of Xyl*p*), 5.40 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1 of Man*p*), 5.32 (dd, 1H, $J_{1,2} = 3.8$ Hz, $J_{2,3} = 4.9$ Hz, H-2 of Xyl*p*), 5.14 (m, 1H, H-4 of Xyl*p*), 4.94 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1 of Xyl*p*), 4.50 (dd, 1H, $J_{1,2} = 1.4$ Hz, $J_{2,3} = 3.3$ Hz, H-2 of Man*p*), 4.40–3.33 (m, 5H, H-5 of Xyl*p*, H-5, 6 of Man*p*). Anal. Calcd for $C_{53}H_{44}O_{16}$: C, 67.95; H, 4.70. Found: C, 68.04; H, 4.72.

4.7. 2,3,4-Tri-*O*-benzoyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosoyl trichloroacetimidate (7)

A mixture of **6** (4.02 g, 4.30 mmol), trichloroacetonitrile (1.0 mL, 10.0 mmol) and 1,8-diazabicyclo[5.4.0]-undecene (DBU) (0.5 mL) in dry CH_2Cl_2 (50 mL) was stirred under nitrogen for 3 h and then concentrated. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to give **7** (3.71 g, 89%) as an amorphous solid. $[\alpha]_D$ −17.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H, $CNHCCL_3$), 8.18–7.12 (m, 30H, 6*Ph*), 6.44 (d, 1H, H-1 of Man*p*), 6.03 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4 of Man*p*), 5.68 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 10.1$ Hz, H-3 of Man*p*), 5.66 (dd, 1H, $J_{2,3} = J_{3,4} = 5.1$ Hz, H-3 of Xyl*p*), 5.40 (dd, 1H, $J_{1,2} = 3.8$ Hz, $J_{2,3} = 5.1$ Hz, H-2 of Xyl*p*), 5.13 (m, 1H, H-4 of Xyl*p*), 5.09 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1 of Xyl*p*), 4.75 (dd, 1H, $J_{1,2} = 0.6$ Hz, $J_{2,3} = 3.2$ Hz, H-2 of Man*p*), 4.50 (ddd, 1H, $J_{4,5} = 10.1$ Hz, $J_{5,6a} = 6.3$ Hz, $J_{5,6b} = 4.6$ Hz, H-5 of Man*p*), 4.44 (dd, 1H, $J_{4,5a} = 2.8$ Hz, $J_{5a,5b} = 12.1$ Hz, H-5a of Xyl*p*), 4.34 (dd, 1H, $J_{4,5b} = 3.2$ Hz, $J_{5a,5b} = 12.1$ Hz, H-5b of Xyl*p*), 4.16 (dd, 1H, $J_{5,6a} = 5.8$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6a of Man*p*), 3.44 (dd, 1H, $J_{5,6b} = 5.0$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6b of

Man*p*). Anal. Calcd for $C_{55}H_{44}Cl_3NO_{16}$: C, 61.09; H, 4.10. Found: C, 61.19; H, 4.18.

4.8. Allyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(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-mannopyranoside (9)

As described in the general procedure, **7** (2.33 g, 2.44 mmol) and **8** (2.27 g, 2.60 mmol) were coupled, and the product was purified by column chromatography with 2:1 petroleum ether–EtOAc as the eluent to give **9** (2.72 g, 62%) as an amorphous solid. $[\alpha]_D$ −92.9 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16–7.31 (m, 55H, 11*Ph*), 5.85 (m, 1H, CH_2=C*H*CH_2O), 5.84 (dd, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.76–5.62 (m, 3H, H-4 of Man*p*, H-3 of Xyl*p*), 5.49 (m, 1H, H-4 of Xyl*p*), 5.45 (dd, 1H, $J_{1,2} = J_{2,3} = 5.7$ Hz, H-2 of Xyl*p*), 5.35–5.07 (m, 3H, H-3 of Man*p*, H-2 of Xyl*p*, H-4 of Xyl*p*), 5.10 (d, 1H, $J_{1,2} = 0.7$ Hz, H-1 of Man*p*), 5.07 (d, 1H, $J_{1,2} = 4.2$ Hz, H-1 of Xyl*p*), 4.87 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1 of Man*p*), 4.43 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1 of Xyl*p*), 4.35–3.88 (m, 15H, H-5 of Xyl*p*, H-2, 3, 5 of Man*p*, CH_2=CHC*H*$_2$O), 2.92–2.81 (m, 2H, H-6a, H-6b of Man*p*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 166.1, 165.6, 165.6, 165.5, 165.4, 165.3, 165.3, 165.1, 164.8, 164.7 (11C, 11Ph*CO*), 118.3 (*C*H_2=*C*HCH$_2$O), 100.0 ($J_{C1\text{-}H1}$ 163.4 Hz), 99.3 ($J_{C1\text{-}H1}$ 163.4 Hz), 99.1 ($J_{C1\text{-}H1}$ 176 Hz), 96.7 ($J_{C1\text{-}H1}$ 172 Hz) (4C, 4C-1), 78.2, 76.0, 75.9, 70.3, 69.8, 69.8, 69.7, 69.3, 68.8, 68.7, 68.7, 68.6, 68.1, 67.6, 64.0, 63.7, 60.6, 60.5 (C-2 to C-6). Anal. Calcd for $C_{102}H_{86}O_{30}$: C, 68.37; H, 4.84. Found: C, 68.52; H, 4.89.

4.9. 2,3,4-Tri-*O*-benzoyl-β-D-xylopyranosyl-(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-mannopyranose (10)

Deallylation of tetrasaccharide **9** (2.69 g, 1.5 mmol) under the same conditions as that used for preparation of **6** from **5** gave a residue, which was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to give **10** (2.36 g, 89%) as an amorphous solid. $[\alpha]_D$ −69.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17–7.21 (m, 55H, 11*Ph*), 5.82 (dd, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.79–5.67 (m, 3H), 5.51 (m, 1H, H-4 of Xyl*p*), 5.40 (dd, 1H, $J_{1,2} = J_{2,3} = 5.7$ Hz, H-2 of Xyl*p*), 5.33–5.15 (m, 3H), 5.20 (d, 1H, $J_{1,2} = 0.7$ Hz, H-1 of Man*p*), 5.09 (d, 1H, $J_{1,2} = 4.2$ Hz, H-1 of Xyl*p*), 5.06 (m, 1H, H-4 of Xyl*p*), 5.00 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1 of Man*p*), 4.87 (dd, 1H, $J_{2,3} = 5.4$ Hz, $J_{3,4} = 6.0$ Hz, H-3 of Xyl*p*), 4.36 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1 of Xyl*p*), 4.36–2.86 (m, 13H), 2.92–2.81 (dd, 2H, H-6a H-6b of Man*p*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 166.1, 165.6, 165.5, 165.5, 165.3, 165.2, 165.2, 165.1, 164.7, 164.6 (11C, 11Ph*CO*), 99.5, 99.1, 98.2, 92.2 (4C, 4C-1), 77.9, 77.3, 75.6, 75.8, 70.0, 69.8, 69.7, 69.6, 69.4, 69.2, 68.6, 68.3, 68.0, 67.9, 64.2, 63.9, 60.5, 60.0 (C-2 to C-6). Anal. Calcd for $C_{99}H_{82}O_{30}$: C, 67.89; H, 4.69. Found: C, 67.99; H, 4.63.

4.10. 2,3,4-Tri-*O*-benzoyl-β-D-xylopyranosyl-(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 trichloroacetimidate (11)

A mixture of **10** (1.5 g, 0.86 mmol), trichloroacetonitrile (200 μL, 2 mmol) and 1,8-diazabicyclo[5.4.0]-undecene (DBU) (200 μL) in dry CH_2Cl_2 (30 mL) was stirred under nitrogen for 3 h and then concentrated. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **11** (1.47 g, 89%) as an amorphous solid. $[\alpha]_D$ −71.2 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H, CN*H*CCL_3), 8.13–7.31 (m, 55H, 11*Ph*), 6.40 (s, 1H, H-1, Man*p*), 5.82 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4, Man*p*), 5.78 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 9.8$ Hz, H-3, Man*p*), 5.74 (dd, 1H, $J_{1,2} = J_{2,3} = 4.1$ Hz, H-2, Xyl*p*), 5.72 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4, Man*p*), 5.51 (m, 1H, H-4, Xyl*p*), 5.45 (dd, 1H, $J_{1,2} = J_{2,3} = 4.0$ Hz, H-2, Xyl*p*), 5.36 (dd, 1H, $J_{2,3} = 4.0$ Hz, $J_{3,4} = 6.5$ Hz, H-3, Xyl*p*), 5.16 (d, 1H, $J_{1,2} = 4.1$ Hz, H-1, Xyl*p*), 5.06 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1, Man*p*), 5.04 (m, 1H, H-4, Xyl*p*), 4.58 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1, Xyl*p*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 166.0, 165.4, 165.4, 165.4, 165.3, 165.2, 165.1, 165.0, 164.6, 164.5 (11C, 11Ph*CO*), 100.0, 99.0, 98.9, 94.9, (4C, 4C-1), 75.9, 75.6, 75.3, 71.4, 69.9, 69.5, 69.5, 69.5, 69.2, 69.2, 68.8, 68.4, 68.2, 68.0, 67.9, 64.0, 63.4, 60.5 (C-2 to C-6). Anal. Calcd for $C_{101}H_{82}Cl_3NO_{30}$: C, 63.98; H, 4.36. Found: C, 63.75; H, 4.41.

4.11. Methyl 3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (13)

To a solution of **12** (4.35 g, 21.2 mmol) in CH_3OH (50 mL) was added Bu_2SnO (5.22 g, 21.2 mmol), and the mixture was heated under reflux for 2 h, then concentrated to dryness. The residue was diluted with toluene (60 mL), and then allyl bromide (17.1 mL, 200 mmol), Bu_4NI (7.38 g, 20.0 mmol) were added to the mixture. The reaction was carried out at 60 °C for 24 h, at which time TLC (1:1 petroleum ether–EtOAc) showed that the reaction was complete. The solution was concentrated to dryness. The residue was passed through a short silica gel column to give **13** (5.22 g, 85%) as an amorphous solid. $[\alpha]_D$ +12.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.35 (m, 5H, 1*Ph*), 5.96–5.86 (m, 1H, –CH_2–C*H*=CH_2), 5.59 (s, 1H, *Ph*C*H*), 5.32–5.17 (m, 2H, –CH_2–CH=CH_2), 4.77 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1), 4.31–3.80 (m, 8H, H-2, H-3, H-4, H-5, H-6, –CH_2–CH=CH_2), 3.38 (s, 3H, OCH_3). Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.35; H, 6.83. Found: C, 63.44; H, 6.80.

4.12. Methyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (14)

Compound **13** (4.14 g, 12.86 mmol) was dissolved in pyridine (30 mL), and Ac_2O (6.00 mL, 50 mmol) was added. The mixture was stirred at rt for 12 h, then was concentrated to give a residue. Purification of the residue by column chromatography (3:1 petroleum ether–EtOAc) gave **14** (4.17 g, 90.0%) as a syrup. $[\alpha]_D$ +10.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.33 (m, 5H, 1*Ph*),

5.90–5.82 (m, 1H, –CH_2–C*H*=CH_2), 5.59 (s, 1H, *PhCH*), 5.31 (dd, 1H, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 5.32–5.13 (m, 2H, –CH_2–CH=C*H*$_2$), 4.66 (d, 1H, H-1), 3.91 (dd, 1H, $J_{3,4}$ = 9.9 Hz, H-3), 4.66–3.92 (m, 6H, H-4, H-5, H-6, –C*H*$_2$–CH=CH_2), 3.37 (s, 3H, OC*H*$_3$), 2.14 (s, 3H, C*H*$_3$CO). Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.64; H, 6.59. Found: C, 62.81; H, 6.52.

4.13. Methyl 2-*O*-acetyl-3-*O*-allyl-α-D-mannopyranoside (15)

A mixture of **14** (4.1 g, 11.26 mmol) and 90% HOAc–H_2O (50 mL) was stirred for 2 h at 70 °C, then concentrated to dryness. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave **15** (2.95 g, 90%) as an amorphous solid. $[\alpha]_D$ +14.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.93–5.83 (m, 1H, –CH_2–C*H*=CH_2), 5.26 (dd, 1H, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.31–5.19 (m, 2H, –CH_2–CH=C*H*$_2$), 4.68 (d, 1H, H-1), 3.69 (dd, 1H, $J_{3,4}$ = 9.9 Hz, H-3), 4.66–3.92 (m, 6H, H-4, H-5, H-6, –C*H*$_2$–CH=CH_2), 3.38 (s, 3H, OC*H*$_3$), 2.12 (s, 3H, C*H*$_3$CO). Anal. Calcd for $C_{12}H_{20}O_7$: C, 52.17; H, 7.25. Found: C, 52.30; H, 7.20.

4.14. Methyl 2-*O*-acetyl-3-*O*-allyl-6-*O*-benzoyl-α-D-mannopyranoside (16)

Compound **15** (2.90 g, 10.51 mmol) was dissolved in anhyd CH_2Cl_2 (30 mL) containing pyridine (4.1 mL, 50 mmol), then under N_2 protection and stirring, a solution of benzoyl chloride (1.23 mL, 10.51 mmol) in anhyd dichloromethane (6 mL) was added dropwise within 20 min at 0 °C. The reaction temperature slowly raised to rt. After stirring the mixture for 8 h, TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated to dryness. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave **16** (3.60 g, 90%) as an amorphous solid. $[\alpha]_D$ +13.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–7.40 (m, 5H, 1*Ph*), 5.93–5.83 (m, 1H, –CH_2–C*H*=CH_2), 5.27 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.31–5.18 (m, 2H, –CH_2–CH=C*H*$_2$), 4.71 (d, 1H, H-1), 4.64–4.61 (m, 2H, H-6a, H-6b), 3.73 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 4.18–3.87 (m, 4H, H-4, H-5, –C*H*$_2$–CH=CH_2), 3.39 (s, 3H, OC*H*$_3$), 2.08 (s, 3H, C*H*$_3$CO). Anal. Calcd for $C_{19}H_{24}O_8$: C, 60.00; H, 6.32. Found: C, 60.18; H, 6.30.

4.15. Methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-acetyl-3-*O*-allyl-6-*O*-benzoyl-α-D-mannopyranoside (17)

As described in the general procedure, **16** (3.00 g, 7.89 mmol) and **2** (4.86 g, 8.00 mmol) were coupled, and the product was purified by column chromatography with 2.5:1 petroleum ether–EtOAc as the eluent to give **17** (5.85 g, 90%) as an amorphous solid. $[\alpha]_D$ −25.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98–7.17 (m, 20H, 4Ph*H*), 5.96–5.87 (m, 1H, CH_2=C*H*CH_2O–), 5.78 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.3 Hz, H-3 of Xyl*p*), 5.43 (dd, 1H, $J_{1,2}$ = 6.4 Hz, H-2 of Xyl*p*), 5.32 (m, 1H, H-4 of Xyl*p*), 5.26 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2 of Man*p*), 5.34–5.18 (m, 2H, C*H*$_2$=CHCH_2O), 5.03 (d, 1H, $J_{1,2}$ = 6.4 Hz, H-1 of Xyl*p*), 4.62 (d, 1H, H-1 of Man*p*), 4.60 (dd, 1H, $J_{5,6a}$ = 1.6 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a of Man*p*), 4.47 (dd, 1H, $J_{4,5a}$ = 4.8 Hz, $J_{5a,5b}$ = 12.0 Hz, H-5a of Xyl*p*), 4.36 (dd, 1H, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b of Man*p*), 4.20–4.06 (m, 2H, CH_2=CHC*H*$_2$O), 4.14 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4 of Man*p*), 3.90 (d, 1H, $J_{2,3}$ = 3.4 Hz, H-3 of Man*p*), 3.81 (m, 1H, H-5 of Man*p*), 3.60 (dd, 1H, $J_{4,5a}$ = 8.2 Hz, $J_{5a,5b}$ = 12.0 Hz, H-5b of Xyl*p*), 3.32 (s, 3H, OC*H*$_3$), 2.03 (s, 3H, C*H*$_3$CO). Anal. Calcd for $C_{45}H_{44}O_{15}$: C, 65.53; H, 5.34. Found: C, 65.67; H, 5.32.

4.16. Methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzoyl-α-D-mannopyranoside (18)

Deallylation of tetrasaccharide **17** (5.22 g, 6.33 mmol) under the same conditions as that used for the preparation of **6** from **5** gave a residue. Then the residue was passed through a short silica gel column to give **18** (4.21 g, 85%) as an amorphous solid. $[\alpha]_D$ −14.5 (*c* 1.3, H_2O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96–7.27 (m, 20H, 4Ph*H*), 5.85 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3 of Xyl*p*), 5.55 (dd, 1H, $J_{1,2}$ = 7.3 Hz, H-2 of Xyl*p*), 5.40 (m, 1H, H-4 of Xyl*p*), 5.17 (dd, 1H, $J_{1,2}$ = 1.7 Hz, H-2 of Man*p*), 4.86 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1 of Xyl*p*), 4.68 (d, 1H, H-1 of Man*p*), 4.50 (dd, 1H, $J_{4,5a}$ = 5.2 Hz, $J_{5a,5b}$ = 11.7 Hz, H-5a of Xyl*p*), 4.36–4.30 (m, 2H, H-6a, H-6b of Man*p*), 4.12 (dd, 1H, $J_{2,3}$ = 3.6 Hz, H-3 of Man*p*), 3.90 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4 of Man*p*), 3.83 (m, 1H, H-5 of Man*p*), 3.67 (dd, 1H, $J_{4,5b}$ = 9.6 Hz, $J_{5a,5b}$ = 11.7 Hz, H-5b of Xyl*p*), 3.33 (s, 3H, OC*H*$_3$), 2.08 (s, 3H, C*H*$_3$CO). Anal. Calcd for $C_{42}H_{40}O_{15}$: C, 64.29; H, 5.10. Found: C, 64.10; H, 5.15.

4.17. Methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(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→4)]-2-*O*-acetyl-6-*O*-benzoyl-α-D-mannopyranoside (19)

As described in the general procedure, **11** (1.00 g, 0.53 mmol) and **17** (0.60 g, 1.00 mmol) were coupled, and the product was purified by silica gel column chromatography with 1:1 petroleum ether–EtOAc as the eluent to give **19** (0.91 g, 70%) as an amorphous solid. $[\alpha]_D$ −46.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.14–7.03 (m, 75H, 15Ph*H*), 5.90 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.2 Hz, H-3 of Man*p*), 5.86 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 5.8 Hz, H-3 of Xyl*p*), 5.85 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 7.0 Hz, H-3 of Xyl*p*), 5.84 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.2 Hz, H-4 of Man*p*), 5.71 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4 of Man*p*), 5.69 (m, 1H, H-4 of Xyl*p*), 5.57–5.46 (m, 4H), 5.32 (m, 1H, H-4 of Xyl*p*), 5.30 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1 of Xyl*p*), 5.28 (s, 1H, H-1 of Man*p*), 5.19 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1 of Xyl*p*), 5.17 (s, 1H, H-1 of Man*p*), 5.02 (m, 1H, H-4 of Xyl*p*), 5.00 (dd, 1H, H-2 of Man*p*), 4.68 (d, 1H, $J_{1,2}$ = 4.8 Hz, H-1 of Xyl*p*), 4.56 (s, 1H, H-1 of Man*p*), 4.75–2.87 (m, 20H), 3.26 (s, 3H, OC*H*$_3$), 1.81 (s, 3H, COC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.3 (*C*OCH$_3$), 166.2,

166.0, 165.9, 165.7, 165.5, 165.4, 165.3, 165.3, 165.3, 165.3, 165.2, 164.6, 164.6, 164.5, 164.5 (15C, 15*C*OPh), 102.6 ($J_{C1\text{-}H1}$ 163.4 Hz), 99.7 ($J_{C1\text{-}H1}$ 163.4 Hz), 98.6 ($J_{C1\text{-}H1}$ 164.1 Hz), 98.5 ($J_{C1\text{-}H1}$ 173.4 Hz), 97.7 ($J_{C1\text{-}H1}$ 174.2 Hz), 97.0 ($J_{C1\text{-}H1}$ 176.1 Hz) (6C, C-1), 78.9, 77.6, 75.2, 74.7, 74.6, 74.6, 74.1, 72.4, 71.6, 71.0, 70.3, 70.2, 69.9, 69.7, 69.7, 69.6, 69.3, 69.2, 68.7, 68.4, 67.7, 63.8, 63.2, 63.1, 62.6, 60.5, 59.7 (C-2 to C-6), 54.9 (O*C*H$_3$), 20.51 (CO*C*H$_3$). Anal. Calcd for $C_{141}H_{120}O_{44}$: C, 67.25; H, 4.77. Found: C, 67.50; H, 4.82.

4.18. Methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(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→4)]-6-*O*-benzoyl-α-D-mannopyranoside (20)

To a solution of **19** (600 mg, 0.24 mmol) in anhyd CH_2Cl_2 (10 mL) was added anhyd MeOH (40 mL), then acetyl chloride (3.5 mL) was added to the reaction mixture at 0 °C. The mixture was stirred at rt for 3 days, TLC (1:1 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was neutralized with Et_3N, then concentrated to dryness. Purification of the residue by silica gel column chromatography (1:1.2 petroleum ether–EtOAc) gave **20** (210 mg, 45%) as an amorphous solid. $[\alpha]_D$ −47.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17–7.05 (m, 75H, 15Ph*H*), 5.93 (dd, 1H, $J_{3,4} = J_{2,3} = 5.6$ Hz, H-3 of Xyl*p*), 5.92 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 10.1$ Hz, H-3 of Man*p*), 5.89 (dd, 1H, $J_{3,4} = J_{4,5} = 7.2$ Hz, H-3 of Xyl*p*), 5.82 (dd, 1H, $J_{2,3} = J_{3,4} = 10.2$ Hz, H-4 of Man*p*), 5.73 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4 of Man*p*), 5.71–5.48 (m, 5H), 5.39–5.33 (m, 2H), 5.27 (s, 1H, H-1 of Man*p*), 5.08 (d, 1H, $J_{1,2} = 4.8$ Hz, H-1 of Xyl*p*), 5.08–5.00 (m, 2H, H-4 of Xyl*p*), 4.65 (d, 1H, $J_{1,2} = 4.9$ Hz, H-1 of Xyl*p*), 4.64 (s, 1H, H-1 of Man*p*), 4.53 (d, 1H, $J_{1,2} = 4.9$ Hz, H-1 of Xyl*p*), 4.51 (s, 1H, H-1 of Man*p*), 4.55–2.98 (m, 19H), 3.16 (s, 3H, OC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 166.3, 166.0, 166.0, 165.5, 165.4, 165.4, 165.4, 165.3, 165.2, 165.2, 165.2, 164.8, 164.8, 164.6, 164.6 (15C, 15*C*OPh), 102.4, 100.0, 99.1, 99.0, 98.0, 97.3 (6C, C-1), 77.2, 76.2, 74.8, 74.8, 74.8, 72.1, 71.5, 70.9, 70.3, 70.2, 70.1, 69.8, 69.7, 69.6, 69.5, 69.2, 69.1, 69.0, 68.7, 68.3, 67.6, 64.3, 64.1, 63.3, 62.9, 60.2, 59.9 (C-2 to C-6), 54.8 (O*C*H$_3$). Anal. Calcd for $C_{139}H_{118}O_{43}$: C, 67.42; H, 4.77. Found: C, 67.68; H, 4.72.

4.19. Methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(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)-[methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1→2)][2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)]-6-*O*-benzoyl-α-D-mannopyranoside (22)

To a cooled solution (0 °C) of **20** (200 mg, 0.09 mmol), **21** (92 mg, 0.24 mmol), and 2,4-lutidine (10 μL, 0.10 mmol) in anhyd CH_2Cl_2 (20 mL), was added silver triflate (45 mg, 0.24 mmol). The mixture was stirred at this temperature for 6 h, TLC (1:1.5 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was concentrated to dryness. Purification of the residue by silica gel column chromatography (1:1.3 petroleum ether–EtOAc) gave **22** (210 mg, 78%) as an amorphous solid. $[\alpha]_D$ −61.7 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20–6.90 (m, 75H, 15Ph*H*), 6.09 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4, Man*p*), 6.00 (dd, 1H, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4, Man*p*), 5.92 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 9.8$ Hz, H-3 of Man*p*), 5.27 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1, Man*p*), 5.16 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1, Man*p*), 5.10 (d, 1H, $J_{1,2} = 1.1$ Hz, H-1, Man*p*), 4.88 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1, Xyl*p*), 4.80 (d, 1H, $J_{1,2} = 7.0$ Hz, H-1, Xyl*p*), 4.72 (d, 1H, $J_{1,2} = 7.1$ Hz, H-1, Xyl*p*), 4.44 (dd, 1H, $J_{1,2} = 1.1$ Hz, $J_{2,3} = 3.3$ Hz, H-2, Man*p*), 4.13 (dd, 1H, $J_{1,2} = 1.0$ Hz, $J_{2,3} = 3.2$ Hz, H-2, Man*p*), 4.00 (d, 1H, $J_{1,2} = 7.0$ Hz, H-1, Glc*p*A), 3.79 (dd, 1H, $J_{1,2} = 1.2$ Hz, $J_{2,3} = 2.9$ Hz, H-2, Man*p*), 3.65 (s, 3H, COOC*H*$_3$), 3,24 (s, 3H, OC*H*$_3$), 2.13, 2.12, 2.03 (3s, 9H, 3COC*H*$_3$). ^{13}C NMR (100 MHz, $CDCI_3$): 171.2, 171.0, 169.8, 169.2 (4C, 3*C*OCH$_3$, *C*OOMe), 167.3, 167.2, 166.1, 166.0, 165.8, 165.8, 165.4, 165.3, 165.3, 165.3, 165.3, 165.2, 165.2, 165.1, 164.6 (15C, 15*C*OPh), 102.7 ($J_{C1\text{-}H1}$ 163.2 Hz), 99.3 ($J_{C1\text{-}H1}$ 164.1 Hz), 99.0 ($J_{C1\text{-}H1}$ 163.2 Hz), 97.9 ($J_{C1\text{-}H1}$ 176.2 Hz), 96.4 ($J_{C1\text{-}H1}$ 173.8 Hz), 96.4 ($J_{C1\text{-}H1}$ 173.4 Hz), 95.7 ($J_{C1\text{-}H1}$ 166.8 Hz) (7C, 7C-1), 54.8 (O*C*H$_3$), 52.8 (COO*C*$_3$), 20.8, 20.8, 20.5 (3C, 3CO*C*H$_3$). Anal. Calcd for $C_{152}H_{134}O_{52}$: C, 65.13; H, 4.85. Found: C, 65.32; H, 4.91; Negative-ESI MS calcd for $C_{152}H_{134}O_{52}$: [M] 2790.1. Found: [M+Na] 2812.7, [M+K] 2828.7.

4.20. Methyl β-D-xylopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[(β-D-glucopyranosyluronic acid)-(1→2)][β-D-xylopyranosyl-(1→4)]-α-D-mannopyranoside, ammonium salt (23)

Heptasaccharide **22** (200 mg, 0.07 mmol) was dissolved in a satd methanolic ammonia (50 mL). After 96 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 heptasaccharide **23** (63 mg, 64.5%) as an amorphous solid. $[\alpha]_D$ +56.9 (*c* 0.5, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.05 (s, 1H, H-1, Man*p*), 4.97 (s, 1H, H-1, Man*p*), 4.65 (s, 1H, H-1, Man*p*), 4.59 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1, Glc*p*A), 4.38 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1, Xyl*p*), 4.34 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1, Xyl*p*), 4.30 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1, Xyl*p*), 3.31 (s, 3H, OC*H*$_3$); ^{13}C NMR (100 MHz, D_2O): 173.9 (–*C*OONH$_4$), 103.5, 103.5, 103.3, 102.7, 100.6, 99.1, 96.0 (7C, 7C-1), 78.4, 78.2, 76.2, 76.1, 76.0, 75.7, 74.1, 73.5, 73.4, 72.8, 72.2, 70.5, 70.2, 69.4, 66.9, 66.5, 66.2, 65.3, 65.3, 61.5, 60.4, 60.4 (C-2 to C-6), 54.5 (O*C*H$_3$). MALDI-TOF MS Calcd for the ammonium salt of **22**, $C_{40}H_{69}NO_{34}$: 1107.9 [M]. Found: 1107.9 (M); 1113.0 ($M - NH_4^+ + Na^+$).

4.21. Allyl 2,3,4,6-tetra-*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 (25)

As described in the general procedure, **24** (2.00 g, 2.70 mmol) and **8** (2.26 g, 2.60 mmol) were coupled,

and the product was purified by column chromatography with 2.5:1 petroleum ether–EtOAc as the eluent to give **25** (3.60 g, 95%) as an amorphous solid. $[\alpha]_D$ −82.9 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.06–7.21 (m, 45H, 9*Ph*), 6.00 (dd, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.98 (dd, 1H, $J_{2,3} = J_{3,4} = 5.1$ Hz, H-3 of Xyl*p*), 5.80 (m, 1H, $CH_2{=}CHCH_2O$), 5.80–5.69 (m, 2H, H-3 of Man*p*, H-2 of Xyl*p*), 5.50 (m, 1H, H-4 of Xyl*p*), 5.42–5.39 (m, 2H, H-4 of Man*p*, H-2 of Man*p*), 5.34 (s, 1H, H-1 of Man*p*), 5.12 (d, 1H, $J_{1,2} = 4.2$ Hz, H-1 of Xyl*p*), 5.20–5.11 (m, 2H, $CH_2{=}CHCH_2O$), 4.96 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1 of Man*p*), 4.86–3.86 (m, 12H, $CH_2{=}CHCH_2O$, H-5 of Xyl*p*, H-2, 3, 5, 6 of Man*p*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 166.0, 165.7, 165.4, 165.3, 165.3, 165.1, 164.9, 164.4 (9C, 9Ph*CO*), 118.2 ($CH_2{=}CHCH_2O$), 99.8, 98.6, 96.4 (3C, 3C-1), 77.6, 76.2, 75.9, 70.3, 69.8, 69.4, 69.0, 68.9, 68.6, 68.1, 67.6, 63.9, 63.9, 63.3 (C-2 to C-6). Anal. Calcd for $C_{83}H_{70}O_{24}$: C, 68.69; H, 4.83. Found: C, 68.62; H, 4.90.

4.22. 2,3,4,6-Tetra-*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-mannopyranose (26)

Deallylation of tetrasaccharide **25** (3.22 g, 2.22 mmol) under the same conditions as that used for preparation of **6** from **5** gave a residue, which was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **26** (2.66 g, 86%) as an amorphous solid. $[\alpha]_D$ −30.4 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.06–7.14 (m, 45H, 9 Ph*H*), 6.03 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 10.1$ Hz, H-3 of Man*p*), 5.96 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4 of Man*p*), 5.77 (dd, 1H, $J_{2,3} = J_{3,4} = 5.1$ Hz, H-3 of Xyl*p*), 5.76 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.52 (m, 1H, H-4 of Xyl*p*), 5.44 (dd, 1H, H-2 of Man*p*), 5.38 (dd, 1H, $J_{1,2} = 6.4$ Hz, H-2 of Xyl*p*), 5.37 (s, 1H, H-1 of Man*p*), 5.34 (d, 1H, $J_{1,2} = 6.4$ Hz, H-1 of Xyl*p*), 4.94 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1 of Man*p*), 4.97–3.35 (m, 12H, $CH_2{=}CHCH_2O$, H-5 of Xyl*p*, H-2, 3, 5, 6 of Man*p*); ^{13}C NMR (100 MHz, $CDCl_3$): 166.1, 165.7, 165.5, 165.2, 165.1, 165.0, 164.9, 164.9, 164.8, 164.6, (10C, 10*C*OPh), 99.6, 98.3, 92.1 (3C, 3C-1), 75.6, 75.1, 70.4, 69.6, 69.4, 69.0, 68.8, 69.3, 68.0, 67.7, 63.9, 63.4, 60.4, 60.2 (C-2 to C-6). Anal. Calcd for $C_{80}H_{66}O_{24}$: C, 68.09; H, 4.68. Found: C, 68.25; H, 4.72.

4.23. 2,3,4,6-Tetra-*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 (27)

A mixture of **26** (2.60 g, 1.84 mmol), trichloroacetonitrile (0.4 mL, 4.0 mmol) and 1,8-diazabicyclo[5.4.0]-undecene (DBU) (0.3 mL) in dry CH_2Cl_2 (10 mL) was stirred under nitrogen for 3 h and then concentrated. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **27** (2.58 g, 89%) as an amorphous solid: $[\alpha]_D$ −71.2 (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H, CN*H*CCl$_3$), 8.05–7.19 (m, 45H, 9*Ph*), 6.46 (s, 1H, H-1, Man*p*), 6.10 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4 of Man*p*), 6.04 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 9.7$ Hz, H-3 of Man*p*), 5.79 (dd, 1H, $J_{2,3} = J_{3,4} = 5.4$ Hz, H-3 of Xyl*p*), 5.76 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4 of Man*p*), 5.52 (d, 1H, H-2 of Man*p*), 5.52 (m, 1H, H-4 of Xyl*p*), 5.48 (dd, 1H, $J_{1,2} = 4.0$ Hz, H-2 of Xyl*p*), 5.39 (s, 1H, H-1 of Man*p*), 5.25 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1 of Xyl*p*), 4.80–4.03 (m, 10H, $CH_2{=}CHCH_2O$, H-5 of Xyl*p*, H-2, 3, 5, 6 of Man*p*). ^{13}C NMR (100 MHz, $CDCl_3$): 166.1, 165.9, 165.6, 165.4, 165.3, 165.2, 165.1, 165.0, 164.8, (9C, 9*C*OPh), 99.8, 98.6, 94.9 (3C, 3C-1), 75.6, 75.4, 71.7, 70.3, 69.7, 69.5, 69.8, 68.6, 68.0, 67.3, 63.3, 62.9, 60.3, 60.2 (C-2 to C-6). Anal. Calcd for $C_{82}H_{66}Cl_3NO_{24}$: C, 63.28; H, 4.24. Found: C, 63.43; H, 4.19.

4.24. Methyl 2,3,4,6-tetra-*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→4)]-2-*O*-acetyl-6-*O*-benzoyl-α-D-mannopyranoside (28)

As described in the general procedure, **27** (1.21 g, 0.78 mmol) and **17** (0.72 g, 0.92 mmol) were coupled, and the product was purified by silica gel column chromatography with 1:1 petroleum ether–EtOAc as the eluent to give **28** (1.36 g, 80%) as an amorphous solid. $[\alpha]_D$ −16.9 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.16–7.04 (m, 65H, 13Ph*H*), 6.15 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 6.05 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 10.0$ Hz, H-3 of Man*p*), 5.98 (dd, 1H, $J_{2,3} = J_{3,4} = 6.1$ Hz, H-3 of Xyl*p*), 5.85 (dd, 1H, $J_{2,3} = J_{3,4} = 8.0$ Hz, H-3 of Xyl*p*), 5.81 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.66 (m, 1H, H-4 of Xyl*p*), 5.62 (dd, 1H, $J_{1,2} = 7.2$ Hz, $J_{2,3} = 8.0$ Hz, H-2 of Xyl*p*), 5.56 (dd, 1H, $J_{1,2} = 4.0$ Hz, $J_{2,3} = 6.1$ Hz, H-2 of Xyl*p*), 5.53 (dd, 1H, H-2 of Man*p*), 5.50 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1 of Xyl*p*), 5.40 (m, 1H, H-4 of Xyl*p*), 5.40 (s, 1H, H-1 of Man*p*), 5.35 (s, 1H, H-1 of Man*p*), 5.24 (dd, 1H, H-2 of Man*p*), 4.77 (d, 1H, $J_{1,2} = 7.2$ Hz, H-1 of Xyl*p*), 4.55 (s, 1H, H-1 of Man*p*), 4.95–3.45 (m, 17H, H-5 of Xyl*p*, H-2, 3, 4, 5, 6 of Man*p*), 3.15 (s, 3H, OCH_3), 2.16 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.1 (*C*OCH$_3$), 166.1, 166.0, 165.9, 165.7, 165.6, 165.4, 165.3, 165.3, 165.2, 164.8, 164.5, 164.5, 164.3, 5 (13C, 13*C*OPh), 102.8, 99.9, 98.2, 98.2, 97.5 (5C, 5C-1), 77.1, 77.1, 76.1, 74.7, 72.3, 71.6, 71.2, 70.5, 70.4, 70.0, 69.8, 69.6, 69.4, 69.0, 68.9, 68.7, 68.5, 66.9, 63.9, 63.3, 62.5, 62.1, 60.3 (C-2 to C-6), 54.9 (O*C*H$_3$), 20.0 (CO*C*H$_3$). Anal. Calcd for $C_{122}H_{104}O_{38}$: C, 67.28; H, 4.78. Found: C, 67.18; H, 4.81.

4.25. Methyl 2,3,4,6-tetra-*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→4)]-6-*O*-benzoyl-α-D-mannopyranoside (29)

Deacetylation of **28** (1.00 g, 0.46 mmol) under the same conditions as that used for preparation of **20** from **19** gave the crude product, which was purified by flash chromatography (1:1 petroleum ether–EtOAc) to furnish **29** (390 mg, 42%) as an amorphous solid. $[\alpha]_D$ +17.2 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.11–6.89 (m, 65H, 13*Ph*), 6.14 (dd, 1H,

$J_{3,4} = J_{4,5} = 10.0$ Hz, H-4 of Man*p*), 5.95 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 10.0$ Hz, H-3 of Man*p*), 5.67 (dd, 1H, $J_{2,3} = 4.5$ Hz, $J_{3,4} = 5.3$ Hz, H-3 of Xyl*p*), 5.58 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4 of Man*p*), 5.55 (dd, 1H, $J_{2,3} = 4.5$ Hz, $J_{3,4} = 5.3$ Hz, H-3 of Xyl*p*), 5.51 (dd, 1H, $J_{1,2} = J_{2,3} = 5.1$ Hz, H-2 of Xyl*p*), 5.44 (dd, 1H, H-2 of Man*p*), 5.30 (m, 1H, H-4 of Xyl*p*), 5.26 (dd, 1H, $J_{1,2} = J_{2,3} = 4.5$ Hz, H-2 of Xyl*p*), 5.11 (m, 1H, H-4 of Xyl*p*), 5.04 (d, 1H, $J_{1,2} = 1.1$ Hz, H-1 of Man*p*), 5.01 (d, 1H, $J_{1,2} = 0.6$ Hz, H-1 of Man*p*), 5.02 (d, 1H, $J_{1,2} = 4.5$ Hz, H-1 of Xyl*p*), 4.53 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1 of Man*p*), 4.50 (dd, 1H, $J_{2,3} = 2.9$ Hz, $J_{3,4} = 9.9$ Hz, H-3 of Man*p*), 4.44 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1 of Xyl*p*), 4.42–3.86 (m, 18H, H-5 of Xyl*p*, H-2, 3, 4, 5, 6 of Man*p*), 3.16 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 166.0, 166.0, 165.6, 165.6, 165.4, 165.4, 165.4, 165.3, 164.9, 164.9, 164.7, 164.5 (13C, 13Ph*CO*), 102.5, 100.1, 99.6, 98.8, 98.1 (5C, 5C-1), 76.1, 75.2, 72.1, 71.6, 70.6, 70.3, 70.0, 69.9, 69.6, 69.5, 69.3, 69.2, 69.1, 68.7, 66.9, 65.5, 64.2, 63.0, 62.9, 62.3, 60.7 (C-2 to C-6), 54.9 (O*C*H_3). Anal. Calcd for $C_{120}H_{102}O_{37}$: C, 67.48; H, 4.78. Found: C, 67.62; H, 4.70.

4.26. Methyl 2,3,4,6-tetra-*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)-[methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1→2)][2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)]-6-*O*-benzoyl-α-D-mannopyranoside (30)

To a cooled solution (0 °C) of **29** (200 mg, 0.11 mmol), **21** (96 mg, 0.26 mmol), and 2,4-lutidine (7 μL, 0.07 mmol) in anhyd CH_2Cl_2 (20 mL), was added silver triflate (50 mg, 0.26 mmol). The mixture was stirred at this temperature for 6 h, TLC (1:1.5 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was concentrated to dryness. Purification of the residue by flash column chromatography (1:1.2 petroleum ether–EtOAc) gave **30** (208 mg, 76%) as an amorphous solid. $[\alpha]_D$ −66.7 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24–6.80 (m, 65H, 13Ph*H*), 6.25 (dd, 1H, $J_{1,2} = J_{2,3} = 7.7$ Hz, H-2 of Xyl*p*), 6.11 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4 of Man*p*), 5.74 (dd, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.21 (d, 1H, $J_{1,2} = 1.1$ Hz, H-1 of Man*p*), 5.13 (d, 1H, $J_{1,2} = 0.8$ Hz, H-1 of Man*p*), 5.11 (d, 1H, $J_{1,2} = 1.1$ Hz, H-1 of Man*p*), 4.80 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1 of Xyl*p*), 4.73 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1 of Xyl*p*), 4.50 (dd, 1H, H-2 of Man*p*), 4.33 (dd, 1H, $J_{1,2} = 1.1$ Hz, $J_{2,3} = 3.2$ Hz, H-2 of Man*p*), 4.14 (dd, 1H, $J_{1,2} = 0.8$ Hz, $J_{2,3} = 2.8$ Hz, H-2 of Man*p*), 4.06 (d, 1H, $J_{1,2} = 7.2$ Hz, H-1 of Glc*p*A), 3.55 (s, 3H, COOCH_3), 3.28 (s, 3H, OCH_3), 2.14, 2.12, 2.01 (3s, 9H, 3COCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 171.2, 171.0, 169.5, 169.4 (4C, 3*C*OCH$_3$, *C*OOMe), 167.2, 167.2, 166.1, 166.1, 165.8, 165.4, 165.4, 165.3, 165.3, 165.2, 165.1, 165.1, 164.2 (13C, 13*C*OPh), 102.6, 100.6, 100.6, 99.6, 98.9, 98.2 (6C, 6C-1), 54.9 (O*C*H_3), 52.6 (COO*C*H_3), 20.5, 20.4, 20.1 (3C, 3CO*C*H_3). Anal. Calcd for $C_{133}H_{118}O_{46}$: C, 65.13; H, 4.85. Found: C, 65.32; H, 4.90. Negative-ESI MS calcd for $C_{133}H_{118}O_{46}$: [M] 2450.2. Found: [M+Na] 2472.7. [M+K] 2488.6.

4.27. Methyl α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[(β-D-glucopyranosyluronic acid)-(1→2)][β-D-xylopyranosyl-(1→4)]-α-D-mannopyranoside, ammonium salt (31)

Hexasaccharide **30** (245 mg, 0.10 mmol) was dissolved in a satd methanolic ammonia (50 mL). After 96 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 **31** (63 mg, 64.0%) as an amorphous solid. $[\alpha]_D$ +56.9 (*c* 0.5, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.00 (s, 1H, H-1, Man*p*), 4.94 (s, 1H, H-1, Man*p*), 4.63 (s, 1H, H-1, Man*p*), 4.50 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1, Glc*p*A), 4.38 (d, 1H, $J_{1,2} = 8.8$ Hz, H-1, Xyl*p*), 4.20 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1, Xyl*p*), 3.20 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, D_2O): 173.9 (–*C*OONH$_4$), 103.5, 103.5, 102.7, 100.6, 99.1, 97.0 (6C, 6C-1), 78.4, 78.2, 76.2, 76.1, 76.0, 75.7, 74.1, 73.5, 73.4, 72.8, 72.2, 70.5, 70.2, 69.4, 66.9, 66.5, 66.2, 65.3, 65.3, 61.5, 60.4, 60.4 (C-2 to C-6), 55.0 (O*C*H_3). MALDI-TOF MS calcd for the ammonium salt of **31**, $C_{35}H_{61}O_{30}N$: 975.8 [M]. Found: 975.8 (M); 980.9 ($M - NH_4^+ + Na^+$).

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References and notes

1. Evans, E. E. *Proc. Soc. Exp. Biol. Med.* **1949**, *71*, 644–652.
2. Wilson, D. E.; Bennett, G. E.; Bailey, J. W. *Proc. Soc. Exp. Biol. Med.* **1968**, *127*, 820–827.
3. (a) Bhattacharjee, A. K.; Kwon-Chung, K. J.; Glaudemans, C. P. J. *Carbohydr. Res.* **1979**, *73*, 183–192; (b) Bhattacharjee, A. K.; Kwon-Chung, K. J.; Glaudemans, C. P. J. *Carbohydr. Res.* **1981**, *95*, 237–245; (c) Bhattacharjee, A. K.; Kwon-Chung, K. J.; Glaudemans, C. P. J. *Carbohydr. Res.* **1980**, *82*, 103–111; (d) Bhattacharjee, A. K.; Kwon-Chung, K. J.; Glaudemans, C. P. J. *Mol. Immunol.* **1979**, *16*, 531–540.
4. (a) Zhang, J.; Kong, F. *Tetrahedron Lett.* **2003**, 1839–1850; (b) Zhang, J.; Kong, F. *Bioorg. Med. Chem.* **2003**, *11*, 4027–4037; (c) Zhang, J.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 1719–1725.
5. Zhao, W.; Kong, F. *Carbohydr. Res.* **2004**, *339*, 1779–1786.
6. (a) Garegg, P. J.; Olsson, L.; Oscarson, S. *J. Carbohydr. Chem.* **1993**, *12*, 195–203; (b) Garegg, P. J.; Olsson, L.; Oscarson, S. *Bioorg. Med. Chem.* **1996**, *4*, 1867–1878.
7. Zegelaar-Jaarsveld, K.; Smits, S. A. W.; van der Marel, G. A.; van Boom, J. H. *Bioorg. Med. Chem.* **1996**, *4*, 1819–1823.
8. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–125.
9. (a) Byramova, N. E.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, c8; (b) Zhu, Y.; Kong, F. *Chin. J. Chem.* **2001**, *19*, 119–123.