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Synthesis of a mannose nonasaccharide existing in the exopolysaccharide of *Cryphonectria parasitica*

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Abstract

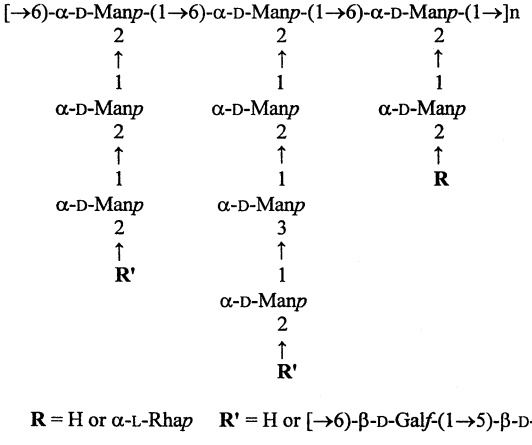
α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)[α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)]-α-D-Man*p*-(1→6)-[α-D-Man*p*-(1→2)]-α-D-Man*p*, existing in the exopolysaccharide of *Cryphonectria parasitica* was synthesized as its allyl glycoside in a regio- and stereoselective manner.

Keywords: Oligosaccharide; Mannose; Synthesis

1. Introduction

Exopolysaccharides (EPSs) of fungi are thought to be agents of phototoxicity and can play a role in plant–fungal interactions.[1] *Cryphonectria parasitica* (Murr.) Barr is the causal agent of chestnut blight,[2] which is characterized by the formation of a 'gelatinous zone' beyond the advancing edge of the mycelium. The structure of a new EPS from the virulent strain of *C. parasitica* was elucidated[3] by means of 2D NMR spectroscopy and mild hydrolysis and acetolysis. The polysaccharide has a rather complex structure that can be idealized as follows:

As an ongoing project for investigation of the structure–function relationships of oligosaccharides, we have reported the synthesis of a variety of biologically important oligosaccharides, such as (1→3)-branched (1→6)-linked glucans,[4] (1→6)-branched (1→3)-linked glucans,[5] (1→2)-branched (1→6)-linked mannans,[6] 3,6-branched mannans[7] etc. Now we present the synthesis of the mannose nonasaccharide existing in the idealized structure of the EPSs of *C. parasitica*.



2. Results and discussion

As outlined in Scheme 1, selective acetylation of allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**) with acetyl chloride in pyridine gave allyl 3-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**2**) in satisfactory yield (91%), and no acetyl migration was observed. Meanwhile, selective glycosylation of **1** with perbenzoylated mannosyl trichloroacetimidate afforded the (1→3)-linked disaccharide **7** (76%). The regioselectivity was confirmed by benzoylation of **7** to give **8**, and the ^{1}H

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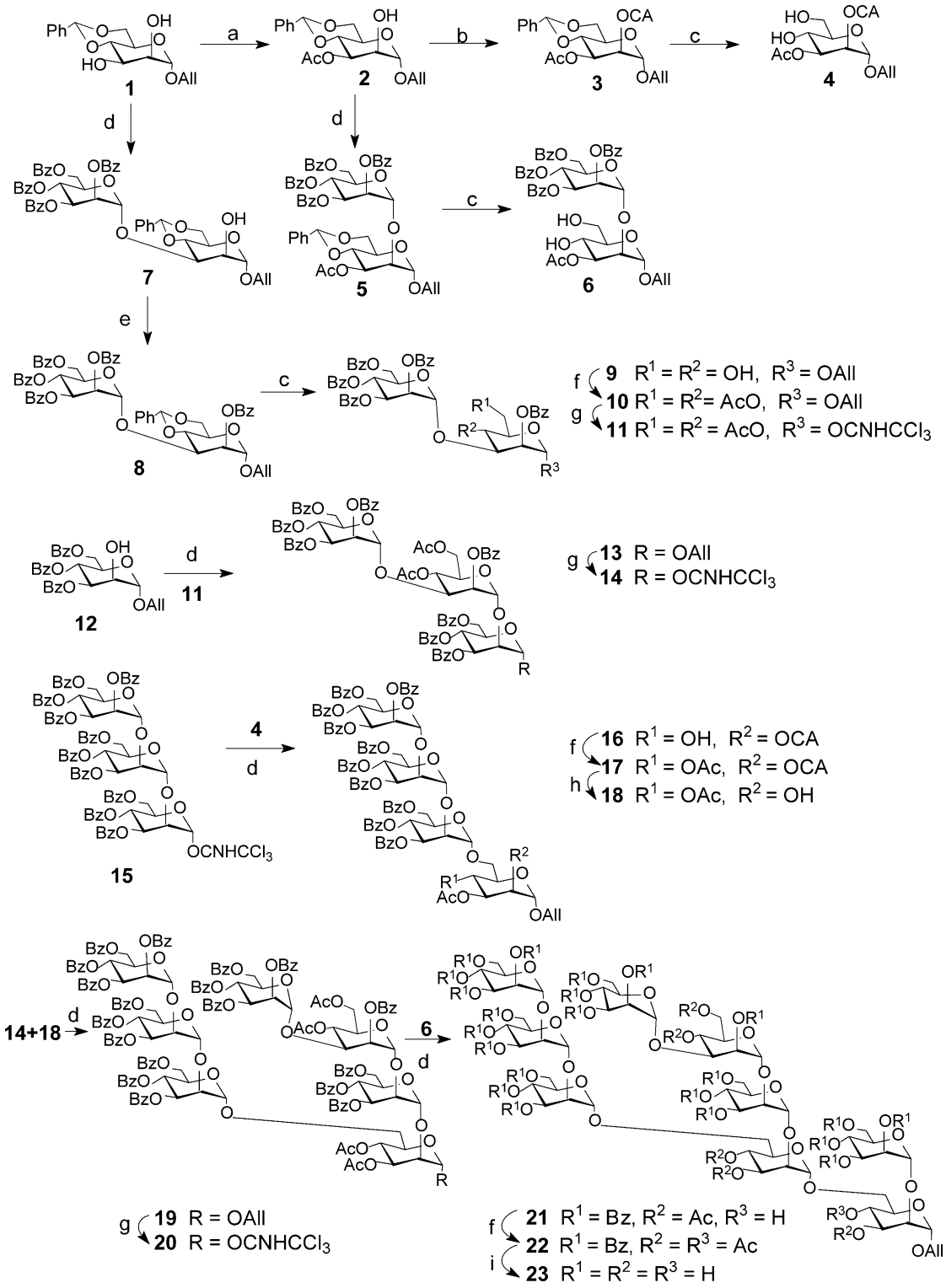

Scheme 1. (a) CH_3COCl, CH_2Cl_2, pyridine; (b) $ClCH_2COCl$, CH_2Cl_2, pyridine; (c) 90% TFA, 2 h, rt; (d) TMSOTf, CH_2Cl_2; (e) BzCl–pyridine (dry); (f) Ac_2O–pyridine (dry); (g) $PdCl_2$, 90% HOAc–NaOAc, rt, 12 h; then Cl_3CN, DBU, CH_2Cl_2 2–4 h; (h) thiourea in EtOH–CH_2Cl_2; (i) satd NH_3–MeOH, rt, 72 h.

NMR spectrum of **8** showed two characteristic signals for H-2 and H-2′ at δ 5.63 ($J_{1,2}$ 1.4, $J_{2,3}$ 3.2 Hz) and 5.76 ($J_{1,2}$ 0.9, $J_{2,3}$ 2.8 Hz) ppm, respectively. Debenzylidenation of **8**, followed by acetylation, furnished **10** (82% for two steps). Subsequent deallylation and trichloroacetimidation yielded the disaccharide donor **11** (83% for two steps). Condensation of **11** with allyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (**12**) gave a trisaccharide

13. Reiteration of the deallylation and trichloroacetimidation transformed **13** to the trisaccharide donor **14**, which was used to build one side chain. For construction of the other side chain, **2** was chloroacetylated to afford **3**, and subsequent debenzylidenation furnished the monosaccharide acceptor **4**. Selective 6-O-glycosylation[8] of **4** with perbenzoylated (1→2)-linked trisaccharide donor **15**[7b] gave (1→6)-linked tetrasaccharide **16** (88%). The selective 6-O-glycosylation was verified by acetylation of **16** to furnish **17**, and the ^{1}H NMR spectrum of **17** showed a newly emerged signal at δ 5.45 with $J_{3,4} = J_{4,5} = 9.9$ Hz for H-4 compared to **16**. Subsequent dechloroacetylation of **17** with thiourea gave the tetrasaccharide acceptor **18** (84%). Condensation of **18** with **14** was carried out smoothly giving the heptasaccharide **19** (68%). Deallylation of **19**, followed by trichloroacetimidation, afforded the heptasaccharide donor **20** (78%). For completion of the assembly of the nonasaccharide, another disaccharide unit was prepared. Thus, coupling of **2** with perbenzoylated mannosyl trichloroacetimidate furnished the disaccharide **5**, and subsequent debenzylidenation afforded the disaccharide acceptor **6** (86%). The nonasaccharide **21** was readily obtained by 6-O-selective glycosylation of the disaccharide acceptor **6** with the heptasaccharide donor **20** (75%). Acetylation of **21** gave **22**, whose ^{1}H spectrum showed one more signal, compared to **21**, at δ 5.32 ppm with $J_{3,4} = J_{4,5} = 9.9$ Hz for H-4, confirming the selective 6-O-glycosylation. Deacylation of **22** was carried out in a saturated solution of ammonia in methanol giving the target nonasaccharide **23**, and the ^{1}H and ^{13}C NMR spectra of **23** showed characteristic signals such as at δ 5.16, 5.16, 5.14, 5.01, 4.99, 4.98, 4.96, 4.91, and 4.88 for 9 H-1s, and δ 104.9, 104.8, 104.8, 104.7, 103.2, 103.2, 100.7, 100.6, 100.2 for 9 C-1s, and δ 81.39, 81.38, 81.37, 81.02, 81.01, and 80.51 for glycosylated 5 C-2s and 1 C-3. Since only six signals were found at δ 80–82, and the rest of the C-2 to C-6 signals were all at $< \delta$ 76, the 6-O-glycosylation of **6** was again confirmed by this; otherwise, if C-4 were glycosylated, a signal at $\delta > 80$ would have appeared.

In summary, a complex branched mannose nonasaccharide was synthesized in a regio- and stereoselective way by a simple procedure. Large-scale preparations should be possible with this method.

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. ^{1}H, ^{13}C, and 2D NMR spectra were recorded with Varian XL-400 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 electrospray-ionization (ESI) mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) sulfuric acid in MeOH 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), differential refractometer (132-RI Detector), 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 Py (8.1 mL, 100 mmol), then under N_2 protection, acetyl chloride (0.8 mL, 11 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise to the solution 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: $[\alpha]_D$ −58.7° (*c* 1.0, $CHCl_3$); ^{1}H 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 Hz, $J_{3,4}$ 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 Hz, $J_{2,3}$ 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, C*H*$_3$CO). Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.70; H, 6.33. Found: C, 61.54; H, 6.24.

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

Compound **2** (3.50 g, 10 mmol) was dissolved in dry CH_2Cl_2 (40 mL) containing Py (8.1 mL, 100 mmol),

then under N_2 protection, chloroacetyl chloride (0.9 mL, 11 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise to the solution within 30 min at 0 °C. The reaction mixture was slowly raised to 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 **3** (3.88 g, 91.1%) as a syrup: $[\alpha]_D$ +29.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.35 (m, 5 H, Ph*H*), 5.91 (m, 1 H, CH_2=C*H*CH_2O), 5.58 (s, 1 H, PhC*H*O_2), 5.36 (dd, 1 H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.42 (dd, 1 H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.5 Hz, H-2), 5.34 (m, 1 H, CH_2=CHCH_2O), 5.27 (m, 1 H, CH_2=CHCH_2O), 4.85 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.30 (dd, 1 H, *J* 4.2, 10.5 Hz, H-6a), 4.23 (m, 1 H, CH_2=CHCH_2O), 4.18, 4.16 (ABq, 2 H, *J* 15.2 Hz, ClCH_2COO), 4.06–3.98 (m, 3 H), 3.84 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 2.03 (s, 3 H, CH_3CO). Anal. Calcd for $C_{20}H_{23}ClO_8$: C, 56.27; H, 5.43. Found: C, 56.50; H, 5.31.

3.4. Allyl 3-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranoside (4)

Compound **3** (4.26 g, 10 mmol) was dissolved in 90% TFA (50 mL), and the mixture was stirred for 2 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with toluene (200 mL) and concentrated in vacuo directly. The residue was passed through a short silica gel column with 1:1 petroleum ether–EtOAc as the eluent to give **4** (3.02 g, 89.1%) as a foamy solid: $[\alpha]_D$ −93.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.87 (m, 1 H, CH_2=C*H*CH_2O), 5.30 (m, 1 H, CH_2=CHCH_2O), 5.29 (dd, 1 H, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 3.1 Hz, H-2), 5.24 (m, 1 H, CH_2=CHCH_2O), 5.22 (dd, 1 H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.8 Hz, H-3), 4.85 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.20 (m, 1 H, CH_2=CHCH_2O), 4.13, 4.12 (ABq, 2 H, *J* 15.2 Hz, ClCH_2COO), 4.03–3.97 (m, 2 H), 3.89–3.87 (m, 2 H), 3.76 (m, 1 H), 2.07 (s, 3 H, CH_3CO). Anal. Calcd for $C_{13}H_{19}ClO_8$: C, 46.09; H, 5.65. Found: C, 46.30; H, 5.38.

3.5. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (5)

To a cooled solution (−20 °C) of **2** (3.50 g, 10 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (8.14 g, 11 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (18 μL, 0.1 mmol). The mixture was stirred at this temperature for 2 h and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue that was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give disaccharide **5** (7.53 g, 81.1%) as a syrup: $[\alpha]_D$ −39.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.29 (m, 25 H, Ph*H*), 6.14 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4′), 5.97 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.0 Hz, H-3′), 5.84 (m, 1 H, CH_2=C*H*CH_2O), 5.29 (dd, 1 H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.3 Hz, H-2′), 5.71 (s, 1 H, PhC*H*O_2), 5.42 (dd, 1 H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.29 (m, 1 H, CH_2=CHCH_2O), 5.21 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1′), 5.19 (m, 1 H, CH_2=CHCH_2O), 5.01 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 2.17 (s, 3 H, CH_3CO). Anal. Calcd for $C_{52}H_{48}O_{16}$: C, 67.23; H, 5.21. Found: C, 67.08; H, 5.50.

3.6. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3-*O*-acetyl-α-D-mannopyranoside (6)

Compound **5** (4.64 g, 5 mmol) was dissolved in 90% TFA (50 mL), and the mixture was stirred for 2 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with toluene (200 mL) and concentrated in vacuo directly. The residue was passed through a short silica gel column with 2:1 petroleum ether–EtOAc as the eluent to give **6** (3.59 g, 85.5%) as a foamy solid: $[\alpha]_D$ −55.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.28 (m, 20 H, Ph*H*), 5.97 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4′), 5.91 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.8 Hz, H-3′), 5.85 (m, 1 H, CH_2=C*H*CH_2O), 5.70 (dd, 1 H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.2 Hz, H-2′), 5.22 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1′), 5.21 (dd, 1 H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.17–5.10 (m, 2 H, CH_2=CHCH_2O), 5.03 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 2.22 (s, 3 H, CH_3CO). Anal. Calcd for $C_{45}H_{44}O_{16}$: C, 64.28; H, 5.27. Found: C, 64.44; H, 5.20.

3.7. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-α-D-mannopyranoside (7)

To a cooled solution (−20 °C) of **1** (3.08 g, 10 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichlroacetimidate (8.14 g, 11 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (18 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue that was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give disaccharide **7** as a syrup (6.76 g, 76.3%): $[\alpha]_D$ −33.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.19 (m, 25 H, Ph*H*), 6.08 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4′), 5.98 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.0 Hz, H-3′), 5.88 (dd, 1 H, $J_{1,2}$ 0.6 Hz, $J_{2,3}$ 3.3 Hz, H-2′), 5.82 (m, 1 H, CH_2=C*H*CH_2O), 5.67 (s, 1 H, PhC*H*O_2), 5.62 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1′), 5.29 (m, 1 H,

CH_2=$CHCH_2O$), 5.18 (m, 1 H, CH_2=$CHCH_2O$), 4.92 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1). Anal. Calcd for $C_{50}H_{46}O_{15}$: C, 67.71; H, 5.23. Found: C, 67.49; H, 5.40.

3.8. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (8)

To a solution of **7** (4.43 g, 5.0 mmol) in Py (20 mL) was added benzoyl chloride (1.2 mL, 10 mmol). The reaction mixture was stirred at rt for 12 h and then quenched with MeOH (2.0 mL). The solvents were evaporated and coevaporated with toluene in vacuo to give a residue that was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give disaccharide **8** as a syrup (4.36 g, 88.1%): $[\alpha]_D$ −63.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.21 (m, 30 H, Ph*H*), 6.06 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4′), 5.85 (m, 1 H, CH_2=C*H*CH_2O), 5.76 (dd, 1 H, $J_{1,2}$ 0.9 Hz, $J_{2,3}$ 2.8 Hz, H-2), 5.73 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.9 Hz, H-3′), 5.71 (s, 1 H, PhC*H*O_2), 5.63 (d, 1 H, $J_{1,2}$ 1.4 Hz, $J_{2,3}$ 3.2 Hz, H-2′), 5.48 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1′), 5.30 (m, 1 H, CH_2=$CHCH_2O$), 5.22 (m, 1 H, CH_2=$CHCH_2O$), 5.04 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1). Anal. Calcd for $C_{57}H_{50}O_{16}$: C, 69.08; H, 5.09. Found: C, 68.94; H, 5.22.

3.9. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranoside (10)

Compound **8** (4.45 g, 5 mmol) was dissolved in 90% TFA (50 mL), and the mixture was stirred for 2 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with toluene (200 mL) and concentrated in vacuo directly. The residue was passed through a short silica gel column with 2:1 petroleum ether–EtOAc as the eluent to give **9** as a foamy solid. Compound **9** was dissolved in Py (50 mL), then Ac_2O (20 mL, 20 mmol) was added. The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, and then the residue was purified by flash column chromatography on a silica gel column (2:1 petroleum ether–EtOAc) to give compound **10** (4.06 g, 82.4% for two steps) as a foamy solid: $[\alpha]_D$ −51.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–7.22 (m, 25 H, Ph*H*), 6.07 (dd, 1 H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4′), 5.84 (m, 1 H, CH_2=C*H*CH_2O), 5.68 (dd, 1 H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.8 Hz, H-3′), 5.61 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 5.58 (d, 1 H, $J_{1,2}$ 0.6 Hz, $J_{2,3}$ 3.0 Hz, H-2′), 5.48 (d, 1 H, $J_{1,2}$ 1.0 Hz, $J_{2,3}$ 3.1 Hz, H-2), 5.31 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1′), 5.27 (m, 1 H, CH_2=$CHCH_2O$), 5.18 (m, 1 H, CH_2=$CHCH_2O$), 5.06 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 2.25 (s, 3 H, C*H*$_3$CO), 2.17 (s, 3 H, C*H*$_3$CO). Anal. Calcd for $C_{54}H_{50}O_{18}$: C, 65.71; H, 5.11. Found: C, 65.90; H, 5.00.

3.10. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (11)

To a solution of **10** (4.93 g, 5 mmol) in 90% HOAc (50 mL) containing AcONa (1.47 g, 15 mmol) was added $PdCl_2$[9] (270 mg, 2.5 mmol), and the mixture was stirred for 12 h, at the end of which time TLC (3:1 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 $NaHCO_3$. The organic layer was concentrated, and the residue was passed through a short silica gel column with 2:1 petroleum ether–EtOAc as the eluent to give crude 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α,β-D-mannopyranose as a syrup. Dried under high vacuum for 2 h, the syrup was dissolved in CH_2Cl_2 (50 mL), and CCl_3CN (2.5 mL, 25 mmol) and DBU (80 μL, 0.6 mmol) were added.[10] The reaction mixture was stirred for 12 h, at the end of which time TLC (3:1 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 3:1 petroleum ether–EtOAc as the eluent, furnished the disaccharide donor **11** (4.53 g, 83.1%) as a foamy solid: $[\alpha]_D$ −38.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1 H, CN*H*CCl_3), 8.26–7.25 (m, 25 H, Ph*H*), 6.52 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 6.14 (dd, 1 H, $J_{3,4}=J_{4,5}=10.1$ Hz, H-4′), 5.77 (dd, 1 H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.2 Hz, H-2′), 5.74 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.69 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.1 Hz, H-3′), 5.53 (d, 1 H, $J_{1,2}$ 1.0 Hz, $J_{2,3}$ 3.1 Hz, H-2), 5.37 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1′), 2.29 (s, 3 H, C*H*$_3$CO), 2.14 (s, 3 H, C*H*$_3$CO). Anal. Calcd for $C_{53}H_{46}Cl_3NO_{18}$: C, 58.33; H, 4.25. Found: C, 58.54; H, 4.22.

3.11. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (13)

To a cooled solution (−20 °C) of **12** (1.60 g, 3 mmol) and **11** (3.60 g, 3.3 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (18 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give trisaccharide **13** as a syrup (3.25 g, 74.2%): $[\alpha]_D$ −40.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–7.24 (m, 40 H, Ph*H*), 6.17 (dd, 1 H, $J_{3,4}=J_{4,5}=$

10.4 Hz, H-4), 5.96 (m, 1 H, CH_2=C*H*CH_2O), 5.95 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.83 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.73 (d, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.68 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.9 Hz, H-3), 5.61 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.58 (d, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 2.9 Hz, H-2), 5.37 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.33 (m, 1 H, CH_2=CHCH_2O), 5.26 (m, 1 H, CH_2=CHCH_2O), 5.22 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.17 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 2.21 (s, 3 H, CH_3CO), 2.13 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.2 (2 C, 2 CH_3*C*O), 166.2, 166.0, 165.8, 165.5, 165.5, 165.4, 165.2, 165.1 (8 C, 8 Ph*CO*), 118.4 (*C*H_2=CHCH_2O), 99.6, 99.3, 97.8 (3 C, 3 C-1), 77.6, 76.3, 71.4, 71.0, 70.4, 69.9, 69.8, 69.7, 69.1, 68.9, 67.5, 67.2, 66.1, 63.6, 62.7, 62.1 (16 C, C-2–6, CH_2=CH*C*H_2O), 20.8, 20.8 (2 C, 2 *C*H_3CO). Anal. Calcd for $C_{81}H_{72}O_{26}$: C, 66.57; H, 4.97. Found: C, 66.74; H, 5.20.

3.12. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (14)

Compound **13** (1.16 g, 0.8 mmol) was deallylated and subsequently trichloroacetimidated under the same conditions as those that were used for the preparation of **11** from **9**, giving **14** (1.07 g, 85.6%) as a foamy solid: $[\alpha]_D$ −30.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1 H, CN*H*CCl_3), 8.14–7.25 (m, 40 H, Ph*H*), 6.60 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 6.17 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 6.08 (dd, 1 H, $J_{3,4} = J_{4,5} =$ 9.9 Hz, H-4), 5.85 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.72 (d, 1 H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.3 Hz, H-2), 5.68 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.9 Hz, H-3), 5.62 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.59 (d, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.0 Hz, H-2), 5.35 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.30 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 2.20 (s, 3 H, CH_3CO), 2.11 (s, 3 H, CH_3CO). Anal. Calcd for $C_{80}H_{68}Cl_3NO_{26}$: C, 61.37; H, 4.38. Found: C, 61.50; H, 4.22.

3.13. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranoside (16)

Compound **4** (676 mg, 2.0 mmol) and **15** (3.38 g, 2.0 mmol) were coupled under the same conditions as those that were used for the preparation of **13** from **11** and **12**, giving **16** as a foamy solid (3.30 g, 88.5%): $[\alpha]_D$ −26.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–7.15 (m, 50 H, Ph*H*), 6.05 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.97 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.97–5.90 (m, 4 H), 5.78 (dd, 1 H, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 9.7 Hz, H-3), 5.77 (d, 1 H, $J_{1,2}$ 0.8 Hz, $J_{2,3}$ 3.1 Hz, H-2), 5.41 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.77 (d, 1 H, $J_{2,3}$ 1.0 Hz, $J_{2,3}$ 2.9 Hz, H-2), 5.32–5.22 (m, 2 H, CH_2=CHCH_2O), 5.28 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.99 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.93 (d, 1 H, $J_{1,2}$ 0.5 Hz, H-1), 4.11, 4.09 (ABq, 2 H, *J* 15.3 Hz, ClCH_2COO), 2.03 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0 (CH_3*C*O), 166.9, 166.6, 166.5, 165.9, 165.8, 165.6, 165.5, 165.4, 165.4, 165.1, 164.9 (11 C, 10 Ph*CO*, $ClCH_2$*C*O), 118.4 (*C*H_2=CHCH_2O), 100.6, 99.7, 98.4, 96.5 (4 C, 4 C-1), 72.3, 72.1, 71.4, 71.1, 70.6, 70.2, 69.8, 69.7, 69.6, 68.7, 68.7, 68.0, 67.5, 66.7, 65.6, 65.6, 65.5, 64.0, 63.7, 62.8, 60.5 (21 C, C-2–6, CH_2=CH*C*H_2O), 29.6 ($ClCH_2$CO), 20.9 (*C*H_3CO). Anal. Calcd for $C_{101}H_{89}ClO_{33}$: C, 65.00; H, 4.81. Found: C, 65.14; H, 5.03.

3.14. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranoside (17)

To a solution of **16** (1.85 g, 1 mmol) in Py (20 mL) was added Ac_2O (10 mL, 10 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, and then the residue was purified by flash column chromatography on a silica gel column (2:1 petroleum ether–EtOAc) to give compound **17** (1.81 g, 94.8%) as a foamy solid: $[\alpha]_D$ −8.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02–7.11 (m, 50 H, Ph*H*), 6.08 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 6.02 (dd, 1 H, $J_{3,4} = J_{4,5} =$ 9.8 Hz, H-4), 6.01 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.95–5.88 (m, 3 H), 5.76–5.73 (m, 2 H), 5.45 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.44–5.35 (m, 2 H), 5.44 (d, 1 H, $J_{1,2}$ 0.5 Hz, H-1), 5.40 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.26 (m, 1 H, CH_2=CHCH_2O), 5.19 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.95 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.16, 4.07 (ABq, 2 H, *J* 15.1 Hz, ClCH_2COO), 2.04 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 169.7 (2 C, 2 CH_3*C*O), 167.0, 166.4, 166.4, 165.9, 165.7, 165.7, 165.5, 165.4, 165.4, 165.1, 164.9 (11 C, 10 Ph*CO*, $ClCH_2$*C*O), 118.7 (*C*H_2=CHCH_2O), 100.7, 99.7, 98.6, 96.2 (4 C, 4 C-1), 71.5, 71.4, 70.5, 70.2, 69.9, 69.7, 69.6, 69.6, 69.4, 69.0, 68.8, 68.8, 67.6, 67.2, 66.6, 66.2, 66.0, 63.8, 63.8, 63.7, 62.8 (21 C, C-2–6, CH_2=CH*C*H_2O), 29.7 ($ClCH_2$CO), 20.9, 20.8 (*C*H_3CO). Anal. Calcd for $C_{103}H_{91}ClO_{34}$: C, 64.83; H, 4.81. Found: C, 64.90; H, 5.02.

3.15. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3,4-di-*O*-acetyl-α-D-mannopyranoside (18)

To a solution of **17** (1.80 g, 0.94 mmol) in EtOH (25 mL)–CH_2Cl_2 (100 mL) was added thiourea (0.36 g), and

the mixture was refluxed for 16 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated. The residue was passed through a silica gel column with 3:1 petroleum ether–EtOAc as the eluent to give **18** (1.44 g, 83.7%) as a foamy solid: $[\alpha]_D$ −16.5° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.09–7.11 (m, 50 H, Ph*H*), 6.06 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 6.02 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.97–5.85 (m, 4 H), 5.76–5.73 (m, 2 H), 5.49 (dd, 1 H, $J_{3,4} = J_{4,5} =$ 9.9 Hz, H-4), 5.42 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 5.35–5.30 (m, 2 H), 5.28 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.19 (m, 1 H, C*H*$_2$=CHCH$_2$O), 4.93 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.91 (d, 1 H, H-1), 2.09 (s, 3 H, C*H*$_3$CO), 2.04 (s, 3 H, C*H*$_3$CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.8 (2 C, 2 CH$_3$*C*O), 166.6, 166.5, 166.1, 165.7, 165.6, 165.5, 165.4, 165.4, 165.1, 164.8 (10 C, 10 Ph*C*O), 118.1 (*C*H$_2$=CHCH$_2$O), 100.2, 99.7, 98.9, 98.7 (4 C, 4 C-1), 72.0, 71.2, 70.6, 70.2, 70.0, 69.9, 69.7, 69.5, 69.4, 68.9, 68.5, 68.5, 67.6, 67.5, 66.6, 66.5, 66.2, 63.9, 63.8, 62.8, 60.5 (21 C, C-2–6, CH$_2$=CH*C*H$_2$O), 21.1, 20.9 (*C*H$_3$CO). Anal. Calcd for $C_{101}H_{90}O_{33}$: C, 66.22; H, 4.95. Found: C, 65.94; H, 4.83.

3.16. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-acetyl-α-D-mannopyranoside (19)

Compound **14** (1.31 g, 0.84 mmol) and **18** (1.40 g, 0.76 mmol) were coupled under the same conditions as that used for the preparation of **13** from **11** and **12**, giving **19** (1.66 g, 67.5%) as a foamy solid: $[\alpha]_D$ −31.5° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), (some characteristic signals are given): δ 6.14 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 6.03 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.96 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.72 (dd, 1 H, $J_{1,2} = 1.0$ Hz, $J_{2,3} = 3.1$ Hz, H-2), 5.68 (dd, 1 H, $J_{2,3} = 3.1$ Hz, $J_{3,4} =$ 9.7 Hz, H-3), 5.59 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.55 (dd, 1 H, $J_{1,2} = 1.4$ Hz, $J_{2,3} = 3.2$ Hz, H-2), 5.48 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.46 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 5.37 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 5.33 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 5.23 (m, 1 H, C*H*$_2$=CHCH$_2$O), 5.15 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.06 (s, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 2.19 (s, 3 H, C*H*$_3$CO), 2.12 (s, 3 H, C*H*$_3$CO), 2.09 (s, 3 H, C*H*$_3$CO), 2.01 (s, 3 H, C*H*$_3$CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 170.2, 169.7 (4 C, 4 CH$_3$*C*O), 166.4, 166.3, 166.2, 165.9, 165.8, 165.7, 165.6, 165.5, 165.4, 164.8 (18 C, 18 Ph*C*O, some signals overlapped), 118.2 (*C*H$_2$=CHCH$_2$O), 100.3, 100.0, 99.7, 99.4, 99.2, 98.7, 97.9 (7 C, 7 C-1), 21.1, 20.9, 20.9, 20.8 (4 C, 4 *C*H$_3$CO). Anal. Calcd for $C_{179}H_{156}O_{58}$: C, 66.45; H, 4.86. Found: C, 66.64; H, 5.05.

3.17. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (20)

Compound **19** (1.50 g, 0.46 mmol) was deallylated and subsequently trichloroacetimidated under the same conditions as those used for the preparation of **11** from **10**, giving **20** (1.21 g, 78.1%) as a foamy solid: $[\alpha]_D$ −36.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), (some characteristic signals are given): δ 8.68 (s, 1 H, CN*H*CCL$_3$), 6.47 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 6.18 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.73 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.47 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.36 (d, 2 H, $J_{1,2}$ 1.0 Hz, 2 H-1), 5.14 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 4.91 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 2.17 (s, 3 H, C*H*$_3$CO), 2.16 (s, 3 H, C*H*$_3$CO), 2.10 (s, 3 H, C*H*$_3$CO), 2.01 (s, 3 H, C*H*$_3$CO). Anal. Calcd for $C_{178}H_{152}Cl_3NO_{58}$: C, 64.02; H, 4.59. Found: C, 64.30; H, 4.40.

3.18. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3-*O*-acetyl-α-D-mannopyranoside (21)

To a cooled solution (−20 °C) of **20** (1.00 g, 0.3 mmol) and **6** (0.50 g, 0.6 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (9 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue that was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give nonasaccharide **21** (898 mg, 74.8%) as a syrup: $[\alpha]_D$ −33.0° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, some characteristic signals are given): δ 6.17 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 6.13 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 6.08 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.57 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.48 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.36 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 5.32 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 5.29 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.24 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 5.18 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.03 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.99 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 2.16 (s, 3 H, C*H*$_3$CO), 2.09 (s, 3 H, C*H*$_3$CO), 2.07 (s, 3 H, C*H*$_3$CO), 1.99 (s, 3 H, C*H*$_3$CO), 1.81 (s, 3 H, C*H*$_3$CO); ^{13}C NMR

(100 MHz, $CDCl_3$): δ 171.6, 170.6, 170.1, 169.9, 169.8 (5 C, 5 CH_3CO), 166.9–164.9 (Ph*C*O, some signals overlapped), 118.0 (CH_2=CHCH$_2$O), 100.2, 100.0, 99.8, 99.6, 99.3, 99.1, 98.6, 98.1, 97.7 (9 C, 9 C-1), 22.8, 21.1, 21.0, 20.8, 20.6 (5 C, CH_3CO). Anal. Calcd for $C_{221}H_{194}O_{73}$: C, 66.06; H, 4.86. Found: C, 65.94; H, 5.01.

3.19. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-acetyl-α-D-mannopyranoside (22)

Compound **21** (161 mg, 0.04 mmol) was acetylated under the same conditions as that used for the preparation of **10** from **9**, giving **22** (145 mg, 89.5%) as a foamy solid: $[\alpha]_D$ −36.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, some characteristic signals are given): δ 6.19 (dd, 1 H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 6.12 (dd, 1 H, $J_{3,4}=J_{4,5}=9.7$ Hz, H-4), 6.10 (dd, 1 H, $J_{3,4}=J_{4,5}=9.7$ Hz, H-4), 6.07 (dd, 1 H, $J_{3,4}=J_{4,5}=9.7$ Hz, H-4), 5.78 (dd, $J_{1,2}=0.6$ Hz, $J_{2,3}=3.2$ Hz, H-2), 5.55 (d, 1 H, $J_{1,2}$ 0.5 Hz, H-1), 5.52 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.42 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.37 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.32 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 5.31 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.16 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 5.13 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 5.05 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 4.95 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 2.17 (s, 3 H, CH_3CO), 2.16 (s, 3 H, CH_3CO), 2.08 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 2.03 (s, 3 H, CH_3CO), 1.99 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 170.5, 170.3, 170.2, 169.9, 169.6 (6 C, 6 CH_3CO), 167.2–164.9 (Ph*C*O, some signals overlapped), 118.3 (CH_2=CHCH$_2$O), 100.3, 100.1, 99.8, 99.7, 99.3, 99.0, 98.8, 98.8, 97.7 (9 C, 9 C-1), 22.8, 20.9, 20.9, 20.8, 20.8, 20.4 (6 C, CH_3CO). Anal. Calcd for $C_{223}H_{196}O_{74}$: C, 65.97; H, 4.87. Found: C, 66.14; H, 5.10.

3.20. Allyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→2)]-α-D-mannopyranoside (23)

Compound **21** (700 mg, 0.17 mmol) was dissolved in a satd ammonia–MeOH solution (50 mL). After a week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **23** (181 mg, 68.6%) as a syrup: $[\alpha]_D$ +10.5° (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O, some characteristic signals are given): δ 5.83 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.22 (m, 1 H, OCH$_2$CH=CH_2), 5.16 (s, 1 H, H-1), 5.16 (s, 1 H, H-1), 5.14 (s, 1 H, H-1), 5.01 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.99 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.98 (s, 1 H, H-1), 4.96 (s, 1 H, H-1), 4.91 (s, 1 H, H-1), 4.89 (m, 1 H, OCH$_2$CH=CH_2), 4.88 (s, 1 H, H-1); ^{13}C NMR (100 MHz, D_2O): δ 121.2 (CH_2=CHCH$_2$O), 104.9, 104.8, 104.8, 104.7, 103.2, 103.2, 100.7, 100.6, 100.2 (9 C, 9 C-1), 81.39, 81.38, 81.37, 81.02, 81.01, 80.51. MALDI-TOF MS Calcd for $C_{57}H_{96}O_{46}$: 1517.34 [M]. Found: 1540.41 [M+Na].

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